Compilation of a series of presentations

delivered at the

IDF World Dairy Summit 2015,

Vilnius, Lithuania

Preamble

Please note that IDF has only received a series of abstracts and speeches delivered at the IDF World Dairy Summit 2015 in Vilnius, Lithuania. Consequently, IDF has decided not to publish these in the form of an IDF Bulletin. In this compilation, you will find the full programme and the full texts when provided by the presenters.

IDF FORUM

Chairman: Dr. Jeremy Hill, IDF President

11:00-11:05	Update on IDF Strategy Refresh Dr. Jeremy Hill, IDF President
11:05-11:20	Work Programme Strategy Michael Hickey, Chair of the IDF Science and Programme Coordination Committee (SPCC)
11:20-11:35	Dairy Safety and Quality Dr. Nico van Belzen, IDF Director General
11:35-11:50	Sustainability Brian Lindsay, SPCC Representative
11:50-12:05	Nutrition Mary Anne Burkman, SPCC Representative
12:05-12:20	Standards Jaap Evers, Sector Leader
12:20-12:30	Questions and Closing Remarks Dr. Jeremy Hill, IDF President

IDF WORLD DAIRY LEADERS FORUM *Fiat lactis:* improving nutrition security with dairy

Moderator: Dr. Nico van Belzen, IDF Director General

Keynote speaker (and panelist): H.E. Gerda Verburg, Chair of the UN Committee on World Food Security (CFS): The challenge of global food security and nutrition **(full text available)**

Introductory remarks by the panel: How is dairy contributing to nutrition security, from your perspective?

- Elaine Sun, CEO, Mengniu
- Rajni Sekhri Sibal, Ministary of Agriculture, Government of India
- Dennis Jönsson, CEO, Tetra Pak
- Dr. Berhe Tekola, Director of Animal Production and Health Division, FAO

GLOBAL DAIRY AGENDA FOR ACTION (GDAA) REPORTING SESSION

16:00-16:05 Chairman's introduction
Donald Moore, GDAA Chairman

16:05-16:15 Collaboration is key
Dr. Jeremy Hill, IDF President and DSF Governor

16:15-16:30 Where have we come from and where are we now?
Brian Lindsay, DSF Development Director

16:30-16:40 Why are we members and what are we doing?
Jaap Petraeus, Manager Corporate Sustainability, FrieslandCampina

16:40-16:50 Why are we members and what are we doing?
Tobie de Villiers, General Manager Milk Procurement, Clover Industries

16:50-17:00 An encompassing approach to DSF Membership
Helen Dornom, Manager Sustainability Including Food Safety and Integrity, Dairy Australia

17:00-17:10 Why are we members and what are we doing?
Jay Waldvogel, Senior Vice President of Strategy and International Development, Dairy Farmers of America

17:10-17:30 Panel discussion – By dairy for dairy
Dr. Jeremy Hill, Jaap Petraeus, Helen Dornom, Tobie de Villiers, Jay Waldvogel, Brian Lindsay, Facilitator – Donald Moore

DAIRY POLICIES AND ECONOMICS

Representative of the Local Organising Committee: Lilija Tepelienė, Ministry of Agriculture of the Republic of Lithuania (Lithuania)

Session 1 - Interdisciplinary Session: Global milk and changes in the dairy sector and forecast
Moderator: Gilles Froment / Canadian Dairy Commission, Standing Committee on Dairy Policies and Economics, FIL-IDF Canada (Canada)

9:00-9:05 Introduction
Gilles Froment
Canadian Dairy Commission, Standing Committee on Dairy Policies and Economics, FIL-IDF Canada (Canada)

9:05-9:30 Dairy sector in Lithuania: strengths and challenges
Indrė Genytė-Pikčienė
DNB Bank (Lithuania)

9:30-9:55 World Dairy Situation 2015 and Dairy Outlook
Veronique Pilet
CNIEL (France)

9:55-10:20 Overview of trade policy / agreement development.
Dr. Stephan Hubertus Gay
Organisation for Economic Co-operation and Development (UK)

10:20-10:30 Q&A session
Gilles Froment
Canadian Dairy Commission, Standing Committee Dairy Policies and Economics, FIL-IDF Canada (Canada)

Session 2 – Recent dairy policy developments

Moderator: Jurgen Jansen, Zuivel NL (The Netherlands)

11:00-11:25 EU (post-quota) dairy policies and the implementation of the "Milk package"
Joost Korte
European Commission (Belgium)

11:25-11:50 Managing risk in the continuing global dairy expansion
Kevin Bellamy
Rabobank (The Netherlands)

11:50-12:15 Dairy policy in India **(full text available)**
Status of the National Dairy Plan in India
Meenesh C. Shah / National Dairy Development Board (India)

12:15-12:30 Q&A session
Jurgen Jansen / ZuivelNL (The Netherlands)

Session 3 – The restructuring of the dairy sector

13:30 -13.50 Restructuring and investment trends in the dairy processing sector around the world
Benoit Rouyer
CNIEL (France)

13.50-14.10 Current status of milk production worldwide and expected development to 2025
Andriy Dykun
Association of Milk Producers (Ukraine)

14.30-14.50 Brazil in the balance between domestic and export market
Marcelo Pereira de Carvalho
MilkPoint – Information services (Brazil)

14.50 – 15.10 Current dairy situation in Russia
Mikhail Mishchenko
The Dairy News (Russia)

15:10- 15:30 Q&A Sessions
Dr. Edvardas Makelis
Lithuanian Agricultural Advisory Service (Lithuania)

Session 4: Dairy sector sustainability from an economic and policy perspective

16:00-16:25 Development of Polish dairy sector after accession to the European Union
Agricultural Market Agency (Poland) **(full text available)**

16:25 -16:50 Sustainable development of the dairy sector in the Sub-Saharan African **(full text available)**
Kobus Mulder
MULDER Consult (South Africa)

16:50 – 17:15 Social/economic sustainability in the dairy sector
Dr Andrew Novakovic
Cornell University (USA)

17:15 – 17:30 Q&A Session
Prof. Andrzej Babuchowski
Agriculture and Fisheries Section of the Republic of Poland to the EU (Poland)

Poster session

Moha. Abul Kalam Azad – Successful dairying in Banglaseh through mik vita

Dennis Bergmann – Price volatility transmission in, and between, skim milk powder and oil markets

Yiping Sun - Borderless Dairy Industry under Global Integration.

Luke York - Dairy policy and climate change – household impacts in Odisha, India.

Luke York - Adapting smallholder dairy to climate change – findings from Odisha, India.

Dr. Sonal Choudhary - The UK dairy industries: challenges and perspectives.

Prof. Dr. Júlio Cesar Valandro Soares – Speculations as to the impact of the ending of production quotas across the dairy industry in the European Union (EU); lesion from Brazil.

Marin Bozic – Would margin protection program for dairy producers work in the European Union?

NUTRITION AND HEALTH

Conference Manager: Laurence Rycken, International Dairy Federation (Belgium)
Representative of the Local Organising Committee: Prof. Habil. Dr. Dalia Sekmokienė, Lithuanian University of Health Sciences (Lithuania)

Session 1: The role of dairy products in under- and malnutrition

Moderators: Prof. Rimantas Stukas, Vilnius University (Lithuania) and Dr. Yvette Soustre, CNIEL (France)

9:00-9:10 Introduction-Dr. Stefanie Oude Elferink
Friesland Campina Innovation (The Netherlands)

9:10-9:30 Vulnerable people in developing countries
Dr. Inge Brouwer
Wageningen UR (The Netherlands)

9:30-9:50 Role of dairy foods to ameliorate malnutrition in developing countries
Dr. Marta van Loan / USDA
Western Human Nutrition Research Center (USA)

9:50-10:10 Dairy for development: how to increase availability, access and affordability of milk based products for poor consumers?
Charlotte Pedersen
The Global Alliance for Improved Nutrition (GAIN) (Denmark)

10:10-10:30 Q&A session

Session 2: Bioactive components in dairy: present and future

Moderators: Doc. Dr. Vaidotas Urbonas, Vilnius University (Lithuania) and Dr. Geoffrey Smithers, GWS Consulting Services Inc. (Australia)

11:00-11:30 Milk-derived bioactive peptides: potential health benefits and applications **(full text available)**
Dr. Anne Pihlanto
Natural Resources Institute Finland (Luke) (Finland)

11:30-11:40 Phylogenetic analysis and selection of lactic acid bacteria with enhanced antimicrobial activity for the manufacture of fermented products
Habil. Dr. Joana Šalomskienė
Kaunas University of Technology (Lithuania)

11:40-11:50 Exopolysaccharides of lactic acid bacteria: perspectives in fermented dairy products production
Prof. Inga Ciprovica
Latvia University of Agriculture (Latvia)

11:50-12:00 Preventive effects of *Lactobacillus gasseri* SBT2055 and *Lactobacillus helveticus* SBT2171 on the infectious and autoimmune diseases
Prof. Dr. Tadaaki Miyazaki

Hokkaido University (Japan)

12:00-12:10 Milk *vs* soybean polar lipids as emulsifier: faster lipid metabolism through greater emulsion lipolysis
Dr. Marie-Caroline Michalski
French National Institute for Agricultural Research (INRA) (France)

12:10-12:30 Q&A session

Session 3: Continuation – Bioactive components in dairy: present and future

Moderators: Habil. Dr. Joana Šalomskienė, Kaunas University of Technology (Lithuania) and Mary Anne Burkman, Dairy Council of California (USA)

14:00-14:15 The health effects of fermented milk products
Dr. Vaidotas Urbonas
Vilnius University (Lithuania)

14:15-14:30 Advance application of whey protein in development of nutritious dairy products with exceptional texture and mouthfeel for ageing population **(full text available)**
Dr. Anna Bannikova, Prof. Ivan Evdokimov
Saratov State Agrarian University, North Caucasus Federal University (Russia)

14:30-14:45 The physiology of lactoferrin **(full text available)**
Dr. Jean-Paul Perraudin
Taradon Laboratory (Belgium)

14:45-15:05 Bioactive components in dairy – discovery is only part of the journey
Dr. Geoffrey Smithers
GWS Consulting Services Inc. (Australia)

15:05-15:25 Q&A session

Session 4: Milk products and health through the ages

16:00-16:20 Place of dairy products in a holistic vision of the diet
Dr. Anthony Fardet
French National Institute for Agricultural Research (INRA) (France)

16:20-16:40 Dr. Partap Chauhan
BAMS, Ayurvedacharya Jiva Ayurveda (India)
Assoc. Prof. Goda Denapienė
Vilnius University (Lithuania)

16:40-17:00 Milk consumption among the adult population of Lithuania
Prof. Rimantas Stukas and Dr.Valerij Dobrovolskij
Vilnius University (Lithuania)

17:00-17:20 Dairy products development and its consumption in China
Prof. Guansheng Ma
School of Public Health, Peking University (China)

17:20-17:30 Concluding remarks Mary Anne Burkman
Dairy Council of California (USA)

Poster presentations

R.C. Gupta - Is goat milk superior for longevity, alleviating ageing and sound health? The inside happening Taurine factor.

Prof. Dr.Zubeyde Oner – The hypocholesterolemic effect of a probiotic's yeast in rats fed on a cholesterol-enriched diet.

Nissim Silanikove - Source, composition and potentiel applications of nanosized vesicles frome bovine whey.

Matieny Aicha Maiga – Impact of neonatal mono-colonization of balb/C mice with *Lactobacillus casei* on oral sensitization to cow's milk proteins.

Algirdas Liutkevičius – Fermented buttermilk based product, enriched by milk protein concentrate impact on human health. **(full text available)**

Ivana Hyrslova – The cholesterol-lowering effect of milk containing synbiotics in rats fed on a cholesterol-enriched diet.

Dr. Bjørn Petrat-Melin – Exploring the bioactive potential of purified bovine beta and kappa casein variants during in vitro digestion.

Dr. Marcus Persson - Improving the nutritional profile of traditional dairy products.

Agnes Fekete – Can milk and dairy consumption improve cardiovascular health? Association between dairy intake, blood pressure and vascular function in UK adults with mild hypertension: a cross-sectional study.

ANIMAL HEALTH AND WELFARE

Conference Manager: Dr. Olav Østerås, TINE Advisory Services, Standing Committee on Animal Health and Welfare of IDF (Norway)

Representative of the Local Organising Committee: Prof. Dr. Antanas Sederevičius, Lithuanian University of Health Sciences (Lithuania)

Session 1: Responsible and prudent use of antimicrobials in the dairy sector

Moderator: Dr. Vidmantas Paulauskas, State Food and Veterinary Service (Lithuania)

9:00-9:30 Responsible and prudent use of antimicrobials in dairy
Dr. Elisabeth Erlacher-Vindel
OIE (France)

9:30-9:45 Involving the dairy supply chain in responsible and prudent use of Antimicrobials
Dr. Robin Condron
Dairy Australia (Australia)

9:45-10:00 The use of antibiotics and penicillin resistance of *S.aureus* in Norway
Dr. Olav Østerås
TINE Advisory Services, Standing Committee on Animal Health and Welfare of IDF (Norway)

10:00-10:15 Antimicrobial resistant *Escherichia coli* in faeces from preweaned dairy Calves **(full text available)**
Dr. Ylva Persson
National Veterinary Institute (Sweden)

10:15-10:30 Antibiotic stewardship in the United States dairy industry
Dr. Jamie Jonker
National Milk Producers Federation (USA)

Session 2: Epidemiology of infectious diseases of importance for dairy production

Moderator: Dr. Elisabeth Erlacher-Vindel, OIE (France)

11:00-11:30 The epidemiology of infectious diseases importance for dairy production
Dr. Petras Mačiulskis
Deputy Director of National Food and Veterinary Risk Assessment Institute (Lithuania)

11:30-11:45 Paratuberculosis and Tb situation
Dr. Robin Condron
Dairy Australia (Australia)

11:45-12:00 CD109 gene: a potential candidate of immune response **(full text available)**
Bianca Moioli
The Agricultural Research Council (Italy)

12:00-12:15 Bulk tank bacterial count and parity group cow somatic cell count in *Strep. agalactiae* positive herds **(full text available)**
Michael Farre
SEGES (Denmark)

12:15-12:30 Animal health control in dairy herds-Lithuanian experience
Dr. Vidmantas Paulauskas
State Food and Veterinary Service (Lithuania)

Session 3: Animal welfare: problems and solutions

14:00-14:30 Animal welfare: problems and solutions
Prof. Daniel Weary
The University of British Columbia (Canada)

14:30-14:50 Global experience on ketosis screening by FTIR technology **(full text available)**
Dr. Daniel Schwarz
FOSS (Denmark)

14:50-15:10 Addressing animal care concerns and building consumer trust through "responsible sourcing" guidelines for dairy producers
Emily Meredith
National Milk Producers Federation (USA)

15:10-15:30 What can we learn from on-farm monitoring of "low-resilience" behaviours?
Roi Mandel
Hebrew University (Israel)

Session 4: Continuation – Animal welfare: problems and solutions

Moderator: Dr. Olav Østerås, TINE Advisory Services, Standing Committee on Animal Health and Welfare of IDF (Norway)

16:00-16:30 Best poster presentations

16:30-17:30 Poster section with discussion – summing up panel discussion
Dr. Olav Østerås
TINE Advisory Services, Standing Committee on Animal Health and Welfare of IDF (Norway)

Poster presentations

Dr. Vita Krunglevičiūtė - The influence of lactic acid bacteria on the alkylresorcinols and lignans content in fermented calves feed stock.

Dr. Danguolė Urbšienė - The optimal number of days for estimation of the average milk yield on a test day.

Dr. Juan Kruze - Challenge trial to evaluate the efficacy of a new vaccine (Masti-Vac) against Staph.aureus mastitis.

Dr. Giuseppe Ragona - Health investigation and milk quality in native amiata donkeys (equus asinus).

Lynette Chew - Reduced somatic cell counts in response to a standardized high activity proprietary garlic powder administered to lactating cows – a pilot trial. **(full text available)**

Dr. Ylva Persson -Bulk milk somatic cell count in open and zero-grazed dairy herds in urban and peri-urban kampala, Uganda.

Dr. Roynston Albert - Improved faecal consistency in calves administered with standardised high activity proprietary garlic powder. **(full text available)**

Dr. Rufino Lopez - Effects of net energy density on feed intake body weight changes of Holstein – Friesian cows during a dry-off period.

Dr. Rufino Lopez - Effect of yeast enriched with trace mineral and vitamin supplemented prepartum on milk production and health of dairy cows in early lactation.

Dr. Reyes Lopez-Ordaz - Impact of organic supplementation in replacement of inorganic sources of Se, Cu and Zn during prepartum on milk yield and metabolic dysfunctions in Holstein-Friesian cows.

Dr. Daniel Schwarz - Distribution of SCC in DHI samples around the globe. **(full text available)**

Ass. Prof. Rūta Budreckienė -The influence of probiotics additives and multienzyme composition on milk quality of Lithuanian black-and-white cattle. **(full text available)**

Dr. Mitch Hockett - On-farm milk leukocyte differential diagnosis of subclinical mastitis and intervention improves productivity and ensures precise antibiotic use.

Mitch Hockett - Quarter-level selective dry cow therapy guided by on-farm milk leukocyte differential diagnosis

G. Palubinskas - The possibility to use low intensity laser irradiation to the superovulatory treatment of dairy cows-donors

DAIRY FARMING

Conference Manager: Bronius Markauskas, Chamber of Agriculture of the Republic of Lithuania (Lithuania)

Session 1: Milk production, structure of farms and tendencies of changes
Moderator: Mindaugas Maciulevičius, Chamber of Agriculture of the Republic of Lithuania (Lithuania)

9:00-9:05 Introduction
Bronius Markauskas
Chamber of Agriculture of the Republic of Lithuania (Lithuania)

9:05-9:35 Dairy farm structure and its changes the last 10 years – what trends and drivers
Lukasz Wyrzykowski
International Farm Comparison Network (Germany)

9:35-9:55 The structure of dairy farms, cooperation (Lithuania, Latvia, Estonia)
Juratė Dovydėnienė
Chairwoman of Lithuanian Association of Agricultural Cooperatives Kooperacijos Kelias (Lithuania)

9:55-10:15 Dairy farming in Belarus: structure, internal and external challenges
Aliaksandr Serzhanovich
Analitycs Agency BUSINESS NEWS (Belarus)

10:15-10:30 Concluding remarks Mindaugas Maciulevičius
Chamber of Agriculture of the Republic of Lithuania (Lithuania)

Session 2: Application of innovations in dairy farms

11:00-11:20 Impact of genomic innovations on future dairy herd
Dr. Erwin Koenen
CRV (The Netherlands)

11:20-11:40 Feeding the cow of the future – nutrition, management and environmental considerations **(full text available)**
Prof. Larry E. Chase
Cornell University (USA)

11:40-12:00 Highly productive genetic resources in dairy farming in Stavropol region **(full text available)**
Prof. Serhii Oliinyk
Stavropol State Agrarian University (Russia)

12:00-12:20 Integrated approach of advisory services to farm development
Gintarė Kučinskienė and Reda Milčiuvienė
Lithuanian Agricultural Advisory Service (Lithuania)

12:20-12:30 Concluding remarks
Dr. Eglė Stonkutė
Baltic Institute for Research and Development (Lithuania)

Session 3: Risk management in dairy farms

Moderator: Ron Maynard, Dairy Farmers of Canada (Canada)

14:00-14:10 Assuring food safety and quality in Europe **(full text available)**
Vytenis Andriukaitis
European Commission (Lithuania)

14:10-14:30 What is the future for the EU dairy sector after the abolition of quotas
Luis Carazo Jimenez
European Commission (Belgium)

14:30-14:50 Milk futures – lessons learned from European dairy farmers experiences
M.Sc. Lukas Steinmann and Prof Dr. Holger D. Thiele
University of Applied Sciences Kiel (Germany)

14:50-15:10 Cooperation experience
Rafal Stachura
Polish Federation of Cattle Breeders and Dairy Farmers (Poland)

15:10-15:30 The dynamics of milk farms incomes and expenses **(full text available)**
Dr. Eglė Stonkutė
Baltic Institute for Research and Development (Lithuania)

Session 4: Risk management in dairy farms (farmers' perspective).

Moderator: Dr. Jamie Jonker, National Milk Producers Federation (USA)

16:00-16:20 The Dairy Farmer Margin Protection Program: U.S. Safety Net For Dairy Farmers
Shawna Morris
National Milk Producers Federation (USA)

16:20-16:40 The end of milk quota system, EU "Milk Package" (farmers' perspective)
Udo Folgart
IDF Germany, German Dairy Association (Germany)

16:40-16:55 The example of creating additional value in small dairy farms
Audrius Jokubauskas
Farmer (Lithuania)

16:55-17:15 Concluding remarks. Presentation of IDF Dairy Farmers Forum Report by facilitators Dr. Jamie Jonker
National Milk Producers Federation (USA)

Poster presentations

Dr. David Gleeson - On-farm milk quality in Ireland.

Kristina Morkūnienė -Genomic selection in dairy cattle – application of innovations in dairy farms. **(full text available)**

Prof. Dr. El-Sayed El-Tanboly - Recovery of cheese whey, a by-product from the dairy industry for use as an animal feed.

Dr. Giovanni Brajon / Andrea Lombardo -Risk assessment in a dairy farm in Italy for the development of an integrated system of sensors for cow milk quality monitoring. **(full text available)**

Dr. Jūratė Rudejevienė -Predict of cow's reproductive ability by milk progesterone concentration.

Kristina Liucvaikienė -Selection for milkability traits by genetic markers.

Dr. Ramutė Miseikiene - The influence of farm size on cow's milk quality. **(full text available)**

Snorri Sigurdsson – Develoment of ams in the Nordic countries 1996-2014.

Snorri Sigurdsson - Low TBC in bulk milk with ams.

Per Justesen - Thermo-resistant bacteria count vs. total bacterial count.

Iben Strøm - The use of thermographic cameras in the milk quality advisory service.

Prof. Vladimir Trukhachev – Highly productive genetic resources in dairy farming in Stavropol region

Prof. Dr. Yong Suk Son - Effects of automatic milking system and dietary fat supplementation on milk fat properties.

Dr. Jamie Jonker - A secure milk supply plant for a foot-and mouth disease outbreak in the United States.

DAIRY SCIENCE AND TECHNOLOGY

Conference Manager: Dr. Geoffrey Smithers, GWS Consulting Services Inc. (Australia)
Representative of the Local Organising Committee: *Dr. Jonas Damašius, Kaunas University of Technology (Lithuania)*

Session 1: Introduction and background

Moderators: *Dr. Geoffrey Smithers, GWS Consulting Services Inc. (Australia)* and *Dr. David Everett, Riddet Institute, New Zealand Centre of Research Excellence (New Zeland)*

9:00-9:05 Introduction and link to N&H Conference on dairy bioactives
Dr. Geoffrey Smithers
GWS Consulting Services Inc. (Australia)

9:05-9:30 World market for dairy bioactives: what are consumers demanding and how will the industry deliver?
Dr. Tage Affertsholt
3A Business Consulting (Denmark)

9:30-9:50 Isolation of immunoglobulins from colostrum and milk derived from specially immunized cows – modern separation approaches
Prof. Dr.-Ing. Ulrich Kulozik
Technical University Munich (TUM) (Germany)

9:50-10:10 Milk proteins and peptides for mineral binding and encapsulation
Dr. Thom Huppertz
NIZO food research (The Netherlands)

10:10-10:30 Impact of structural factors on the digestion of whey protein-stabilized emulsions
Prof. Dr. Daiva Leskauskaitė
Kaunas University of Technology (Lithuania)

Session 2: Proteins and peptides II

Moderator: *Dr. Thom Huppertz, NIZO food research (The Netherlands)*

11:00-11:25 Membrane technology for isolation of bioactive peptides
Prof. Laurent Bazinet
Université Laval (Canada)

11:25-11:50 Isolation of proline-rich bioactive peptides from dairy streams for food functional and biomedical applications
Dr. Louise Bennett
CSIRO Food and Nutrition Flagship (Australia)

11:50-12:10 Lactoferrin production from bovine milk or cheese whey **(full text available)**
Dr. Léo de Valck
Mirakal (Belgium)

12:10-12:30 The cheese matrix impacts protein in vitro digestion
Prof. Sylvie Turgeon

Université Laval (Canada)

Session 3: Lactose and lipids: the forgotten "bioactives" in dairy?

14:00-14:30 Oligosaccharides from lactose – update on production and applications
Prof. Michael Gaenzle
University of Alberta (Canada)

14:30-15:00 Sustainable valorization of buttermilk: Nutritional benefits, functional value in food products, and consumer perception
Dr. Marie-Caroline Michalski
INRA (France)

15:00-15:30 Isolation of functional milk fat globule membrane proteins using an advanced coagulation approach
Dr. Wolfgang Holzmüller
Technical University Munich (TUM) (Germany)

Session 4: What is on and over the horizon: future technologies and future applications

Moderator: *Prof. Marie Paulsson, Lund University (Sweden)*

16:00-16:25 Innovations and opportunities in processes for concentrated and dried dairy products – can we do better to preserve the bioactivity of the dairy components? An overview
Dr. Pierre Schuck
INRA (France)

16:25-16:45 Novel technologies for isolation of bioactive components from dairy
Phil Clarke
CSIRO Food & Nutrition Flagship (Australia)

16:45-16:50 Announcement of top 3 posters / winner declared / prize(s) presented
Prof. Marie Paulsson
Lund University (Sweden)

16:50-17:00 Concluding remarks and wrap up
Dr. David Everett
Riddet Institute (New Zealand)

Poster presentations

Dr. Mazri Chafiaa - Effect of pressure on the biological bovine whey proteins as functional food ingredients **(full text available)**

Ass. Prof. Zerrin Yuksel Onur – Interaction of gree tea flavonoids with milk proteins and the effect of heat treatment **(full text available)**

Kristina Musayeva - Determination of immunoglobulins G in cows milk in relation with somatic cells count

Ass. Prof. Zerrin Yuksel – Identification of indigenous lactic acid bacteria isolated from ezine cheese (PDO)

Prof. Dr. Zubeyde Oner – Identification of antioxidant activity of peptides of beyaz cheese produced from ovine milk **(full text available)**

Ayşe Mine SARIDAG – Microbiological and physical properties of Turkish white cheese **(full text available)**

Dr. Ralf Zink – Effect of salt reduction in industrially manufactured hard and semi-hard cheese (foil ripened gouda and emmental type) **(full text available)**

Sven-Rainer Doering - A new technology to produce a whey ingredient which is low in bacterial counts **(full text available)**

Mareike Hunold – Influence of packaging on long-term stability of evaporated milk cups **(full text available)**

Dr. Katja Borcherding – Acidic whey – a basic raw material for various food products **(full text available)**

Anja Kappler – Influence of cold filtration for germ reduction on the composition and functional properties of skim milk powder **(full text available)**

Torben Wiegers – Development of a new technology to produce skim milk powder with low termophilic bacteria count, low spore count and high whey protein nitrogen index (WPNI) **(full text available)**

Jan Pommer – Integration of denatured whey proteins into the cheese matrix by means of high-temperature heating in a partial flow method **(full text available)**

Dirk Euwens – Determination of lactose reduction during the cheese production process and subsequent maturation, as well as implementation of a meaningful enzymatic procedure for lactose analysis **(full text available)**

Eddy von Thaden – Studies on mozzarella production: can important final products characteristic (quality and product application) survive the economic stress field of a starter culture changes and reduced production costs? **(full text available)**

Ralf Mankiewitz – Statistical analyzes for process improvements must have an impact on customer satisfaction (a structured six sigma approach in practical use) **(full text available)**

Ass. Prof. Maria Glantz - Genetic parameters for rennet- and acid-induced coagulation properties in milk from Swedish Red dairy cows

Françoise Bafort – The bovine milk lactoperoxidase system, an innovative low impact biopesticide in crop's diseases

Prof. Belinda Vallejo-Cordoba – Technological characterization of specific lactobacillus strains: assessment towards novel probiotics starters

Dr. Aarón F. González-Córdova – Diversity and evolution of the microbial populations during manufacture of poro de balancán, a Mexican artisanal cheese

Dr. Adrian Hernandez-Mendoza – Response evaluation of antioxidant properties of lactic acid bacteria strains to different environmental stress conditions

Fanny Lazzaro – Forming and stability of dairy emulsions prepared with gradually demineralized casein micelles

Dr. Giovanni Brajon / Andrea Lombardo - A QR-code labeling system guarantees milk origin in the “pecorino” production chain in Tuscany (Italy) **(full text available)**

Dr. Andrea Adami – Microtechnologies for rapid detection of aflatoxin M1 in milk

P. Shuck/ Eve-Anne Norwood – Strutural markers of the evolution of WPI powders during ageing and impact on foaming and interfacial properties

Dr. Marco Nocetti – Cyclopropane fatty acid as molecular marker of authenticity for grated parmigiano-reggiano cheese

Dr. Krzysztof Bohdziewicz – Enzymatic agglomeration of whey protein concentrate

Elise Vanbergue – Effects of different sources of omega 3 fatty acids on cow milk spontaneous lipolysis

Dr. Dalia Čižeikienė – The evaluation of chemical composition of biologically active lipids from cow colostrum

Dr.Oğuz Aydemir - Traditional Turkish Yogurt

Fanny Lazzaro – Rheological properties of milk derived peptides in high-concentrated casein matrices

Prof. Dr. Seung-Yong Park - Fatty acid compositions of cheeses fed with condensed feed and grass from alpage pasture

Dr. Vivi Gregersen - Good coagulation properties of milk are not completely linked to the genetic B-variant of CSN3

Dr. Nina Poulsen – New genetic markers strongly affect rennet-induced milk coagulation

Dr. Antanas Šarkinas – Characteristics of lactic acid bacteria growth and multiplication in milk cherry extracts

Guillaume Gillot – Insights into *Penicillium roqueforti* morphological, genetic and functional diversity

Dr. Kouichirou Shin – Effects of lactoferrin on the production of the interferon lambda by human intestinal epithelial cell line HT-29

Sarah Nasser – Evolutions of functional properties of native phosphor casein (NPC) powder during storage

Prof. Dr. Savaş Atasever - Assessing milk yield, somatic cell count and some milk components in Czech holstein cows

Prof. Vladimir Trukhachev - The role of somatic cells in rennet clotting

Prof. Vladimir Trukhachev - Milk of goats as an additional source of biologically value raw materials for realization alternative technologies of food products

Dr. Maria Baranowska – Properties of nonfat dry powder with transglutaminase

Andrea Mühlhansová – Undesirable microflora of ripened cheeses

Nazli Turkmen - Effect of somatic cell count in goat milk on some physical, chemical and sensory properties of vanilla ice-cream

Shoma Ikegami – Control of pH improves the rennet coagulation properties of heated skim milk

Dr. Felicia Ciocia – Bio-active peptides in low-fat cheddar cheese

Hans-Jürgen Heidebrecht – Separation of biologically-active bovine immunoglobilins from milk and colostrums

Dr. Eva Svirakova - Identification of Acinetobacter spp. isolated from dairy products and production facility using pyrosequencing and MALDI-TOF MS

Dr. Wim Engels - CHEF: Towards the next generation cheeses: engineering of excellent flavours for healthy cheeses

Zhdanov Seva - Ultrafiltration of cheese whey with pre-treatment electroflotation

Nissim Silanikove - Nitrite and catalase levels rule oxidative stability and safety properties of milk: a review

Hasmukh patel – Effects of solvent quality and ionic environment on rheological properties and microstructure of acid gels prepared from MPC

Hasmukh patel – Texture and microstructure of high protein acid gels (Greek-style yogurt) as affected by ionic strength and protein interactions

Hasmukh patel - Effect of hydrodynamic cavitation on acid gelation and rennet coagulation properties of skim milk

Hasmukh patel – Hypocholesterolemic effect of *Lactobacillus helveticus* MTCC Y 5463 studied across the adult life span

Sergey Mikhaylin – Impact of pulsed electric fields with short pulse/pause duration and electroconvective vortices on membrane fouling and performances of electrodialysis

Dipl. – Ing. Hans-Jürgen Heidebrecht - Milk protein fractionation with spiral-wound membranes to obtain native protein fractions

MARKETING

Conference Manager: Winnie Pauli, Standing Committee on Marketing of IDF, Danish Agriculture & Food Council (Denmark). Representative of the Local Organising Committee: Arūnas Uleckas, Litamilk (Lithuania)

Session 1: Marketing challenges

Moderator: Dr. Mike Johnston, Dairy Council for Northern Ireland (United Kingdom)

9:00-9:15 Introduction to the Marketing Conference
Winnie Pauli and Arūnas Uleckas
International Dairy Federation (Denmark) and Litamilk (Lithuania)

9:15-9:35 The Russian embargo, a point of no return
Christophe Lafougere
GIRA (France)

9:35-9:55 Marketing challenges in Lithuania due to embargo
Liutauras Našliūnas
Dairy Producers Association (Lithuania)

9:55-10:15 Marketing challenges in Belarus
Alekseij Bogdanov
Ministry of Agriculture and Food of the Republic of Belarus (Belarus)

10:15-10:30 Panel discussion

Session 2: Marketing Practices

Moderator: Christine Leighton, SAMPRO CEP (South Africa)

11:00-11:20 Marketing trends of the Dairy Sector in Russia
Dr. Ludmila Manitskaya
Russian Dairy Union (Russia)

11:20-11:40 Dairy marketing opportunity in India
R. S. Sodhi
GCMMF (AMUL) (India)

11:40-12:00 Promoting milk consumption – Polish Milk Chamber experience in Poland and worldwide
Agnieszka Maliszewska
Polish Milk Chamber (Poland)

12:00-12:20 Revitalising dairy – the new Good of Milk
Oxana Kopytko
Tetra Pak

12:20-12:35 Panel discussion

Session 3: Dairy, nutrition and social media

Moderator: Ida Berg Hauge, Norwegian Dairy Council (Norway)

14:00-14:20 DMI – communication strategy in social media
Dr. Gregory Miller
National Dairy Council (USA)

14:20-14:40 Arla Foods, strategy on social media in Denmark
Tanja Bang Udengaard
Arla Foods (Denmark)

14:40-15:00 Legendairy
Isabel MacNeill
Dairy Australia (Australia)

15:00-15:15 Panel discussion

15:15-15:30 Global consumption trends in sugar reduced dairy
Merel Roes
DSM (The Netherlands)

Session 4: Generic marketing

Moderator: Laurent Damiens, CNIEL (France)

16:00-16:15 Presentation of the work of the IMP group
Laurent Damiens
CNIEL (France)

16:15-16:35 Get enough campaign
Isabelle Neiderer
Dairy Farmers of Canada (Canada)

16:35-16:55 How to move from nutrition to enjoyment?
Dominique Poisson
CNIEL (France)

16:55-17:15 Consumer Education Program of Milk SA
Christine Leighton
SAMPRO CEP (South Africa)

17:15-17:30 Closing remarks
Laurent Damiens
CNIEL (France)

Poster presentations

Prof. Lijun Chen - Impact of “Internet +” on Chinese Dairy Industry

Malin Thors Rosenquist - Marketing of analogue products – finding the right market entry for alternative product ranges

Merel Roes – Global consumption trends in sugar reduced dairy

FOOD SAFETY

Conference Manager: Robert Salter, Charm Sciences, Inc. (USA)
Representative of the Local Organising Committee: Prof. Loreta Šernienė, Lithuanian University of Health Sciences (Lithuania)

Session 1: Food safety risk analysis

Moderator: Robert Salter, Charm Sciences, Inc. (USA)

9:00-9:05 Introduction

9:05-9:25 Risk prioritization as part of risk assessment and current trends in analysis
Claus Heggum
Danish Agriculture & Food Council (Denmark)

9:25-9:45 Sucessful dairy safety messaging: using the right tools with the right message
Allen Sayler
Center for Food Safety & Regulatory Solutions (CFSRS) (USA)

9:45-10:05 Enhancing powder milk safety in China
Prof. Yujun Jiang
Heilongjiang Dairy Industry Technical Development Center (China)

10:05-10:25 Processing, plant integration, creating transparency across the value chain, securing quality & safety
Anders Andrén
Tetra Pak

Session 2: Chemical dairy safety: control of residues and chemical contaminants in dairy

Moderator: Prof. Loreta Šernienė, Lithuanian University of Health Sciences (Lithuania)

11:00-11:25 Chemical contaminants overview and risk prioritization
Dr. Paul Hanlon
Abbott Nutrition (USA)

11:25-11:45 Chemical import-export criteria for dairy products
Dr. Larisa Abdullaeva
Russian Dairy Union (Russia)

11:45-12:05 Official control of residues and chemical contaminants in milk and milk products in Lithuania
Snieguolė Džekčiorienė and Dr. Gediminas Pridotkas
National Food and Veterinary Risk

12:05-12:30 Allergens and detection avoidance strategy
Allen Sayler
Center for Food Safety & Regulatory Solutions (CFSRS) (USA)

Session 3: Microbial dairy safety: interpretation of results of pathogen detection in dairy

Moderator: Dr. Choreh Farrokh, CNIEL (France)

14:00-14:20 Contamination of milk and milk products by *Bacillus cereus* and how to determine the pathogenicity of strains
Dr. Valérie Michel
Actalia (France)

14:20-14:40 Good milking practices, environment monitoring, finished product testing. Where and what to look to mitigate the risk of *Listeria* contamination in dairy?
Francois Bourdichon
Danone (France)

14:40-15:00 Molecular methods for microbiological analysis of food – what they can and cannot do
Dr. Kieran Jordan
Teagasc (Ireland)

15:00-15:20 Food safety modernization still requires analysis of hygiene indicators
Robert Salter
Charm Sciences, Inc. (USA)

Poster presentation

Dr. Antonio Giuseppe Fadda – Own-check plan revision to prevent fresh ricotta cheese contamination by *Pseudomonas fluorescens*

Prof. Dr. Luiz Abreu – Polycyclic aromatic-hydrocarbons in smoked cheeses produced in Brazil

Dr. Giovanni Terrosu – Efficacy on bacteriophage P100 treatment to *L. monocytogenes*

Sigita Urbienė – Impact of technological factors on chloramphenicol concentration in milk

Hanna Castro – The growth of *Yersinia Pseudotuberculosis* in raw drinking milk packaged in bottles and bag – in –boxes.

Jūratė Šlapkauskaitė – Impact of antimicrobial properties of essential oils on curd-type cheese quality.

Dr. David Gleeson - A sampling and advisory strategy used in the reduction of Trichloromethane residues in farm bulk milk.

Dr. Kieran Jordan - Monitoring the occurrence of *Listeria monocytogenes* in sixteen dairy food processing facilities over two years.

Dr. Kieran Jordan - *Listeria ivanovii* in dairy foods and food processing environments in the Republic of Ireland.

Sandile Khoza - Attachment and biofilm formation by *Bacillus cereus*, *Paenibacillus* spp., *Micrococcus luteus* and *Staphylococcus epidermidis* on the nozzles of ESL milk filling machines.

Dr. Irina Kulikova – Comparative study of sweet and acid whey microflora growth during electrodialysis

Dr. Giuseppe Ragona – Hygiene and animal health requirements for donkey milk production. **(full text available)**

Allen Sayler – Private and government-driven dairy product safety management programs

Elna Buys – *Bacillus* and *Paenibacillus* spp. associated with extended shelf life milk.

Elna Buys – Antibiotic resistance patterns and virulence factors of identified O157 and non O157 *E. coli* serotypes in milk.

Jurgita Jovaisiene – Prevalence of aflatoxin M1 in raw milk in Lithuania. **(full text available)**

ANALYTIC TOOLS

Conference Manager: Dr. Saulius Savickis, Pieno Tyrimai (Lithuania)

Moderator: Dr. Harrie van den Bijgaart, Qlip (The Netherlands)

16:00-16:30 Raw milk analysis: matching analytical methods to the needs of farmers and processors
Prof. Dave Barbano
Cornell University (USA

16:30-16:45 Development of centralized raw milk analytical system in Lithuania
Dr. Saulius Savickis
Pieno Tyrimai (Lithuania)

16:45-17:00 Traditional and innovative analytical concepts for Bavarian raw milk
Dr. Christian Baumgartner
Milchprufring Bayern E.V. (Germany)

17:00-17:15 Harmonized analytical workflows for raw milk quality control in multiple markets? **(full text available)**
Lucie Racault
Nestlé (France)

17:15-17:30 Discussion

Poster presentations

Dr. Marco Nocetti - Set up of a NIR portable spectrometer system for the assessment of whole wheels of parmigiano reggiano cheese composition.

Dr. Willem Haasnoot - Immunoassays for bovine milk and bovine rennet whey in products of other species and sources.

Yoshitomo Amo - In-line monitoring of cheese texture by near-infrared spectroscopy.

Wolfgang Holzmüller - Quantification of milk fat globule membrane proteins by means of a novel stain free SDS-PAGE method.

ENVIRONMENT

Conference Manager: Piercristiano Brazzale, Brazzale Spa Company, Orrero a.s. (Italy)

Representative of the Local Organising Committee: Prof. Dr. Laima Česonienė, Aleksandras Stulginskis University (Lithuania)

Session 1: Environmental sustainability along the dairy supply chain in different regions of the world

Moderator: Marcin Preidl, IDFGermany, German Dairy Association (Germany)

8:50-9:00 Introduction

9:00-9:15 Getting dairy ready for 21 century agriculture
Carlos Saviani
WWF-US (USA)

9:15-9:30 Understanding sustainable food systems and what we can do today to take meaningful impacts for healthy people and a healthy planet
Erin Kathleen Fitzgerald
Global Sustainability Innovation Center for U.S. Dairy (USA)

9:30-9:45 Environmental impact of the dairy chain and what the scientists, industry and authorities are thinking to do in China
Prof. Mao Xueying
China Agricultural University (China)

9:45-10:00 The Australian dairy industry sustainability framework and progress report
Helen Dornom
Dairy Australia (Australia)

10:00-10:15 The French environmental action plan on 4000 dairy farms
Jean Baptiste Dolle
The French Livestock Institute (IDELE) (France)

10:15-10:30 Discussion

Session 2: Sustainability in the dairy sector: turning science into practice

Moderator: Sophie Bertrand, CNIEL (France)

11:00-11:15 Production of milk with new pasture silvo pastoral systems in South America
Ernesto Reyes
Agri benchmark (Germany)

11:15-11:30 Dairy Sustainability Framework – a programme of the Global Dairy Agenda for Action
Brian Lindsay
Global Dairy Agenda for Action (UK)

11:30-11:45 Healthy milk in healthy environment

Prof. Bruno Stefanon
University of Udine (Italy)

11:45-12:00 Possible uses of environmental footprinting for the dairy chain – a view from the European project at its mid-term
Helen Simonin
European Dairy Association (EDA) (Belgium)

12:00-12:15 How performance indicators increase resource efficiency through “dilution of maintenance”
Dr. Roger A. Cady
Elanco (USA)

12:15-12:30 Discussion

13:15-13:45 Increasing resource efficiency in dairy production: analysis along the supply chain. Sustainable packaging solutions and renewable materials – a key element of competitiveness.
Erik Lindroth
Tetra Pak

Session 3: Eco friendly dairy technologies: practical examples on how to reduce the impact of dairy chain

Moderator: Piercristiano Brazzale, Brazzale Spa Company, Orrero a.s. (Italy)

14:00-14:15 The "Green dairy" model: a technology and innovation-driven approach for sustainable milk processing
Dr.-Ing. Christoph Glasner
Fraunhofer Institute for Environmental, Safety and Energy Technology UMSICHT (Germany)

14:15-14:30 Energy saving and environmental protection in dairy industry with technological solutions and management of innovative type. Case study at an Italian Group
Prof. Stefano Guercini
University of Padova (Italy)

14:30-14:45 Energy efficient processing for the dairy industry
Dr. Thom Huppertz
NIZO Food Research (The Netherlands)

14:45-15:00 Energy efficient concentration of milk and whey by means of membrane cascades and ways for thermal preservation of concentrates as an alternative to drying
Prof. Dr.-Ing. Ulrich Kulozik
Technical University Munich (TUM) (Germany)

15:00-15:15 Improving the environmental performance of the dairy supply chain
Phil Clarke
CSIRO Food & Nutrition Flagship (Australia)

15:15-15:30 Discussion

Session 4: Migration of nutrients in the environment of the European dairy sector

Moderators: Piercristiano Brazzale, Brazzale Spa Company, Orrero a.s. (Italy) and Prof. Dr. Laima Česonienė, Aleksandras Stulginskis University (Lithuania)

16:00-16:20 The impact of dairy farms activity on the quality of surface water
Prof. Laima Česonienė
Aleksandras Stulginskis University (Lithuania)

16:20-16:40 Nitrogen efficiency in dairy cows diet
Annu Palmio
Natural Resources Institute Finland (Luke) (Finland)

16:40-17:00 Resource efficiency in ecological recycling agriculture with dairy production
Artur Granstedt
BERAS International, Biodynamic Research Institute (Sweden)

17:00-17:20 Towards a common nutrient use efficiency assessment method for livestock supply chains: a case of study of mixed dairy supply chains in Western Europe
Dr. Aimable Uwizeye
Food and Agriculture Organization of the United Nations (Italy)

17:20-17:30 Final discussion with all the speakers

Poster presentation

Dr. Regula Keller (Niels Jungbluth) – Life cycle assessment of milk: comparison of romanian and swiss production.

Dr. Regula Keller – Challenges for environmental assessments based on life cycle thinking in SMEs.

Dr. Vytautas Ribikauskas – Sources of airborne ammonia and microorganisms in naturally ventilated barn for dairy cows.

Ass. Prof. Jonas Damašius - Re-Design of raw mill delivery in the Lithuanian dairy industry using milk pre-concentration.

Prof. Dr. Gražina Juodeikienė - Bio-recylcing of dairy industry residues to organic acids isomers and other metabolites by using bacteriocins producing LAB

Darius Černauskas – Application of biotools for recycling of dairy by-products to sterospecific lactic acid.

Saioa Ramos – Reducing environmental impact of dairy products through ecodesign.

Thomas H. Weißer - Envimodul Biomar modular wastewater treatment plant for dairy industry “Made in Germany”.

Dr. Ditte Hobbs – Can dairy products be part of a sustainable diet?

Sievert Jonasson - A sustainable way of turning waste into value.

THE CHALLENGE OF GLOBAL FOOD SECURITY AND NUTRITION

Gerda Verburg he challenge of global food security and nutrition

CFS Chair

Abstract

The global fight against hunger and malnutrition is gaining ground, but today, an unacceptable number of more than 795 million people are still undernourished, while one third of all food produced is lost or wasted. Furthermore, by 2050 we will have to sustainability feed an additional 2 billion with increasing environmental constraints on water, land and emissions, coping with climate change impacts on productivity, pests and diseases, and natural hazards. Food security and nutrition for all through sustainable agriculture will only be achieved if all stakeholders jointly invest their resources and efforts to enable food producers, including dairy farmers, to play their role. CFS seeks to address this challenge.

Speech

Ladies and gentlemen,

It is an honor to be here with you to address a topic which, as Chair of the UN Committee on World Food Security, I hold particularly close to my heart. As some of you may know, I spent the first 15 years of my life on a dairy farm, among 9 other brothers and sisters; and the role, responsibility and challenges facing farmers and food producers in addressing food security and nutrition, is something I keep very present in mind.

Today, we live in an age of interlinked challenges but also of endless opportunities. Overall, important progress has been made in fighting hunger and malnutrition worldwide. However, 795 million people still go to bed hungry every day, including 161 million children whose physical and intellectual development is impaired by undernutrition and nutrient deficiencies. These numbers, in the twenty-first century, remain unacceptable. We MUST step up our efforts to eradicate hunger and malnutrition globally. And when I say “globally”, I do not only mean on each corner of the planet; I mean, by each and every one of us, stakeholders, farmers, producers, consumers, decision-makers, researchers, industries, etc, through real willingness and ability to collaborate in a multistakeholder way.

Today, I would like to share with you my vision of the challenges we are still facing today that hinder progress, and the paradigm shift we need to tackle these challenges.

I-Challenges

1-Let me start by describing some of the paradoxes and difficulties that stand in the way of food security and nutrition for all.

Food is produced by farmers; paradoxically however, the majority of the hungry people in the world are farmers and their families! Farmers constitute a very heterogeneous category, from tiny smallholders in developing countries to high-tech farms, from crop producers, to livestock farmers, dairy producers, fisherfolk, etc. There is no “one size fits all” recipe, and policies aiming to support food producers worldwide in contributing to global food security must tackle a complex range of factors.

Food producers are entrepreneurs, who aim tomake a living. They invest, take risks, face increasing environmental, hygieneand safety constraints, have to cope with the impacts of climate change on yields or animal welfare. The importance of quality food products for global food security and good nutrition is stressed over and over again. So,farmers must be better supported in their role of providing the world's food and nutritional needs, starting with a decent reward by paying a good price for their work and products! Convincing the young generations to take over farms has become a challenge worldwide and will remain so.Unless farmers' livelihoods worldwide are better supported, and farmers are able to earn a decent income and are recognized for their role in producing public goods.

2- But farmers interact also with other actors.Consumers (and we are ALL consumers) and civil organisationsplay a crucial role in a growing world population of over 7.3 billion people, projected to reach more than 9 billion by 2050. Good diets are a key challenge for the future. As we speak, an affluent middle class is quickly arising in many countries, hungry for more meat, fish and dairy. Recent figures indicate that by 2020, the world will consume 60billion liters more milk than in 2012. Good news for the growth of the dairy sector! But at the same time, it is our common responsibility to ensure that this growth is sustainable, and that indeed we are able to produce more with fewer resources, and in particular, with fewer GHG emissions, and close to no loss and waste. Fortunately, particularly in Western countries, the civil opinion mindsets are slowly changing and public awareness campaigns gain clout, assisted by new technologies and social media. This should encourage the dairy sector to further step up on its path to sustainability and towards a leadership position in sustainable business throughout the whole chain, economically, socially and environmentally.

… As should dairy science, innovation and technology. Research is making progress everyday on how to improve the sustainability of agricultural production, but knowledge transfer should be strengthened. And learning should always be a two-way path, where food producers are also knowledge producers, a knowledge that should be shared for society and a sustainable future, aimed to produce concrete results

An important link between food producers, and consumers is the food industry, or agribusiness. While profit remains the driver of businesses, the attention of many actors of the private sector is increasingly focused on realizing sustainable results through inclusive growth. Building longer-term relationships with suppliers, governments and consumers is essential, and I have witnessed how more and more,big and small companies are willing to cooperate in a transparent and sustainable way in order to make a lasting difference and show leadership.

However, to thrive and play a responsible role, businesses of all sizes need an enabling environment. Governments play a crucial role in this respect. An enabling environment means having predictable and evidence-based policies, programs, governance and institutions, that can support farmers and food producers with public investments, that facilitate private investments with a sound and stable legal framework and through the provision and development of infrastructures. But currently, how many countries are really giving proper attention to the organization and improvement of their food systems? Indeed, some challenges, - and food security and nutrition is one of them- , are too big to be solved by governments alone; and even within a single government,on such interconnected issues, interests between ministries diverge. But, it is important to realize that dairy farmers will only be able to produce in a sustainable way if they have the expectation to sell their milk, butter, cheese, for a higher price than what it cost them to produce it; and if there is a well-functioning market to selltheir products.

Summarizing : all stakeholders have their own perspectives, assets, and particular challenges. And these may sometimes seem mutually exclusive.However, if we want to achieve a world where, as the Global Sustainable Development Goals state, in 2030, "no-one is left behind" on such a topic as hunger and malnutrition, then joining efforts is clearly the only way forward.So, how can we move forward?

II-How can we take action and have more impact together?

Inclusiveness, knowledge and collective ownership, are not only guiding principles of the Post 2015 Sustainable Development Goals or Agenda 2030, to be adopted 4 days from now in New York.They constitute the paradigm shift the world needs to achieve sustainability in all its three dimensions: economic, social, and environmental. And particularly for food security and nutrition of all worldwide.

A traditional African proverbsays: “If you want to go quickly, go alone. If you want to go far, go together”.

This is what guides us at the United Nations’ Committee on World Food Security (CFS), the most inclusive international and intergovernmental platform for all stakeholders to work together towards food security and nutrition for all. And IDF is an appreciated participant of CFS. Just think how many dimensions of food and nutrition issues would be missing, if policy dialogue just took place among States and international organizations? We need to have all relevant actors around the table to contribute to policies, implement them and share responsabilities for concrete solutions and results. Including, given their role in providing food security and nutrition, dairy producers!I congratulate IDF for their exemplary work!Gatheringeverybody, from dairy farmers, dairy processing industry, dairy suppliers, to academics and governments/food control authorities.Youhave made crucial contributions to the Second International Conference on Nutrition, last November, to provide private-sector inputs into the political Rome declaration and the framework for action, adopted by more than 150 countries. And indeed,we can no longer afford to work in silos!

Secondly, knowledge exchange is essential. The key to broadening and deepening our understanding of food insecurity and malnutrition, and of its immediate and underlying causes, is evidence. It requires scientific expertise at different levels and interaction with stakeholders. This is the role, for example, of the CFS High Level Panel of Experts on Food Security and Nutrition (HLPE), a participative and dedicated group of international experts that presents independent, solid, evidence based reports with associated policy implications.Next year’s HLPE report, in parts thanks to effective IDF lobbying, will address “Sustainable Agriculture including the role of livestock”, and will prepare the ground for policy recommendations negotiated among CFS stakeholders.

Thirdly, responsibility and ownership must be felt at all levels. In CFS, member states take decisions; but, only after substantial debate and negotiation with all stakeholders. Whether it is on responsible agricultural investment; food security and nutrition in protracted crises; or tackling food loss and waste, everyone has a part to play in implementing decisions. Countries, of course, through policies. But also civil society, private sector, academia, international organisations, UN agencies, should lead within their own programmes, improve the enabling environment, and hold governments to account. If all CFS participants played their role in implementing CFS recommendations, such a complex challenge as food security and nutrition would be tackled and become history within a generation!

To conclude:

Inclusiveness, evidence-base, and ownership are the paradigms which should guide us to achieve “the future we want”. Only multi-stakeholder collaboration can support us in moving beyond short-term individual interests and silos,to global sustainable solutions in agriculture and food security. This year, moving towards Agenda 2030 and the SDGs, thousands of stakeholders have been solicited to define our common objectives for 2015-2030; and there is a common understanding that, after the endorsement of the SDGs, all stakeholders will be responsible for the implementation of these goals.

The dairy sector has more than once proven to be able to take the lead in building an innovative future.Here in Vilnius, I count on the World Dairy Leaders, the IDF, and on each and everyone of you,

entrepreneurs and other participants, to become a champion of this approach, back home, and to ensure that on our path to global sustainability, “no one is left behind”. Today, 21st September 2015, it is more than ever time to make hunger and malnutrition belong to history.

Thank you very much for your attention.

DAIRY POLICY IN INDIA: STATUS OF THE NATIONAL DAIRY PLAN IN INDIA

Meenesh Shah

Group Head (Cooperative Services), National Dairy Development Board, Anand 388001, Gujarat, India

meenesh@nddb.coop

Abstract

The dairy cooperative movement brought about a paradigm shift in the Indian Dairy Industry. From being a milk deficient nation earlier, India today is world's biggest producer of milk. This transformation is largely attributed to replication of 'Anand Model' in different States of India by the National Dairy Development Board (NDDB) under its 'Operation Flood' programme launched in 1970. The underlying philosophy behind 'Anand Model' was establishment of a vertically integrated three-tier structure which created a direct link between milk producers and the ultimate consumers. 'Operation Flood' created a new policy environment in the dairy sector and linked rural and urban populations, introduced market orientation and technological advancements, developed extension services and supported the growth of cooperatives in a sustainable manner. The strong dairy cooperative framework left behind by 'Operation Flood' was further consolidated by 'Perspective 2010' programme launched by NDDB in 2001 to strengthen the milk business of dairy cooperatives in key areas.

Currently, Indian dairying scenario is characterized by growing domestic demand, which necessitates increase in the pace of milk production in coming years. NDDB is addressing this by introduction of 'National Dairy Plan' (NDP) which aims at enhancing milk production at the required pace through productivity enhancement measures and improved genetics of milch animals. The main expected outcome from the interventions proposed in NDP are increased productivity per milch animal, increase in in-milk animals, improved AI conception rates, improved nutrition, reduction in feeding costs per animal and reduction in methane emissions. Efforts to increase milk production through increase in productivity will also be supported by expanding the setting up of village based milk procurement systems to collect milk in a fair and transparent manner and ensure timely payements.

Dairying in India-Unique Features

Milk production in India has been generally growing at the rate of 4.5% per year double than the World milk production growth. Dairying in India is an occupation of small and marginal farmers. Dairying has not meant just producing milk leading to India emerging as the largest milk producer in the world. It has provided livelihood to millions of poorest in the country, and for many it is sole source of livelihood bringing cash into their hands, twice a day every day of the year. In India, dairying is production that results from the efforts of individuals that constitute masses rather than mass production.

The Indian dairy system has certain characteristics, common to many developing countries in Asia. Indian dairy farmers are predominantly small-holder producers with a majority of them owning less than two hectares of land and one to three animals. Unlike many major developed dairying countries where grain/pasture is used for feeding, the dairy animals in India are largely fed on agricultural by-products and crop residues. Household members carry out most of the dairy farming operations by themselves, with women contributing significantly to these operations. With availability of inputs at farm level itself the occupation remains self-sustainable and eco-friendly.

Dairy contributes more than one-fourth of the gross income of the rural producers and in those households without land it contributes half of the gross incomes. Livestock share in Agriculture and allied GDP is 27% in India. Milk contributes to nearly 65% of the total livestock output, amounting to

about US dollars 62 billion. More than 90% of India's farmers either don't possess any operational land or have less than 2 Ha of land. While these small farmers own about half of the total operational land, they own about 80% of the female bovines. Dairying in India, therefore, represents a more equitable distribution of productive assets, income and wealth than crop husbandry.

During 2014-15, the total milk production in India was 146.3 million tonnes with per capita availability of 322 grams per day which is higher than the world's average. As on March 2015, the cooperative milk unions covered around 160 thousand village dairy cooperative societies with a total membership of 15.4 million milk producers, of which 4.5 million were women. These dairy cooperatives together procured 38 million litres of milk per day in 2014-15 with growth of 11.2% over the last year. The sales of liquid milk reached 31.2 million litres per day in 2014-15, recording a growth of 6.1% compared to the last year.

India has domestic milk production of about 400 million kg/day. Of the milk produced in the villages, about 40% is consumed in the villages and the rest is traded, which means an income of about 23 billion US dollars to the dairy farmers. Among agricultural commodities in India, milk is now the largest contributor to the Gross National Product - even larger than rice and wheat, the principal agricultural crops.

Dairy Co-operative Movement in India

The seeds of dairy cooperative movement were sown more than 70 years back in Anand, a small town in the state of Gujarat in western India. The exploitative trade practices followed by the local trade cartel triggered off the consolidation of farmers and milk became a symbol of protest. Angered by unfair and manipulative practices followed by the trade, the farmers of the district approached the great Indian patriot Sardar Vallabhbhai Patel for a solution. He advised them to get rid of middlemen and form their own milk co-operative, which would have procurement, processing and marketing under their control.

In 1946, the farmers of this area went on a milk strike refusing to be cowed down by the cartel. Under the inspiration of Sardar Patel, and the guidance of leaders like Morarji Desai and Tribhuvandas Patel, they formed their own cooperative in 1946 in Anand.

This cooperative, the Kaira District Cooperative Milk Producers Union Ltd. began with just two village dairy cooperative societies and 247 litres of milk and is today better known as Amul Dairy. Amul grew from strength to strength thanks to the inspired leadership of Tribhuvandas Patel, the founder Chairman and the committed professionalism of Dr Verghese Kurien, who was entrusted the task of running the dairy from 1950.

'Anand Model' envisages creation of cooperative institutions at the Village Level, District Level and State Level to procure , process and market the milk produced by member producer farmers. These institutions are owned and controlled by the milk producers, which have provided them with the necessary support structure to carve out a viable livelihood source for themselves.

Evolution of NDDB and Implementation of Operation Flood

During the 1950s and 1960s Indian dairy sector was characterized by stagnant domestic milk production and declining per capita availability. There was heavy dependence on imports of dairy commodities and domestic milk production was highly unorganized. Milk processing and cold chain infrastructure was absent and dairying was not practiced as commercial activity. The rich dairying heritage of India was being eroded.

In 1964, India's then Prime Minister Shri Lal Bahadur Shastri visited Anand to inaugurate Amul's cattle feed plant. He spent a night in a village and had detailed discussions with the farmers to understand

the reasons for the success of the cooperative. Convinced about the true development through cooperative, he desired that the 'Anand Model' be replicated all over India. To achieve this, the Government of India decided to put in place a national organization to assume this responsibility – the National Dairy Development Board (NDDB) – which would give the farmers the best chance of succeeding in organizing themselves into cooperatives.

NDDB was set up in 1964 as a registered society. The first Chairman of the NDDB was Dr. Kurien. He continued as the Chairman of NDDB for over 30 years and imbued NDDB with the values and a work culture that NDDB is known for. In 1987 the society, NDDB, was merged with the Indian Dairy Corporation, a Government of India Undertaking incorporated to receive and monetize commodities from abroad. The new body, which succeeded the two merged entities, was set up through an Act of the Parliament and retained the name NDDB.

The significant functions entrusted to NDDB are: to promote, plan and organise programmes for development of dairy and other agriculture allied industries and biologicals; promote and set up dairy industries; finance any scheme in the cooperative or public sector to stimulate production and marketing of milk; develop and preserve high yielding cattle; adopt the cooperative strategy in an effective manner; cooperate with international organisations; and conduct research and development.

NDDB was instrumental in implementing Operation Flood (OF) programme. Operation Flood programme was the most comprehensive dairy development project undertaken – it was executed in three phases between 1970 and 1996. Its objective was the development of rural milk production through an extensive network of village milk producers' cooperatives to meet the growing urban demand for milk. The project was financed through commodity aid and loans from the World Bank. In all, about US dollars 1 billion was spent. The reconstituted milk powder and butter-oil helped the cooperative dairies to capture a dominant share of the market. The proceeds thus obtained, combined with World Bank assistance, funded the establishment of a vast infrastructure required to link millions of farmer producers to processing plants and markets.

OF assisted in establishing 73 000 village milk cooperative societies in 170 milksheds covering more than 250 districts in 22 states. 9 million milk producing households came under the fold of OF. From 21.2 million tonnes in 1968-69, total milk production more than tripled to 69.1 million tonnes by the end of 1996-1997. During this period, the per capita milk availability increased from 112 gram per day to 202 gram per day despite a substantial increase in population.

OF had already put a dairy cooperative framework in place. The challenge was to build on this strong foundation, both in quantitative and qualitative terms. To take this work forward, NDDB along with the dairy cooperatives evolved 'Perspective 2010' in 2001. To set goals for the next decade, dairy cooperatives worked with NDDB to evolve perspective plans. These plans covered four main thrust areas – a) Cooperatives Business; which includes procurement and marketing of milk; b) Productivity Enhancement, which would include feeding, breeding and animal health; c) Quality Assurance; and d) National Information Network. Implementation of perspective plans by milk unions further consolidated the gains made during OF.

National Dairy Plan (2011-12 to 2018-19), Rationale, Outlay, and Components

The demand for milk in India is now growing much faster. Amongst others, the key drivers for growth in demand for milk are: i) rising incomes due to high GDP growth; (ii) growing urbanization and changing food habits; (iii) increase in population; (iv) export opportunities, and increase in incomes of village households through rural employment programmes.

Emerging trends indicate that milk demand is likely to be 210 million tonnes in 2021-2022. Unless milk production increases at the pace required there is a possibility of a widening gap in supply of milk and the dependence on imports may increase. On the other hand, some milk producers are not finding

milk production to be sufficiently remunerative and are disengaging from dairying as a source of income and looking at other alternatives.

Meeting projected demand from domestic sources and ensuring that dairying remains a remunerative livelihood option required a focused national initiative, National Dairy Plan is conceptualized to precisely achieve the same. To successfully meet the growing demand for milk, NDP aims at the average incremental annual increase in milk production with an average of 6 million tonnes per annum over the next 15 years compared to existing average of 3 million tonnes over the last 15 years. Further, strengthening and expanding infrastructure (capacities) to procure, process and market milk through existing/new institutional structures is also being supported.

National Dairy Plan is focusing on 18 major milk producing states which accounts for more than 90% of the country's milk production, over 87% of the breedable cattle and buffalo population and 98% of the country's fodder resources. Benefits of NDP however will be across the country.

NDP Financial Outlay

Component	Activity	Outlay (in INR million)	Outlay (in million USD) (@ 1 USD=49 INR)
Component A	Breed Improvement	7150	146
	Animal Nutrition	4250	86
Component B	Village Based Milk Procurement System	4880	100
Component C	Project Management and Learning	1320	27
Sub Total*		17600	359
End Implementing Agencies(EIA) Contribution		2820	57
Grand Total		20420	416
*Source of Funds: World Bank-IDA: INR 15840 million (323 million USD, Govt. of India's contribution: INR 1760 million (36 million USD), NDDB INR 2000 million(41million USD)			

NDP Physical Targets

Component A: Productivity Enhancement

Production of High Genetic Merit (HGM) cattle and buffalo bulls

Production of 2500 HGM bulls

Import of 400 exotic bulls/equivalent embryos

Strengthening of "A" and "B" graded Semen Stations

Production of 100 million semen doses annually in the terminal year

Pilot Model for Viable Doorstep AI delivery Services

3000 Mobile Artificial Insemination Technicians(MAITs) carrying out annual 4 million doorstep AIs by the terminal year

Ration Balancing Programme (RBP)

Coverage of 2.7 million milch animals in 40,000 villages

Fodder Development Programme

Production of 7500 tonnes of certified/ truthfully labeled fodder seed

1365 silage making/ fodder conservation demonstrations

Component B: Village Based Milk Procurement System

Strengthening and Expanding Milk Procurement System at Village level

23800 additional villages to be covered

1.2 million additional milk producers

Component- A Productivity Enhancement

When breedable animals are bred through Artificial Insemination (AI), it becomes possible to use the semen of a few top bulls of high genetic merit over a much larger population and achieve genetic progress. Presently, only 20% of the breedable animals are bred through AI in India, which needs to be increased to 35% to accelerate genetic progress. For achieving this NDP proposes production of 2500 High genetic merit (HGM) bulls in India and import of 400 exotic bulls/equivalent embryos. By March 2015, 262 HGM bulls have been produced and 76 exotic bulls have been imported. These semen doses will primarily be used for breeding crossbred/non-descript cows confirming to the breeding policies of the concerned states.

In order to raise the proportion of milch animals raised through AI, the demand for quality semen dose is estimated at 100 million doses of the required breeds. 22 semen stations are being strengthened under NDP to meet the demand. The semen station strengthening projects have triggered the development of infrastructure, especially for biosecurity and for production and processing of high genetic, disease free semen doses complying with Minimum Standards as laid down by Government of India. During 2014-2015, these 22 semen stations produced 68.04 million semen doses.

A scientific approach to the delivery of AI services at the doorstep of milk producer is needed to ensure animal conceiving better conception rate without spread of disease and the production of a genetically superior calf. NDP envisages setting up the viable model for AI delivery services by inducting about 3000 trained mobile AI technicians (MAITS) in a financially self-sustainable manner. By March 2015, about 685 AI technicians have been inducted.

Breed Improvement Programme need to be supported by feeding a balanced diet so as to have a higher productivity commensurate with their genetic potential. Imbalanced feeding impacts the health of animal and income from milk production, since 70% of the total milk production cost is on account of feed. There is a need to educate milk producers on ration balancing –through trained village based local resource persons(LRPs) – so that the nutrients required by a milch animal is fulfilled in an

optimum manner, thereby improving milk production efficiency and economic returns from dairying. LRPs will provide Ration Balancing Advisory Services to farmers based on centrally developed and standardized user-friendly computerized software. NDP proposes to cover 2.7 million milch animals in 40000 villages. By March 2015, 0.57 million milch animals have been covered in 8632 villages. Ration Balancing Programme (RBP) is helping the milk producers in increasing their net daily income by Rs 24 per animal.

There is about 50% deficit of green fodder in the country, and dry fodder is deficit by 20%. Enhancing green fodder availability and securing available bio-mass from farmers feed is needed to reduce the deficit. NDP proposes production of 7500 tonnes of certified/truthfully labeled fodder seed and 1365 silage making/ fodder conservation demonstrations. NDDB is assisting dairy cooperatives in establishing fodder seed production, processing and marketing units. By 2014-15, 3600 MT of certified seeds have been supplied to the farmers. 557 silage/fodder demonstrations have been organized in villages.

Due to increase in use of combine harvesters, a significant part of the crop residue is left in farmers' fields and usually burnt to prepare the field for next crop thereby causing not only environmental pollution but also affecting quality of soil and reducing availability of feed and fodder resources. Under NDP appropriate mower ranges are being introduced in various milk unions for securing crop residue from fields. In all 121 sets of mowers and pick up devices were introduced by 2014-15. To create awareness on dry fodder storage, construction of biomass bunkers, each of 50 MT capacity, was organized at 20 locations.

Component-B Village Based Milk Procurement Systems (VBMPS)

Efforts to increase milk production through increase in productivity would need to be supported by expanding the setting up of village based milk procurement systems to collect milk. Investments in village level infrastructure for milk collection and bulking such as Automatic milk collection units to ensure fairness and transparency in milk transactions and installation of Bulk Milk Coolers for better quality will be needed. The project proposes to cover 23800 villages and 1.2 million milk producers. VBMPS is helping the dairy cooperatives in the country to reach out to the hitherto uncovered areas and increase coverage of cooperative dairying. A large number of women members have been covered under VBMPS, which has help improve their confidence and economic self-reliance. By March 2015, 12540 villages and 0.38 million milk producers have been covered.

In terms of overall benefits, the NDP will put in place a scientific approach and systematic processes, which would take India on a path to improving the genetics of milk producing animals in a consistent and continuous manner. It will: make prudent use of country's scarce natural resources; Impact on reducing methane emissions; contribute to disease control; help strengthen regulatory and policy measures that will provide an enabling environment for future growth of dairying in the country; and contribute to strengthening livelihoods of small holder and marginal milk producers that form the majority of India's milk production system.

DEVELOPMENT OF POLISH DAIRY SECTOR AFTER ACCESSION TO THE EUROPEAN UNION

Paweł Zaręba

Agricultural Market Agency (ARR), 30 Karolkowa Str., 01-207 Warsaw, Poland

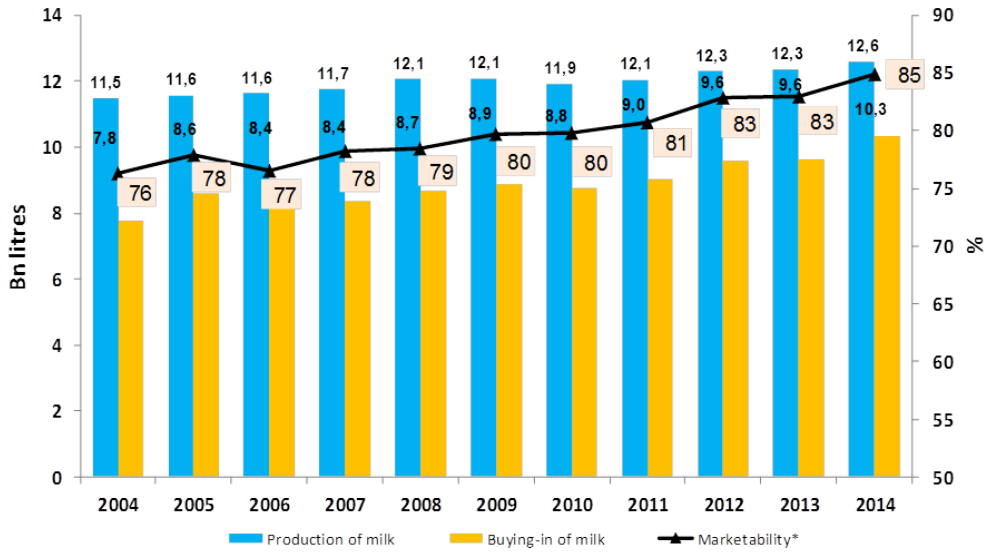
p.zareba@arr.gov.pl

Introduction

Accession to the European Union strongly influenced developments in Polish dairy sector. The most significant changes were noted in raw milk production, milk processing industry as well as in Polish foreign trade in milk products.

Raw milk production

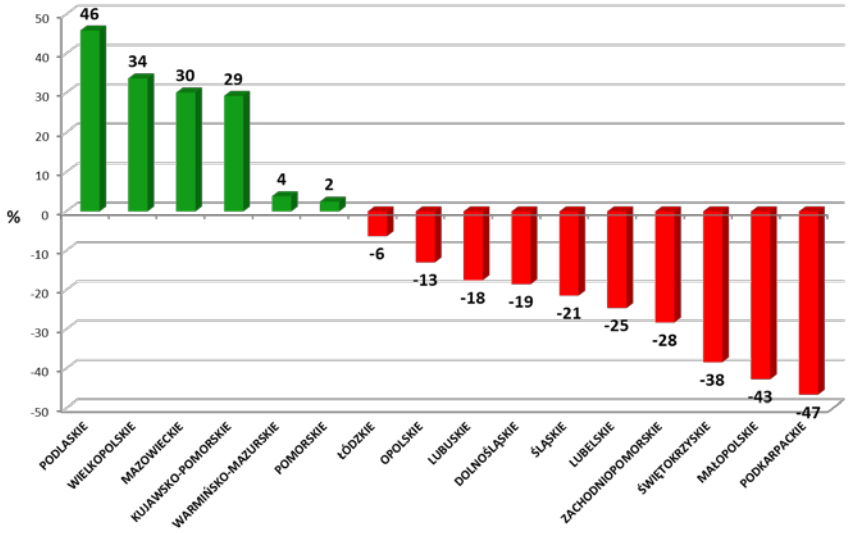
Milk sector represents one of the most important branches of agricultural production in Poland. In 2014 the value of marketed milk production amounted to 19% of the total agricultural output estimated at around 78 bn PLN. Since the accession to the EU raw milk production was covered by the milk quota scheme which expired in spring 2015. Therefore over the last eleven years the levels of raw milk production in Poland were determined by production limits granted in accordance with the EU legislation. Raw milk production was rising slightly since 2004 to reach 12,6 bn litres in 2014. In the same period buying-in of raw milk increased to 10,3 bn litres. As a consequence the level of marketability improved and reached 85% (Graph 1). Currently Poland is 4th milk producer in the EU and 12th milk producer in the World.



Graph 1 Production, buying-in of milk (in bn litres) and its marketability (in %). (*) share of buying-in and other types of sales in milk production. Source: ARR's own study based on data of the GUS and IERiGŻ-PIB

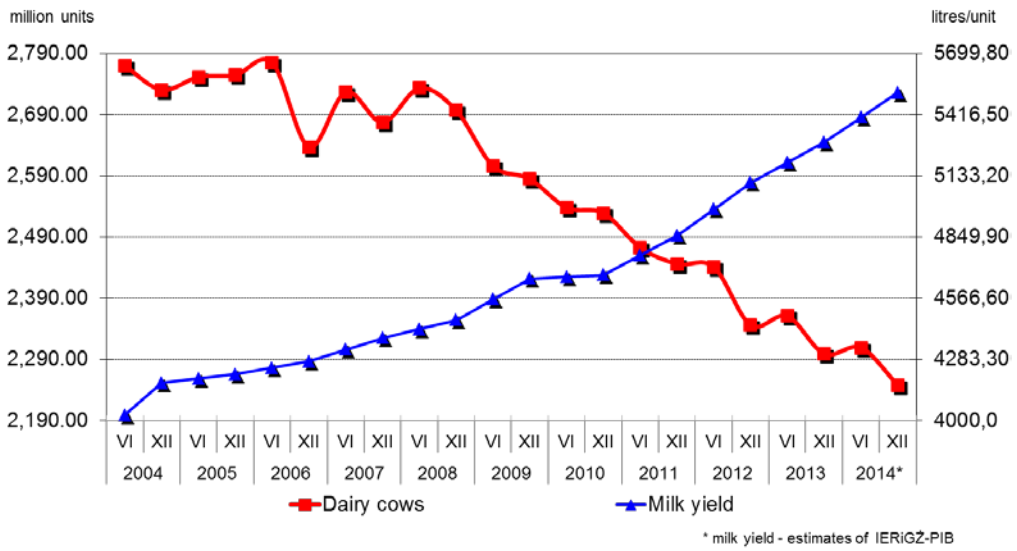
Over the last 10 years regional concentration in raw milk production was progressing. Production increased in podlaskie, wielkopolskie, mazowieckie, kujawsko-pomorskie, warmińsko-mazurskie and pomorskie voivodships (Graph 2). On the other hand milk production decreased in the remaining

regions of Poland. As a consequence raw milk production is now concentrated in central and eastern part of the country. Five most productive regions provide for 70% of raw milk supply in Poland.



Graph 2 Changes in raw milk production in Polish regions between 2004 and 2013. Source: ARR's own study based on data of the GUS and IERiGŻ-PIB

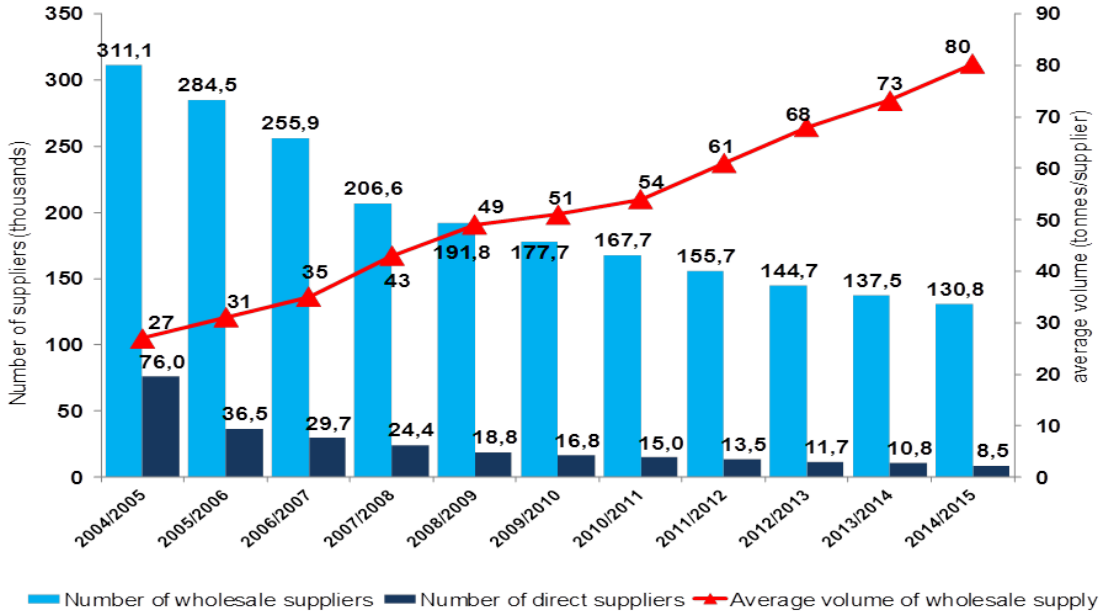
Another interesting change concerns the number of cows and average milk yield. Since the accession to the EU the number of milk cows decreased by approximately 15%. On the contrary, average milk yield per unit was over 30% higher (Graph 3). The process started in the 90-ties of previous century and was associated with transformation of Polish agriculture to market oriented economy. Improved milk yields compensate for a reduction in cows' herd. Increase in productivity was possible thanks to introduction of a more productive breeds, improved feeding programmes and investments in modern production facilities at farm level. Despite its gradual increase the average milk yields in Poland are still below the levels achieved by leading EU producing countries.



Graph 3 Dairy cows herd against milk yield in Poland. Source: ARR's own study based on data of the CSO and IERiGŻ-PIB

Almost entire dairy herd in Poland is being held by individual family farms. Only 5% is in possession of other operators. The accession to the EU accelerated structural changes in Polish milk farming. The number of large scale specialized farms increased. The same applies to the average number of cows per herd. Milk production is gradually gaining commercial character. Nevertheless small milk farms are still predominating in Poland. In 2014 almost 65% of cows were kept in herds less than 30 units.

Moreover structural changes in Polish milk production are visible when looking at the number of raw milk suppliers and average volume of milk supply over the last 10 years (Graph 4). Since the adoption of the EU milk quota regime the number of wholesale suppliers in Poland systematically decreases. In 2004 there were 311 thousands wholesale milk suppliers and in 2014 only 130 thousands of them remained. It means their number has been reduced by approximately 60%. Even more significant decrease was observed in case of direct suppliers. As a consequence the average volume of milk per supplier went up from 27 tonnes in 2004 to 80 tonnes in 2014. Such development is another sign of progressive concentration of milk production in Poland.



Graph 4 Number of raw milk suppliers (in thousands) and average volume of milk supply (in tonnes) in Poland. Source: ARR's own data

Processing industry

At present over 70 percent of milk produced in Poland is being bought by dairy cooperatives. One quarter is being sold to commercial companies and less than 2 percent to private individuals or other operators. Such structure of milk buying-in proves high level of vertical integration of the milk sector in Poland. However the role of private companies on the market increases in recent years.

Over the last 10 years the number of milk processing plants in Poland decreased by 84 (from 265 in 2004 to 181 in 2014). Volume of processed milk per plant, value of sale per processing plant, value of sale per employee as well as volume of processed milk per employee increased since Poland's accession to the EU.

Significant changes were observed in the levels of production of the main milk products, namely butter, skimmed milk powder (SMP), hard cheeses, curd cheeses and fermented milk drinks over the last decade. The most significant increase in production was observed in case of fermented milk drinks as well as in case of hard cheeses and curd cheeses. The volume of production of butter and SMP was rather stable during the period in question, or even declining over several years in case of SMP.

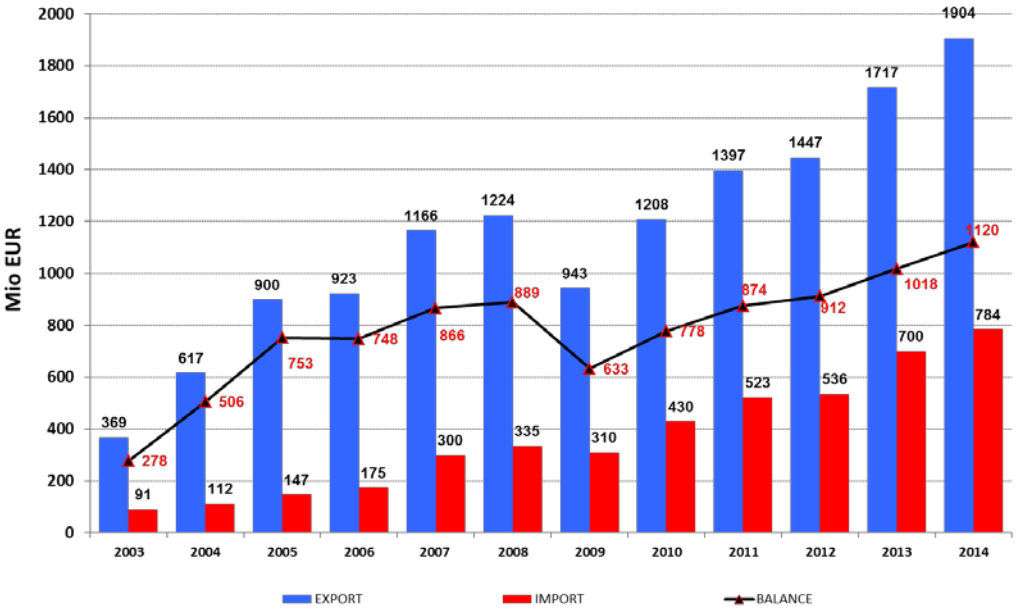
In 2014 the levels of production of all main milk commodities were higher than in 2004 (Table 1). In most cases the share of export in production increased as well. As a result almost 70% of SMP production and almost 50% of hard cheeses production were sent abroad in 2014. The other products were manufactured mainly for domestic market. Currently Poland's own supply remains higher than its internal demand for milk products. As a consequence part of the production needs to be exported.

Table 1 Share of exports in total industrial production of milk products in Poland (2004 vs 2014). Source: ARR's own study based on data of the GUS, IERiGŻ-PIB, Ministry of Finance.

Type of product	2004		2014	
	Production (thousand tonnes)	Share of exports in total production (%)	Production (thousand tonnes)	Share of exports in total production (%)
SMP	135	72	170	69
Butter and milk fats	177	16	181	20
Hard cheeses	219	30	306	46
Curd cheeses	296	6	406	15
Fermented milk drinks, incl. yoghurts	469	9	707	16
Whey	835	6	1 275	20

Trade with third countries

Over the last decade there was a gradual increase of Polish trade in milk products with other countries. Over the entire period Poland was a net exporter of milk products, with the value of exports amounting to 1,9 bn EUR and import value of 784 million EUR in 2014, which was a record year in this respect (Graph 5). As a result we were able to reach a positive trade balance of 1,1 bn EUR.



Graph 5 Polish foreign trade in milk products (2003-2014) (value). Source: ARR's own study based on data of the IERiGŻ-PIB, Ministry of Finance

In 2014 the leading group of products most frequently exported from Poland were hard cheeses, followed by SMP, liquid milk, cream and whey. Together they represented over two thirds of Polish milk exports in terms of value. Other cheeses, fermented milk drinks and butter were also important export products. Compared to 2004 there was an increase in the share of exported liquid milk and cream, whey, other types of cheeses and fermented milk drinks.

The most popular destinations for Polish milk products were the EU Member States. The EU remains Poland's main trading partner to which it has sent 66% of its exports (data for 2014). 8% of milk products exports were sent to Commonwealth of Independent States, 7% to Algeria, 3% to China, 1% to Saudi Arabia and the remaining 15% to other countries outside the EU.

Factors influencing development

Modernization and restructuring of Polish milk sector have been supported by the EU structural funds, which were directed to Polish agriculture and processing industry both before accession and after the accession to the EU. The most important ones were:

Special Accession Programme for Agriculture and Rural Development 2002-2004;

Sectorial Operational Programme – restructuring and modernization of agri-food sector and rural development 2004-2006;

Rural Development Programmes: 2004-2006 and 2007-2013.

Projects co-financed from the abovementioned EU funds were aiming at

- improvement of sanitary and veterinary conditions in milk production;
- market orientation and exploitation of market niches;
- creation of new distribution channels and improvement of existing ones;
- improvement of product's quality;
- increase in value added of production;
- reduction of negative influence on environment;
- improvement of logistics;
- improvement of animal welfare;
- introduction of new production technologies and modernization of existing ones.

For the next financial perspective that is 2014-2020 there are also EU financial resources envisaged for similar purposes. More money should be directed to innovative projects and solutions to increase the competitiveness of Polish agri-food industry.

Further development of Polish dairy sector will depend on number of economic and policy factors, including among others: rate of economic growth and demand for dairy products both at national level and worldwide, input prices, markets volatility, competition from other countries, as well as the results of the EU bilateral and multilateral trade negotiations and effectiveness of the reformed EU Common Agricultural Policy measures.

Summary

Accession to the EU accelerated modernization and restructuring of the Polish milk sector. Over the last ten years Poland experienced deepened regional specialization in milk production and progressive concentration of production in specialized individual farms. It resulted in robust production potential of Polish dairy farmers and processing plants. Investments co-financed from the EU structural funds improved competitiveness of Polish dairy sector and increased export capacity. Nevertheless there is room for further concentration of milk processing and increase in innovations. Future development of the sector will be influenced by many economic and policy issues.

SUSTAINABLE DEVELOPMENT OF THE DAIRY INDUSTRY IN THE SUB-SAHARAN REGION

Kobus MULDER

Consultant / South Africa

Speech

Thank you to the International Dairy Federation for the opportunity to share some dairy information about the continent I live on.

The interest in information about the dairy industry in Sub-Saharan has increased considerably in recent years and the reason is threefold.

Firstly,many regional governments consider milk production as a means to improve the nutritional status and income of the people and they need reliable information to structure policies for the industry.

Secondly,many non-governmental and international aid organizations need good information to plan, structure and execute aid programs in the industry.

Thirdly, and more recent, large international dairy companies are searching for emerging countries which have a shortage of milk and/or in whichthe per capita consumption is showing good growth.

However, the availability of reliable production and consumption information for the Region remain a challenge. Only three countries, Kenya, Zimbabwe and South Africa supply information to the IDF on an annual basis – in the rest of the countries it is gathered by local governments and international aid organizations.

Sub-Saharan African is divided into western, eastern, central and southern. The Region is home to 51 countries with a population of some 969 million people and has a wide variety of ethnic groups, cultures and climate zones. What makes the region important in world population is the UN projected population growth for the region. This projection shows that the region's current contribution to world population, which stands at 13.3%, will grow to 18.6% during the next 25 years and then on to 25.0% in the next 25 years. This will happen while the percentage shares of the other world regions are shrinking. This effectively means that the region will soon become the second biggest geographical group of consumers in the world – and growing. These statistics could be interpreted in more than one way but we see it as a huge market with potential and if we do the right things right, everybody would benefit.

Africa currently produces 5.4% of the cow milk produced in the world, which isironically a largerpercentage than Oceania however; the difference is that nearly 100% of the milk produced in Oceania is processed whereas only a small percentage of what is produced in the Region is processed.Culture, climate, geographical size and government focus all play a part in why some regions produce more or less milk than others. What is important is to see this information in relation to thepopulation, consumption and GDP of each sub-region.

East Africa and Southern Africa are without doubt important dairy regions but the sub-regions are so diverse that quick conclusions should be avoided. The correct approach for making commercial or foreign aid decisionsis to study the countries individually. For instance, the low per capita consumption figure in the West changes dramatically when the large quantities of imported milk powders are factored in.Alternatively, if one considers that Kenya has a per capita consumption of 100 litres.

Per capita consumption is an important statistic in the food industry and it is therefore important to Sub-Saharan African consumption compared to international consumptions.Sub-Saharan African consumption lags far behind that of other world regions but it should be seen in the context that many value added dairy products are not yet part of the African diet.

When one looks at the per capita milk consumption for some individual countries, it is clear that fresh milk is consumed in larger volumes in some Eastern and Southern African countries. It is an indication how the dairy situation differs from country to country and to understand this better, it is wise to look at the history of milk production in the various sub-regions.

The Maasai in East Africa and Fulani group in West Africa have always been traditional owners of dairy cows and utilized the milk as part of their diet. The Maasai are traders and sold the milk whereas the Fulani used it as a product of peace and used it as gifts to friends.

After independence in the 60's, many previous Eastern colonial governments started aid programs to assist the local population to produce more milk to consume and sell. The result of this aid was increased milk production and higher per capita consumption. Many local governments invested in modern dairy processing plants with the help of international aid however, these processing plants were not well maintained and many of them came to a standstill after 35 – 40 years.

In SouthAfrica, it was the Dutch and English settlers who imported the first thoroughbred dairy animals and after WW II, many dairy processing cooperatives were established to add value to milk. With the help of government interventions, the primary and secondary dairy sectors made good progress and established a sound industry. Today, the dairy industry in the sub-region is developed and a number of international investors found it a good sub-region to invest and operate in.

A variety of milk production systems is applied in Sub-Saharan African.

A pastoral system is used by migrating farmerswhoown many animals but only milk once a day for own consumption and to donate to friends and family. Milk is seldom sold. Often the milking of animals is left to young boys and females in the family.

The agro-pastoral system rose from the pastoral system and is practiced by sedentary farmers who also cultivate some crops, the residues of which are given to the cows. These farmers milk twice a day and milk is sold at markets or to cooperative milk collection centres on a volume basis

The semi-intensive system is practiced in peri-urban zones and farmers own their land. Farmers are often retired civil servants who employ staff to cater for the animals. Feeding is done through grazing and feeding concentrates. Breeding is often done with artificial insemination to improve local cows. Milk is sold to individuals and to small cooperative processors on a volume basis.

The intensive system is mainly practiced in South Africa and by a few large farmers in Kenya, Zambia and Zimbabwe. Herd sizes vary between 200 and 7 000 cows and a viable herd is considered to consist of 600 cows. All the milk is sold to processors and is bought on a quality basis with butterfat, protein, total count and somatic cell count being the pillars of such a scheme.

Because the status quo of dairy in Sub-Saharan African is so diverse, it is advisable to give an overview of the west, central & eastern sub-regions and the southern region separately.

Dairy is the fourth most important agricultural sector in the Western, Central and Eastern sub-regions and milk is mostly produced by small farmers. Production per lactation per cow is between600- 1 000 litres with a few larger farms producing up to 3 000 kg per cow. Due to extreme dry and rainy seasons, significant seasonal variations are the norm. Although milk quality has improved in recent years, it

stays a challenge and so does milking efficiency. With the exception of a few larger farms, milking is done by hand, which strangely, helps to produces cleaner milk. Milk collection centres are the backbone of milk production and a number of them have evolved into processing plants. Unfortunately, most milk is still sold as raw milk to individuals and milk shops. Sadly, only 5-25% of milk produced is processed into value added products and this remains a big challenge in these sub-regions. A few large processors in the eastern sub-region process 60% of this milk while the rest is processed by small cooperative processors. An unsatisfactory situation, whichaffects negatively on profitability, is the fact that capacity utilization is only 25-50%. The reasons for this is that recently erected processing plants are fitted with over capacity equipment and that many new plants have been built in the recent past; thereby creating over capacity. Milk is bought on a volume basis except for Kenya and Zambia where processors have started to pay on quality.The main products are pasteurized milk, fermented products and some UHT milk and these products are mainly marketed in urban areas due to underdeveloped distribution chain activities.Many countries understand the value of industry associations and have these in place however, they do not all function effectively.

The dairy industries in Southern Africa countries such as Lesotho, Swaziland, Botswana and Namibia are poorly developed with hardly any milk production. The South African industry is developed. Both primary and secondary sectors are well organized and stable in a liberalized environment.Like in many countries, a concentration at primary level has taken place from 7 000 dairy farmers in 1995 to the current 1 800. A herd size of 600 cows is considered viable and average lactation production is around 6 000 litres per cow. Ninety five percent (95%) of all milk is sold tosome 300 processors who manufacture a wide variety of value added except for super proteins and specialty products. Neighbouring countries such as Lesotho, Swaziland, Botswana and Namibia are largely dependent on South Africa for the majority of its dairy products.

International aid plays and important role in the dairy industries of many Sub-Saharan African countries and the interventions have put the dairy industries of many countries on the road to sustainability.Each of these international role players has its own objectives and strategies but the overall objective is to improve the income of small-scale farmers through increased milk volumes.

The results of these interventions can be seen in many countries as it has lifted many people and communities out of poverty.There are many foreign aid organizations active in Sub-Saharan African dairy industries but the largest andmost visible are:

- USAID
- Land 0'Lakes
- Heifer International
- International Livestock Research Institute
- Technoserve
- Irish Aid
- SNV

They manage projects in the dairy sectors of many countries and their broad focus is to:

- feed the people
- improve livestock quality
- increase and improve milk production
- add value to milk
- develop markets

This focus is executed through the following actions, which mostly benefit small-scale farmers.

- Genetic improvement of dairy animals.
- By supplying grade pregnant heifers to selected farmers the female calf of which is then passed on to the next selected farmer
- Availability of semen and AI service to farmers
- Higher milk yield
- Dry period management, mastitis control, cow comfort and optimum feed intake after calving
- Milk collection centres
- Cooperative milk collection centers are established and erected in strategic locations
- Technicians are trained in milk quality testing
- Connect milk collection centres with milk buyers
- Supply contracts are facilitated with reliable milk buyers/processors
- Boost income
- Farmers are trained in economical herd management and milk production
- Milk for money

The result of all these foreign interventions and government support is that milk production has become an attractive career for new small-scale farmers and an incentive for existing dairy farmers to increase their herds.

The many knowledge-sharing programs presented by international aid organizations have resulted in improved milk efficiency and more quality milk. The overall result of these outcomes is not only increased incomes for the farmers but also for secondary beneficiaries such milk transporters, milk sellers and technicians employed by processing plants. These are the kind of outcomes which we are convinced will make dairy more sustainable in Sub-Saharan African.

We understand that sustainable results will take time and that the dairy industries have to be supported for much longer with further actions such as:

- improved genetics of dairy animals
- availability of financial services such as credit for farm inputs
- reliable veterinary and extension service
- good manufacturing methods
- the promotion for dairy consumption

This last action is especially daunting because as traditional dietsare changing, we need to offer the correct type of dairy products.

Another aspect, which has, and will bring more sustainability to dairy in the Region, is investment by large international dairy processors. The Region attracted a number of early of investors but it is the recent investors, which see economic growth and strong potential for dairy products in the Region, which is exciting.

Arla inthe Ivory Coast Nigeria and South Africa. FrieslandCampina also in Nigeria; Danone’s latest Sub-Saharan African ventures were into Ghana, Kenya and Uganda. Lactalis, with its acquisition of Parmalat, is now in South Africa, Mozambique, Botswana and Swaziland. We believe that sustained growth will come from the private sector and increased integration with the global economy.All these dairy companies are searching for new markets to grow in and they have realized that it would come from emerging markets. This has become more feasible because of trade liberalization and governmental investment in infrastructure and education.

The World Bank's investment of $5.3 billion during 2013 and further investments of $600 million in agribusiness all show the confidence in the Region.

The confidence, whichGreenfield investors have in the future of the continent, is clearly shown in this table.Many world regions show a negative growth whereas Africa is showinggood growth.

What is it that draws international dairy investors to the Region? More available milk for processing is being produced thanks to the work of international aid programs and government support. More and more consumers are including dairy products in their diet and per capita consumption is increasing as shown earlier. Strong growth in the dairy and retail sectors are evidence of this. The fact that Africa accounts for 15% of world imports of dairy products is an opportunity, which makes it attractive for companies such as Arla, FrieslandCampina and Lactalis to invest in the Region.

The ongoing challenges for all of us who are operative in the Region remain the relative small milk volumes produced by farmers and who do not always have the necessary skills to manage their dairy operations as a profitable business. Many local processors still lack adequate processing knowledge; a fact, which will improve with more knowledge sharing and the entry of international processors. Improved marketing and distribution will assist in growing the per capita consumption of dairy products.

What is needed to ensure a sustainable dairy industry in Sub-Saharan African?Our ongoing efforts will focus on the following:

- the enhancement of livelihoods
- the improvement of the health of people and animals
- minimize environmental impact

It is unknown how many people are employed directly in Sub-Saharan African dairy but we know it is millions and it is one of the objectives of all international aid and government programs to invest in these people and to improve their lives. Further, the Sub-Saharan African dairy industries can only be sustainable if we create industry prosperity, improve the lives of local and regional communities.

Sub-Saharan African knows the value of milk and milk products as a nutritional food and sustainability is possible if we produce quality dairy products complying with food safety and quality standards.

However, the dairy value chain does not consist of people only, dairy animals are part of it and their wellbeing is a constant part of our current and future focus.

Sub-Saharan African is fortunate to be a large geographical area with manageable cow and human population however, this will change in future. To ensure the sustainability of the dairy industry in the Region we will have tomanage its impact on the environment.

Thank you.

MILK-DERIVED BIOACTIVE PEPTIDES: POTENTIAL HEALTH BENEFITS AND APPLICATIONS

Anne Pihlanto

Natural Resources Institute Finland (Luke), Myllytie 1, FI 31600 Jokioinen Finland

Abstract

During food digestion, proteins are hydrolysed into a large variety of peptides. Some of these peptides are structurally similar to endogenous peptides that play a crucial role in the organism as hormones, neurotransmitters or antibiotics. Therefore, food peptides can interact with the same receptors than endogenous peptides and exert an agonistic or antagonistic effect in the organism. The activity of the peptides depends on the ability of these peptides to reach the target tissue in an active form, which depends on their structure. Especially, milk protein derived bioactive peptides has received growing interest. At present, evidence of the potential of these dairy peptides to modulate numerous physiological conditions has been mainly achieved using in vitro assays; however, it is accepted that the health evidence has to be based on in vivo trials (animal and human). For example, there is evidence for the antihypertensive effects of fermented milk products containing bioactive peptides, which inhibit angiotensin converting enzymes and are absorbed. Recently, industrial scale technologies suitable for the industrial production of bioactive peptides have been developed. This communication will review the current scientific knowledge of milk-derived peptides with emphasis on the evidence in animal and human studies as well as commercial applications of bovine milk protein-derived bioactive peptides.

Introduction

Milk is very often described as the “ultimate” food which fulfills the nutritional needs of the mammal newborn and ensures safe development and growth during the first stages of its life [1]. Individual milk components contribute to the high biological and nourishing value of this food. Milk proteins, for instance, are known to exert various actions that promote human health. Recently, milk proteins have attracted extensive interest in terms of their bioavailability following ingestion. In vivo digestion of milk proteins within the gastrointestinal tract results in the release of peptides with new biological properties compared to the ones exhibited by the intact, precursor molecule [2]. Many of these protein fragments, also known as bioactive peptides (BAPs), consist of approximately 3–20 amino acids, which are encrypted within the parental protein and become activated following their release, induced by the action of gastric and pancreatic enzymes. The activity of peptides is based on their inherent amino acid composition and sequence [3]. The multifunctional properties of biologically active milk peptides are increasingly acknowledged.

Biologically active peptides derived from milk are initially found in inactive form within the sequence of the precursor molecules but it can be released in three ways; (i) enzymatic hydrolysis with digestive enzymes like pepsin, trypsin, chymotrypsin etc; (ii) fermentation of milk with proteolytic starter cultures; (iii) proteolysis by enzymes derived from proteolytic microorganisms. Once these bioactive peptides are liberated, they may serve to influence numerous physiological responses including cardiovascular, digestive, endocrine, immune and neurological activity etc. Because of such physiological versatility, milk-derived BAPs have drawn the attention of many researchers worldwide in order to formulate several potential drugs with nutraceutical supplement properties, health promoting functional foods or other pharmaceutical products [4,5].

This review focuses on the occurrence and potential health benefits and applications of bioactive peptides derived from milk. A Particular emphasis is given to peptides with properties relevant to cardiovascular health including the effects on blood pressure and oxidative stress.

Release of bioactive peptides

In the search for new peptides with biological activity, bioinformatics constitutes an important tool. Bioinformatics enables the prediction of protein structure-function relationships, the identification of protein domains and computer simulation of proteolytic processes. All this information can be extracted from the vast number of bioactive peptides that have already been isolated and identified. The structural motifs in active peptides serve as a source of information to be used in the search for new bioactive molecules [3-6]. Databases, such as BIOPEP, can be utilized to determine the occurrence frequency of known BAPs and structure-function patterns in the primary sequence of food proteins and to simulate the proteolysis of dietary proteins based on the knowledge of the specific cleavage sites of certain enzymes [7]. The resulting peptides from the simulated hydrolysis treatment can be matched with the BAPs with known bioactivities, and the remainder of the purportedly “inactive” peptides can be analyzed with quantitative structure activity relationship model (QSAR) to predict biological activities by physicochemical descriptors associated with known bioactivities. However, there are certain limitations with the in silico approaches. For example, it is not certain that in silico peptides can be reproduced empirically due to the complex nature of protease-substrate interactions. Also, the information included to databases is limited and often does not include constantly accumulating information on new BAPs. Thus, an integrated approach utilizing in silico analysis in combination with experimental validation with hydrolysates and synthetic peptides is the most useful for mining and discovering of BAPs.

Enzymatic hydrolysis has been used to liberate BAPs from intact proteins sequences. A variety of different enzymes have been used to hydrolyse milk proteins generating hydrolysates with different degree of hydrolysis, containing a diverse array of peptides with different activities. The most prominent enzymes are pepsin, trypsin, chymotrypsin and Alcalase that have been shown to release a number of antihypertensive, antioxidant, antibacterial, immunomodulatory and opioid peptides both from caseins and whey proteins [4]. The release of BAPs by enzymatic hydrolysis is summarized in Table 1. Several lactic acid bacteria (e.g. Lactococcus lactis, Lactobacillus helveticus) have been reported to liberate BAPs during fermentation. This system consists of a number of distinct intracellular peptidases including endo-peptidases, amino-peptidases, di-peptidases, and tripeptidases [8]. The production of various bioactive peptides including antimicrobial, immunomodulatory, antioxidative and ACE-inhibitory through microbial proteolysis has been reviewed in several articles [4, 9, 10].

Bioactive peptides may be released in vivo during gastrointestinal digestion by the action of digestive enzymes like pepsin, trypsin or chymotrypsin. Gastrointestinal digestion permits the consequent action of the enzymes present in the small intestine such as pepsin, trypsin or chymotrypsin, which are responsible for protein hydrolysis [4]. Several bioactive peptides (antibacterial, immunomodulatory, anti-hypertensive and opioid peptides) are known to be released from casein and/or whey proteins by gastrointestinal digestion [5, 11, 12]. The formation of bioactive peptides in vivo following the digestion of milk proteins has been documented in the past both in human [13, 14] and animal [15] models. According to these studies, although evidence exists to support their presence in the human and piglet gastrointestinal tract, further research is required to investigate the physiologic conditions which would allow the BAPs to exert their bioactivity.

Bioavailability of bioactive peptides

Bioavailability is a major issue when establishing correspondence between in vitro and in vivo activities of BAPs. The capacity to reach target organ in an active conformation determines the physiological effect of BAPs. Various processes take place after oral administration of a BAP and need to be considered on the final activity. It's highly likely that putatively antihypertensive reported peptide sequences are subjected to alteration before the final activity in vivo after the various steps, such as attack of gastrointestinal enzymes and brush border peptidases, absorption through the intestinal barrier, attack of intracellular peptidases in the transcellular absorption and plasma enzymes after the peptides have entered the circulation [16, 17].

As human studies are time-consuming, costly and restricted by ethical concerns, the development of in vitro models for investigating the effects of digestion on the BAPs has attracted much attention. A plethora of models have been implemented to simulate gastrointestinal digestion; most typically models simulate digestion in the oral cavity, the stomach and the small intestine. There is substantial variability among the conditions, such as the time of digestion, agitation, enzymes and concentrations of the salts and bile acids used. Moreover, there is great differentiation in the inclusion of various digestion stages and whether the chosen conditions are static with fixed concentrations of enzymes and bile acids etc., or dynamic with varying concentrations of these constituents. For instance, human digestive liquids have been utilized to model digestion in vitro [18]. and meanwhile several reports have concerned the implementation of porcine enzyme mixtures [19, 20]. The diversity of model conditions has hampered the ability to compare results across the different studies. Thus, a consensus concerning the basic parameters would be relevant in order to harmonize the various in vitro digestion models. Recently, in vitro models for studying the digestion of secondary plant metabolites (such as polyphenols) were reviewed by Alminger et al. [21]). A set of parameters for static in vitro models was suggested based on relevant in vivo data. Thus far, such a harmonization of parameters based on in vivo data has not been reported for modeling the bioavailability of peptides.

Study of intestinal absorption in vitro is another common aim when elucidating the bioavailability. It has been indicated that a small portion of peptides can pass the intestine barrier and although it is usually too small to be considered nutritionally important, it can present the biological effects in tissue level [22, 23]. Research findings indicate that peptides with 2–6 amino acids are absorbed more readily in comparison to protein and free amino acids. As the molecular weight of peptides increases, their chance to pass the intestinal barrier decreases. Peptides are transported by active transcellular transport or by passive process [24]. The absorption studies are commonly performed with the monolayer of intestinal cell lines, such as Caco-2 cells, simulating intestinal epithelium, and analysis of peptides and metabolites in serum after in vivo and clinical studies. Foltz et al. [25] investigated the transport of Ile-Pro-Pro and Val-Pro-Pro by using three different absorption models and demonstrated that these tri-peptides are transported in small amounts intact across the barrier of the intestinal epithelium. In another study, the absolute bioavailability of the tri-peptides in pigs was below 0.1%, with an extremely short elimination half-life ranging from 5 to 20 min [26]. In humans, maximal plasma concentration did not exceed picomolar concentration [14]. Milk-derived peptide Leu-His-Leu-Pro-Leu-Pro is an interesting example of a peptide with evaluation of bioavailability. This peptide resisted gastrointestinal simulation, but cellular peptidases digested the peptide to His-Leu-Pro-Leu-Pro before crossing Caco-2 cell monolayer [27, 28]. The degradation product, His-Leu-Pro-Leu-Pro, has been demonstrated to absorb in human intestine as it has been identified in human plasma after oral administration [29].

Impact of milk protein-derived peptides on non-communicable diseases

Metabolic syndrome (MetS) consists of a combination of cardiometabolic risk determinants, including central obesity, insulin resistance, glucose intolerance, dyslipidemia and hypertension, that have been linked to an increased risk of developing cardiovascular disease and diabetes mellitus type 2. The

prevalence of MetS is about 20% to 30% among adults worldwide and is increasing due to the consequence of unhealthy diet, physical inactivity, tobacco use, and the ongoing obesity epidemic. Cardiovascular disease (CVD) is the single leading cause of death for both males and females in technologically advanced countries in the world. In lesser-developed countries it generally ranks among the top five causes of death. The World Health Organization estimates that by 2020, heart disease and stroke will have surpassed infectious diseases to become the leading cause of death and disability worldwide [30]. Consequently, there has been an increased focus on improving diet and lifestyle as a strategy for CVD risk reduction. Milk derived peptides have gained interest because of their notably anthypertensive, antioxidatiive, anti-inflammatory and hypocholesterolemic effects. The most current scientific information regarding in vitro and in vivo studies on the role of milk protein-derived peptides on CVD reduction is summarized and discussed below.

Milk peptides with antihypertensive activity

Elevated blood pressure is one of the major independent risk factors for CVD [31]. Angiotensin I-converting enzyme (ACE) plays a crucial role in the regulation of blood pressure as it promotes the conversion of angiotensin I to the potent vasoconstrictor angiotensin II as well as inactivates the vasodilator bradykinin. Thus, ACE inhibition is regarded as one of the best strategies for hypertension treatment. During the last decade, many food proteins have been identified as a source of ACE inhibiting peptides which have become the best known class of bioactive peptides. In the last two decades antihypertensive effects of some of these peptides have been evaluated in spontaneusouly hypertensive rats (SHR) and hypertensive humans, and the peptide sequences, doses and maximum decrease of systolic blood pressure (SBP) have been summarized in several reviews [12, 32-34] and in Tables 2 and 3.

Casein hydrolysates have in some studies [35] produced higher ACE-inhibitory activity than whey protein hydrolysates but also whey peptides such as Ala-Leu-Pro-Met-His-Ile-Arg (ALPMHIR) from tryptic digest of -lg have been identified with strong antihypertensive activity [36-39]. Other proteolytic enzymes, such as Alcalase, Thermolysin, Subtilisin and successive treatment with pepsin and trypsin in order to simulate gastrointestinal digestion have been employed to release ACE inhibitory peptides. The most commonly known peptides of milk origin are the ACE-inhibitory tripeptides Val-Pro-Pro (VPP) and Ile-Pro-Pro (IPP), derived from - and -caseins. Some structural features, like amino acids with cyclic or aromatic rings (Tyr, Pro, Trp), are found at the C terminal of the ACE-inhibitory peptides.

Numerous studies in spontaneously hypertensive rats (SHR) as well as in hypertensive human volunteers have been performed to determine the antihypertensive effect of milk-derived peptides (for reviews see 12, 32-34]. These studies demonstrated that several ACE-inhibitory peptides significantly reduces blood pressure, either after intravenous or oral administration. More than twenty human studies have been published linking the consumption of products containing the tripeptides, IPP and VPP, with a significant reduction in both SBP (systolic blood pressure) and DBP (diastolic blood pressure). Maximum blood pressure reductions approximate 13 mmHg (SBP) and 8 mmHg (DBP) after active treatment compared to placebo, and are likely to reached after 8-12 weeks of treatment (Table 3). Effective dosages of tripeptides range from 3.07 to 52.5 mg/d . Four meta-analyses performed with the published data of 17 [40], 12 [41], 28 [42] and 30 [43] clinical trials have reported an average decrease in SBP of 5.1, 4.8, 1.7 and 2.95 of mmHg, respectively. No effects were found in Dutch and Danish subjects consuming fermented milk containing peptides VPP and IPP [44, 45]. Sub-group analyses showed that participants with elevated BP at baseline appeared to have a greater SBP and DBP reduction after LTP supplementation [43]. A meta-analysis including 18 trials has reported higher antihypertensive effects for these two tripeptides in Asian than in Caucasian people [46]. It is known that there are ethnic differences in response to cardiovascular drugs, specifically BP-lowering

drugs, which might be due to genetic polymorphism or environmental factors such as diet. Another possible explanation for the differences in the response in the Japanese studies, compared with European studies, may be the participants' habitual milk and dairy consumption. The European Food Safety Authority (EFSA) Panel on Dietetic Products, Nutrition and Allergies (NDA) has concluded that the evidence presented to date on the antihypertensive effects of peptides VPP and IPP is insufficient to establish the relationship between the consumption of these peptides and the maintenance of normal blood pressure [47].

Other peptides derived from β-casein during milk fermentation with Enterococcus faecalis, in which sequences are LHLPLP and HLPLP, have also shown antihypertensive effects in SHR [48]. In recent studies, fermented milk with Lactococcus lactis NRRLB-50571 and NRRLB-50572 has presented important SBP, DBP, and heart rate-lowering effects in SHR [49, 50] although the peptides responsible for the activity have not been identified. Consumption of whey protein hydrolysate for 6 weeks resulted in reduction of SBP and DBP, of hypertensive subjects. However, peptides responsible for the observed effect have not been identified. More recently, yoghurt enriched with other milk-derived peptides, Arg-Tyr-Leu-Gly-Tyr and Ala-Tyr-Phe-Tyr-Pro-Glu-Leu, reduced blood pressure in hypertensive humans [51].

Antioxidant and anti-inflammatory milk-derived peptides

Oxidative stress, resulting from an increased production of reactive oxygen species (ROS) in combination with outstripping endogenous antioxidant defense mechanisms, is another significant causative factor for the initiation or progression of several vascular diseases. ROS can cause extensive damage to biological macromolecules like DNA, proteins and lipids.

Neither the structure-activity relationship nor the antioxidant mechanism of peptides is fully understood. Saito et al. [52] have screened 40 peptides structurally related to Leu-Leu-Pro-His-His, which is an antioxidative peptide isolated from soybean protein digests, Pro-His-His was identified as the active center. The antioxidative properties of peptides varied depending on their structure and assay system. The majority of the studies carried out to characterize antioxidant peptides derived from casein and whey proteins have only used in vitro chemical assays. However, their limited similarity to physiological conditions makes the in vitro assays very restrictive and reported effects need to be confirmed by animal models and/or human trials. Nevertheless, to date, just few in vivo trials have been carried out to demonstrate the antioxidant effects of milk derived peptides related to benefits on cardiovascular health. Zommara et al. [53] reported the antiperoxidative action of fermented milk on rats fed a vitamin-E deficient diet. The consumption of fermented milk by healthy subjects has been demonstrated to lower the levels of oxidized low-density lipoprotein, isoprostanes, and the glutathione redox ratio. Improvements of total plasma antioxidant activity and of the resistance of the lipoprotein fraction to oxidation have resulted in enhanced antiatherogenicity [54].The compounds responsible for the observed effects have not been identified yet, although milk peptides liberated during fermentation process might have a crucial role.

Chronic inflammation is another responsible factor for the development of CVD. The down regulation of cytokines involved in the inflammation-associated endothelial dysfunction by food components, including peptides, may delay or alleviate inflammation, thus exerting favorable effects against CVD [55]. A recent study using lipopolysaccharide- (LPS-) stimulated mouse macrophages showed that a yak casein hydrolysate reduced the secretion of proinflammatory cytokines and the production of nitric oxide and to scavenge free radicals. The results thus suggest a potential role as preventive agent against inflammation related disorders [56]. To date, only one human trial has been conducted to demonstrate the anti-inflammatory properties of milk peptides. This study reported an improvement in the vascular function through modulation of the glucose levels and inflammation and oxidative stress

biomarkers after the consumption of the commercial whey derived peptide NOP-47 by healthy individuals [57]. This finding opens a new door towards searching of new milk-derived peptides with antioxidant and anti-inflammatory activity.

Hypocholesterolaemic Milk Peptides

Blood lipids are represented in various forms including total cholesterol, triglycerides, lipoproteins (high-density lipoproteins or HDL, low-density lipoproteins or LDL, and very-low-density lipoproteins or VLDL), and free fatty acids. An inappropriate ratio of these lipids is one of the most important risk factors for developing CVD. Therefore, CVD therapy/prevention strategies focus on reaching an optimal lipid balance in order to achieve a positive cardiovascular health. Those therapies aim at increasing the physiological levels of desirable lipids (e.g., HDL cholesterol) while reducing the others associated with atherogenic functions (e.g., LDL cholesterol, triglycerides). Milk proteins, mainly whey proteins and derived hydrolyzates or peptides, have been reported to exert hypocholesterolaemic effects in different animal models. The ingestion of whey protein was correlated with a significant reduction of total cholesterol levels in rats fed with cholesterol-free and cholesterol-enriched diets [58, 59]. Nagaoka et al. [60] have reported similar effects for a β-lactoglobulin tryptic hydrolyzate administered to rats fed with a diet rich in cholesterol. The hydrolyzate reduced total cholesterol and increased HDL cholesterol and fecal steroid excretion. The Lactostatin Ile-Ile-Ala-Glu-Lys, has been reported as the main factor responsible for the observed effects. β-Lactotensin, another β-lactoglobulin peptide, released by chymotrypsin hydrolysis, decreased total cholesterol, LDL, and VLDL cholesterol content in mice fed with a cholesterol enriched diet [61]. Although the mechanism of action of those peptides has not been completely elucidated, preliminary results suggest a key role played by the amino acid composition [6]. Further studies are clearly needed to corroborate those results.

Milk-derived Peptides against Diabetes

Diabetes mellitus is considered one of the most common metabolic disorders and one of the major health problems worldwide. It affects almost 6% of the world's population, with type 2 diabetes representing approximately 90–95% of the diagnosed cases [62]. Epidemiological evidence supports that consumption of milk and dairy foods is associated with a lower incidence of type 2 diabetes. These beneficial effects on metabolic and inflammation factors linked to diabetes and insulin resistance have been also demonstrated by cell and animal models, being multiple milk components, such as calcium, medium-chain fatty acids, linoleic conjugated acid, lactose, citrate, proteins, and peptides characterized as the main responsible factors for the observed effects acting through different mechanisms of action [63]. During the ingestion of a meal, the presence of nutrients at gastrointestinal level stimulates the secretion of two incretins hormones, the glucagon-like peptide-1 (GLP-1) and the glucose-dependent insulinotropic polypeptide (GIP). Both hormones are implicated in the stimulation of the insulin secretion from the pancreatic β-cells, secretion of gastric and pancreatic enzymes, and modulation of gut motility and nutrient absorption, allowing the clearance of the absorbed glucose. Type 2 diabetes is characterized by different disorders including progressive dysfunction of pancreatic cells, insulin resistance, and augmented production of hepatic glucose. Continuous intravenous administration of GLP-1 has been demonstrated to normalize blood glucose levels in diabetic subjects [64]. However, the rapid degradation of this hormone by the enzyme dipeptidyl peptidase-IV (DPP-IV) and its consequent inactivation makes this type 2 diabetes treatment strategy impracticable. Currently, specific DPP-IV inhibitors are thus incorporated to GLP-1 analogues in new oral therapies against this metabolic disease [65]. Oral administration of the whey protein hydrolyzates to obese mice evoked an improvement of blood glucose clearance, reduction of

hyperinsulinemia, and restoration of the pancreatic capacity to secrete insulin in response to glucose. The main mechanism of action suggested for these hydrolyzates is the DPP-IV inhibitory activity exerted by the peptides contained in them [66]. Recent in silico studies have shown that both caseins and whey proteins might serve as precursors of DPP-IV inhibitory peptides because of the high number of fragments contained within them that match DPP-IV inhibitory sequences [67, 68]. Moreover, both in vitro DPP-IV inhibitory and in vivo hypoglycemic effects have been reported for peptides released from caseins. Lacroix and Li-Chan [66]] used pepsin to prepare hydrolysates from whey protein isolate or -LA, whereas Uchida et al. [69] and Silveira et al. [70]) prepared tryptic digestions of -LG and whey protein concentrate enriched in -LG, respectively. Among the bioactive peptides described to date, sequences derived from β-lg Ile-Pro-Ala and Ile-Pro-Aala-Val-Phe are the most potent as DPPIV inhibitors [70, 71]. Another β-lg fragment with sequence Val-Ala-Gly-Thr-Trp-Tyr has been also demonstrated to exert hypoglycemic effects in the oral glucose tolerance test in mice [69]. Thus, this research area holds a great potential, and currently a number of investigations are focused on the identification of new milk proteins-derived peptide with capacity to prevent diabetes and associated metabolic syndromes.

Other health promoting properties

Opioid peptides are small molecules which are synthesized in vivo and may function both as hormones and neurotransmitters. These peptides are classified as opioid agonists or antagonists. β-casomorphins derived from β-casein, and particularly β-casomorphin-5 (BCM5) and β-casomorphin-7 (BCM7), are the best-known opioid peptides [72]. The release of BCM7 from commercial milk-based infant formulas following simulated gastric digestion was confirmed [73]. The peptides identified were released from genetic variants A1 and B of β-casein. According to the European Food Safety Authority, there is no established relationship between the dietary intake of BCM7 and non-communicable diseases [74].

Casein phosphopeptides (CPPs) deriving from the digestion of milk proteins may act as mineral carriers and affect their bioavailability in the human body [75]. Recent studies document the presence of bioactive peptides with mineral carrier properties in infant formulas [76]. According to the authors, calcium could be bound preferentially to CPPs with the cluster sequence SpSpSpEE and iron and zinc to CPPs with the phosphorylated cluster and phosphoserine residues. The release of CPPs with metal-chelating properties following digestion of infant formulas was also reported in previous studies [77].

The antimicrobial activity of milk is mainly associated with minor whey proteins, namely lactoferrin. Tomita et al. [78] found out that pepsin digestion of bovine lactoferrin produces potent bactericidial peptide, and that the antimicrobial potency of hydrolysate was higher than that of undigested lactoferrin. Dionysius and Milne [79] have identified two peptides from the N-terminal of lactoferrin which displayed antimicrobial activity toward a number of pathogenic and food spoilage microorganisms [80, 81]. Casein-derived peptides, caseicidins, exhibits antimicrobial activity against Staphylococcus spp., Sarcina spp., Bacillus subtilis, Streptococcus pyogenes.. Iscardin an -casein derived antimicrobial peptide has a strong protective effect against S. aureus, S. pyogenes and Listeria monocytogenes [82].

Applications

There certainly is a potential for the application of BAPs as functional foods or pharmaceutical products. Health-conscious and people with specific demands or opportunities may be target groups for foods enriched with antihypertensive, antidiabetic and antioxidative peptides, substances, that

stimulate mineral absorption and immunomodulatory peptides. At present, milk proteins are the best known source of such ingredients but until recently the commercial production of milk derived bioactive peptides has been limited by a lack of suitable large-scale technologies. Membrane separation techniques seem to provide the best technology available for the enrichment of peptides with a specific molecular weight range [83, 84]. A novel membrane technology known as electrodialysis-ultrafiltration is useful for separating cationic, anionic and neutral peptides with defined molecular size on a large scale [85]. Recently, this method has been utilized to concentrate and separate low molecular-weight bioactive peptides with a net positive or negative charge[86, 87]. Absorbent materials, such as activated carbon, are applicable for enriching particular amino acids in food protein hydrolysates [88]. This processing method can result in sufficient yield if the amino acid residues of the target are prevalent in the peptides of interest. In addition, there are extensive bioassay-guided purification methods for the isolation of pure peptides for further analysis, especially for structure-function investigations. However, low peptide yield is still a limiting factor of the feasibility of using food protein-derived bioactive peptides. Therefore, it would be relevant to develop large-scale applicable food-grade processing methods for the production of a high yield of highly active peptide fractions. Understanding the unique structural characteristics of peptides with targeted bioactivity and exploitation of these characteristics in the concentration of the particular peptides is a crucial requirement for this approach.

Nanofiltration and ultrafiltration techniques are now employed industrially to produce ingredients which contain specific bioactive peptides based on casein or whey protein hydrolysates. Such preparations are commercially available and are being introduced into different consumer products, such as dairy and fruit based drinks, confectionery, chewing gum, pastilles and capsules. Currently marketed products contain peptides with anticariogenic, antihypertensive, mineral-binding, stress relieving and satiety inducing properties.

Conclusions

There are plenty of reports on the bioactivity of peptides in vitro. Such data, however, are insufficient in claiming an effect on human health since the active compound may be degraded during digestion, may not be absorbed or not attain the appropriate concentrations in blood and target tissues that are required for acting significantly. Certain processing procedures, especially heating, may influence the bioactivity and may also lead to the formation of undesired toxic, allergic or carcinogenic substances. In addition, the bioactivity may be reduced through molecular alteration during food processing or interaction with other food ingredients. The bitter taste of protein hydrolysates prevents the use of many products as food additives. The challenge for food technologists will be to develop functional foods and nutraceuticals without the undesired side effects of the added peptides, and to retain the stability of the added peptides within the shelf life of the product.

Moreover, the safety of all novel peptides intended for food or pharmaceutical uses should be tested in accordance with international and national food safety regulations. In cases of products intended to be marketed in the EU member states, the novel food legislation has to be observed. Other challenges with dietary bioactive peptides are posed by health claims, which in the EU countries are strictly regulated and require science-based documentation before approval by the European Commission. Therefore, controlled trials in humans are mandatory when claiming a health effect for a food. So far, evidence for such health effects exists only for a few peptides. The sour milk product Calpis™, containing antihypertensive tripeptides, an is example for products available on the market .At present, there are worldwide efforts to harmonize these regulations so as to develop fair global food marketing and protect consumers against false or misleading product information.

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ADVANCE APPLICATION OF WHEY PROTEIN IN DEVEOPLEMT OF NUTRITIOUS DAIRY PRODUCST WITH EXPECTIONAL TEXTURE AND MOUTHFEEL FOR AGEING POPULATION

Anna Bannikova[1] and Ivan Evdokimov[2]

[1]Saratov State Agrarian University named after N.I Vavilov, Saratov, Sokolovaya street, 335, Russia; [2]North Caucasus Federal University, Stavropol, Pushkin street, 1, Russia

annbannikova@gmail.com

Abstract

It is universally agreed than a greater intake of protein is required for ageing individuals to prevent the degenerative loss of muscle mass seen in Sarcopenia. Dairy is a superior protein source since it delivers higher levels of leucine which is the most potent in the elderly due to its specific action on the production of ribosoms in the cell. This work deals with the applications of whey protein in dairy products in order to develop a wide range of protein-enriched dairy products with maintaining an exceptional texture and mouthfeel. The final formulations content more than double amount of protein of a high biological value with increasing the amount of essential amino acids up to 80% as compared to the commercial counterparts. Thus, development of a range of protein-enriched dairy products will meet consumer's nutritional expectations and high likelihood of acceptance that will deliver commercial benefits for dairy industry.

Key words: Sarcopenia, branch chain amino acids, leucine, texture, sensory evaluation

Introduction

World's population is ageing while population growth is expected to slow. This presents the challenge of an increasing and changing demand for access to quality and affordable aged care services through subsidies and grants as well as industry assistance. Older adults experience reductions in metabolism and changes in physiology that significantly affect their nutritional needs. According to the research surveys, 55% of the people 60+ feel the aged-related impact of Sarcopenia, i.e. muscle loss, reduced stamina, strength and suppleness, and 53% will change their diet for maintaining wellbeing and achieve health benefits [1]. Wise food choices and a balanced diet are essential for older adults to maintain a healthy lifestyle and to promote longevity [2]. Enhancing the older people's health is a national priority since a good vigor of people 60+ will help reduce demands for health and aged care services, that indeed will stimulate economical growth of most of the countries.

One of the key-factors to prolong the vigorous lifestyle for older people is consumption of adequate amount of protein, since it is necessary for a healthy immune system and for repair and maintenance of body tissues. Research revealed that a dietary plan which includes 25–30 g of high quality protein per meal will maximize muscle protein synthesis while being cognizant of total energy intake [3]. One of the principle to address the issue of utilizing the recommended amount of protein for elderly individuals is using the fortified with protein foods.

Quality and quantity of the protein are key-establishment since by consuming the right type of protein at the right times healthy agers can achieve more consistent physiological responses and optimize their protein intake [5]. In this context, dairy is a superior protein source where even its smaller amounts deliver higher levels of the Branch Chain Amino Acids (BCAA) which are important for muscle protein synthesis. Dairy protein is available in flexible formats that are easy to digest and incorporate into diet [6].

Among three BCAA (leucine, isoleucine and valine) that shown to stimulate protein synthesis in the heart muscle to a similar extent as a combination of amino acids [7], it appears that leucine is the most potent in the elderly due to its specific action on the production of ribosoms which are the site of protein manufacture in the cell [8]. This work deals with the development of a range of dairy products (creams, beverages and desserts) with improved nutritional profile and acceptable texture and mouthfeel. The approach was confirmed by the application of plastic flow behavior that imitates chewing in the mouth, and viscoelastic flow curve that provides information on the structure and texture of materials.

Materials and methods

The following protein materials were utilised in preparation of dairy products: whey protein concentrate Alacen ^{TM} 392, calcium caseinate Alanate ^{TM} 385, sodium caseinate Alanate ^{TM} 180 and whey protein isolate were from Fonterra, New Zealand; whey protein concentrate Lacprodan DI-7017 (Arla Foods Ingredient Group, Denmark); hydrolysed gelatin (Peptiplus XB, Gelita, Australia).

This work utilises a range of stabilisers, delivered by different suppliers (carrageenan, lecithin, inulin, xanthan gum, hydroxy propyl distarch phosphate (HPDP), gelatin high bloom 25, vegetable shortening, low-methoxy pectin (LMP); sodium alginate), colours and flavours.

Sample preparation of dairy products included mixing of dry and milk ingredients using an electric mixer for 20 min at room temperature. Temperature of the product was raised up to 50°C and held there for 6 min to ensure proper dissolution of the ingredients followed by “pasteurisation” at 85°C for 5 min. All samples were cooled down to 55°C and filled into 120 ml plastic containers. Dairy beverages and creams were stored at 4°C, dairy desserts at -20°C to produce the frozen desserts of this investigation.

Samples were taken from the refrigerator or freezer just before the physicochemical measurements and sensory analysis.

Steady shear viscosity was carried out on a controlled strain rheometer (Anton Paar, Austria) with a 40 mm diameter parallel-plate geometry and 1 mm gap at 4 and 22°C. The viscosity of the samples was analysed as a function of shear rate ranging from 0.1 to 100 s^{-1}.

Hardness and adhesiveness of the samples were determined using CT 3 Texture Analyser (Brookfield, USA) with a load cell of 5 kg. The measuring geometry consisted of a cylindrical aluminium probe (25 mm diameter), which was driven into a larger ring (40 mm diameter) to compress the sample. Tests were carried out at 1 mm/s with a trigger force of 10 g and a compressive deformation up to 80% of the original height. The mechanical behaviour (hardness) was evaluated by expressing data in stress (kPa) versus strain. Following the compression cycle, force was removed from the sample as the machine's crosshead moved back to its original position. Since dairy desserts are adhesive, the force becomes negative. The area of this negative peak was taken as a measure of the adhesiveness of the sample. There are no real units for this parameter, which is expressed in the internal integrator units of the computer. Each experiment was repeated three times and an average was taken. All experiments were conducted at room temperature (22 ± 1°C) and open atmosphere.

The overrun of the cream was determined by whipping the cream and measuring the overrun every 30 seconds until maximum overrun was obtained (approx 3 min). For percentage overrun by volume, the following formulation was used:

OR (%) = [(fixed aerated volume/fixed unaerated volume) -1] x 100

After the foam overrun was determined, the foam stability was evaluated. However, the whipping cycle (using the same mixer conditions) was continued until the cream was either severely over-whipped (as judged by liquid [syneresis] that began to appear at the bottom of the bowl) or the cream had turned into 'butter'. Foam stability was measured every 30 minutes for 7 hours from the maximum overrun. Whipping curves of percentage overrun against whipping time as well as curves for foam stability dependence on time were constructed from the data obtained. Each curve was based on triplicate results which had been averaged and with error bars.

Sensory analysis taste panel trial including a hedonic taste panel of preference test was carried out with panellists (n = 40) from RMIT students and staff (aged 18-65). Commercial dairy products with low protein and dietary fibre levels versus developed dairy products enriched with protein and dietary fibre were trialled by the panellists. Panellists were asked to identify panellists were asked to identify how much they liked the samples on a hedonic scale ranging from extremely dislike (1) to extremely like (9). The sampling codes were selected from a random design number table, and the form and plates were numbered so that neither the panellists nor the presenter knew the product codes. The panellists were instructed to cleanse their palette before tasting with lemon juice followed by water and to do so in between each sample, and to taste the samples from left to right.

Results and discussion

Functional and physicochemical characterisation of protein enriched dairy products

In order to investigate the role of protein in development of structure in different products, the principle of protein gradation was chosen as a basis for producing structured dairy products. This principle is based on the production of dairy products comprising about 4% (whipped products), 6% (dairy beverages), and 12% (dairy desserts) of protein. This choice was due to characterisations of structural properties of various systems comprising an increased amount of protein and evaluation of texture formation in the structured dairy products depending on the protein content. It was assumed that the implicated principle will allow us to develop a range of structures and dairy products.

Creams with reduced amount of fat (20%) and increased amount of protein produced a very similar trend for steady shear viscosity to that produced by commercial creams (data not shown). This behaviour indicates that developed creams can meaningfully 'mimick' the flow (and creaminess) of full-fat creams producing semi-structured systems, which is of interest to us. Once the texture for thickened cream was achieved, then the foam properties of overrun and foam stability of the associated whipped creams was investigated. Interestingly, for the full-fat products desired overrun of the systems with addition of protein was greater and obtained faster than for the commercial products (Figure 1). However, overrun (Figure 2) of the reduced fat whipped cream was similar to that of the full fat whipped cream, whereas the foam stability of reduced fat whipped cream was 30% lower than that of the full fat products. After creaming serum separation was not detected for all the creams tested.

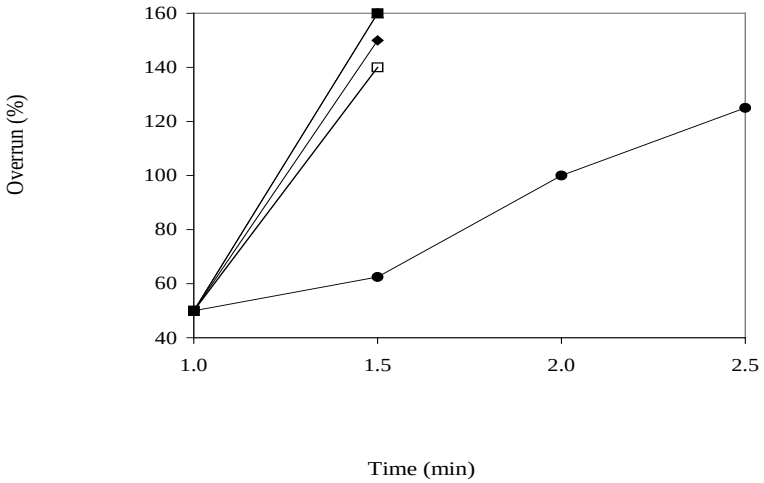

Figure 1. Overrun curves at 5 ^{0}C for full fat whipped cream (●); full fat thickened cream with addition of protein and stabilised with a mixture 1 (◆), 2 (▲) 3 (■), 4 (□), with the overrun being measured every 30 seconds

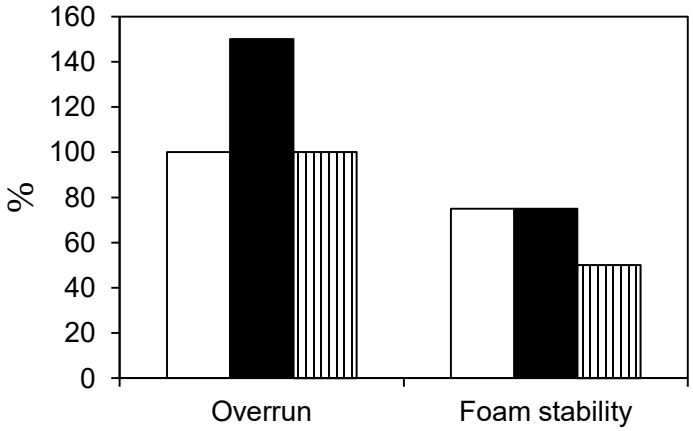

Figure 2. Foam properties (as a percentage): overrun and foam stability (after 7 hours) for full fat commercial whipped cream (empty bar), full fat whipped cream with addition of protein (solid bar), and reduced fat whipped cream with addition of protein (line bar).

Novel formulations of dairy beverages enriched with protein produced a congruent trend in the steady-shear dependence of viscosity to that recorded for the commercial product (Figure 3). This rheological profile indicated that there is an effect of the addition of extra protein on the flow behaviour of dairy beverages showing higher viscosity values, as compared to the commercial product, due to the differences in solids content. Moreover, sodium caseinate and whey protein concentrate have a high water holding capacity and create intermolecular interactions with heat processing that affect the flow characteristics of the enriched products.

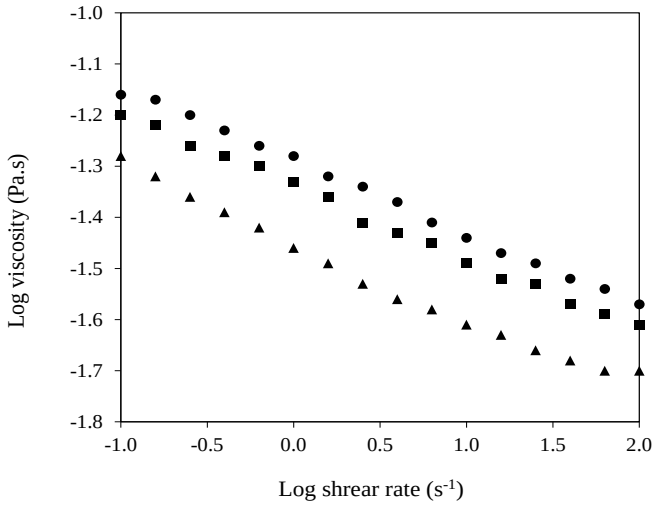

Figure 3 Steady shear viscosity for the commercial dairy beverage (▲), and dairy beverages enriched with protein: calcium caseinate (●) and whey protein concentrate (■) at 4°C.

The protein enriched dairy-based beverages with addition of fruit juice, as well as protein enriched dairy-based beverages with addition of grain components were also developed in this work. The quantitative analysis of the texture of the developed products show that flow behaviour depends on the temperature of storage, amount of protein and extra addition of dietary fibre and (Figure 4). Thus, we can see from the experimental results that developed protein-enriched dairy beverages produce similar values of viscosity to that of the commercial products with no addition of protein and dietary fibre at low temperatures.

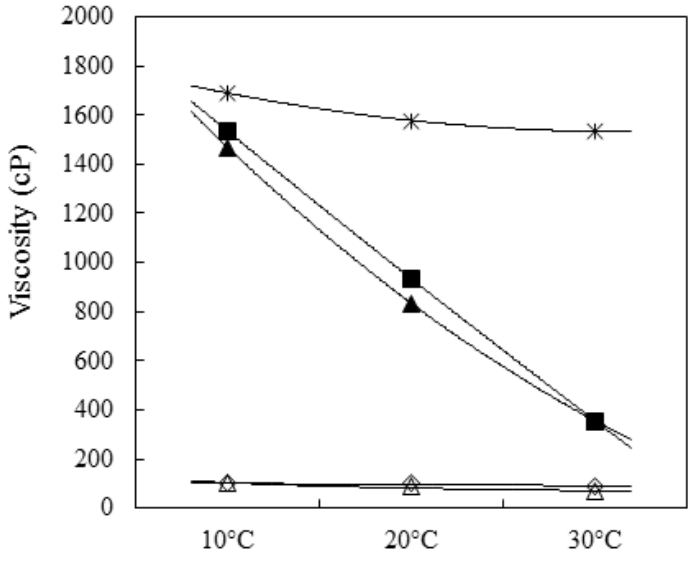

Figure 4 - Viscosity dependence from the temperature and addition of protein for the protein-enriched dairy beverages with grain components with 2.5 (◊) and 3% (Δ) of calcium casienate, and 2.5 (▲) and 3% (■) of whey protein concentrate at 22 ºC.

Commercial dairy dessert and the four novel formulations with a high protein content showed similar patterns of shear thinning behaviour with comparable viscosity values in Figure 5. The three formulations containing 2.6 to 3% WPI and 7 to 7.8% WPC (Lacprodan or Alasen 392) show similar values in hardness (around 0.9 kPa) depicted in Figure 6. Thus the gels of these preparations are relatively weaker than the fourth novel formulation containing 3.2% WPI and 7.5% WPC in the form of Lacprodan (hardness of around 1.7 kPa). Findings are associated with the increasing strength of the continuous network, at higher level of protein solids, being able to form supporting tertiary structures and aggregates that affect gel firmness.

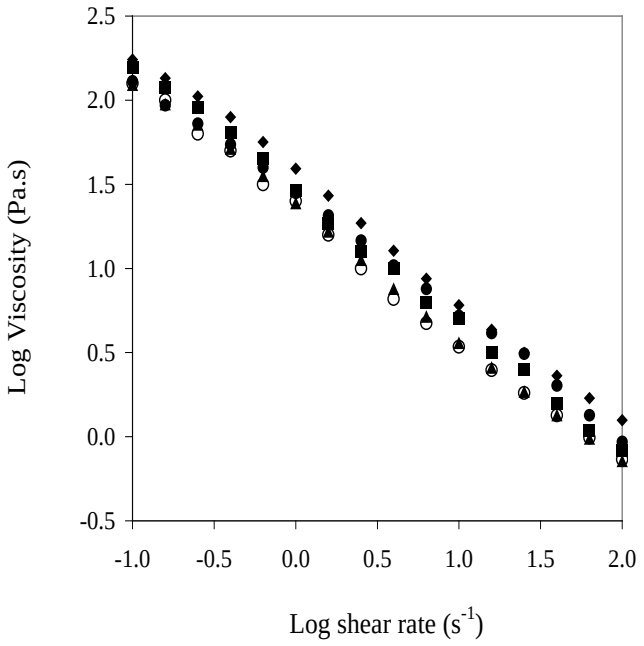

Figure 5 - Steady shear viscosity for the commercial dairy dessert (●), and protein-enriched formulations with 2.6% WPI, 7.8% Lacprodan (♦), with 3.2% WPI, 7.5% Lacprodan (○), with 3% WPI, 7.1% Alacen 392 (■), and 3% WPI, 7% Alacen 392 (▲) at 22°C .

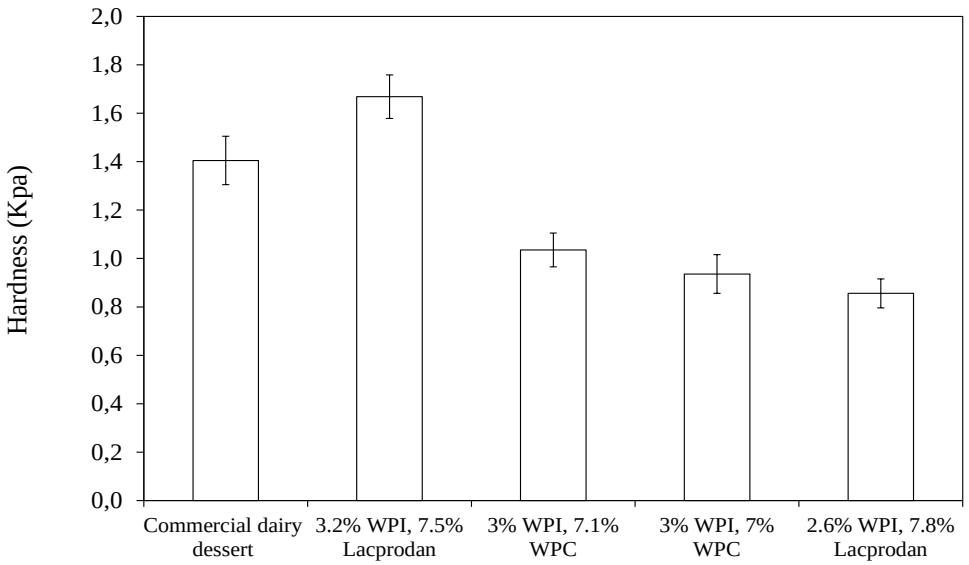

Figure 6 - Hardness for the commercial dairy dessert, and protein-enriched formulations with 2.6% WPI, 7.8% Lacprodan, with 3.2% WPI, 7.5% Lacprodan, with 3% WPI, 7.1% Alacen 392, and 3% WPI, 7% Alacen 392 at 22^{o}C .

Sensory evaluation of the protein enriched dairy products

Once the similarity of the dairy products was established in terms of their instrumental flow, structure and textural properties, the question has been asked at this stage on what is the sensory perception and biological quality of the developed products. Thus, sensory test of the samples of commercial full fat thickened creams and reduced fat thickened cream with addition of protein was performed. As mentioned above, the panellists were asked to rank how much they liked the samples on a hedonic scale (1 extremely disliked to 9 extremely liked). In this test 18% of panellists had no preference (i.e. rated both equally), 36% preferred the reduced fat cream and 45.5% full fat cream. According to ANOVA (one way statistical test), there was no significant ($p > 0.05$) difference in the preference level for the two creams trialled. The average score for the developed protein-enriched cream was 6,60 (±0,88) which refers to “Like” by the potential consumers.

In the sensory evaluation of the dairy beverage according to ANOVA statistical test (one way), there was no significant ($p > 0.05$) difference in the preference level of the two beverages (overall acceptance of the commercial and prototype formulations were 6.67 and 6.55, respectively), which further argues that both dairy beverage are acceptable by the consumer with a score of “liked”. Therefore, addition of 6% protein creates an acceptable mouthfeel that is assessed similarly to the commercial product with only 3% protein in the formulation.

In a sensory panel of dairy desserts, out of 20 panellists, 20% had no preference providing the same score for both products tested (commercial and developed ones). The average score (± SD) for the protein enriched dairy dessert was 6.75 (± 1.16). Thus, novel formulations were met with organoleptic acceptance by the panelists and maintained the high nutritional requirement to prevent Sarcopenia in the elderly population.

Biological quality of the developed protein enriched dairy products

Following identification the texture and sensory parameters, the nutritional analysis show that application of our working protocol on the development of a range of protein-enriched products results in delivery of biologically valuable dairy products containing 11% of protein which can cover about 30% of recommended protein daily intake. Thus, developed formulations deliver up to 14 g total protein and 1.4 g of leucine per 120 g product serving and liked by the panellists. Experimental results on the amino acid content shows that the sum of the essential amino acids in the developed samples exceeds the values of the commercial samples. Thus, the amount of the essential amino acids in the novel creams increased by 76.9%, in the dairy beverages – by 80%, and in the desserts – by 80.7%, which ultimately increases the total content of amino acids in the cream by 82.2%, in the dairy beverages – by 82.9%, and in the desserts – by 65.7%, as compared to the commercial samples (Figure 7).

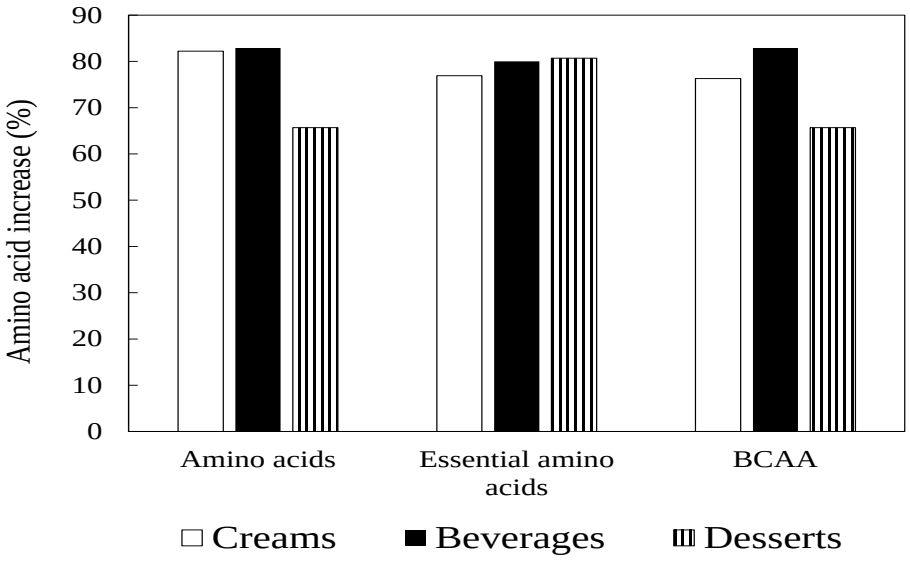

Figure 7 – Increase in amino acids (%) for the developed dairy products as compared to commercial formulations

Increase in leucine was in average of 61.9%, while increase in another branched-chain amino acid, isoleucine, which is necessary for the hemoglobin synthesis, was up to 87% [9]. Such an increase in the amount of the essential amino acids, especially of the branched-chain amino acids, is beneficial for the utilizing of these products in all groups of population, since the developed products have whole spectrum of biological properties required for the protein synthesis. Moreover, the principle of protein gradation successively increased the concentration of the essential amino acids with preservation of the acceptable texture and mouthfeel of the developed products.

Conclusions

Research and market analysis reveal that products that deliver a dose of protein which can help combat Sarcopenia, will be a niche, but a high value niche and one that will grow significantly in the

years ahead. Delivering protein density will be a key to execution in dairy formulations that maximize sensory, nutritional and economic value. Thus, development of a range of protein-enriched dairy products will meet consumer's nutritional expectations as well as high likelihood of acceptance that will deliver commercial benefits for dairy industry. In this work our efforts were on the characterization of texture and mouthfeel of the protein-enriched products via wide range of physicochemical techniques and sensory tests to prove the consumer acceptance. Inclusion of such products into the diet of the adult population will provide real benefits associated with the high level of leucine, and therefore maximize muscle protein synthesis. Besides nutritional properties, novel products liked by consumers considering a high level of acceptance for future implementation.

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THE PHYSIOLOGY OF LACTOFERRIN

Jean-Paul Perraudin

Taradon Laboratory, 4 Allée de la Recherche 1070 Brussels – Belgium

jp.perraudin@taradon-laboratory.com

Abstract

Working on the secretion liquids and on the main molecules that are contained in these liquids, many scientists have tried to understand why these molecules are part of the preliminary defenses of the human and animal health. In counterpart, emerging knowledge on diseases and the role of these natural compounds contained in the secretions liquids, it is possible to lower the risk of such disease processes. Lactoferrin, one of the most important molecule present in many secretions from exocrine glands and in specific granules of neutrophils, is an iron binding glycoprotein. From the scientists, lactoferrin is considered as one of the best sentries of the non-specific defense of the organism. Lactoferrin possesses various biological functions, including antibacterial, antiviral and antiparasitic activity, roles in iron metabolism, immunomodulatory, antioxidant, host protective effects against infection, cancer and inflammation in adult animal and human, as well as in infants, cell proliferation and differentiation.

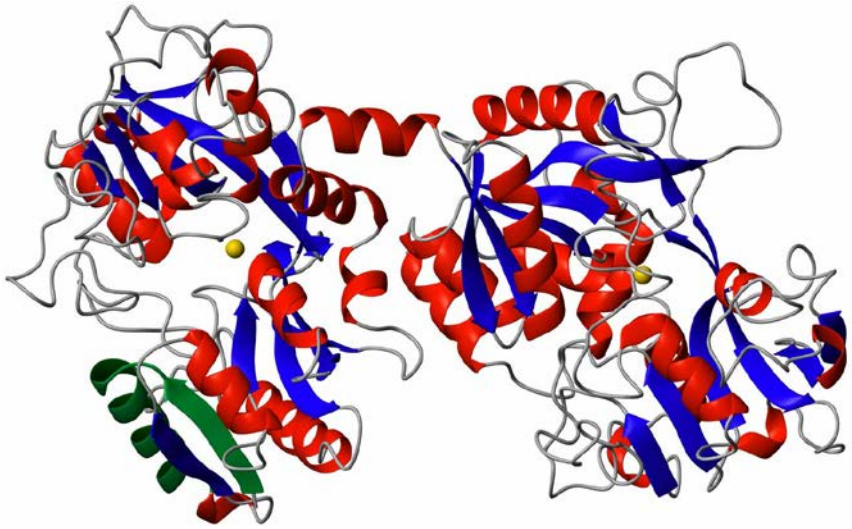
Introduction

Lactoferrin (Lf) is a single chain, iron-binding glycoprotein of the transferrin family that is expressed and secreted by glandular cells and found in the secondary granules of neutrophils from which it is released in infected tissues and blood during the inflammatory process. Lf is present to the major secretion liquids that bathe mucosal surfaces such as milk, tears, nasal, saliva, bronchial mucus, gastrointestinal fluids, cervicovaginal mucus, seminal fluid (Figure 1). Additionally, Lf is produced in polymophonuclear leucocytes and is deposited by circulating cells in septic sites.

Initially described as an iron-binding molecule with bacteriostatic properties, Lf is now known to be a multifunctional or multi-tasting protein. It is a major component of the innate immune system of mammals. Its protective effects are ranged from direct anti-microbial activities, against a large panel of microorganisms including bacteria, viruses, fungi, and parasites, to anti-inflammatory and anti-cancer activities. While iron chelation is central to some of the biological functions of Lf, other activities involve interactions of Lf with molecular and cellular components of both hosts and pathogens. Combined with *in vitro* and *in vivo* data, the powerful antimicrobial activities, immunomodulatory properties and prevention of septic shock, anti-carcinogenic functions and its growing importance in iron delivery and bone growth, make the Lf a very promising and fascinating molecule for health applications.

Chemical structure of lactoferrin

Lf is a single chain glycoprotein folded into two globular units, each of which can bind one ferric ion (Fe^{3+}) together with bicarbonate ions (figure 1).



Lactoferrin is a single chain glycoprotein

- Two globulars units
- Each unit can bind one ferric ion (Fe^{3+}) together with a bicarbonate ion (HCO^{3-})
- 4 glycan chains

Figure 1: Structure and activity of bovine Lf

Lf has a very high affinity for iron. Most of proposed biological activities of Lf are related to the binding of iron even if non-iron related functions have been also described.

In a “natural state”, bovine Lf is only partly saturated with iron (15 to 25%) and has a salmon pink colour, the intensity of which depends on the degree of iron saturation. Iron depleted Lf with less than 10% iron saturation is called apo-lactoferrin. Iron saturated is referred to as holo-lactoferrin.

Bovine Lf has a molecular weight of 77 kDa. The complete amino acid sequence of the molecule has been determined and found to contain 690 amino acids. The chemical analysis reveals 4N-linked glycans. The 3-D structures and the glycan structures of both human and bovine lactoferrin are now known in great detail.

Their 3-D structures between the human and the bovine lactoferrin, are similar, but not exactly identical.

Lf is composed of two homologous lobes, called N- and C-lobe, referring to the N-terminal and C-terminal part of the molecule respectively. Each lobe further consists of two sub-lobes or domains, which form a cleft where the ferric ion (Fe^{3+}) is tightly bound in synergistic cooperation with bicarbonate anions (figure 1).

Lf is mainly present in the exocrine glands located in the gateways of the digestive, respiratory and reproductive systems to provide mucosal protection against invading microorganisms and toxic insults. The physiological distribution of Lf in human body and secretions is schematically described in the figure 2. Molecular dysfunctionality or deficiency of Lf levels in the body could cause physiological disorders and predispose various infections [1]. Several studies, carried out with a high level quality of Lf, have established that Lf supplementation could provide exceptional health and benefits and a powerful protection against several illness [2, 3, 4].

Biological fluid	Amounts
Colostral breast milk	5 – 7 mgr/ml
Mature breast milk	1 – 2 mgr/ml
Tear fluid	2 – 2,5 mgr/ml
Seminal plasma	0,4 – 1,9 mgr/ml
Synovial fluid	50 – 80 µgr/ml
Saliva	7 – 10 µgr/ml
Bovine colostrum	1 - 2 mgr/ml
Cow's milk	100 – 200 µgr/ml
Uterine secretion	0,5 – 1 mgr/ml
Neutrophils	0,1 µgr / 10^6 cells
Amniotic fluid	2 – 30 µgr/ml
Blood	0,3 µgr/ml
Nasal secretions	0,2 mgr/ml
Urine	1,5 µgr/ml

Figure 2 : Biological sources of Lf

Sources of Lactoferrin

Breast milk, one of the secretion liquids, is the original delivery system for the essential nutrients to the newborn. This natural system provides several bioactive ingredients for regular management of gastrointestinal functions including innate defense, gut maturation and repair, nutrient diffusion and transport across the mucosal barrier. The molecule has also been identified in the milk of other mammalian species like cow, pig, horse, mouse, etc.

There are 2 reasons for using lactoferrin from cow's milk, the first one is the availability of quantities of the raw materials to produce Lf, and the second one was noted that in demonstrating the different physiological activities of the molecule, it was reported that the lactoferrin from human or bovine origin showed not only an extreme homology comparing their amino acid sequence (69%) but also comparing their numerous activities. This similarity was particularly evident when it was demonstrated that the bovine lactoferrin is able to bind specifically the lactoferrin receptor located in the human cells [5]. It was demonstrated by X-ray crystallographic analysis that the tridimensional structure of the peptides originating from human and from bovine lactoferrin, that we called lactoferricin (Lfcin) which are involved to the binding of the lactoferrin receptor, are identical (Figure 3). Human lactoferrin receptors have been identified on different target cells such as enterocytes, activated human lymphocytes, platelets and breast epithelial cancerous cell lines. Due to the similarity of the peptides, it was demonstrated that bovine is able to recognize human lactoferrin receptors and to compete with human lactoferrin molecules [6]. There is no doubt actually for the scientists that bovine lactoferrin can be used for human applications.

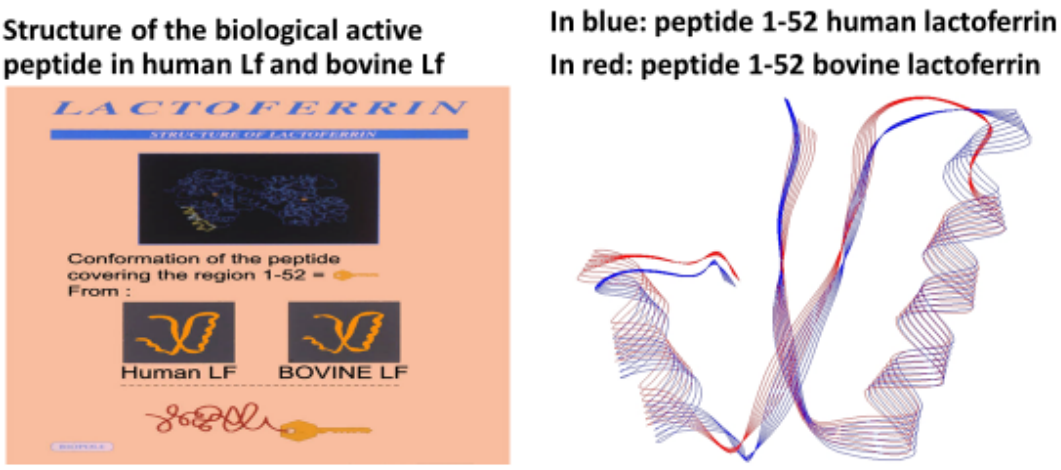


Figure 3 : Comparison of the conformational structure of the peptide 1-52 (lactoferricin) between the human Lf and the bovine Lf

Biological activities

As explained here above, the activity of Lf is associated to iron, so before to describe the biological activities of Lf, it is important to describe the role of iron in the human body.

If we consider that iron is an essential element for our life, it is important to know that an excessive iron is a poison. Iron is a double-edged sword. In moderate quantities and bound to proteins or other iron binding species, it is an essential element in the metabolism and growth of all cells, but toxic when it is not bound. The unique abilities of iron to vary oxidation state, potential redox, and electronic spin configuration in response to different ligand environments, are important criteria enabling iron to play multifunctional roles as a protein cofactor. As benefit role of iron, we can mention some examples such as the fundamental role of the iron-containing proteins in respiration, oxygen delivery to tissues, DNA synthesis, and the regulation of the citric acid cycle. Concurrent with the evolution of complex iron-containing proteins, nature needed to design an efficient and nontoxic means of controlling iron absorption, transport, and storage. This is because the same physical properties that allow iron to act as an efficient cofactor in controlled redox chemistry, also allow iron to act as a potent toxin, when not shielded from oxidatively susceptible biomolecules.

A) Lf and intestinal iron homeostasis

Iron deficiency is worldwide the most common cause of anemia. The condition is associated with many unpleasant symptoms such as fatigue, decreased work performance, increased susceptibility for infections and retardation of mental and psychomotor development and of growth. Moreover, the iron deficiency anemia and inflammatory either related to infection or sterile inflammatory response represent also risks factors for preterm delivery. Extensive efforts are needed to prevent the devastating results of serious iron deficiency on personal and socio-economic development of the population. Although nutritional and socio-economic factors play a role, in most cases one should consider accelerated growth and blood loss as the main causes.

It has been proposed that Lf is involved in intestinal iron delivery, although this has been the subject of many debates over the year [1]. The high ironbioavailability and abundant concentration of Lf in breast milk suggested that Lf may play a role in intestinal absorption in the neonate [1]. In addition, Lf has been shown to be relatively resistant to proteolysis in the gastrointestinal tract [7], and specific receptors have been identified for Lf in the brush border membrane of enterocytes from many species [8]. Furthermore, Caco-2 intestinal cells transfected with a human Lf enterocyte receptor demonstrated an increased uptake of Lf-bound iron [9]. Unfortunately, it was demonstrated that the divalent metal transporter (DMT-1) pathway is a major mechanism for non-heme iron uptake in the gut [10,11], this suggests that milk-derived Lf may have functions alternative to intestinal iron delivery. In this regards, it has been proposed that the iron-binding properties and stability of Lf function is to sequester and remove free iron at the intestinal mucosa barrier. [7]. Further studies using genetic mouse model of Lf deficiency confirmed that Lf is not required for iron delivery to the neonate [12].

Taking together, this argues against an essential role of Lf in intestinal iron delivery and suggests that the iron-binding activity of Lf may function primarily to sequester free iron in the gut, thus controlling microbial pathogenesis and iron-induced cellular oxidative damage [7].

Nevertheless, recently, Lönnerdal and his collaborators [13] have characterized a receptor of the Lf (Intelectine) at the surface of the fetal intestinal mucous cells and moreover, a study carried out by Paesano in 2010 [14] has shown that taking orally, 30% iron saturated bovine Lf has resulted an improvement in pregnant women suffering iron deficiency and iron deficiency anemia. The results of the treatment have shown also that Lf was more effective and safer alternative than ferrous sulfate for treating iron deficiency and iron deficiency anemia. Therefore, it is imperative to consider new safe and efficient therapeutic interventions to cure iron deficiency and iron deficiency anemia associated or not to the inflammation. In this subject, Lf is emerging as an important regular of both iron and inflammatory homeostasis. These results provide strong evidence for a role of bovine Lf in preterm delivery treatment, and preterm brain protection.

B) Antimicrobial activity

Antibacterial activity

Lf has both bacteriostatic and bactericidal activities.

Lf has a strong capacity to bind iron and this power of sequestration confers to the molecule the ability to inhibit the growth of a wide variety of bacteria, virus, yeast, fungi and parasites. A non-exhaustive list has been described by Pierce in 2009 [15].

The bacteriostatic activity of Lf is known to be dependent on its iron-free state and is commonly attributed to its ability to bind and sequester iron, producing an iron-deficient environment that limits microbial growth [16,17]. However, several studies suggest that lactoferrin has also bactericidal activity and kills sensitive microorganisms by a mechanism distinct from sequestering of iron that involves its direct interaction with the bacterial cell surface [18,19,20]. This antibacterial action involves the mechanism of cell binding by the lactoferrin to the cells and the liberation of the lipopolysaccharide (LPS) from the outer membrane of the microorganisms, followed by permeabilization of the cell wall and cytoplasmic membrane.

The bactericidal activity is able also to inhibit the attachment of the bacteria to the host cells. So, fixing to the glycoaminoglycanes and integrins of the host cell, Lf neutralize the pathogens since the first steps of the infection.

Other antibacterial mechanisms have been reported. These include prevention of biofilm formation by Pseudomonas aeruginosa [21] and oral pathogens [22] and proteolysis of virulence factors of *Haemophilus influenza* [23]. It was demonstrated that in this case, Lf can prevent the formation of biofilm.

The bactericidal activity is concentrated in the amino terminal peptide (figure 2), which is the same that the one which binds to the Lf receptors (Lfcin). This peptide has a bactericidal activity much stronger than the native Lf [24,25]. So, outside the major part of its polypeptide chain, the Lf produces an antibacterial effect much stronger than itself. The efficacy of the Lfcin has served as model for new antimicrobial peptides such as lactoferrampin (Lfampine) [26, 27]

Antiviral activity

The Lf has efficacious antiviral activity against both naked and enveloped DNA and RNA viruses, in particular hepatitis virus, herpes virus and HIV (human immunodeficiency virus) [28,29, 30]. In its antiviral pursuit, LF acts predominantly at the acute phase of the viral infection or even at the intracellular stage, as in hepatitis C virus infection. LF inhibits the entry of the viral particles into host cells, either by direct attachment to the the glycoaminoglycans and the integrins used by the virus to penetrate the host cells or by blocking their cellular receptors. This wide range of activities may be attributed to the capacity of LF to bind iron and its ability to interfere with the cellular receptors of both hosts and pathogenic microbes [31].

Antiparasitic and antifungal activity

In many cases, Lf acts against parasites by breaching their membranes integrity. Among fungal pathogens responsible for opportunistic infections, species of the genus *Candida* have a central contribution. Lf secreted in mucosal surface has antifungal activity against Candida due to membrane perturbation [32]. Intestinal amoebiasis is caused by *Entamoeba histolygica* . Lf is able to kill *E. histolytica* by binding the lipids of their membrane, and disrupting the membrane structure [33]. On the other hand, Lf induces tolerance to infection of *Toxoplasma gondii* by inhibiting the intracellular growth of *T. gondii* [34]. The anti-parasitic activity of Lf against *Pneumocystis carinii* is dependent on its iron chelating activity [35].

C. Activity anti-oxidant

Here also iron plays a key role in oxygen radical biochemistry. In numerous diseases the final common pathway, leading to tissue damage, is the iron-catalysed Haber-Weiss reaction, in which the relatively harmless oxygen products, superoxide and hydrogen peroxide, are transformed into the highly aggressive hydroxyl radical. As described in the figure 5, if iron is present in ferrous form, no superoxide generating system is needed, as the ubiquitous hydrogen peroxide can directly be reduced to hydroxyl radical, which can immediately destroy a wide variety of molecular structures in its close proximity.

Nowadays, much attention has been given to the effects of oxidative stress in relation to increased risks to life-threatening and chronic or degenerative diseases. Oxidative damage by reactive oxygen species (ROS) may promote carcinogenesis [36].

Several results of tests strongly suggest that lactoferrin may serve as an antioxidant defence mechanism at least two levels, as described in the figure 4. Firstly by binding the bacterial LPS or the endotoxin CD14 complex with the lipopolysaccharide-binding protein [37], which activating the monocytes/macropages, strigger the phagocyte activity and led to an important production of oxygen reactive species and secondly as described in the figure 5, by binding any catalytic iron [38], which

may be generated during the course of cell destruction or overproduced by activated granulocytes. Owing to its iron-binding stability at a low pH, iron-free Lf released from polymorphonuclear cells at inflammatory sites may chelate ferric ions and prevent the formation of hydroxyl radicals and subsequent lipid peroxidation [39]. During an inflammatory response it may serve to prevent tissue injury caused by the excess presence of free radical (OH°). The ability of lactoferrin to inhibit OH° formation can be also extended to those cells, which do not contain lactoferrin via the ability of these cells to bind lactoferrin through a specific surface receptor for the protein. One of the consequence of the presence of the hydroxyl radical will be the peroxidation of lipids (lipoperoxidation = lipid free radical formation)

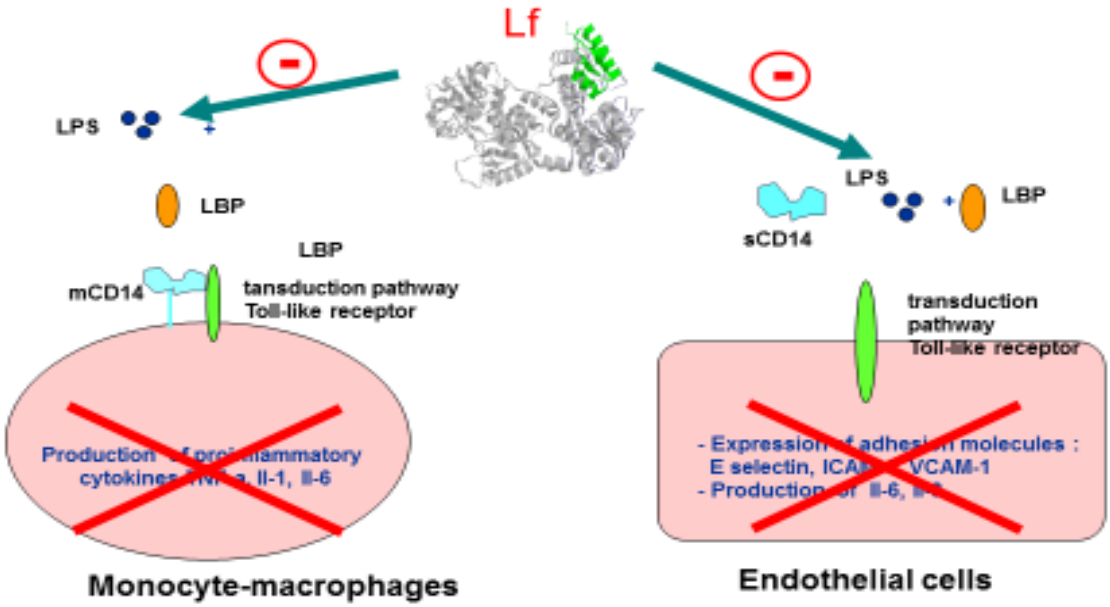


Figure 4 : LPS-binding properties of Lf play an important role in the immunodulatory activity. LPS is a potent initiator of an inflammatory response and serves as an indicator of bacterial infection. LPS interacts with LPS-binding protein (LBP) and CD14. CD14 exists in two forms mCD14 is a membrane form and sCD14 is the soluble form which can be detected in the circulation. In monocytes and macrophages, mCD14 acts as LPS receptor. LPS form a complex withLBP.

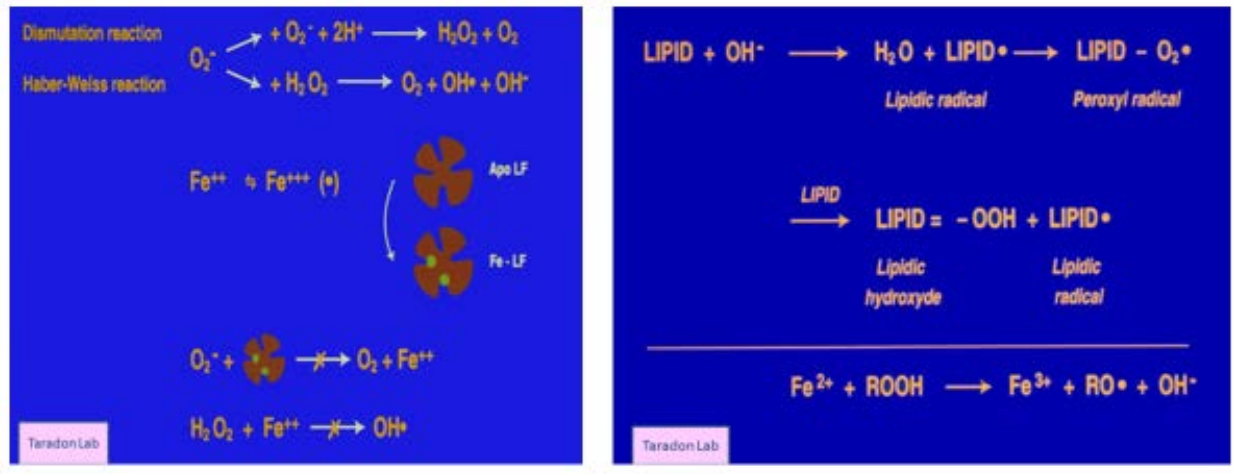


Figure 5: The production of hydroxy radical is only possible in presence of iron as catalyseur. The binding of this iron bt Lf can avoid the production of the free readical

Important studies showed that this free radical formation is one of the important causes of the aging process, either the lipid free radical can be scavenged by melanin creating dark spots, or the free radical will cause damages to the cells (figure 6). An important study, performed on rats fed with diet containing lactoferrin during 6 months, showed a lower concentration of the peroxidised lipids in the blood compared to the control group, which received the same diet without the presence of lactoferrin (1,1 nmole/ml against 1,8 nmole/ml). Recently, a clinical study on 90 patients having chronic Hepatitis C has shown that the patients who have ingested Lf from bovine origin had a better oxidant hepatic status [40].

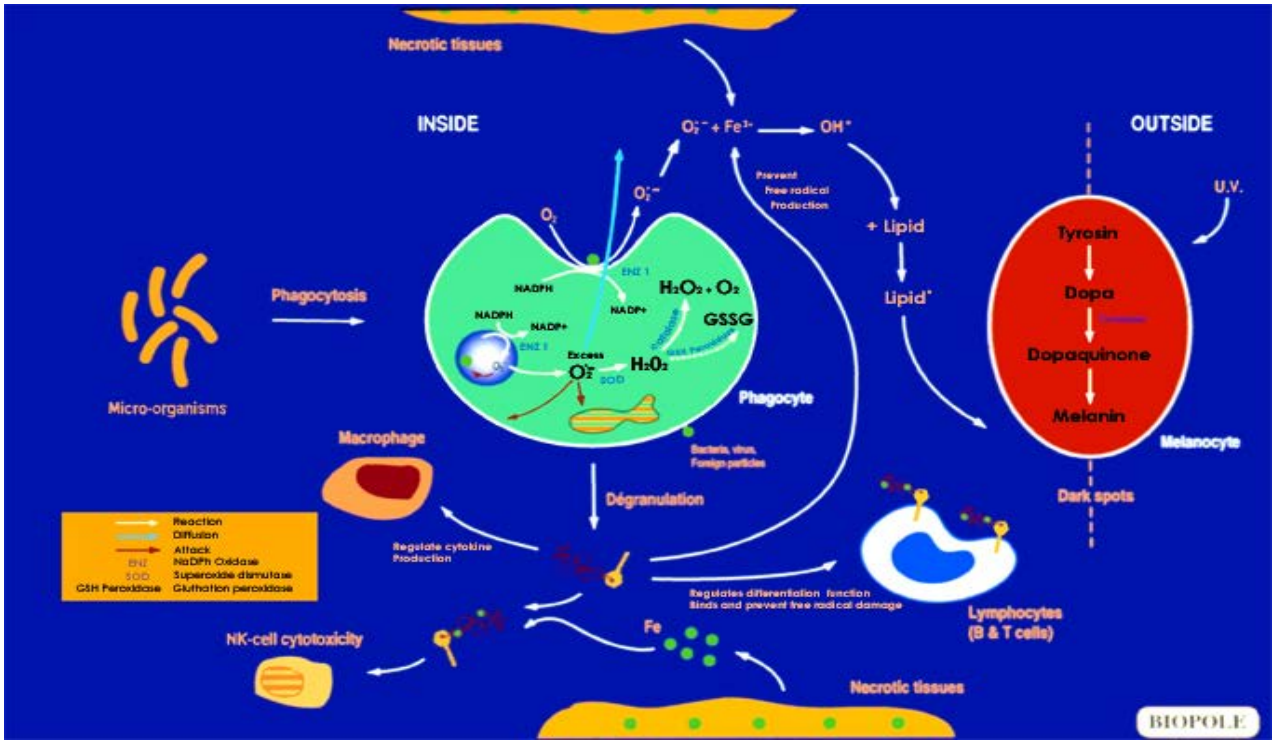


Figure 6: Ability of Lf to bind iron with high affinity as antioxidant protective molecule. During inflammation, reactive oxygen species (ROS) are produced either generated by free iron released from necrosed tissues or overproduced by activated granulocytes. This reaction will produce hydroxyl radicals and subsequent the formation of lipid radicals (peroxidation of lipids) which can be scavenged at the melanin to produce the dark spots on the skin (part of the aging process).

In summary, as it was explained earlier, the addition of inorganic iron can cause a lot of trouble for the organism. It was proved that the ingestion of iron salts leads to the formation of free radicals such as OH° in the gastrointestinal tract. These experiments were prompted by the knowledge that poisoning by oral iron preparation is a major cause of accidental death in young children. In addition, intolerance to oral iron can be a serious problem when iron is prescribed for anaemic situations or other medical reasons. The mechanism for the toxicity of oral iron includes the production of free radical as described in the figure 5.

The radical peroxide is a highly reactive molecule for which the presence is usually associated to a destroying of the cells with pathologic consequences.

Almost all oral iron preparations contain a ferrous salt as the source of elemental iron. The reason is that iron is absorbed from the gastrointestinal tract most efficiently. Many preparations additionally contain ascorbic acid, which is intended to maintain the iron in the ferrous state. In the experiment described by Cohen, they showed that the ferrous salt dissolved in phosphate buffer solution at neutral pH, are oxidized rapidly by molecular oxygen (less than 15 seconds for a 0,1 mM solution in 50 mM phosphate at pH 7). The stomach exhibits a strongly acidic pH, while the small intestine is mildly alkaline. These experiments show that OH° is observed under naturally occurring conditions of pH and in the presence of various endogenous biomolecules when the ferrous salt is instilled into the stomach. The results of the tests carried out in rats, showed that hydroxyl radicals is a mediator of damage to the gastrointestinal tract by oral inorganic iron. It was demonstrated by studies carried out on the knee-joint synovial fluid that the presence of iron in free form is responsible on the rhumatoid arthritis. In fact the doses of iron detected were sufficient to allow the production of toxic substances as hydroxyl radical, but it was proved that this effect can be stopped by addition of a chelator of iron as lactoferrin. Even if the supplementation of an infant formula with human or bovine lactoferrin has obtained a similar iron retention as the supplementation of the formula with ferrous sulphate, never the protein will participate to the formation of toxic substances for the tissues. Even if the price of Lf as a carrier for iron supplementation, is more expensive compared to any other iron supplements, it deserves to be used to avoid secondary effects.

D) Immunoregulatory

In response to tissue infection or inflammation, both innate leukocytes (macrophages, dentritic cells and natural killer cells) and adaptive immune cells (T-cells and B-cells) are exposed to high concentration of lactoferrin as result of degranulation of neutrophils. Accumulating evidences suggest that lactoferrin can control hot immune response by regulating their maturation, migration and secretion of cytokines and chemokines [41, 42]. In patient suffering from recurrent infections, lactoferrin expression in neutrophil is deficient whereas its expression in glandular secretions is normal [43]. Regarding the results of animal study, it was suggested that the Lf can play a regulatory role in immune response. Several examples have demonstrated that Lf could exhibit enhanced T helper type 1 (Th 1) response to *Staphylococcus* [44], that taking orally, Lf diminished the susceptibility to tuberculosis in β2-microglobulin knockout mice [45].

Phagocytose is a central component of the innate immune response. Phagocytes (neutrophils, macrophages, and dentritic cells) recognize and seize foreign extracellular material. Even if the effect of Lf on their phagocytic activity is well characterized, Lf expression is observed in resting neutrophils and involved in its binding to microorganisms [46]. LF interact with neutrophils and promotes their phagocytic activity and ROS production. [47].

Monocyte is the first mammalian cell whose Lf receptor has been identified [48]. LF stimulates phagocytosis of monocytes, and promotes their migration and ROS production. [49]. Concerning macrophages, phagocytes involved in innate immune response through phagocytosis of infected microorganisms, Lf can interact with them and regulate their phagocytic activity. This was demonstrated by oral administration in mice that Lf increases phagocytic activity of macrophages injected with inactivated *Candida albicans* [50]. Promoting the phagocytosis of macrophages, Lf promotes also IL-8, TNF-α and nitric oxide (NO) production *in vitro* [51]

Lf stimulates the cytotoxic activity of natural NK cells [52] and in addition to innate response, Lf can regulate antigen specific adaptive immune response. Lf can also regulate the levels of the co-stimulatory molecules on the surfaces of macrophages [42]. Many studies indicate that Lf can regulate the maturation and differentiation of T-cells and Th1/Th2 cytokine balance [53].

E) Anti-inflammatory role of Lf

The protective role of Lf against inflammation is partially depends on its ability to bind free iron and bacterial endotoxin such as LPS. In support of this, animal studies have shown that Lf administration diminishes inflammation induced with bacteria or LPS and protects against gastritis induced by *Helicobacter felis* [54], gut mucosal integrity during endotoxemia induced by LPS in mice [55]. The results of *in vitro* experiments in mononuclear cells and *in vivo* studies in mice suggest that the protective effect of Lf may involve an inhibition of production of several pro-inflammatory cytokines, including tumor necrosis factor (TNFα), interleukin-1β (IL-1β) and Interleukin-6 (IL-6) [56] as it is described in the figure 4.

Interacting also to the surfaces of epithelial and immune cells, the Lf modules the cytokine production and the recruitment of the immune cells to the inflammatory site and so protects against the septic shock [57,58,59]. Several studies on animals show that Lf taken orally is able to protect the animal against lethal shock of LPS. The Lf is able also protect against some diseases such rheumatoid arthritis, chronic inflammation of the intestine, neurodegenerative diseases. This modulation of the inflammatory process cause also a decrease of the recruitment of the immune cells notably the leucocytes [60].

F) Cancer

Here also iron represents an essential element for the growth of the tumor cells. It influences also the development of some cancers and plays a role without ambiguous in the development of the arthritis. Jeremy Brock [71] has also shown that there is a relation between the metal and the immunity. The cells of the immune system protect the tissues against the iron toxicity, playing a primordial role in the control of the concentration of the metal. This mechanism show that such cells synthesize and release the proteins as lactoferrin, transferring and ferritin, able to neutralize the iron and to eliminate so its toxicity in binding it.

Several experimental carcinogenesis studies in rodents performed by Tsuda and his team have demonstrated protective effects of orally applied bovine lactoferrin [61]. In a model of colon carcinogenesis, it was shown that the tumor incidence and multiplicity of small and large intestinal tumors were significantly decreased by bovine lactoferrin [62,63]. The highest concentration of bovine lactoferrin was shown to have the strongest inhibiting effect on tumor formation. Lf was also found to reduce spontaneous intestinal polyp formation in APC knock out mice, a model for sporadic colon carcinoma [64]. The chemopreventive effects of bovine Lf supplementation was also demonstrated in a rat model of lung and esophagus carcinogenesis [65]. This effect of Lf has also been demonstrated in case of tumor of bladder and tongue [65,66].

Part of the antitumor effect of Lf is also due to the inhibition of cancer cell proliferation. It was demonstrated *in vitro* (70) that LF inhibits the growth of human breast carcinoma cell. Dietary Lf reduced cell proliferation in colon epithelium.

It was also demonstrated that Lf is able to inhibit the development of metastasis in experimental mouse models [67]. The effect of parenterally applied bovine Lf on inhibition of tumor metastasis by highly metastatic murine tumor cells was examined in several mouse models [68,69]. In these models tumor metastasis was inhibited by subcutaneous administration of Lf. Furthermore, Lfcin has also been reported to inhibit carcinogenesis in a rat model where the colon cancer has been initiated by azoxymethane. The results of these experiments indicate that several properties of lactoferrin could be involved in its role in the defense against tumorigenesis. It was demonstrated that has cytotoxic activity against three different tumor cell lines *in vitro* and has reduced the size of the tumors.

Studying the anti-cancer effect of Lf, we have got the feeling to associate this LF's activity with its immune stimulating effects. Performing *in vitro* and *in vivo* experiments, it was demonstrated that Lf is able to stimulate different cell types of the immune system. As it was shown in the figure 6, it was reported that Lf is able to stimulate natural killer cells (NK cells), neutrophils, lymphocyte activated

killer cells and to induce colony stimulating activity and macrophage cytotoxicity [72]. The effect of lactoferrin on NK cells appears to be the most important for anti-tumor activivity, since NK cells play an essential role in the detection and elimination of tumor cells.

As demonstrated, Lf stimulates NK cell activity *in vitro* [73] and *in vivo* [74]. A treatment with anti-NK cell antibody indicates that the anti-tumor effect of lactoferrin depends on NK cell activity [68]

In general, the major assignment of Lf, either expressed at the epithelial barrier level or released from polymorphonuclear cells (PMNs) with the inflammatory sites, is to limit the proliferation of microbes. This is achieved through different mechanisms including the chelating of ferric ion to avoid the growth of bacteria, parasites and yeast, the destabilisation of microbial membranes and the modification of microbial adherence to host cells independent of its iron-binding property [60].

The ability of Lf to modulate the immune and inflammatory processes makes the molecule a set-apart member of the antimicrobial protein family. In addition to its multifunctional antimicrobial activities on epithelia and infected tissues, Lf was found to interfere with almost all major steps of the innate and adaptive immune responses, including immune cell recruitment, activation, migration, differentiation, and functions.

G) Inhibition of the angiogenesis

In *vivo,* the Lf from bovine source is able to inhibit the angiogenesis mechanism which promote the neovascularisation of the growth of the tumors and of the development of metastases. This activity involve the role anti-angiogenic of the cytokine Il-18 and taking orally Lf, lead to the maturation of the Il-18. This effect seems specific to the bovine Lf. From another side, knowing the important role of the Vascular Endothelial Growth Factor (VEGF), which is a key angiogenic factor in the tumor formation [75], it was demonstrated *in vivo*, that bovine Lf is able to inhibit VEGF-mediated angiogenesis and that *in vitro* bovine Lf suppressed endothelial cell proliferation [76]. In mouse model of tumor metastasis *in vivo* analysis for tumor-induced angiogenesis demonstrated that that bovine Lf reduced the number of tumor-induced blood vessels and suppressed tumor growth [69]. In such case, we can think that the inhibition of angiogenesis may be due to a direct effect of Lf or its digestion products on endothelial cells [36,77].

Quality of lactoferrin

A major issue in elucidating the exact roles of Lf is paradoxically connected to the plethora of results reported in the literature. Depending on whether the experiments were conducted with Lf of different species, under *in vitro* and *in vivo* conditions, and under any other conditions, the conclusions may greatly differ and can be controversial. If we add to these several experimental parameters, the variable of Lf concentrations and also the possible presence of contaminants such as lipopolysaccharides (LPS), proteases and angiogenin in the Lf preparations, can lead to non-physiological and artifactitious responses as it was presented by Dr Perraudin in 2009 at the IXth International Lactoferrin Conference [78].

Every scientists mention the word “Lf” and its origin but in the material and methods of all published scientific papers, the authors do not describe the specifications of the Lf that they used, and the methods of analyses that they used to determine the quality of the Lf. So the comparison between results obtained is not very easy

As we have mentioned in this article, the most part of the biological activities is represented on the peptide 1 – 52 that we call Lfcin. It is also on the same part that the LPS are bound, allowing to the Lf to have a better control on the inflammatory process decreasing the production of the cytokines. As

described by Lönnerdal [79], before to start the experiments, we have to analyze the presence of minor components contained in the commercial Lf and the LPS content, knowing that its presence bound on Lf structure can affect its biological activities. Nevertheless, if working at lab scale, the investigators can isolate their own preparations, this can be difficult when the quantity of Lf necessary is too important when we want to carry out *in vivo* tests on human or animals.

In fact, purifying the Lf in one step from bovine milk or whey as it is done for industrial Lf, it will never be possible to obtain a Lf free of LPS. From the milking of the cows in the farmers to the tanks in the milk dairies, we know that the milk is no more sterile and that it contain a large number of microorganisms. The role of the Lf and of some other molecules consists to protect the milk against the increase of the microorganisms. Then, we cannot be surprised that the Lf extracted from the milk contains LPS bound on its molecular structure. If we want to study the biological activities of the Lf from the one produces industrially, and mainly in the case of *in vivo* tests for which the quantity necessary for the tests is too important and cannot be produced in the laboratory, we have to guarantee about the quality of the Lf carrying out some analyses to assess the impurities such as angiogenin, proteases and other molecules and mainly the presence of the LPS on the lactoferrin's structure. So, it is important to add a 2^{nd} step which consists to the purification the Lf.

From a pure Lf, it will be easier to determine the biological activities of the Lf without to think that our results would give artifactitious responses. In such case, we could compare our results with the ones obtained by other scientists in a complete objectivity, allowing to have more precise conclusions on the biological activities of the Lf.

Conclusions

Lactoferrin can be considered as a multifunctional protein. Initially, described as an iron-binding molecule with bacteriostatic properties, Lf is now known to be a multifunctional or multi-tasking protein. It is major component of the innate immune system of mammals. Its protective effect range from direct anti-microbial activities against a large panel of microorganisms including bacteria, viruses, fungi, and parasites, to anti-inflammatory and anti-cancer activities. While iron chelation is central to some of biological functions of Lf, other activities involve interactions of Lf with molecular and cellular components of both hosts and pathogens. Its powerful antimicrobial activities, immunomodulatory properties and prevention of septic shock, anti-carcinogenic functions and its growing importance in iron delivery and bone growth, combined with the data obtained either by *in vivo* studies or clinical trials, make this molecule and its derivatives very promising tools for health or nutritional applications.

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FERMENTED BUTTERMILK BASED BERVERAGE: IMPACT ON HUMAN HEALTH

A. Liutkevičius[1], V. Speičienė[1], G. Alenčikienė[1], A. Mieželienė[1], R. Narkevičius[1] , A. Kaminskas[2], A. Abaravičius[2]

[1]Food Institute, Kaunas University of Technology, Taikos av. 92, LT-51180 Kaunas, Lithuania, email:, [2] Faculty of Medicine, Vilnius University, M. K. Čiurlionio str. 21, LT-03001 Vilnius, Lithuania.

Algirdas.Liutkevicius@ktu.lt, vilma.speiciene@ktu.lt, gitana.alencikiene@ktu.ltarvydas, kaminskas@mf.vu.lt, algis.abaravicius@mf.vu.lt aldona.miezeliene@ktu.lt, Raimondas.narkevicius@ktu.lt;

Abstract

The objective of this study was to determine the quality indicators of fermented beverages from buttermilk, and establish the impact of the consumption of a product on biochemical blood parameters of volunteers.

Three functional beverages were made on the basis of buttermilk which varied from each other with regard to composition: buttermilk, buttermilk-milk protein concentrate (MPC) and buttermilk-skim milk-MPC). The best quality characteristics (acidity, syneresis, rheological properties, sensory properties) were determined in the beverage containing buttermilk-milk-0.3% MPC). This particular beverage of increased biological value was selected for further studies regarding the impact on human health. The experiments of medical nutrition showed only tendency of decrease of total, low density, high density cholesterol, and triacylglycerol concentrations in the blood of 25 volunteers after a 21-day period of consumption of fermented beverage with MPC.

Key words: buttermilk, milk protein concentrate, quality, lipid metabolism.

Introduction

The use of healthy or the so-called functional foods with a nutritional value and a positive physiological effect could reduce the incidence of diseases which are heavily influenced by nutrition problems. The beneficial impact of these healthy products, including beverages is determined by active substances incorporated in these products.

Particularly high scientific interest is focused on buttermilk due to its unique composition as high biological value components released from destroyed milk fat globules during churning of butter. Evidence in cholesterol lowering, anti-inflammatory, chemotherapeutic and anti-neuro-degenerative effects of milk fat globule membrane lipids, mainly through the action of phospholipids was established by many authors [1, 2, 3, 4].

Beside buttermilk products, milk protein concentrates (MPCs) obtained by drying unfiltered milk, are prospective in terms of functional food production. In MPCs, the casein is in a micellar form, similar to that found in milk, and the whey proteins are also in their native form, but a large part of the lactose is removed. The MPCs are multifunctional ingredients and is characterized by a good water binding, gelling, emulsification and heat stability properties [5].

The aim of study was to develop a technology of a functional fermented buttermilk based beverage enriched by MPC and determine its influence on human health.

Materials and methods

Buttermilk obtained from manufacturing sweet cream butter and skim milk (UAB Rivona, Lithuania), MPC, with a dry matter concentration 85 % (UAB MG Baltija, Lithuania) were used for beverages preparation. Mesophilic culture FD-DVS Flora Danica Normal (Lactococcus lactis subsp. cremoris, Lactococcus lactis subsp. lactis, Leuconostoc mesenteroides subsp. cremoris, Lactococcus lactis subsp. lactis biovar.diacetylactis) (Chr. Hansen, Denmark) was used.

Production of fermented beverages. Three different formulations of fermented beverages were prepared: 1) control from buttermilk, 2) from butter milk and 0.3 % of MPC, and 3) from buttermilk-skimmilk mixture (60:40) and 0.3% of MPC. MPC was dissolved in buttermilk or buttermilk-skimmilk mixtute. Prepared samples were pasteurised at 85 ± 2°C, cooled down to fermentation temperature (24 ± 2°C), fermented with added starter (0.025%) up to pH 4.6–4.7, stirred, distributed into plastic bottles (0.5 l) and stored at 4 ± 2°C.

Apparent viscosity was measured by a rotational viscometer Rheotest-2 (Germany), using the S/S_2 cylinder measuring system at 27 s^{-1} shear rate, at 10º C temperature.

Syneretic properties were determined by centrifugation. The amount of whey discharged after centrifugation (2000 min^{-1}, 20 min, 20 g of sample) was measured and expressed in %.

Sensory analysis. A quantitative descriptive analysis (QDA) was carried out by group of 6 trained assessors. A 9-point scales (where 1 equal to “low intensity/absent”, and 9 equal to “high intensity”) were used to evaluate each sensory attribute.

Medical study design. Product made in pilot plant was supplied for medical investigations. Study was performed in a randomly selected 20-24 year old group of volunteers (n = 25). The participants modified their diet by 0.5 l of the beverage consumed daily over a period of 21 day, at the same time, they were encouraged not to change their dietary habits. Before and after the product consumption blood samples were taken and investigated.

The study protocol was approved by the Lithuanian Bioethics Committee (2012-11-29; Order No 158200-12-227-158).

Biochemical analyses. Cholesterol and triglyceride concentrations were analyzed by enzymatic colorimetric methods (Architect ci8200, Abbott, USA), low density lipoprotein (LDL) cholesterol concentration was calculated using Friedewald formula, high density lipoprotein (HDL) cholesterol was analyzed by accelerator selective detergent method (Architect ci8200, Abbott, USA), glucose concentration was analyzed by hexokinase enzymatic method (Architect ci8200, Abbott, USA), insulin was measured by chemiluminescent micro particle immunoassay (Architect ci8200, Abbott, USA), oxidized low density lipoproteins (OxLDL) were detected by ELISA (Mercodia, Sweden) based on the direct sandwich technique in which monoclonal antibodies are directed against antigenic determinants on the oxidized Apo lipoprotein B molecule, fibrinogen concentration was analyzed by Clauss coagulometric method (STA-Compact, Diagnostica Stago, France), C-reactive protein (CRP) was analyzed by latex enhanced immunoturbidimetric assay (Architect ci8200, Abbott, USA).

Statistical analysis

The data were analyzed by ANOVA, if significant interactions were determined, multiple comparisons were made. The differences were classified by a Duncan multiple comparison test ($P \leq 0.05$). SPSS software, version 15.0 (Chicago, IL, USA, 2006) was used for the statistical analysis of the data.

Results

Enrichment of fermented buttermilk beverages with MPC (0.3%) and partly buttermilk replacement by skimmed milk is characterized by increased viscosity that was instrumentally and sensory evaluated (Table 1). Increased viscosity had a positive impact on the syneretic properties of beverages. Beverage composition of buttermilk-skimmed milk-MPC showed significantly higher overall odor intensity and lactic acid taste when compared with other formulations and as having the best quality characteristics and acceptable sensory properties was produced and supplied for medical nutrition experiments.

Dietary intervention with MPC enriched buttermilk-skimmed milk beverage had some positive influence on the total cholesterol, LDL-cholesterol, DTL-cholesterol and triacylglycerol concentrations in the blood of volunteers: the reductions were of 5.6%, 8.3%, 1.3% and 6.0%, respectively. (Table 2). The absence of significant changes can be associated with a young, healthy group of persons under investigation, when the body is able to maintain a stable and normal metabolic indicators.

Increase in C-reactive protein and fibrinogen concentration in serum (respectively 24.5% and 1%) took place after 3 weeks of taking the beverage but it did not exceed the normal range and medically did not show disorder.

Insulin and glucose level (reflection of the carbohydrate metabolism) in blood plasma of volunteers almost unchanged after diet supplementation.

Many scientific studies have indicated that increased level of oxidized low-density lipoproteins (oxLDL) is a hallmark of atherosclerosis. OxLDL plays a direct role in the initiation stage and progression of cardiovascular diseases [6, 7]. Our study showed insignificant decrease concentration of oxidized LDL. So, undoubtedly is relevant search of components which is able to reduce blood level of oxLDL.

Conclusions

The best quality characteristics (increased viscosity, improved syneresis and sensory properties) were determined in fermented buttermilk-skimmed milk beverage containing 0.3% MPC.

Medical nutrition experiments showed that the beverage positively affected lipid metabolism in blood of volunteers (reductions of total cholesterol, LDL-cholesterol, DTL-cholesterol and triacylglycerol concentrations were of 5.6%, 8.3%, 1.3% and 6.0%, respectively).

Acknowledgments

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ANTIMICROBIAL RESISTANT ESCHERICHIA COLI IN FAECES FROM PREWEANED DAIRY CALVES

A. Duse[1,2], K. P. Waller[1,2], U. Emanuelson[2], H. E. Unnerstad[1], Y. Persson[1,3], and B. Bengtsson[1]

[1]Department of Animal Health and Antimicrobial Strategies, National Veterinary Institute, Uppsala, Sweden. [2]Departmentof Clinical Sciences, Swedish University of Agricultural Sciences, Uppsala, Sweden. [3]Växa Sverige, Uppsala, Sweden

anna.duse@sva.se, karin.persson-waller@sva.se, ulf.emanuelson@slu.se, ylva.persson@sva.se, bjorn.bengtsson@sva.se

Abstract

This thesis investigated the prevalence, risk factors, and spread of resistant E. coli on Swedish dairy farms, with special emphasis on quinolone resistant E. coli (QREC). The occurrence of faecalresistant E. coli in calves was strongly age-dependent, but was also associated with herd size, milking system, calf housing, and geographic location of the farm. Treatment with broad-spectrum antimicrobials increased the prevalence. Feeding waste milk from cows treated with antimicrobials increased the proportion of resistant E. coli. Feeding such colostrum or milk to calves was shown to be a common practice on Swedish dairy farms. On farms where QREC is common in faeces of calves, QREC were also widespread in the farm environment. Genotyping of QREC suggests contagious spread of QREC within and between farms. Fluoroquinolone treatment, waste milk feeding, group calving, poor farm hygiene, purchasing cattle or shared animal transports were risk factors for increasing QREC on the farm.

Introduction

Antimicrobial resistance is an increasing threat for human and animal health. FaecalE. coli from preweaned dairy calves is often multiresistant, even without exposure to antibiotics (1; 2). Antimicrobial resistant Escherichia coli from the gut of calves is normally harmless for the calf itself, but may cause intractable infections in the animal or its herd-mates. There is also a risk that resistant E. coli strains in the cattle population are transferred to humans by direct contact or via contaminated milk or meat products. Hence, calves may act as reservoirs for resistant E. coli causing intractable infections in animals and humans. The epidemiology of faecalresistant E. coli in calves is not yet fully understood and more knowledge is needed to define measures that could reduce the burden of resistant E. coli on dairy farms. This thesis investigates the prevalence, risk factors, and spread of faecalresistant E. coli in preweaned dairy calves in relation to farm and calf characteristics, usage of antimicrobials in the herd, management factors such as the feeding of milk from antimicrobial-treated cows to calves, and factors associated with within- and between- farm biosecurity.

Materials and Methods

This thesis is made up of four part. The first part was a web-based survey investigating the farm practices related to the feeding of waste colostrum and milk from cows treated with antimicrobials. This questionnaire was returned by 457 Swedish dairy farmers.

The second part was a cross-sectional study investigating the occurrence of resistant *E. coli* in faeces from three preweanedcalves on each of 243 of the above farms that voluntarily agreed on taking part

(3). The faecal samples were cultured on agar plates with and without antibiotics at selected breakpoints and the within-sample prevalence of resistant *E. coli* was defined as the proportion of resistant E. coli out of the total number of E. coli. A random E. coli from each sample was also tested for resistance against twelve antimicrobials using broth microdilution. In the meantime, another web-based questionnaire was sent out to the farms collecting samples to obtain information on the use of antibiotics in their herds. Questionnaire data from both questionnaires and data from national databases were used together with the results from the bacteriological analyses to find risk factors for the occurrens of resistant *E. coli*.

The third and fourth parts were conducted in 23 of the above farms with varying prevalence of QREC in faeces from calves. For part three, faecal samples were collected from 15 preweaned calves and five post-partum cows. The occurrence of QREC in these samples were investigated using similar techniques as in part two. While sampling, the overall farm hygiene was determined and an interview with the farmer was conducted to obtain information on herd management. This was used to find out why QREC was common on some farms but not on others. For the fourth part, samples were also collected from the farm environment in order to investigate how QREC is spread on the farm. On some farms, genotyping of QREC from different samples was conducted to determine the genetic diversity within and between farms. The genetic diversity was then correlated to the number of purchased animals and the distance between farms.

Results and Discussion

The first questionnaire study farms showed that such colostrum or milk at least occasionally was fed to calves on 89% and 79% of the farms, respectively (4). Feeding such colostrum or milk to calves was a common practice on Swedish dairy farms, in particular on farms in southern Sweden, on non-organic farms, and on farms with tie stall housing.

In the second part, increasing calf age was found to be a protective factor against resistant E. coli, with highest prevalence around one week of age followed by a gradual decline. The microbiota of the gastrointestinal tract of the new-born calf is less diverse than in older cattle (5). Many resistant *E. coli* strains carry factors that enhance their colonizationability (6; 2) and competitiveness over susceptible E. coli (7), which may explain their successful establishment in the young calf gut. The decline in resistant *E. coli* with increasing age may be due to a combination of acquired immunity to certain strains (8) and increased competition from an increasingly diverse gastrointestinal microbiota (5).

Feeding colostrum from cows treated with antibiotics at drying off did not affect the shedding of antibiotic resistant E. coli by calves. In contrast, feeding calves milk from cows treated with antibiotics during lactation increased faecal streptomycin resistant *E. coli* and QREC. Hence, feeding milk from antibiotic-treated cows cannot be recommended from a resistance point of view. Treatment with broad-spectrum antibiotics in both cows and calves increased the occurrence of faecal resistant *E. coli*. Hence, prudent use of antibiotics likely reduces the overall burden of resistant E. colion dairy farms and should include not only treatment of calves, but also treatment of cows. Resistant *E. coli* was also more common on largethan on small farms, which is a concern with the current development towards larger farms in Sweden. Calves on farms with parlour milking compared to farms with ties tall milking or automatic milking systems, and calveson farms in South and East compared to North Sweden were also more likely to carry resistant *E. coli*.

In the third (9) and fourth part (Duse, submitted manuscript) it was concluded that if QREC was commonly found in faeces from calves, QREC was also frequently isolated from calf feed, water and milk troughs, in the calving pen and in faecal samples from newly calved cows.Thus, it was assumed that QREC is maintained in the calf group by faecal-oral circulation through contamination of feed, water and milk troughs and that transit ofcows and calves via the calving area may be important forthe

dissemination of QREC between cows and calves. Onmost farms, two to four genotypes were found throughout the farm, indicating contagious spread of QREC within the farm. Poor farm hygiene, group calving and infrequent use of the calving pen as a sick pen were risk factors for faecal QREC in cows and calves. Measures to reduce the burden of QREC on farms may be related to factors that decrease contamination and spread of faecal material, such as proper cleaning of feed, water and milk troughs as well as the use of single calving pens.

The same clone of QREC was also found on more thanone farm, suggesting contagious spread between farms. Farmsthat were located closer to each other were more likelyto share the same QREC clone, possibly due to an epidemiologicalconnection between those farms. Quinoloneresistant E. coli was also more common on farms thatpurchase cattle or share animal transporter with otherfarmers. A positive correlation was also found betweenthe number of purchased cattle and the genetic diversityof QREC within the farm, suggesting that new QRECgenotypes are introduced to the farm via purchase ofcattle. These results indicate that QREC is spread betweenfarms via the movement of cattle and equipment.

Conclusion

The results of this thesis indicate that antibiotic resistant E. coli are commonly found in faeces from Swedish dairy calves and that there are many factors that affect the occurrence of these. However, minimizing feeding of milk from antibiotic-treated cows to calves and the use of broad spectrum antibiotics may, together with proper biosecurity and good hygiene, lower the prevalence of resistant E. coli on dairy farms.

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CD109 GENE: A POTENTIAL CANDIDATE OF IMMUNE RESPONSE

Bianca Moioli [1], Luigi De Grossi[2], Roberto Steri[1], Fabio Pilla[3]

[1]Consiglio per la ricerca in agricoltura e l'analisidell'economiaagraria, via Salaria 31, 00015 Monterotondo, Italy; [2]Istituto ZooprofilatticoSperimentale, StradaTerme 01100 Viterbo, Italy; [3]DipartimentoAgricoltura, Ambiente e Alimenti, UniversitàdegliStudi del Molise, 86100 Campobasso, Italy

bianca.moioli@entecra.it

Abstract

Paratuberculosis is a chronic enteritis of ruminants which causes substantial reduced production, higher susceptibility to acquire other diseases and infertility [1]. Because little overall consensus has emerged, in terms of resistance loci, from the existing studies on immune response to infections [2], a genome-wide scan was performed with the Ovine SNP50K Beadchip on 50 negative and 50 positive sheep at paratuberculosis serological assessment. The results indicated the CD109, a co-receptor of TGF-β cytokine, as potential candidate gene of immune response, because located in proximity of the markers with different allele frequency in the two groups of sheep [3]. In silico comparison of the expressed region of the ovine and bovine genes showed complete dissimilarity in the first part, the bovine presenting one exon where the ovine had two exons; on the contrary, 100% similarity was found for the remaining 32 exons. Direct sequencing was then performed on the highly dissimilar region; in sheep, a missense mutation in the second exon was detected, leading to an aa change from Glu to Arg, this mutation showing a strong linkage disequilibrium with the anonymous marker. In cattle, a missense mutation leading to an aa change Pro>Ser was detected in exon 1. The presence of a mutation in both cattle and sheep corroborates the hypothesis of the CD109 gene as candidate of immune response, this gene having a role in binding TGF-β to its receptors, and in facilitating GF-β-receptor degradation, so inhibiting TGF-β signaling [4].

Introduction

Paratuberculosis, or Johne's, disease is a chronic granulomatous enteritis, which affects ruminants, caused by Mycobacterium avium subsp. Paratuberculosis (MAP). It causes substantial reduced production, higher susceptibility to acquire other diseases and infertility [1].Both diagnosis, especially in the early asymptomatic stages of the disease, as well as protective vaccination are notoriously difficult. Although no compulsory eradication program exists for this zoonosis, the infection is estimated to affect 20% of ruminants in Europe, and the probable link between MAP and Crohn's disease in humans stimulated the search for selecting genetically resistant animals.

Moioli et al. [3] performed a Genome Wide Association Analysis (GWAS), with the Ovine Bead chip 54K, on 100 ewes, representing the extreme divergent animals for the S/P ratio obtained from MAP serological assay, assessed on ~700 sheep. These authors hypothesized the presence of 30 putative candidate genes of disease susceptibility, these genes being in close proximity of the polymorphic markerswhich showed a significant effect on the obtained serological values.

One marker on chromosome 8 (OAR8_270360.1) appeared more interesting than the others, being located within the CD109 gene, a TGF-β co-receptor and a negative regulator of TGF-β signaling, playing therefore an important role in immunity.The aim of this study was then to study the CD109 gene in both sheep and cows to detect mutation potentially responsible of disease resistance.

Materials and methods

The genomic scaffolds encoding the ovine CD109 gene was obtained from the NCBI data base (http://www.ncbi.nlm.nih.gov/genome/?term=ovis+aries); the size and the position of the coding regions of the CD109 gene was identified by performing a standard nucleotide blast [5] of the genomic scaffolds against the expressed sequence of the gene under investigation in the NCBI (www.ncbi.nlm.nih.gov/nuccore).A standard nucleotide blast was also used to compare the genomic sequences and the expressed sequences of the CD109 in sheep and cows. Because the hypothesized marker of disease resistance in sheep (OAR8_270360.1) was located in intron 2, the search for the putative mutation in linkage disequilibrium (LD) with the marker was performed by sequencing the genomic regions encompassing exon 1 and 2 in sheepin 10 sheep – 5 positive to MAP and 5 negative. Once the novel SNP had been detected, direct sequencing of the amplicon was performed on 42 sheep, equally distributed between serologically positive and negative. DNA of 10 cows of the Holstein breed wasalso sequenced in the genomic regions encompassing exon 1 and 2. Primer pairs to amplify the expressed regions of the CD109 gene weredesigned on the ovine and bovine sequence by using Primer3Plus software (www.bioinformatics.nl/cgi-bin/primer3plus/primer3plus.cgi) and were reported in Table 1.

Table 1. Primersused to amplify the CD109 gene

GenBank	Covered region	Ampliconsize (bp)	Forwardprimer	Reverse primer
Ovine Oarv3.1 NW_004080171.1	Exon 1 + exon 2	952	TTCCTGGAGAGGGAAACAGA	GGCCACACCTTATCATTGCT
Ovine Oarv3.1 NW_004080171.1	Exon 2	499	ACTGCTGAGCCGGGAGTCT	GGCCACACCTTATCATTGCT
Bovine UMD3.1 NW_003104167.1	Exon 1	303	CTTGGTTTGGAGCCCTCG	AGGCTCAAATCTCCAGGCC

Direct sequencing of the coding regions of the putative candidate genes was performed on the 3500 Genetic Analyzers (Applied Biosystems, Foster City, CA, USA).

Allele frequencies and LD measures, for both the anonymous markers and the novel detected polymorphisms, were estimated using the Allele Procedure in SAS (SAS Inst.Inc., Cary, NC).

Results

The nucleotide blast of the genomic sequences (NC_019465.1 and with the expressed sequences in sheep (XM_004011463.1) and cow (XM_010808366.1) showed that this gene is composed by 54 exon in sheep and 53 exons in cows. Moreover, the nucleotide blast of the expressed sequences in the two different species showed that the cDNA encompassing exon 3 to the end the 3'UTR presented 98% similarity between sheep and cattle, indicating that the difference in this gene between the two species were located in the first part of the gene, where sheep have two exons and cows only one exon. Sequencing of ten sheep - 5 positive and 5 negative to MAP – allowed the detection of a novel missense mutation in exon 2 (g.128 A>G; XM_004011463.1) producing the aa change from Glu to Arg. The sequencing of further 42 sheep allowed to assert that LD extent between the mutation and the anonymous markerOAR8_270360.1, amounting to 0.74, was highly significant ($P<0.0001$).

The possible presence of a missense mutation in the CD109 gene was investigated also in cows, where paratuberculosis is also considered an emerging disease and has been the object of several Genome-Wide association studies between infected and non-infected animals[6; 7].

Sequencing of ten cows allowed to detect a missense mutation in exon 1 (g.230 C>T; XM_010808366.1) producing the aa change from Pro to Ser.

Discussion

The present study offered a twofold result. First, the effect of an ovine marker (OAR8_270360.1),identified through a GWAS and hypothesized as a potential marker of disease resistance wascorroborated by the detection of a missense mutation in LD with the marker in the CD109 gene, known to play a role in the immune system. The second result was the discoveringof completedissimilarity between the CD109 gene in sheep and cattle, only in the first part of the gene, which needs to be further investigated, also because of the presence, in cows, of a missense mutation in exon 1 which might be associated to immunological parameters.

CD109gene has been identified as a co-receptor for transforming growth factor (TGF)-β and a negative regulator of TGF-β signaling[4]. TGF-β is a multifunctional cytokine that regulates a wide variety of cellular processes including proliferation, differentiation, and extracellular matrix deposition. Dysregulation of TGF-β signaling is associated with several diseases. The detection of a missense mutation also in cattle in the first part of the gene opens new opportunities of investigating genes involved in the immune system, so contributing in the future to selection of disease resistant animals.

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BULK TANK BACTERIAL COUNT AND PARITY GROUP COW SOMATIC CELL COUNT IN STREP. AGALACTIA POSITIVE HERDS

Michael Farre

SEGES P/S, Team of health and reproduction, Agro Food Park 15, 8200 Aarhus N, Denmark

mifa@seges.dk

Danish milk production

The Danish dairy sector has undergone a tremendous expansion starting before the financial crisis, and is considered as part of the European “milk belt”. The average herd size is now 174 cows with an average production at 10.147 kg energy corrected milk (ECM). In 2014 bulk tank SCC was 207.000, with 0,33 cases of treated mastitis and 27% of dry cow were treated with antibiotics at dry off. The data recorded on treatments are compulsory, and the milk recording is done in 93% of the dairy herds in Denmark. The quality of data is therefore regarded as good and solid basis for statistic.

The Danish Strep.Agalactia program

Since 1963, Denmark has had a continuous national surveillance program for *Strep. agalactiae*. In the latest annual screening from November 2014 until February 2015, 6,7% of the 3383 dairy herds were tested positive. The number of positive herds has been increasing the last 10 years, for various reasons. The introduction of cows from different herds due to expansion was the major driver, limited DCT and the increase in robotic milking, which in 2015 were milking 26% of the dairy cows in Denmark. In 2009 PCR testing was introduces in the annual test, and the first year both PCR and microbiology was applied in the annual screening. The challenge in sampling on bulk tank milk, where the sample is collected though the loading processes is considered an issue. When a herd is identified as new infected, two additional samples are collected, manually from the bulk tank. The reason for additional tests is the risk of carry-over from the previous farm on the collecting route.

Aim

The objective of the study was to analyze correlation in bulk tank bacterial count (TBC), and the status in the national *Strep. agalactia*. Additionally the purpose was analyzing the SCC at cow level to demonstrate a potential significant difference in SSC in herd negative from *Strep. agalactia* at bulk tank, compared to herds positive in the annual bulk tank screening.

Methods

Data from the national cattle database was analyzed, comparing bacterial count in *Strep.agalactia* positive and negative herds. The data consisted of 495.000 cows, which were enrolled in the national milk recording. The cut off for infection was set at 200.000 SCC, and the cut off for positive PCR was Ct value <40. The analysis was based on a t-test with a bacterial count log transformed.

The first analysis was carried out comparing *Strep.agalactia* positive and negative herds, and TBC at herd level.

Next the correlation between the status in the surveillance program, and SCC in different parities was compared.

The cows were group in;

1. lactation cows

2. lactation cows

3. lactations cows and older cows

Results

Overall, the results revealed major differences in herds tested positive in *Strep agalactia*, compared to the group of herds negative at the annual test. Analyzing the TBC revealed a significant higher level in positive herds.

The results indicated:

Significant higher geometric bacterial count in positive herds positive on *Strep agalactia* 10.524, compared to negative herds negative 8.477

When the comparing the SCC between the two groups of herds, the herds positive and negative on *Strep. agalactia*, were done, the result indicated higher SCC in herds positive on *Strep. agalactia*.

In first lactating cows, there was no significant difference in SCC

In second lactating cows significant higher SCC in cows from *Strep agalactia* positive herds with ($p < 0,01$)

In third lactation and older cows, significant higher SCC in cows from *Strep agalactia* positive herds with ($p < 0,05$)

Conclusion

From a milk quality point of view it is beneficial to enforce an internal disease management and eradication program in herds positive on *Strep.agalactia*. In Denmark the premium scheme provided by the processor, favor TBC below 30.000 and SCC < 200.000. Therefor there is also a direct economic driver to improve overall milk quality.

GLOBAL EXPERIENCE ON KETOSIS SCREENING BY FTIR TECHNOLOGY

Daniel Schwarz[1], Daniel M. Lefebvre[2], Harrie van den Bijgaart[3], Jean-Bernard Daviere[4], Rene van der Linde[5] & Steen Kold-Christensen[1]

[1]FOSS Analytical A/S, Foss Alle 1, 3400 Hilleroed, Denmark; [2]Valacta, 555 Boul. des Anciens-Combattants, H9X 3R4, Ste-Anne-de-Bellevue, QC, Canada ; [3]Qlip BV, Oostzeestraat 2a, 7200 Zutphen, The Netherlands; [4]CLASEL, 141 Bd des Loges, 53940 Saint-Berthevin, France; [5]CRV BV, P.O. Box 454, 6800 AL Arnhem, The Netherlands

das@foss.dk

Abstract

This study summarises the latest global experience on the application of a fairly new service – ketosis screening – that dairy herd improvement (DHI) organisations can offer their customers.

Ketosis is a metabolic disorder of dairy cattle and has profound negative effects on subsequent performance (i.e. milk production, reproduction).

High-throughput Fourier transform infra-red (FTIR) spectrometry analysers (FOSS, Denmark) can be used for measuring ketosis indicators in DHI samples.

The ketosis screening tool is offered in Canada by Valacta, in France by CLASEL, and in Belgium (Flanders) and the Netherlands by Qlip and CRV since more than three years.

Briefly, the prevalence of ketosis could be reduced by 10%. This development is mainly explainable by the regular availability of data and thus a new management tool.

Ketosis screening offers high value to milk recording clients and elevates awareness of an otherwise undetected problem. This in turn can help reduce the incidence of the problem.

Introduction

Ketosis is one of the most frequent metabolic disorders in early lactating dairy cows. It is caused by a severe negative energy balance, where energy demands for milk production and body maintenance exceed energy intake. To compensate, cattle mobilise their body fat. In this process ketone bodies (e.g. acetone (Ac), β-hydroxybutyrate (BHB)) originate and accumulate. The costs per case of ketosis has been estimated to be $289 [1].

The common type of the disorder is subclinical ketosis without any clinical signs [2]. The diagnosis of the subclinical form depends solely on the measurement of ketone bodies in blood, milk, or urine. Electronic hand-held blood BHB meters with high accuracy [3] are available. However, implementing a ketosis surveillance programme using monthly available milk recording samples in the frame of DHI testing offers a more practical and less labour-intensive approach. Fourier Transform Infra-red (FTIR) spectrometry can be used for predicting milk BHB and Ac levels [4, 5]. Investigating this service using regular DHI samples, ketosis could be detected with a good sensitivity (87%) and a very high

specificity of 95% [5, 6]. Hence, milk Ac and milk BHB are valuable parameters for screening herds on the occurrence of subclinical ketosis [5, 6].

The objective of this study is to compile an overview on the latest global experience on the application of ketosis screening as a new tool offered by DHI organisations.

Material and methods

The observations are based on routinely performed DHI testing in Canada (Valacta, region Quebec), France (CLASEL, regions Pays de la Loire and Centre), Belgium (region Flanders) and the Netherlands (Qlip and CRV) from January 1, 2012 to December 31, 2014.

Milkoscan FT+ (FTIR) instruments (FOSS Analytical A/S, Denmark) with a FOSS calibration for Ac and BHB measurements were used for analysis of regular DHI samples.

Results and Discussion

Usage of the ketosis screening service

Ketosis screening on herd level is widely used in Canada, France, Belgium and the Netherlands (Table 1). Between 48% (France) and 85% (Belgium and the Netherlands) of farms are enrolled for the ketosis screening service, thus leading to 51 to 90% of cows that were screened for ketosis.

Table 1. Overview on the proportion of samples, farms and cows under ketosis screening from January 1, 2012 to December 31, 2014.

Country	Total number of DHI samples analysed	Proportion of samples with milk BHB analysis (%)	Proportion of farms using ketosis screening (%)	Proportion of cows under ketosis screening (%)
Canada	7,600,000	54	71	54
France	9,600,000	100	48	51
NL and BE*	35,000,000	100	85	90

*NL = the Netherlands, BE = Belgium

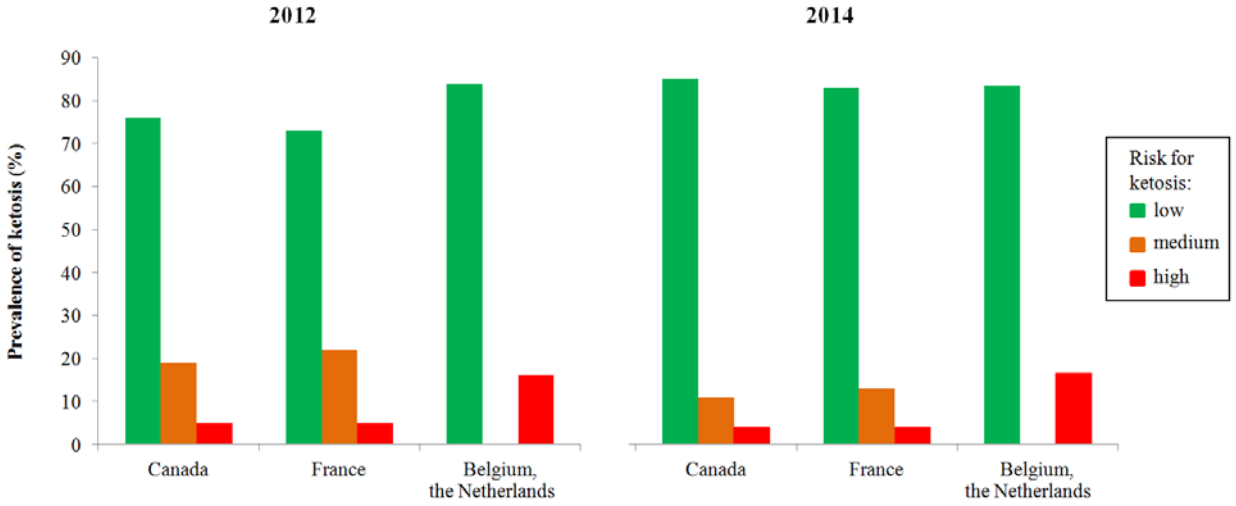

Figure 1. Prevalence of ketosis (low, medium, high risk) in Canada (Valacta), France (CLASEL) and Belgium (region Flanders) and the Netherlands (Qlip and CRV) in 2012 and 2014, respectively. Data for Belgium and the Netherlands are expressed as ketosis yes (high risk) or no (low risk).

Development of the prevalence of ketosis over time

Valacta and CLASEL indicate the risk for ketosis (low, medium, high) in the DHI report provided to their customers enrolled for ketosis screening. CRV processes the data generated at Qlip and indicates ketosis 'yes' or 'no' for individual cows in the DHI report. The occurrence of high risk cows decreased slightly from 5 to 4% in Canada and France, respectively, between 2012 and 2014 (Figure 1). Medium risk cows occurred evidently less frequently in Canada (2012: 19%; 2014: 11%) and France (2012: 22%; 2014: 13%).

The increased awareness and associated interventions due to ketosis screening might be an explanation for lower prevalences seen in 2014 (Figure 1). Nonetheless, experience from France shows that the quality of feed also contributed to this positive development. While the quality of corn silage harvested in 2011 was exceptionally poor, it was fairly high in 2013. The occurrence of cows classified as 'ketosis yes' in the Netherlands and Belgium, however, was steady at a level of 16.1% and 16.7% in 2012 and 2014, respectively (Figure 1). This observation might be explainable by the fact that the prevalence of ketosis was already quite low when the ketosis screening service was implemented in 2011.

Implications of ketosis on performance of dairy cows

Negative implications of ketosis in the early stage of the lactation on the subsequent performance of dairy cows were observed in all countries analysed. Cows with a high risk for ketosis produced between 2 and 6 kg and those with a medium risk between 1 to 3 kg less milk per day compared to the low risk group. Somatic cell count values were evidently high in the groups of high (>360,000 cells/ml) and medium (>318,000 cells/ml) risk cows (low risk group: 230,000 cells/ml). The risk of subsequent diseases such as clinical ketosis and displaced abomasums was also clearly increased in medium and high risk cows. Moreover, reproductive performance (e.g., days open) of high and medium risk cows

was clearly worse than of low risk cows. These observations are in accordance with the literature [7, 8].

Conclusions

The experience from 3 years of ketosis screening in Canada, France, Belgium and the Netherlands using FTIR technology on regular DHI milk samples clearly shows that this is a valuable service for farmers. Ketosis screening offers high value to milk recording clients and elevates awareness of an otherwise undetected problem. This in turn can help reduce the incidence of the problem.

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REDUCED SOMATIC CELL COUNTS IN RESPONSE TO A STANDARIZED HIGH ACTIVITY PROPRIETARY GARLIC POWDER ADMINISTERED TO LACTATING COWS – A PILOT TRIAL

L Chew[1], P De Costa[1], J Zonderland[2]

[1]InQpharm Group SdnBhd, Kuala Lumpur, Malaysia; [2]Veterinary Health Research, Hamilton, New Zealand

lynette@inqpharm.com

Abstract

Elevated somatic cell counts (SCC) during lactation could only be treated at the end of lactation with long acting intramammary preparations, usually with a host of antibiotics. Natural alternatives are constantly sought to better the health of cows and increase returns to the farmer. A Standardized High Activity Proprietary Garlic Powder (IQP-AS-106-1) was administered to lactating cows in a research farm in New Zealand to assess its efficacy in lowering SCC in milk. Eighty cows with composite SCC over 300 000 cells/ml were randomly allocated into 4 groups of 20 cows each. Group 1 was the control and was given water. Each cow in Groups 2, 3 and 4 received 150 ml, 300 ml and 450 ml respectively of IQP-AS-106-1 dissolved in water. All cows were dosed for 5 days. The efficacy of IQP-AS-106-1 in lowering SCC was evaluated using geometric means for the SCC before and after dosing.

Group 3 showed the best response with a 61% reduction of SCC after the last day of dosing versus 46% and 54% reduction in Groups 2 and 4 respectively. Meanwhile the control group had only 41% reduction of SCC. After just 3 days of dosing, Groups 3 and 4 had their SCC reduced to below 300 000 cells/ml. No adverse events were reported during the study. This study showed that at 300 ml, IQP-AS-106-1 (a component in Vetrinol) was able to lower high SCC in cows during lactation to less than half of their starting SCC.

Introduction

High SCC impacts the profit of farmers by causing lower milk quality and reduced productivity. The prevalence of high SCC ranges from 15% to 75% of a herd [1]. Treatment methods include intramammary therapy with broad-spectrum antibiotics. The obvious downside to this therapy is the discharge of the milk as it is unfit for human consumption. The search for an alternative remedy for high SCC could thus contribute to safeguarding our food quality as well.

A standardized liquid garlic extract (IQP-AS-101) was reported capable to reduce SCC in a previous study [2], where SCC was 36% lower when cows received this extract. In the present study, aStandardized High Activity Proprietary Garlic Powder(IQP-AS-106-1) is used to investigate its efficacy in reducing SCC in lactating cows.

Objective

To determine effects of different concentrations of Standardized High Activity Proprietary Garlic Powder, a component of Vetrinol^{TM}, in lowering somatic cell counts (SCC) in lactating cows.

Method

Eighty cows with quarters showing elevated SCC from Tokanui Research Farm, New Zealand, were selected. They were stratified based on their SCC and randomly allocated into 4 groups. Dosing of the IQP-AS-106-1lasted 5 days. Group 1 was the negative control group and was given 300 ml of water. Groups 2, 3 and 4 received 150 ml, 300 ml and 450 ml of the IQP-AS-106-1 respectively. All cows were further monitored for another 28 days after dosing.Milk samples were analysed for SCC by a third-party laboratory.

Results

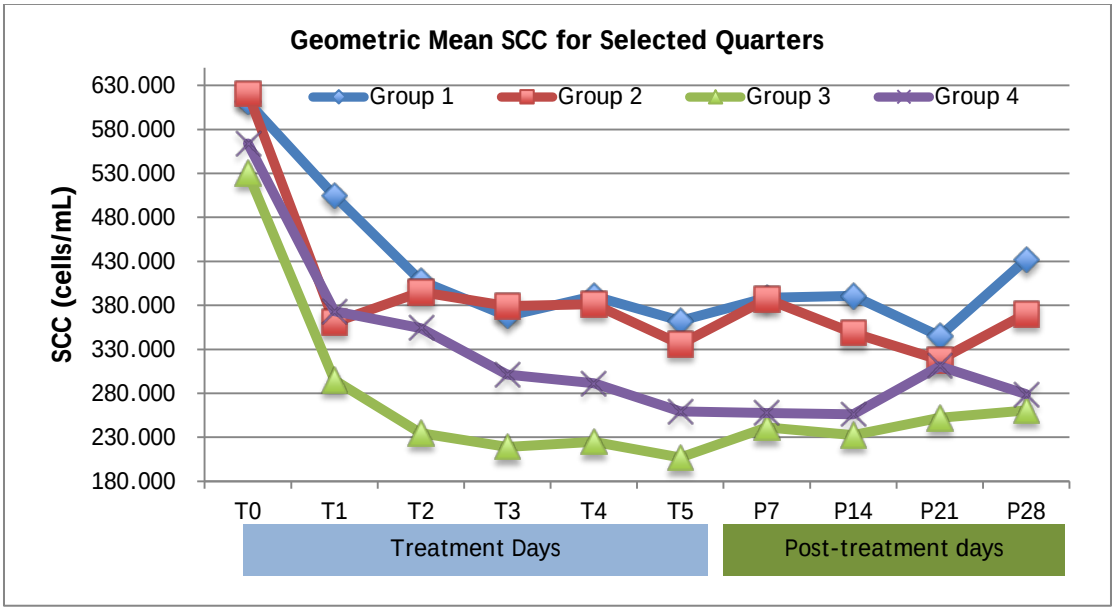

Figure 1: Geometric mean SCC for selected quarters

Error! Reference source not found.shows the geometric mean SCC of the milk samples taken at baseline (T0), during treatment (T1 – T5) and post treatment 7 days apart (P7, P14, P21, P28). At baseline T0, there were no significant differences between groups.

Geometric mean of the selected quarters in Group 2 did not differ much from the control group (Group 1). Groups 3 and 4 showed a treatment effect where their geometric mean SCC were lower than control after receiving theIQP-AS-106-1.

Group 3 SCC fell below 300 000 cells/ml after 1st dosing, SCC in Group 4 was reduced to 300 000 cells/mlafter 3 days of dosing.

The SCC reduction efficacy was calculated using the following formula:

$$\frac{Geomean_{T0} - Geomean_{Tx}}{Geomean_{T0}} \times 100\%$$

Where

$Geomean_{Tx}$ = Geometric mean SCC on day of treatment

$Geomean_{T0}$ = Geometric mean SCC on Day 0

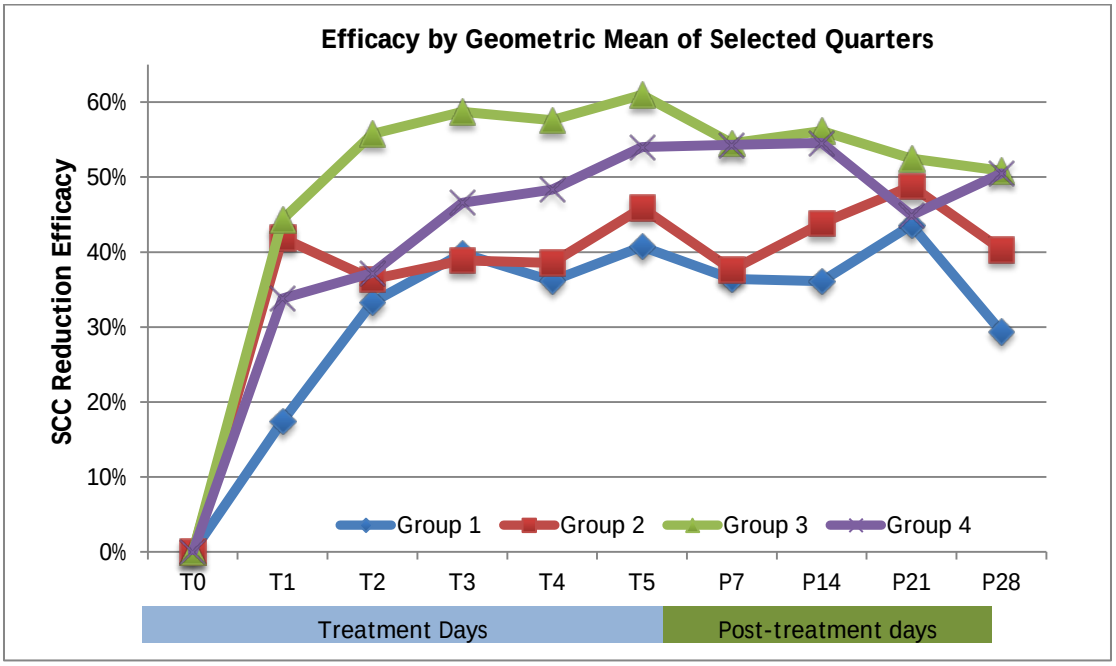

Figure 2: Efficacy as calculated by geometric mean of SCC from the selected quarters

Error! Reference source not found. gives the trend of the efficacy of SCC reduced in each group starting from Day 0 before treatment, until 28 days post treatment. Group 3 had the highest efficacy by achieving 61% reduction after 5 days of treatment, whileGroups 2 and 4 recorded efficacies of 46% and 54% respectively. The control group had an efficacy of 41%. In just 3 days of treatment efficacy recorded for Group 3 was 59% versus 39% and 47% in Groups 2 and 4 respectively. The control group's efficacy at Day 3 of treatment was 40%.

Discussion

At start of this trial the use of stratification randomization ensured that the means between all four groups were equal. The laboratory that provided the milk analysis services was also blinded to avoid biasness.

The results from Group 3 was very encouraging as the group achieved the highest efficacy in just 3 days and at the same time the geometric mean SCC for this group dropped below 300000 cells/ml. The 300000 cells/ml is a significant threshold as farmers could avoid the high SCC penalties if the milk

they sell has less than 300000 cells/ml.The geometric mean SCC of the control group was not reduced to this threshold SCC throughout the study.

All 60 cows did well tolerate the drenching of theStandardized High Activity Proprietary Garlic Powder(IQP-AS-106-1). No adverse events were reported throughout the study.

Conclusion

The Standardized High Activity Proprietary Garlic Powder(a key essential component in Vetrinol^{TM}) demonstrated its capability in reducing SCC in lactating cows. The dose of 300 ml for 5 days was able to lower high SCC by 61% from its starting count.

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IMPROVED FAECAL CONSISTENCY IN CALVES ADMINISTERED WITH STANDARIZED HIGH ACTIVITY PROPRIETARY GARLIC POWDER – A PILOT TRIAL

R Albert[1], M Musthapa[1],P De Costa[1], J.J.C. van Hattum[2]

[1]InQpharm Group Sdn. Bhd, Kuala Lumpur, Malaysia; [2]Farma Research Animal Health B.V., Nijmegen, the Netherlands

Abstract

Calf diarrhoea is a major cause of economic loss in the dairy industry. Common practice on farms is to start treatment with electrolytes. Antibiotics are used when calves have elevated temperatures, an indication of viral or bacterial infection. Consistent use of antibiotics for treatment could potentially contribute to resistance and may alter ruminal and gut flora [1]. The objective was to assess the efficacy of a Standardized High Activity Proprietary Garlic Powder, IQP-AS-106-2 (a component in Vetrinol), as a natural alternative for the treatment of diarrhoea.

Twenty-two male Holstein-Friesian calves, aged 2 to 6 weeks were randomized into 3 groups within 24 hours of the first onset of diarrhoea: Control, Treatment (VET) and Oral Antibiotic (OA) group. A daily dose of 20 mL of IQP-AS-106-2 was administered orally to VET group for 5 days. All calves received their normal supply of electrolytes.

Calves appearance and faecal consistency were scored. Faecal samples that were normal (semi-formed or pasty), fluid (some solid) or watery (no solids) were assigned 1, 2 or 3, respectively. Faecal samples were collected and tested for E.coli K99, Cryptosporidium spp., rotavirus & coronavirus using Bovidiar, an on-site immunoassay test kit.

In faecal consistency score, 91% of the calves in VET group recovered to normal consistency (score=1) compared to only 67% in the OA group on day 8. There were no adverse events reported in the trial. The trial has identified clinically important findings, which warrant further research into the effects of IQP-AS-106-2 in calf diarrhoea.

Introduction

Neonatal calf diarrhoea is a major cause of economic loss in the dairy industry[1]. Common practice on farms is to start treatment with oral rehydration solutions (ORS). Antibiotics are used when calves have elevated temperatures, an indication of viral or bacterial infection. Consistent use of antibiotics for treatment could potentially contribute to resistance and may alter ruminal and gut flora.

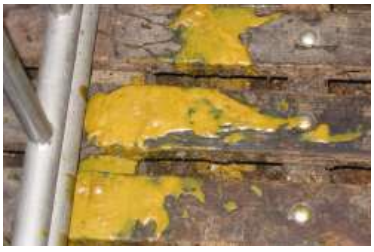
Objective

The objective of this study was to assess the efficacy of a Standardized High Activity Proprietary Garlic Powder, a key essential component of Vetrinol™Neo, as a natural alternative for the treatment of diarrhoea.

Method

Twenty- two Holstein Friesian male calves, age 2 to 6 weeks, were enrolled within 24 hours of the first onset of diarrhoea based on 2 inclusion criteria: the diarrhoea faecal consistency score, and the presence of either Rotavirus, Coronavirus, E. coli or Cryptosporidium by Bovidiar (an on-site immunoassay test kit). Scores of 2 and 3 are indications of diarrhoea and a return to score 1 indicates recovery (refer Table 1).

Table 1: Faecal consistency score chart[2]

Score	0	1
	Normal	Semi-formed or pasty[a]
		
Faecal consistency	2	3
	Fluid, some solid (Loose but stays on top of bedding)	Watery, no solid (Sifts through bedding)
		

[a]Calf on a diet of milk replacer produce semi- formed or pasty faeces

Calves were randomized into 3 groups: Treatment group (VET), Oral Antibiotic group (OAG), and Control group (CON). The proprietary Garlic Powder (IQP-AS-106-2)was dissolved in water. Ten calves in the VET group received a daily dose of 20 mL of the solution for 5 days, administered orally via graduated drench gun.

Faeces were inspected and scored daily. Faeces samples were collected on Days 1, 4, 6, 8, and were quantitatively analysed for presence of pathogen by a third-party laboratory. Other parameters taken and monitored were body weight, rectal temperature &general appearance.

Results

The group of calves that received GP showed an average faster improvement in faecal consistency scores, compared to the calves that received the antibiotic treatment. This is markedly seen in Day 3. At the end of the observation period of 8 days, 91% of the calves in the GP group were fully recovered versus only 67% of the calves in the OA group.

Qualitative faeces examination showed there was more elimination of rotavirus in the VET group compared to the OAG group. There were no notable differences in body weight, rectal temperature nor in general appearance of calves in all groups over the 8 days. There were no adverse events reported in the trial.

Discussion

The trial has identified clinically important findings of proprietary Garlic Powder (IQP-AS-106-2) administration in calves with diarrhoea. The trial is in agreement with findings of in-vitro minimum inhibitory concentration study on common diarrhoea causing bacteria & viruses:E. coli, coronavirus & rotavirus which showed the proprietary Garlic Powder has good antimicrobial and antiviral properties. Further research is warranted to confirm the effects of the product within a larger sample size.

Conclusion

Standardized High Activity Proprietary Garlic Powder (a component in Vetrinol™Neo) showed promising results as a natural and safe alternative to improve faecal consistency for calves with diarrhoea.

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DISTRIBUTION OF SCC IN DHI SAMPLES AROUND THE GLOBE

Daniel Schwarz[1]

1- FOSS Analytical A/S, Foss Alle 1, 3400 Hilleroed, Denmark

das@foss.dk

Abstract

Somatic cell counts (SCC) in milk are a valuable component for udder health monitoring in dairy cows.

The objective of this study was to investigate the distribution of SCC.

A data set (n = 91,866,300) consisting of SCC results from routinely performed dairy herd improvement (DHI) testing in Canada, France, Germany, Japan, New Zealand, and the US between 2010 and 2012 was analysed.

Looking at the distribution of SCC across all countries, 23% of all samples indicated a risk for an intramammary infection (>200 000 cells/ml). The majority of samples (77%) had SCC in a normal range (≤200 000 cells/ml), suggesting a proper level of udder health.

Similar SCC distribution patterns across all countries were found although dairy farming situations varied widely (e.g., herd size, milk yield per cow and year, husbandry system).

The distribution of SCC seems to underlie a certain pattern regardless of the factors considered in our study.

Introduction

Somatic cells in milk provide an indication of the inflammatory response in the mammary gland and can be used as a proxy for measuring intramammary infection and milk quality [1]. Somatic cell counts are typically available on a regular (e.g. monthly) basis for herds on dairy herd improvement (DHI) testing and are a valuable component of udder health monitoring. A cut-off value of 200 000 cells/ml is currently recommended to differentiate between infected and uninfected at cow level [2]. The objective of this study was to investigate and compare the distribution of SCC in samples originating from DHI testing in Canada, France, Germany, Japan, New Zealand, and the US.

Materials and Methods

A data set (n = 91 866 300) consisting of SCC results from routinely performed DHI testing in Canada, France (regions Pays de la Loire and Centre), Germany (federal state Bavaria), Japan (region Tokachi), New Zealand, and the US (North East) within 2010 and 2012 was analysed [3].

Results and Discussion

Dairy Farming Situation

The dairy farming situation in the countries studied was quite different. Average herd sizes varied from small (40, Germany) to large (393 dairy cows, New Zealand). The predominating husbandry system was tie-stall in Canada, Germany and Japan, straw yard in France, free-stall in the US, and pasture in New Zealand. The average cow milk yields ranged between 4 128 (New Zealand) and 9 564 (Japan) kg per year.

Distribution of SCC

Looking at the distribution of SCC across all countries, 23% of all samples indicated a risk for an intramammary infection (>200 000 cells/ml, Figure 1). The majority of samples (77%) had SCC in a normal range (≤200 000 cells/ml), suggesting a proper level of udder health. A proportion of 63% of samples with ≤200 000 cells/ml was reported in a previous study from the US [2]. The higher values observed in our study might be explainable by continuous and substantial improvement of udder health management over time.

Similar SCC distribution patterns across all countries were found although dairy farming situations varied widely (Figure 1). However, highest proportions of events in the range ≤50 000 cells/ml occurred for the US, where free-stalls were the predominating husbandry system. In contrast, France revealed the lowest proportions in this SCC range. This might be explainable by the fact that straw yards, which were the predominating husbandry system in France, were less hygienic.

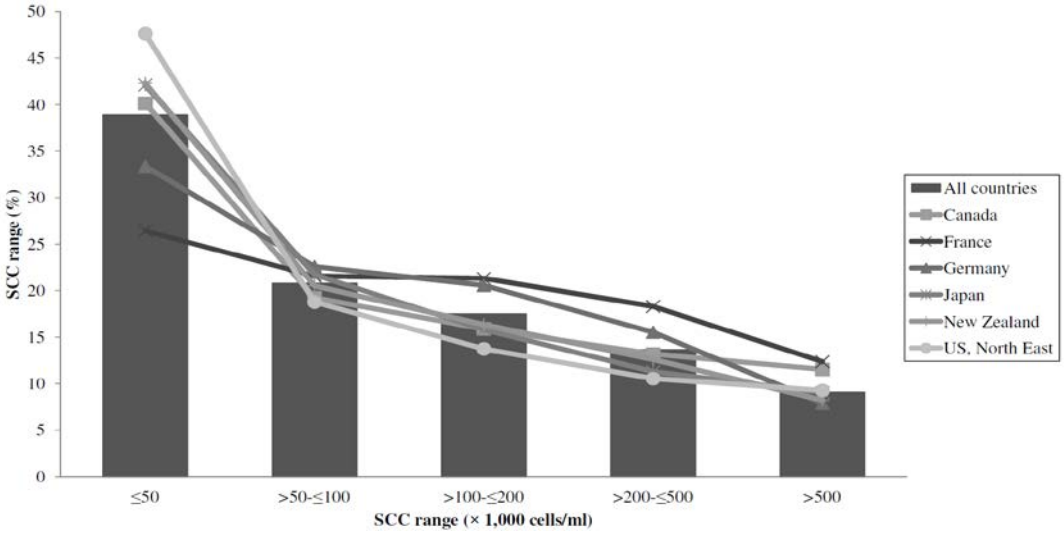

Figure 1. Distribution of SCC of all cow-composite samples (n = 91 866 300) analyzed in Canada, France, Germany, Japan, New Zealand, and US within 2010 and 2012 by classification in 5 SCC ranges. The bar charts show the distribution of SCC observed in all countries, the lines show the distribution of SCC observed in the individual countries.

Conclusions

The distribution of SCC at population level seems to underlie a certain pattern regardless of the factors considered in our study (herd size, husbandry system or cow production data).

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THE INFLUENCE OF PROBIOTIC ADDITIVES AND MULTIENZYME COMPOSITION ON MILK QUALITY OF LITHUANIAN BLACK-AND-WHITE CATTLE

R.Budreckienė[1], J.Dailidavičienė[2], R.Gružauskas[3], S.Kerzienė[4], S.Makauskas[3]

[1]Department of Biochemistry, Lithuanian University of Health Science, Tilzes str. 18, Kaunas, Lithuania; [2]Department of Animal Anatomy and Physiology, Lithuanian University of Health Science, Tilzes str. 18, Kaunas, Lithuania; [3]Institute of Animal Rearing Technologies, Lithuanian University of Health Science, Tilzes str. 18, Kaunas, Lithuania; [4]Department of Physics, Mathematics and Biophysics, Lithuanian University of Health Science, Tilzes str. 18, Kaunas, Lithuania

Ruta.Budreckiene@lsmuni.lt, Jurgita.Dailidaviciene@lsmuni.lt, Romas.Gruzauskas@lsmuni.lt, Saulius .Makauskas@.lsmuni.lt, Sigita.Kerziene@lsmuni.lt

Introduction

Lithuanian Black-and-White cattle account is about 60% of bred cattle in Lithuania. Improving the breed, the most important tasks are to increase the milk production, milk fat and protein content; to improve the udder morphological and physiological characteristics and to preserve meat quality [1, 2]. The most important factor in development of these qualities is feed. The fodder enrichment with various additives is very important and useful, consequently. Carotenoids are present in animal systems, but animals cannot synthesize them de novo and depend on intake from their feed.

The probiotic additives of live yeast cultures (Saccharomyces cerevisiae) with organic selenium have been added to diets for lactating dairy cows to attempt to improve ruminal fermentation, potentially increasing dry matter intake and milk yield [3, 1]. Selenium plays a crucial and ubiquitous role in the organism. The health benefits of selenium supplementation in ruminants are well recognized. In dairy cows, this is directly reflected by the potential of selenium supplementation to reduce somatic cell count (SCC) in milk and prevent sub-clinical mastitis [4, 5]. A universal multienzyme composition increases productivity, feed energy exchange and digestibility of protein and amino acids [6, 7], improves feed conversion, and reduces viscosity of the digestive tract and the incidence of diarrhea.

Objectives

The study was curried out to know the effects of probiotic additives Biogrom® SC and Biogrom® Lux and multienzyme composition Vilzim® on milk quality of Lithuanian Black-and-White cattle during the 90 days. The 28 cattle were divided into 4 groups of 7 cows each (Control and three Experimental – A, B, C). The cattle had a standard, commonly balanced ration with the 40 g supplement of additive Biogrom® SC for the Group A, 40 g supplement of additive Biogrom® Lux for the Group B and 0.2 g supplement of additive Vilzim® for the Group C daily. The milk samples of the researched cows were taken in every 30 days.

Results and Discussion

The results indicated that cows consuming diets supplemented with yeast culture tended to increase their milk yield in all milking periods. Milk yield was 2.64%, 1.75% and 1.4% higher in Group A, B and

C respectively comparing with the Control group. The average values and dynamics of milk yield (kg) of cows of each investigated group during experimental period is shown in Table 1. and Figure 1.

Table 1. The average values of milk yield (kg) of cows of Control and every Experimental group originally, after 30, 60, 90 days.

	Control group	Group A	Group B	Group C
0 days	24.57±0.37	25.03±1.10	24.46±0.88	24.86±0.86
30 days	24.67±0.44	27.57±0.81**	26.57±0.67*	26.43±0.57
60 days	24.36±0.60	27.93±0.66**	26.60±0.66*	26.03±0.67
90 days	24.09±0.56	27.71±0.62**	27.04±0.65*	26.03±1.46

*, ** – average means differed significantly at p<0.05

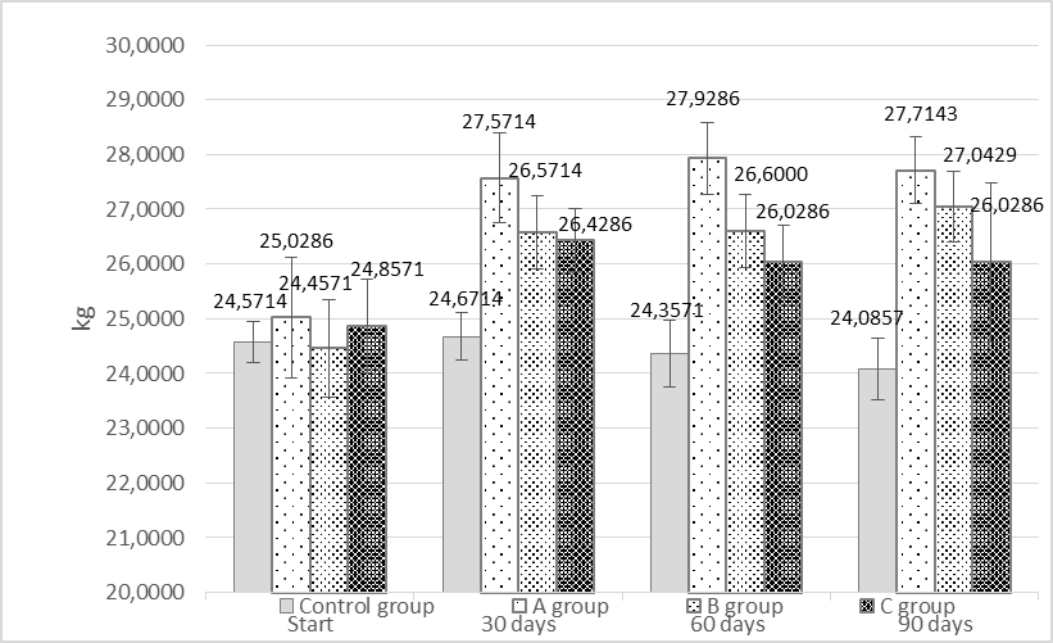

Fig. 1. The dynamics of milk yield (kg/day) in all groups during experimental period

The percentage of milk fat was significantly higher in cows of Group A – 0.33 %, B – 0.31 % and C – 0.16 % comparing with the Control group. Milk proteins did not change significantly with yeast and multienzyme composition supplementation. The milk fat/proteins ratio in each investigated group during experimental period illustrates Figure 2.

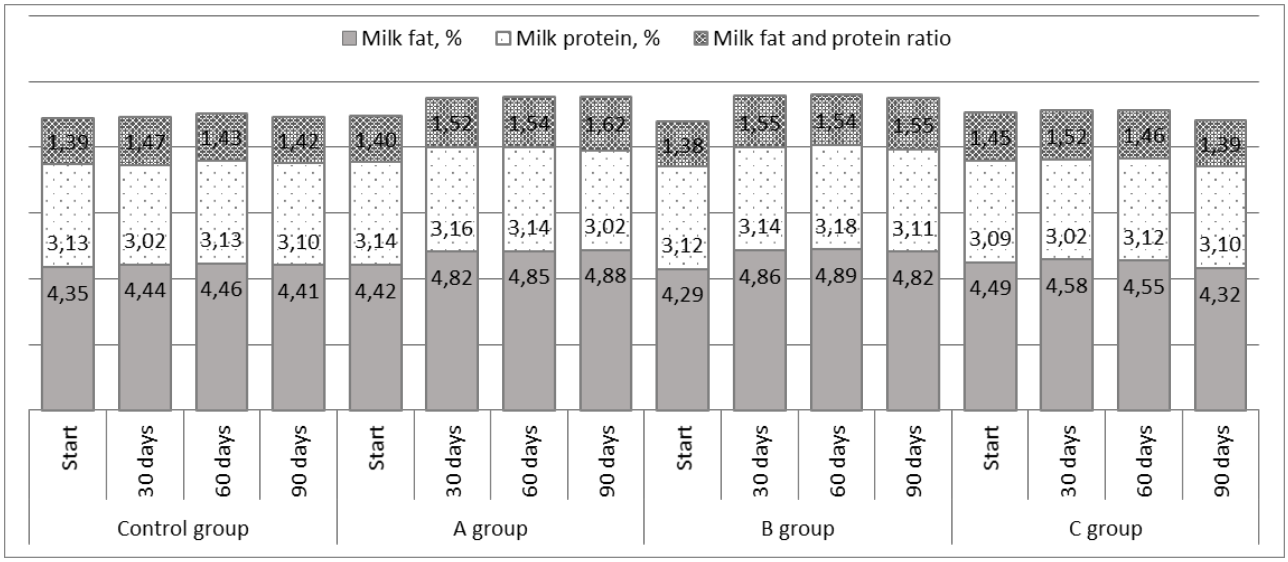

Fig. 2. The milk fat/proteins ratio in all groups during experimental period

The dynamics of SCC in each investigated group during experimental period is shown in Figure 3. The SCC in milk in all experimental groups was lower comparing to the Control group. The lowest SCC values were evaluated in the B group after 60 days of experiment as well as there were the highest values of SCC in the Control group after the 60 days of experiment. There were minimal dynamics of SCC values in all other groups.

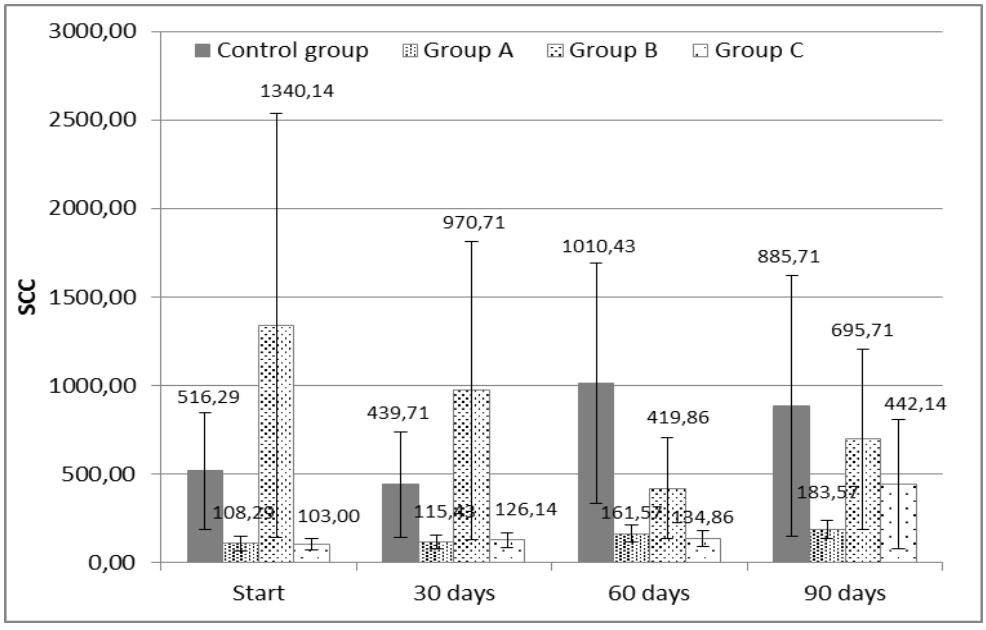

Fig. 3. The dynamics of SCC in all groups during experimantal period

Milk yield increased by 1.75% to 2.64% in cows supplemented with probiotic additives is in agreement with other authors who reported a relatively low responses of milk production ranging from 3% to 9% (Maamouri et al., 2014). As shown in Table 1, the major finding in this study was that average milk production in each experimental period was higher ($p<0.05$) in a probiotic additives supplemented diet than in control cows. Our experiment is in agreement with the work of Maamouri et al. (2014) and

Kalmus P. et al. (2009) which shows an increase in the fat content while the protein is not altered. In this study, milk proteins of all experimental groups and during all experimental periods was not affected significantly by yeast culture supplementation (Fig. 2). However, the average fat percentage was 0.33%, 0.31% and 0.16% higher in the A, B, C groups comparing to the control group, respectively.

Conclusions

The results indicate, that probiotic additives and multienzyme composition supplementation to dairy cows during the different examination periods improved the rumen environment in a way that increased the dry matter intake and in consequence enhanced the productivity and efficiency, and decreased the SCC in milk also.

Acknowledgements

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FEEDING AND MANAGING THE DAIRY COW OF THE FUTURE

Dr. L. E. Chase

Department of Animal Science, Cornell University, Ithaca, NY 14850 USA

Abstract

The average dairy cow in the US is projected to produce 10,684 kg of milk by 2020 and many dairy herds will produce greater than 14,000 kg per cow. The challenge is developing nutrition and herd management programs to support these levels of milk and maintain cow health and reproduction. There are currently herds that are producing greater than 14,000 kg of milk that provide some information of value in developing future programs. The basic principles of forage quality forage testing, ration formulation and optimizing rumen fermentation will be keys to attaining these higher levels of milk production. Two additional important considerations are cow comfort and daily herd management. All of these need to be integrated to support these higher milk production levels.

Introduction

The productivity of dairy cows continues to increase. Milk production for the average dairy cow in the US has increased from 7,131 kg. in 1993 to 9,898 kg. in 2013. This is a 39% increase with an average increase of 138 kg. per year. This rate of increase may double or triple as the use of genomic selection becomes more routinely used by the industry. If the current rate of increase continues, the average dairy cow in the US is projected to produce 10,864 kg. in 2020. This is a 52% increase since 1993. Table 1 contains an overview of the changes that have occurred in the US since 1980. The challenge is how dairy producers will manage for this higher level of productivity while considering animal care, animal health, sustainability, environmental considerations and profitability. At the same time, the US dairy industry has made a commitment to lower greenhouse gas emissions throughout the total dairy supply chain 25% by 2020 (Tricarico, 2012). This is an additional factor that needs to be considered in developing and managing rations in the future. The US dairy industry has already taken a step in this direction by lowering total methane emissions from dairy cows by 62% in 2007 compared with 1944 (Chase, 2010).

What Are the Biological Limits to Milk Production?

The biological limit for milk production appears to be a moving target. At one point, it was assumed that an individual cow could not produce more than 22,000 kg. of milk in a lactation. This has been proven wrong many times.. The current milk production records for individual cows in the US are:

Holsteins – 32,735 kg. milk containing 3.86% milk fat and 3.17% milk true protein in a 365 day lactation. This is an average of 89.7 kg. of milk per day for the entire lactation.

Jerseys – 25,215 kg. milk with 4.58% milk fat and 3.18% milk true protein. Average milk production per day was 69 kg.

A Holstein cow has produced more than 216.821 kg. of milk in her lifetime. This cow averaged 42 kg of milk for every day she was in production.

Input and Output Dynamics for Dairy Cattle

The dairy cow can be thought of as a biological manufacturing plant. Feed and ware are the primary inputs while milk, manure and gases are the outputs. Table 2 contains data on the inputs and outputs for a dairy cow at 3 levels of milk production. Key points from this table are:

Milk nitrogen efficiency increases with higher levels of milk production. Higher producing cows are more efficient in converting feed N to milk N.

Total methane and carbon dioxide production increases with higher levels of milk production.

Methane and carbon dioxide emissions per kg of milk produced decrease as milk production increases.

The percent of the ME (metabolizable energy) intake used to support milk production increases with higher levels of milk production.

Herd Management Parameters in High Producing Dairy Herds.

Table 3 contains data on Holstein herds in the East and Midwest sections of the US that are on the DHI (Dairy Herd Improvement) milk testing program. The table contains for all herds and those producing >13,608 kg of milk per cow. Key points from this table include:

The higher producing herds tend to be larger herds with a lower average age at 1st calving.

Higher producing herds have slightly more 1st lactation cows and fewer 3rd+ lactation cows.

The percent cows died is similar for both groups of herds.

Culling rate is higher in the higher producing herds. However, it is not possible from this data to separate this into voluntary and involuntary culling rates.

Higher producing herds have lower somatic cells and better reproductive performance.

These higher producing herds represent 2.2% of the total herds in this database.

Table 4 contains descriptive parameters in 5 Wisconsin dairy herds that average 15,493 kg of milk per cow. Milk components and somatic cells are similar to other herds. Ration nutrient composition values are similar to many other herds. All of these herds milk the cows 3 times per day and feed a total mixed ration (TMR). On herd feeds the TMR 5 times per day, one herd feeds once per day and the other 3 herds feed twice a day. One herd does not push up feed during the day but they also feed 5 times per day. One herd pushes feed up 12 times per day while the other herds push feed up 4 to 6 time per day.

What Are the Keys to Feeding High Producing Dairy Herds?

This can be answered using a combination of information from high producing herds and direct on-farm observations. A survey was done of the herd management practices used in high producing Kentucky herds (Smith et.al, 2013). Seventy-four percent of these herds milked the cow twice a day while the other herds milked the cows three times per day. The top 4 management practices listed by these dairy producers were attention to detail, nutrition, cow comfort and quality forages. A survey was done of 50 high-producing herds in Minnesota that were housed in free-stalls (Endres and Espejo, 2010). Key results from this survey are:

70% of the heeds fed the cows once per day, 22% fed twice per day and 8% fed three times per day.

The frequency of feed push-ups was 5.4 ± 2.3 times per day.

Feed bunk space was 0.45 ± 0.11 meters of linear space per cow.

Rations contained an average of 52% of total dry matter as forage (range was 38.6 to 68.2%).

Ration crude protein averaged 17.9% with a range of 16.9 to 18.4%.

Ration NDF averaged 29.8% with a range of 25.4 to 32.8%.

The majority of these practices are just continuations of the feeding and nutrition practices that have been evolving in the last 10 – 20 years. Some of the key consideration areas for feeding and managing future herds are:

Ration formulation – Rations are a key component of the total system in dairy herds. However, there are a large number of variables that can influence the milk production attained from a specific ration. A trial was done in Spain to examine some of these (Bach et. al., 2008). Forty-seven herds were fed the same TMR that was mixed and delivered on a daily basis from a TMR mixing center. Average milk production in these herds was 29.3 kg with a range of 20.6 to 33.8 kg per day. There were 4 factors that accounted for more than 50% of the variation in milk production between these herds. These are:

The higher producing herds had a lower age at first calving.

There was a positive relationship between milk production and stalls per cow.

Herds that fed for feed refusals averaged 1.6 kg more milk per cow than herds that did not feed for refusals.

Herds that pushed up feed during the day averaged 3.9 kg more milk than herds that did not push feed up.

There are other examples of herds or cows producing different levels of milk when fed the same ration. One is when a dairy producer sells a TMR to another farm. These 2 farms never have the same level of milk production. As an example, a herd averaging 36 kg of milk per cow per day sold a TMR to another farm. The farm that purchased the TMR produces 46 kg of milk per cow. A second example is cows within the same herd fed a TMR. The group of cows is averaging 40 kg of milk per day. However, there is a cow within this group producing 73 kg per day consuming the same TMR. These examples just emphasize that a number of factors can influence the quantity of milk that can be produced when feeding the same ration. Most nutrition consults indicate that the ration explains only 5-15% of the difference in milk production between herds.

Forage quality – Forage quality available on farms continues to increase due to improved forage genetics, better agronomic practices and improved harvesting and storage programs. The end result has been improvements in both forage quality and quantity. This has permitted higher levels of forages being fed in rations.

Forage testing – There have been improvements in the analytical tests available which assist in increasing the use of forages in rations. One has been the determination of NDF digestibility. Research at Michigan State has indicated that a 1 unit increase in NDF digestibility is associated with an increase of 0.17 kg of dry matter intake and 0.25 kg of 4% fat corrected milk (Oba and Allen, 1999). The recent availability of a 7-hour starch digestibility has been helpful in improving the use of corn (maize) silage in rations. New developments to watch for include separating the NDF fraction into indigestible and potentially digestible fractions. A second development is dividing the potentially

digestible NDF fraction into fast and slow pools. This additional information will permit fine tuning of rations to improve the efficiency of feed use and lower emissions of nutrients and gases to the environment.

Feed delivery and mixing – The goal is to have the ration delivered and consumed by the cow as close to the formulated ration as possible. One tool that has been helpful to assess this is the use of TMR audits (Oelberg, 2011). These audits examine the accuracy and consistency of the mixing and delivery process. A recent paper from Canada reported that feeding a TMR twice a day increased milk production by 2 kg per day compared to feeding once per day (Sova et. al., 2013).

Optimizing rumen fermentation – Improving the efficiency of rumen fermentation increases the overall efficiency of nutrient use, increases microbial protein production and lowers methane emissions. Computer models can be helpful to balance the ammonia available in the rumen with rumen fermentable carbohydrates. Factors related to improving rumen function have been described (Oba and Allen, 2003; Weakley and Reutzel, 2013).

Cow comfort and behavior – A key factor in many high producing herds is cow comfort. Grant (2011) reported that an additional hour of resting activity was associated with an increase of about 1.6 kg of milk for each hour of additional resting time. Grant (2003) presented information on the daily time budget for dairy cows. Cows spend 3 to 5 hours per day eating 9 to 14 meals. Rumination time is 7 to 10 hours per day while drinking time is about 30 minutes. They spend 2 – 3 hours outside of the housing pen (milking, travel time and standing time) and 10 – 12 hours lying or resting. Social interactions are 2 to 3 hours per day. Higher producing cows tend to increase resting time and spend less time standing. The importance of cow comfort can also be assessed by changes in milk production when dairy herds make housing changes. A herd in central New York built a new free-stall barn to lower the number of cows per stall from 1.2 to 1. Milk production in the whole herd increased by 3.6 to 4.5 kg per day. At Cornell, we moved from a 40 year old free-stall barn into a new free-stall with sand bedding. Milk production increased by 3.2 to 4.1 kg per cow. Other farms have reported milk production increases of 2.2 to 5.4 kg per cow when cow comfort was improved.

Daily management – Higher producing herds tend to do the daily management activities on a more consistent basis. They also do a better job of observing and monitoring cows for potential health problems. They spot problems earlier and take corrective actions before a real problem exists. Someone in the herd has an “eye for cows” and can read the various signals from cows that can alert them to potential problems. This is especially critical at calving to minimize the incidence of metabolic disorders and off feed cows.

Conclusion

Developing nutrition and management programs for higher producing dairy cows and herds in the future will be a continuation and expansion of the tools and concepts currently utilized in the industry. Data available from current high producing herds provides a base of information to use in developing future programs. One area that will require more consideration in the future is the environmental and greenhouse gas emissions regulations. Currently available information indicates that higher producing cows are more efficient in converting feed into milk, have lower emissions per unit of milk produced and are generally more profitable. There are 5 key factors that are common to higher producing dairy herds. These are:

- Availability of high quality and highly digestible forages.
- Well balanced rations.
- High levels of dry matter intake.
- Comfortable cows.

- A “cow person” that is ab excellent observer and manager of cows.

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HIGHLY PRODUCTIVE GENETIC RESOURCES IN DAIRY FARMING IN STAVROPOL REGION

Trukhachev V.I. [1], Oliinyk S.A. [2], Zlidnev N.Z.[3]

[1]Department of animal nutrition and general biology of Stavropol State Agrarian University; [2]Department of private animal husbandry, animal breeding and selection of Stavropol State Agrarian University; [3]Department of animal nutrition and general biology of Stavropol State Agrarian University

Keywords: International Committee for Animal Recording (ICAR), dairy cattle

Introduction

Increasing production efficiency and product quality and further specialization of production are the main trends of the modern dairy farming.

Marketing Research of International Research Center IFCN (Dairy Research Center) for the period of 1996-2012 showed that the milk yield per cow (the average for controlled agricultural companies) increased by 29.3% and was equal to 7,500 kg of milk per year. There is also a consolidation of farms, the average number of cows on farms increased by 54% to 51 cows, while the total number of farms producing milk decreased by 2.2 times. As a result of genetic progress, efficient technological management and ensuring full feeding there is an improvement of productive qualities of animals resulting in increased conversion of feed nutrients and 1.5 reduced feed costs for production of 1 liter of milk [3].

Thus, the introduction of modern methods of herd management proven by international practices which are based on the application of the recommendations of ICAR will be an important factor in ensuring a steady trend of positive development of dairy farming in Russia and Stavropol Territory [4, 12].

Purposeful work on the harmonization of national regulatory frameworks with international law is carried out in the Russian Federation. One of the stages of this process is implementation of research project at Stavropol State Agrarian University which is particularly significant for the agro-industrial complex of the Russian Federation in 2015 in the context of ensuring import substitution in animal husbandry (genetic material).

The research aim was to develop a regional model of the formation and management of highly productive livestock genetic resources (on the example of Stavropol Territory). For this purpose, there was established the Center of management of highly productive genetic resources of livestock with the structure: expert-appraisal service; control-assistance service; reference-laboratory; genetic control laboratory.

The objectives of the study were to develop guidelines for the animal recording in compliance with the requirements of ICAR to provide national report [4], to improve interaction between different departments of the Centre and milk producers.

Works were carried out on the following farms of Stavropol Territory: joint venture “Chapaevskoe” Ltd. of Shpakovsky Region; collective farm-breeding plants “Kazminsky”, “Kuban” and “Collective farm-breeding plant named after Chapayev” Ltd. of Kochubeevsky Region; collective farm-breeding plant “Russia” of Novoalexandrovsky Region; collective farm named after Voroshilov of Trunovsky Region.

Materials and methods of research

The material for the research was the Russian [1, 2, 5-9, 12-14] and foreign [4] standard documentation for dairy cattle recording. Based on the use of methods of animal husbandry and comparative analysis there have been developed guidelines for the organization of work of the departments of the regional selection and technology center which include conducting linear measurement of animal body (expert-appraisal service), recording milk productivity of cows (control-assistance service), evaluation of the quality of milk using Russian and foreign reference methods (reference laboratory), evaluation of genetic abnormalities using cytogenetic methods (genetic control laboratory).

Results and discussion

It has been found that dairy cattle recording at the regional level should be carried out according to the following forms: Form 01. National milk productivity; Form 02. State of milk recording (techniques and organization); Form 03. Cost and financing; 04. Results of milk recording.

Recording milk productivity of cattle is carried out by experts of the control-assistance service. During the control milking the following factors are taken into account: date of the control milking which is the date of preparation of the relevant act; name; animal ID; one-time milk yield per milking; milk quality. In determining the rate of milk ejection the following factors are taken into account: the date of determining the rate of milk ejection which is the date of preparation of the relevant act; name, animal ID; number of the current lactation; one-time milk yield per milking; time spent for milking with milking machine; milking machine brand. Recording level of productivity and quality of milk per lactation or a certain period of lactation of each cow is carried out by summarizing the results of control milkings conducted in the prescribed manner, in accordance with existing requirements [1, 2].

Control milking is carried out simultaneously for all animals kept in the same yard, except for dry cows and calved cows until 6 pm the day after calving.

To determine the amount of milk produced by cows, engineering tools are used – milk gages as well as electronic devices. All technical facilities are subject to the established procedure of controlling the accuracy by the organizations of the State Standard of the Russian Federation at least once a year.

The amount of milk is determined with an accuracy of up to 0.1 kg. The yield for the control period is calculated with an accuracy of up to 1 kg.

The content of fat, protein, somatic cells, and if necessary other components in the milk of tested cows is determined by examining the specially selected milk samples in accordance with the applicable regulations and procedures in the laboratory [5-9].

For the preservation of milk the special broad-spectrum preservative Microtabs II is used.

For recording milk productivity, automatic milk counters of three types are used: Waikato, DeLaval MM6 and USM-1A of which the first two models are approved by ICAR [4].

Introducing the given recording system into practice of animal husbandry will enhance the image of Stavropol Territory as a region with a high culture of animal husbandry, improve the quality of products and create conditions for a reliable progeny testing of leading bulls.

1. Indicators of milk productivity of breed genetic resources of Stavropol Territory (Russian reproduction)

Breed	Productivity of cows for 305 days of lactation									
	Highest productivity				Last completed lactation					
	Number of lactations	Milk yield, thousand kg	Fat, %	Protein, %	Number of lactations	Milk yield, thousand kg	Fat, %	Protein, %	Rate of milk ejection, kg/min	Udder shape
Holstein black-and-white	2.3	10.3	3.78	3.13	3.5	9.2	3.78	3.17	2.72	1
Holstein red-and-white	2.0	8.0	3.70	3.17	1.8	7.7	3.68	3.15	2.51	1
Black-and-white	2.3	8.9	3.86	3.2	3.0	8.3	3.94	3.2	1.93	1; 2
Ayrshire	2.9	8.8	3.85	3.10	3.5	8.5	3.85	3.03	2.12	1
Yaroslavskaya	3.2	8.6	3.81	3.17	3.7	8.3	3.80	3.16	2.05	1; 2
Red steppe cow	2.2	6.5	3.77	3.17	3.0	6.8	3.85	3.11	1.87	1; 2

As a result of purposeful work of scholars and practitioners of livestock breeding in Stavropol Territory on the basis of the best breeding farms there were established highly productive genetic resources of the most technological dairy breeds (Table. 1).

Analysis of the data shows that the milk yields of the given animal populations are at the level of the best achievements of dairy farms of the EU, USA and Canada [4].

Thus, the genetic potential of the world's best dairy cattle breeds enables development of regional programs to increase milk production and, thereby, increase the food security of the region.

Comprehensive assessment of pedigree and economically useful parameters of dairy and dual-purpose cattle is carried out by experts of the expert-appraisal service of the regional breeding and technology center and includes linear measurement of body type and appraisal [1, 2, 4].

Nowadays the main method of dairy cattle body estimation is linear measurement of the conformation, which is carried out for the active part of the population and in the progeny testing of bulls [1, 2, 11]. The evaluation of bulls on the base of body type of daughters is carried out on the data of at least 30 daughters, and that of sires used as fathers of young bulls – during the whole period of using their sperm. All daughters, except for ill ones, those which aborted, ones with full atrophy of two or more quarters of the udder, are to be estimated and recorded. Linear profile of sires on the base of body type of daughters is made on the data of all (at least 30) daughters.

The Russian system of linear measurement of body describes 18 main parameters of the conformation where each parameter has independent significance and is estimated from 1 to 9 points. For each parameter, the arithmetic mean and mean square deviation are determined. The vertical center line of the profile corresponds to the exterior ground level or 5 points, i.e. the normal development of the figure. The assessment takes into account the biological extremes (- , +) for its development. Points 1 and 9 represent extreme deviation feature. With an average value of the feature at least 5 points, it is written to the left with the sign -; more – to the right with +.

When appraisal is carrying out, assessment and selection of animals is followed by class, and in some cases individual selection. Appraisal of livestock is held every autumn. In order to determine the value and purpose of breeding animals on farms, artificial insemination stations, pedigree farm hold annual appraisal of bulls, cows, heifers and breeding bulls.

According to ICAR recommendations (2014) in a linear estimation of body type it is necessary to include an additional 5 exterior features: angular edges; characteristic of the movement; fatness (fat deposition); the thickness of the metatarsal bones (back and side); the thickness of the nipple.

Assessment of the quality of milk from animals is carried out under the control of members of the reference laboratory in accordance with the relevant regulations of the Russian Federation and with ICAR recommendations [2, 4] (Table. 2).

Data analysis of Table 2 shows that domestic and foreign reference methods substantially coincide for determining quality indicators of milk protein, fat and somatic cells. At the same time, when determining somatic cells in the Russian Federation it is allowed using indirect methods using drugs «Mastoprim» and viscosity measurement [9].

Modern analytical laboratory during mass analysis can be completed with equipment for the instrumental determination of milk quality [4]:

- determination of fat: devices MilkoTester (Foss Electric, DK);

- determination of fat and protein: devices MTA-PMA (Foss Electric, DK), Milkoscan (Foss Electric, DK), Multispec (Multispec, UK), Bentley (Bentley, USA), Lactoscope (Delta Instruments), Aegys (Anadis Instruments, F);

- determination of somatic cells: Coultronic (UK), Foss Electric (DK), Anadis (F), Bentley (USA), Chemunex (D), Delta Instruments (NL), Foss Electric (DK).

2. Methods of determining milk quality

Indicator	Normative documents of the RF	ICAR reference-methods (2014)
Fat	GOST R ISO 2446-2011 - Milk. Method for determination of the fat content (The Gerber Method)	Gravimetric method, Röse-Gottlieb ISO 1211 Butyrometric method, Gerber ISO 2446
Protein	GOST 23327-98 MILK and dairy products (The Kjeldahl method)	Titrimetric method, Kjeldahl ISO 8968 Dye-binding, Amido Black ISO 5542

Somatic cells	GOST R 54077-2010 - Milk. Methods for determination of the number of somatic cells by the changing of viscosity GOST R ISO 13366-1-2010 «Milk. Determination of the number of somatic cells. Part 1. Microscope method (Control method)»	Microscope Reference method ISO 13366-1 Electronic particle counter ISO 13366 Fluoro-opto-electronic method (Rotating disk) ISO 13366-3

Genetic control laboratory assesses the extent of the genetic anomalies among controlled cattle population in accordance with the relevant regulations [10, 13-15]. Features of modern animal husbandry and the widespread use of foreign breeding material created the conditions for the possibility of hereditary diseases that cause significant economic damage to domestic livestock. The most frequently reported are three types of genetic mutations: BLAD (Bovine Leucocyte Adhesion Deficiency), DUMPS (Deficiency of uridine monophosphate synthetase), CVM (Complex vertebral malformation).

The main objective of Cytogenetics of farm animals is the analysis of causes of the spread of chromosomal anomalies in the population and prevention the spread of hereditary diseases. It is planned to conduct the genomic evaluation of dairy cattle.

Discussion

The developed model of formation and management of genetic resources in highly productive dairy cattle at the regional level is designed for the possibility of applying in all regions of the Russian Federation.

The developed method of selection and organization of a regional technology center for dairy farming, taking into account the number of controlled cattle dairy efficiency, can be used in the organization of the national accounting system in dairy farming in the interaction-control assistant and expert-appraisal services, laboratory of genetic and reference control that will meet the requirements of the Russian legislation in the field of livestock breeding and show the way of harmonization with international ICAR recommendations.

Analysis of the dynamics of milk productivity of cows ICAR member countries during the period of their membership shows strong positive results. For example, when considering the figures of the former Soviet republics – Latvia, Lithuania and Estonia, the increase in various species was 24,5-58,3%. In 2001, the annual yield of the Holstein breed in these countries amounted to 4970 - 5712 kg of milk, and in 2012-2013 this figure was 7376 - 8611 kg of milk.

Registration for milk production is carried out by methods of AT (independent registration, carried out by experts of the regional center separately in the morning or evening milking) and B (registration is carried out directly in the farm, for example, in automatic mode according to the appropriate software); the cost of annual registration using AT method in Lithuania – 34 euro (2013), using B method the cost is in the range of 9-15 euro.

The principles of payment for registration have national features [4], for example, if in Argentina registration methods A4, A6 and AT are completely (100%) paid by the manufacturer, in Estonia the manufacturer pays 80% of method B registration; in Latvia registration methods A4 and B (6-7 euro) are fully paid by the manufacturer; in Lithuania the manufacturer pays 31-39% of registration practices A4 and AT (25-28 euro), the method B of registration, costing 8 euro, is paid only for 30-35% by the manufacturer.

The state applies the leverages to stimulate the development of dairy farming and the introduction of an independent registration of indicators of milk production of cows and milk quality.

According to the ideology of ICAR, quality certificates, which give the right to hold trade genetic material, are of national character and therefore cannot be issued to individual regions, areas or agricultural enterprises. At the same time, it is necessary to prove the right to sell in the face of fierce international competition and audits over three years [4].

Therefore, implementation of ICAR recommendations should be carried out centrally on the basis of national legislation to ensure the safety and quality of animal products, and especially of raw milk.

Of course, the access to the ways of implementation of a domestic genetic material in ICAR member countries would greatly increase the efficiency of livestock, and would strengthen the confidence of domestic producers to the prospects of development of the industry. In turn, this will contribute to the further development of dairy farming in the region and enhance its food security.

Conclusion

The implementation of Interbull and ICAR recommendations in the practice of domestic livestock will increase the accuracy of registration, increase individual productive qualities of animals and total milk production, and strengthen the image of Stavropol Territory as the region with high culture of animal husbandry.

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ASSURING FOOD SAFETY AND QUALITY IN EUROPE

Vytenis Andriukaitis

European Commission

Speech

Ladies and Gentlemen,

IntroductionI am very pleased to have been invited to address the International Dairy Federation's "World Dairy Summit 2015"

It is an even a greater pleasure because it takes place in Vilnius - beautiful capital of the country I know best. I hope that delegates will have the time to explore this wonderful city.

It is symbolic for me, that this event takes place in Vilnius, where famous Doctor CemachSzabad (TsemakhShabad), at the beginning of the twentieth century, created his program called Pienolašas (or Drop of milk in English), to help unprivileged women with babies with providing free milk and food.

The Juncker Commission

As you know, the current European Commission, under the leadership of President Juncker, came into office last November. Nevertheless it has hit the ground running and set out very clearly its modus operandi.

In particular,the Commission aims to be more collaborative than in the past, to be bigger and more ambitious on big things and smaller and more modest on small things – with a strong focus on better regulation, effective implementation and follow-up on the ground.

Food Safety

As you know, I am in charge of Health and Food Safety.

Therefore, I am well aware that the European dairy industry is in anadjustment phase, following the ending of the EU milk quota regime earlier this year (1 April).

So I hope it will come as welcome news that the Commission has no plans to radically alter the European Food Safety System, which has served us well since the radical overhaul which began back in 2002.

Indeed, our food safety system is a true European success story ensuring the highest standards of food safety for European citizens.

My task is to enhance the regulatory environment by modernising or simplifying wherepossibleand where appropriate making it easier for European operators and businesses to succeed, but never compromising the high safety standards.

At the same time, I want to ensure that small, traditional producers are not over-burdened so they canmake sure that Europeans continue enjoying safe, varied, quality products.

Ongoing dossiers

On my appointment, Iinheriteda number of legislative dossiers already passing through the institutional process –and in particular the animal health, plant health and food controls package.

Political agreement has already been reached- earlier this year - on a new Animal Health law, and I am confident that we can conclude remaining initiatives in the near future.

Let me just mention three items of particular interest to this audience.

Official Controls Regulation

First –a new Official Controls Regulation, whichI am very keen to see in place as soon as possible.

This will enable Member States, and the EU as a whole, to enforce more efficiently our comprehensive legal requirements for the food chain and its related sectors – both in relation to our own food operators and to imported products.

The new rules will seek to allow the EU to adopt a more strategic approach to official controls, also in relation to preventing the entry into the EU of non-compliant products from third countries.

Fitness Check of the General Food Law

Second –let me mention in parallel the ongoingFitness Check of the General Food Law Regulation – the cornerstone of the EU regulatory framework for the entire agri-food sector.

The purpose of the exercise – which is due to be completed the first half of 2016 –is to assess in detailwhether this framework is properly"fit for purpose".

Crisis management

Third, safe food is of course of paramount importance to European consumers and an essential foundation for trade.

I will give high priority tofurther improving the structures and tools for managing crisesso we can be better prepared whenfood-related crises emerge – as they inevitably will from time to time.

To start with, an external study will look into the functioning of the current crisis procedures within the fitness-check on the General Food Law. We will also evaluate the Rapid Alert System for Food and Feed (RASFF).

European Commission's Food and Veterinary Office carries regular audits in the dairy sector in Member States and in third countries, as regards hygiene norms, diseases, etc.But of course, responsibility for dealing with food crisescannot fall to the Commission alone. I have therefore called on the food industry to join us in our preparations.

Only when all stakeholders work closely and trustfully togethercanpotential crisis situations be managed and containedwith maximum speed and efficiency.

International issues

Ladies and Gentlemen,

I would like to devote the remainder of this short intervention to international matters of particular relevance to the dairy sector.

Codex Alimentarius has set a number of standards which range from the hygienic code of practice for milk and milk products to the use of dairy terms. It also applies to an array of specific dairy products.

The European Commission – and my services in particular – has always participated activelyin the development of these important Codex standards.

Lately, Codex has held discussions related to Maximum Residue Levels (MRLs) for a substance used to increase milk yields from cows –recombinant bovine somatotropin (rBST) – and, separately, the development of a standard on processed cheese.

As regards recombinant bovine somatotropin, to date, the EU has consistently opposed the adoption of international standards in Codex for growth promoting substances because of the general EU policy banning their use.

As regards to this substance a series of other considerations were at play, including outstanding concerns related to antimicrobial resistance. The latter is a very important issue on the top of my list of priorities in food area.

At its last session, the Codex Alimentarius Commission (CAC) did not reach consensus on the adoption of the Maximum Residue Levels for recombinant bovine somatotropin. It agreed, however, to keep these on hold to provide further time to facilitate a possible consensus in future.

Turning to processed cheese, a standard for this is currently under discussion. The EU position is that an international standard is not necessary- there are no particular trade issues with this commodity, which is easily traded on the international market.

TTIP

Finally, just a few wordsabout the ongoing EU-US negotiations on the Transatlantic Trade and Investment Partnership (TTIP).

The European Union has high expectations for TTIP and I personally am very ambitious concerning the aspects within my portfolio – the Sanitary and Phytosanitary (SPS) Chapter.

The negotiations offer an excellent opportunity to renew and set out a positive tone and visionfor EU-US relations in relation to SPS matters as we go forward into the future.

They are also a unique opportunity for us to promote the export of our products to the United States, as there are still many products that we cannot currently export to the US.

One of the priorities for my negotiating team is to eliminate the barriers that prevent us from exporting pasteurised dairy products to the US – the so-called "Grade A Dairy Rule".

And let me reiterate,what I have said before on many occasions. Our EU food safety standards are not up for negotiation. Not in TTIP – not in any trade negotiation.

As this is an international event, I can assure you that this partnership agreement will not be the one to weaken our other trading partners. On the contrary, it should be seen as an example on how two countries or trading blocs with different regulatory systems can work together in order to promote mutual trade.

Conclusion

Ladies and Gentlemen,

You may know that the topic of this year's World exhibition taking place in Milan, Italy, is 'Feeding the Planet'. I believe that this is an extremely topical issue for your industry, also in light of the millennium goals', as you are also the 'feeders' of this planet. I am sure that you take this role very seriously and i thank you for that.

I think it is useful to remind ourselves, that the dairy is not just a commodity that we produce as in a factory. Behind, there is an animal- living and sentient being- which we cannot reduce to a machinery. We should not lose from our field of vision the animal welfare. We should not lose the sense of humanity.

I also hope that you will enjoy the rest of this conference; and of course your stay here in Vilnius.

Thank you.

THE DINAMICS OF MILK FARMS' INCOME AND EXPENSES

Prof.Dr. Eglė Stonkutė

Baltic Institute for Research and Development, J. Savickio g. 4, Vilnius, Lithuania

egle.stonkute@balticinstitute.eu

Abstract

The paper focusses on the dairy farming income and expenses in EU with special focus on Lithuania's case treating it as the case of the dairy farming survival in the periphery of the global milk market. The discussion starts from the short overview of the dynamics in milk, milk farming input and output prices in the EU. The outlines of the EU milk farming being drawn the discussion turns to Lithuania's milk farming economics. The dynamics of milk farming economic success is revealed by the historical dynamics in safety margin (calculated using break event point analysis of FADN data for Lithuania 2007-2013) and by comparing the impact of different milk prices on the safety margin of Lithuanian milk farming through three hypothetical scenarios.

Introduction

The decreasing world milk prices are initiating discussion on emerging new range of milk prices to become permanent and even on the restructuring of the whole milk production sector itself. Milk prices in leading milk producers' countries and region, such as New Zealand, USA an EU, are decreasing and contribute to the increase in the adversity in milk market, put stress on the whole supply chain all around the world. If the biggest producers in leading countries are dominating in the milk market, small milk farms are struggling for their survival. The question rises, how they do survive or not and what is the future for these small milk farms in EU (after the abolition of milk quota) taking as an example Lithuania's milk farming.

Raw milk prices around the world

The EU milk prices for the close of higher to the USA milk prices and started to decrease rather sharply at the beginning of 2014. The decrease still continues and in the middle of the 2015 was marking somewhere the middle of price range among milk prices in USA, New Zealand and EU itself (Figure 1). The lowest milk prices are still those in New Zealand.

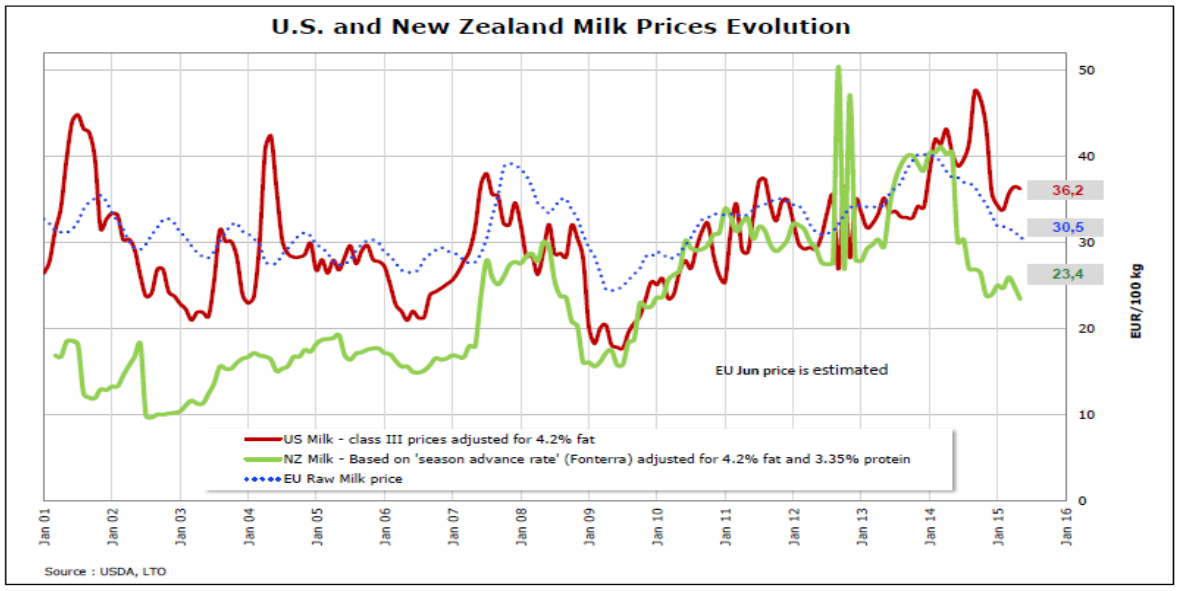


Figure 1. World milk price dynamics [1]

The average milk price in EU in May 2015 was 30.5 EUR/100 kg and that price was lower than the price a year ago (Figure 2). This year is the year where all EU countries are experiencing the decrease in milk prices; however, in some countries that decrease is rather sharper than in another. The rather sharp decrease in milk prices is observed in Belgium, Denmark, Estonia, Germany, Luxemburg, and the Netherlands. In Cyprus, Malta and Greece just minor decrease is observed. Even if it is important to analyze and detect the dynamics in each of the EU country and the potential development in the future, the focus in the paper is on the end of the range, i.e. on the lowest milk prices and farms surviving in these conditions. The lowest milk prices among EU countries are observed in Latvia and Lithuania in 2015.

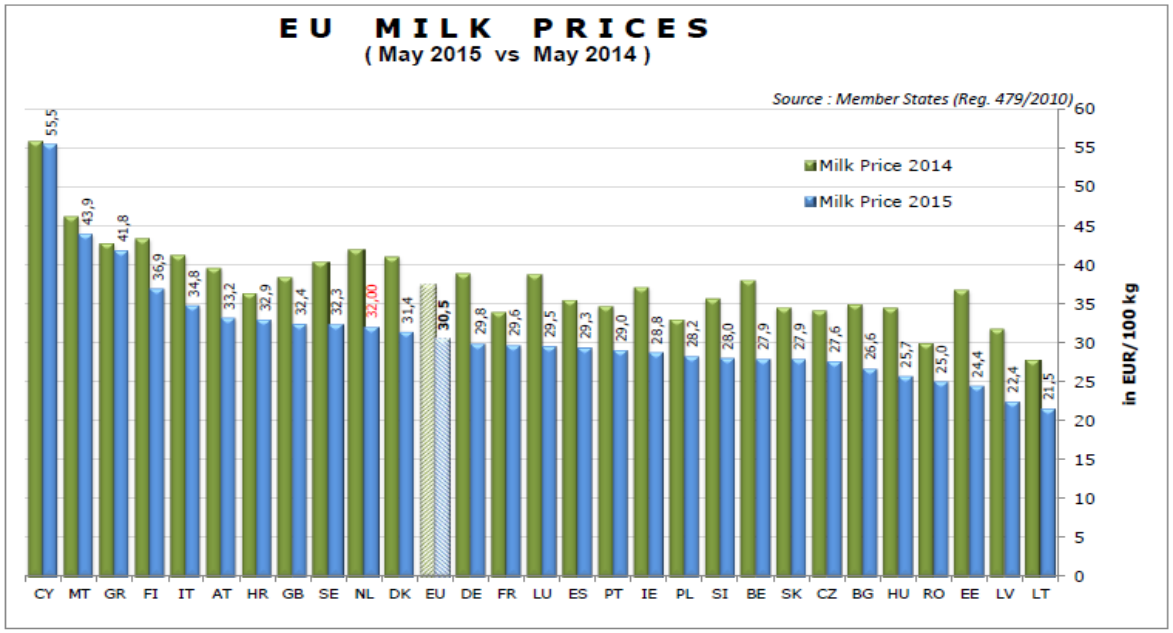


Figure 2. EU milk prices (EUR/100 kg) [2]

The biggest EU milk producers for many years were and still continue being Germany and France. These two countries produces more than a third of all EU milk (Figure 3). Among 28 EU counties just seven of them are producing more than five percent of all EU milk production. The rest are producing less than five percent and among those nine produce less than one percent of all milk production.

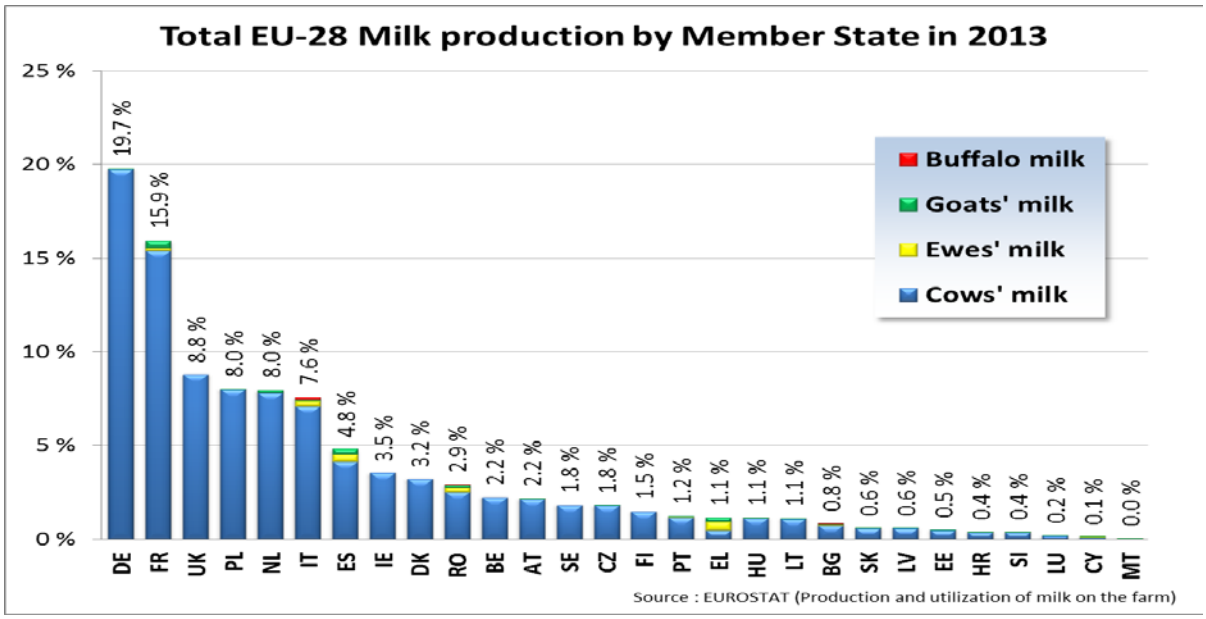
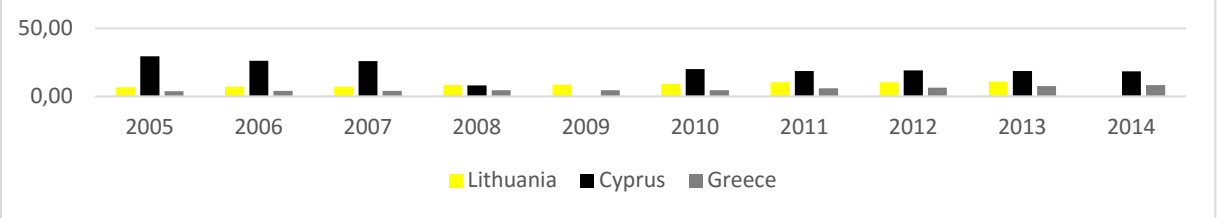


Figure 3. Milk production in Member States [3]

EU milk farming economics

The analysis of EU milk farming economics with the focus of Lithuanian milk farming economics is based on Eurostat data on agriculture in the period 2005-2014. The data analysis is organized in the manner to draw the interval of highest during the period 2005-2014 and lowest levels of a selected indicator and reveal the levels in Lithuania as the case under the focus (the dynamics in a EU country with the highest and lowest average rates in EU, and that rate in Lithuania).

The highest prices of electricity are observed in Cyprus, the lowest – in Greece and in Lithuania the prices of electricity were closer to the lowest ones in the period 2005-2009 and they had started to mark the middle range prices in EU from 2010 to 2014 (Figure 4). The prices of heating gaz in Lithuania remained close to lowest prices among EU member states (Cyprus). The highest heating gaz prices are observed in Bulgaria in 2005-2014.



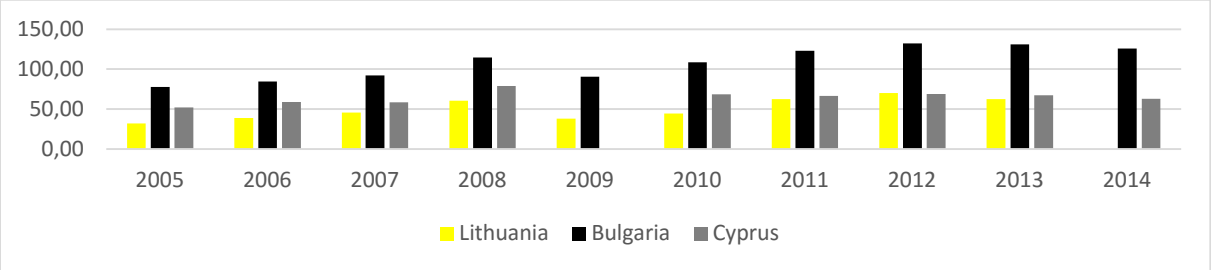
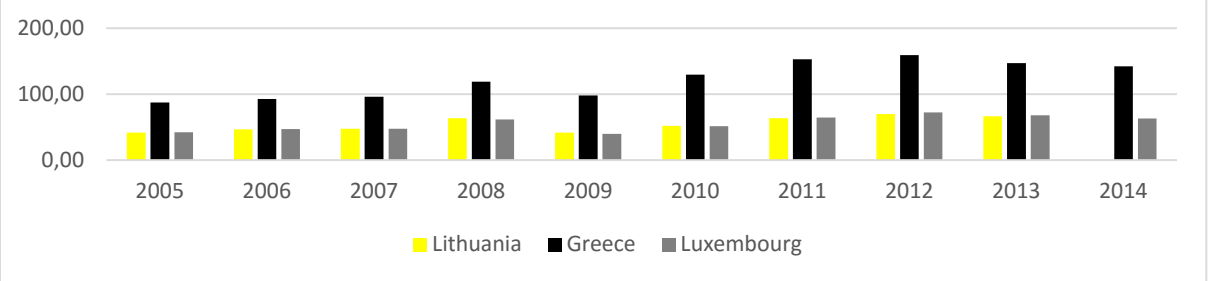


Figure 4. Input prices: electricity (EUR/100 Kw) and heating gaz oil (EUR/100 l) [4]

The highest prices of diesels oil were observed in Greece, while the lowest – in Luxemburg, and those in Lithuania – close to those the lowest ones in the period 2005-2014 (Figure 5). Fodder wheat prices as one of the input were highest in Cyprus, the lowest – in Czech Republic. The prices for that input in Lithuania were closer to the lowest prices in respective period.



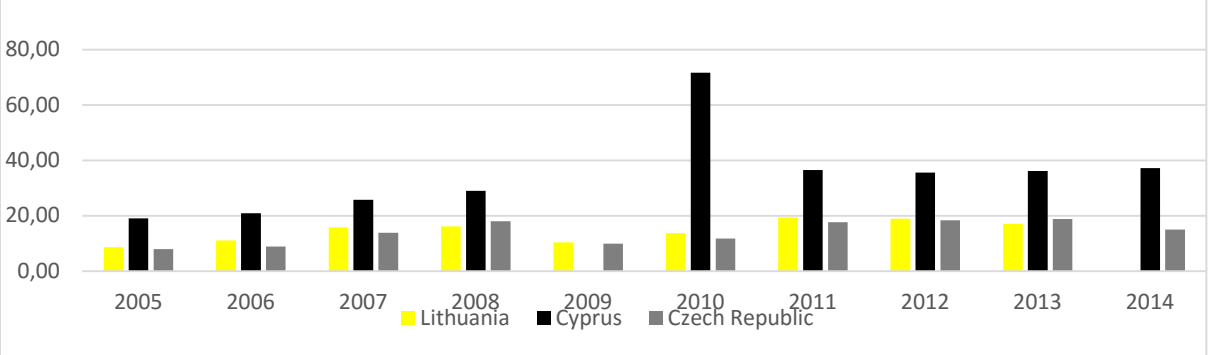


Figure 5. Input prices: diesel oil (EUR/100 l) and fodder wheat (EUR/100 kg) [5]

The prices for fodder (oats and maize) need to feed cows were rather low in Lithuania or close to the lowest among EU countries (Figure 6). The highest prices for oats observed in Cyprus, for maize – in Ireland in 2005-2014. In the same period the lowest prices for the same grains, respectively were – in Czech Republic and Slovakia.

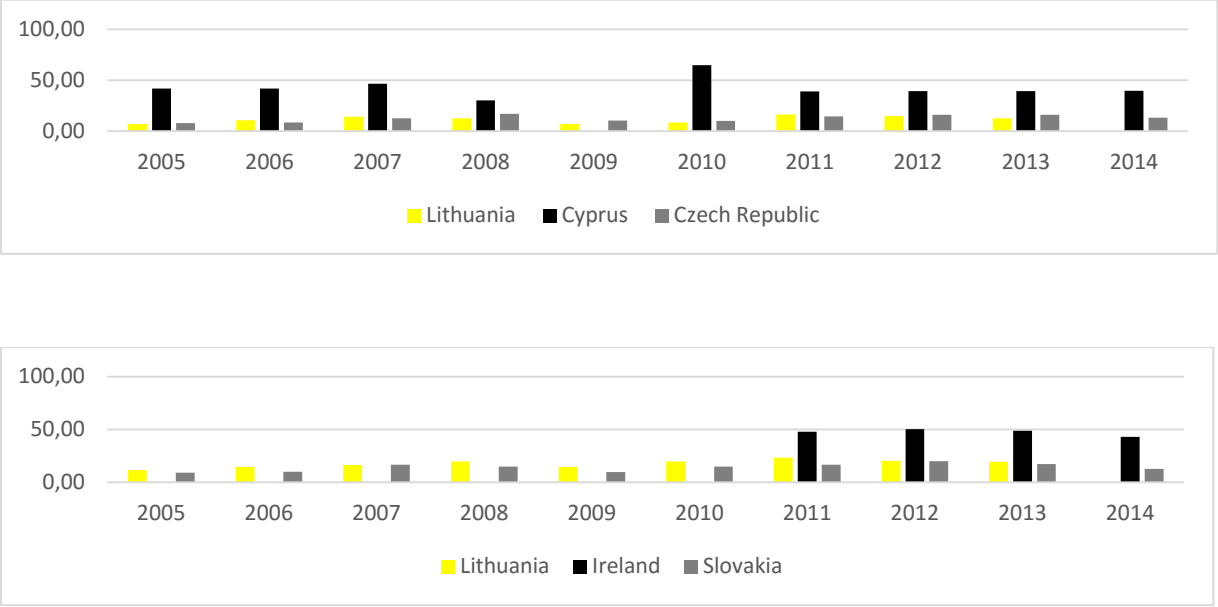


Figure 6. Input prices: oats (EUR/100 kg) and maize (EUR/100 kg) [6]

The next six indicators are compared among three EU countries: Germany and France, two biggest milk producers in the EU and Lithuania as the case in focus. The price indices (nominal) of goods and services currently consumed in agriculture were increasing and the dynamics observed in the interval from some 80 in 2005 to 120 index points in 2014, where 2010=100 index points (Figure 7). The pattern of the dynamic in index was the same in all three countries, i.e. leading countries as Germany and France and Lithuania as small and rather peripheral country.

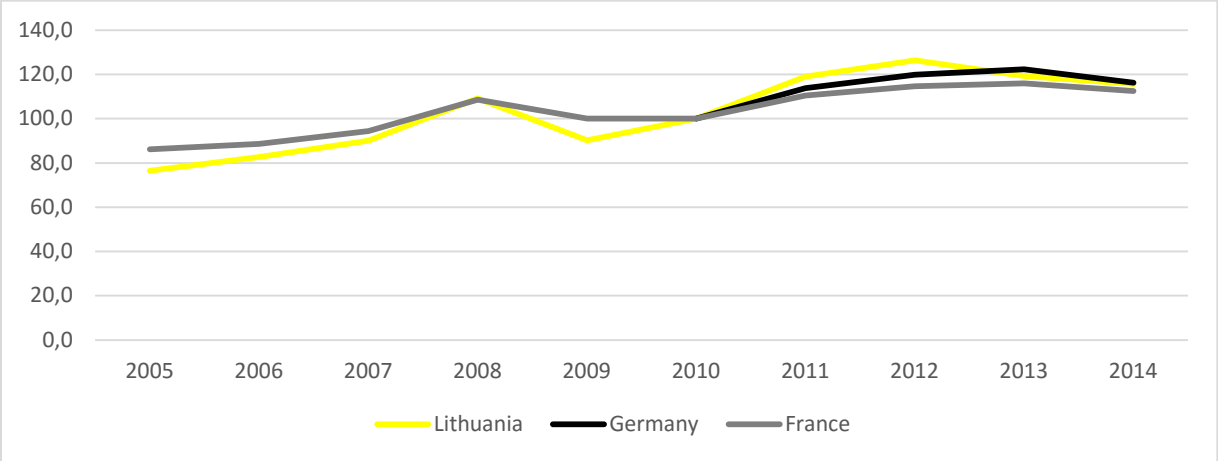


Figure 7. Input price indices (nominal; 2010=100): Goods and services currently consumed in agriculture [7]

The price indices of veterinary expenses in Lithuania were the most dynamics and unpredictable in 2005-2014 (Figure 8). The price index was falling in the period 2005-2007 and then started to increase still marking some important drops in 2011 and 2013 compared to previous year. The dynamics of the

index in Germany and France is rather limited, oriented towards moderate continuous growth in 2005-2014.

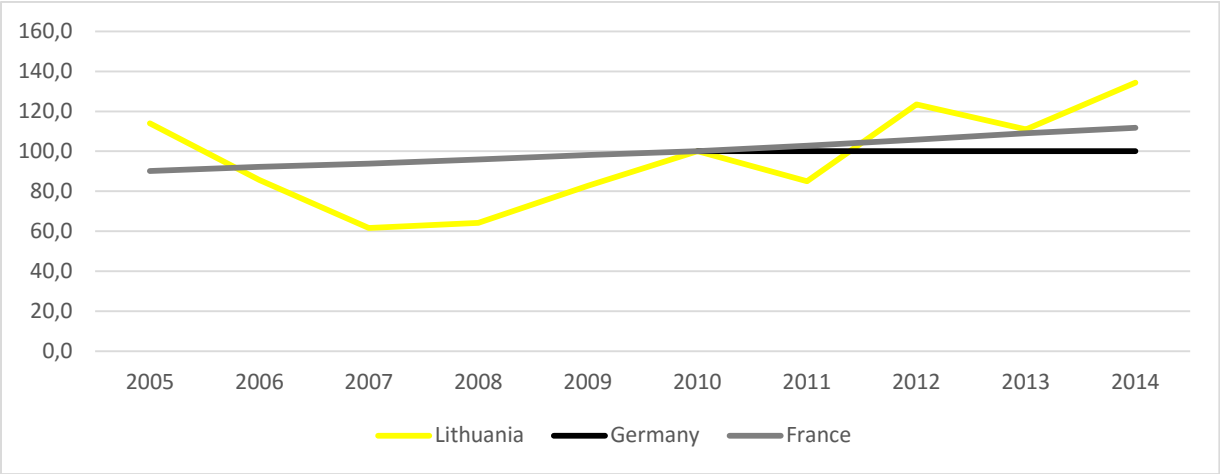


Figure 8. Input price indices (nominal; 2010=100): Veterinary expenses [8]

Animal feed stuffs price indices in Germany, France and Lithuania followed rather the same tendency and marked the increase in the index (Figure 9). Germany and France (their milk farms) were dominating countries in EU milk farming and Lithuanian milk farming sector reflected that pattern.

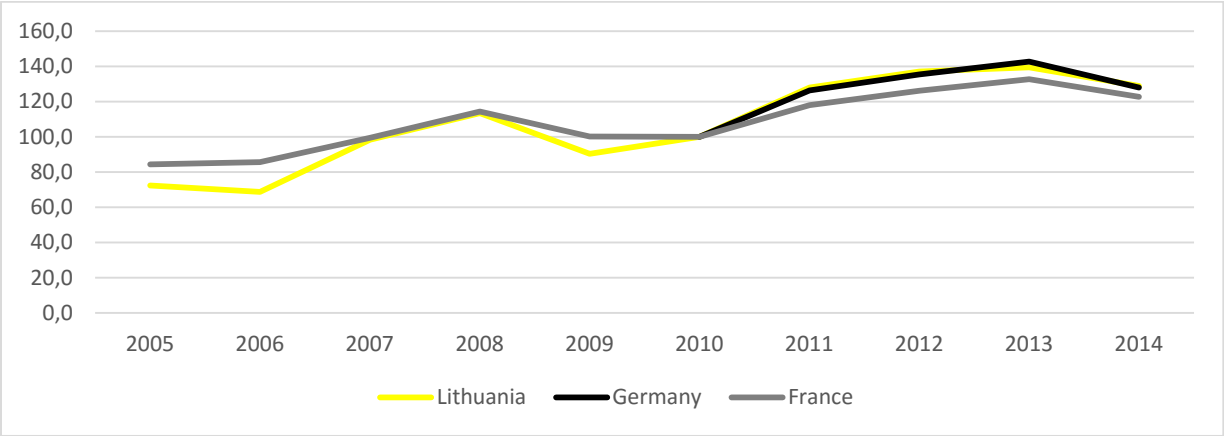


Figure 9. Input price indices (nominal; 2010=100): Animal feeding stuffs [9]

The input prices were increasing in 2005-2014. The agricultural goods output price indices were following the same pattern and were increasing in all three countries, Germany, France and Lithuania with some a deeper dip in 2009 (reflecting global economic and financial crisis; Figure 10).

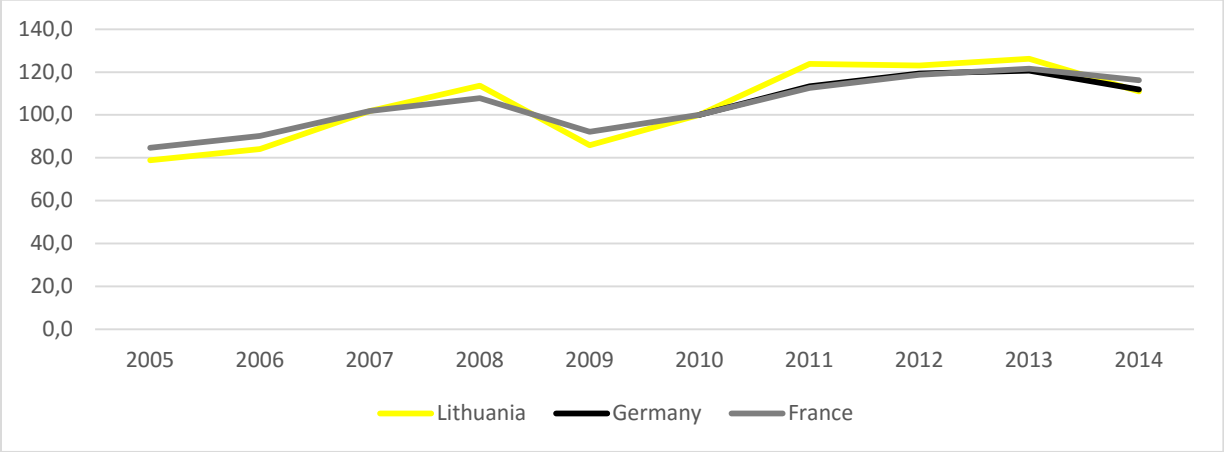


Figure 10. Output prices indices (nominal; 2010=100): Agricultural goods output [10]

If the output price indices were lower in Lithuania in the period 2005-2009 compared to the indices in Germany and France, from 2010 they were converging in all three countries (Figure 11).

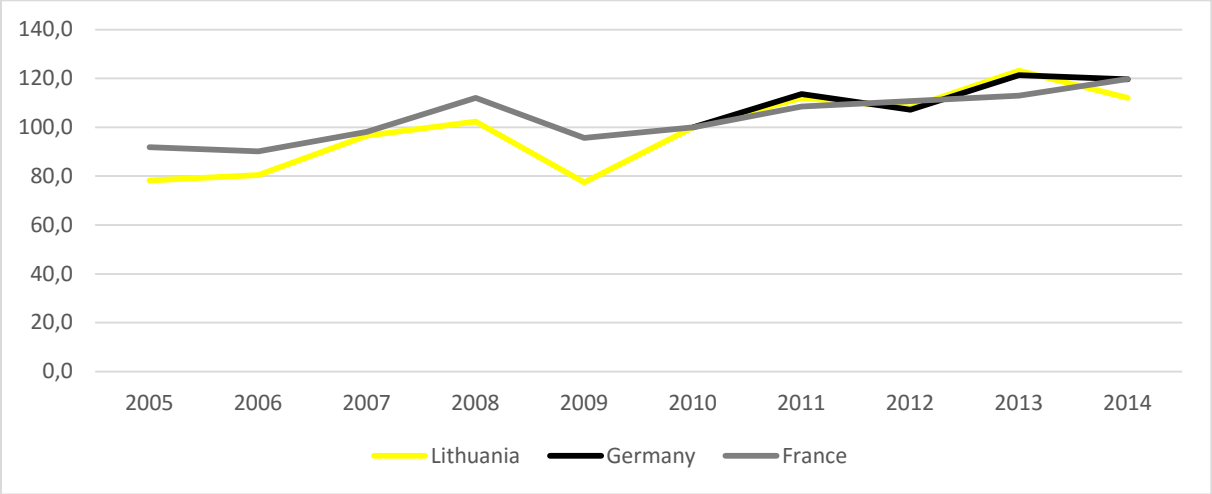


Figure 11. Output prices indices (nominal; 2010=100): Animal products [11]

The cow's milk price indices followed the same patterns as those of animal products except cow's milk price index in Lithuania in 2009 (the index experienced a sharp decrease; Figure 12). The interval of cow's milk price indices was locked in the interval of 80 and 120 in the period 2005-2014 (with the exception of the year 2009 for Lithuania).

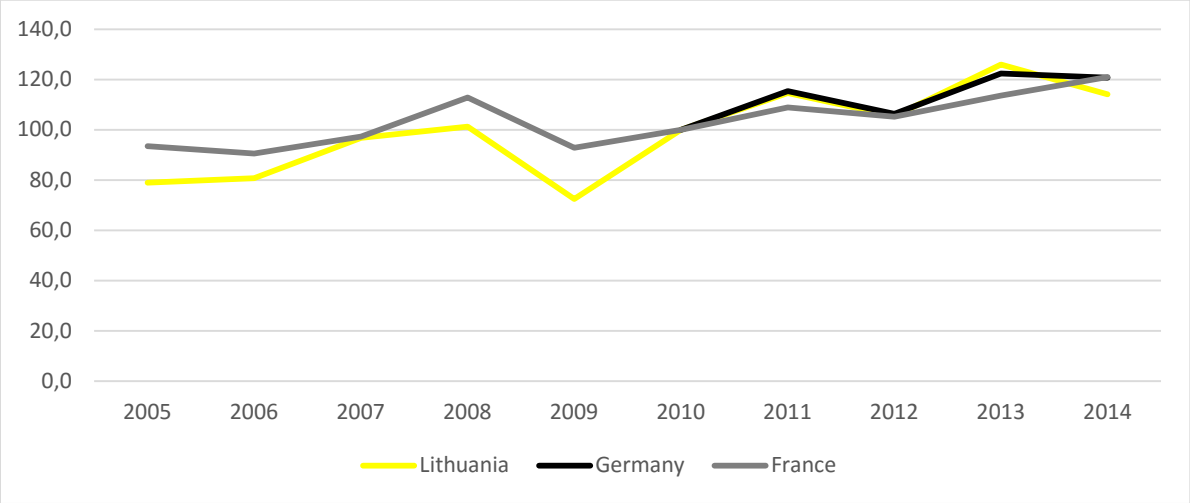


Figure 12. Output prices indices (nominal; 2010=100): Cow's milk [12]

Resuming on the prices of inputs and outputs of agricultural production and milk production especially, the following could be stated: the input and output price indices were increasing all around the EU; however, input prices in Lithuania still remained close to lowest in the period under the analysis (2005-2014).The reason of these lowest prices and lowest of milk prices in Lithuania can be the structural deficiencies of the milk farming sector in Lithuania. Milk farms in Lithuania are almost three times smaller than those in EU-29 and even five times smaller than those in EU-15 (Table 1). The milk farms in Lithuania are smaller than the average in the EU and milk yield per cow lower than in the average in EU-10. The milk farming sector in Lithuania is experiencing its own structural deficiencies that created disincentives in the local milk market.

Table 1. Main structural indicators [13]

	Dairy cows, LU	Forage area, ha	Total lobour, AWU	Milk yield, kg/cow
EU 27	29	29	1.82	6905
EU15	54	51	1.93	7337
EU10	19	24	2.28	5695
Lithuania	11	22	1.81	5482

Lithuanian milk farming economics

The analysis of Lithuanian mil farming economic is based on FADN data for Lithuania in 2003-2013 [14]. The answer to the questions on how peripheral countries' milk farmers survives in the conditions of current milk market development is developed on the basis of breakeven analysis of Lithuanian milk farming activity and especially taking into account the dynamics of safety margin of milk farming.The output of milk farming as well as total costs and contribution margin in Lithuania were increasing in 2003-2013 with some disruptions of the year 2008-2009 – the year of the world economic and financial

crisis (Table 2). Milk output development in the same period was even more modest and was never higher than that in 2005 – some very long time ago. The fixed cost doubled from 2003 to 2008 and continued to increase till 2013. That increase was linked to structural investments into milk farming implemented in the programming period of 2004-2013 and revealing its full impact with some time lag. The safety margin of milk farming in Lithuania shrank from some 42% in 2003 to some 24% in 2013 with the sharp decrease in 2008.

Table 2. Milk family farms economics in Lithuania, EUR [14]

	2003	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Output	17073	20333	28791	21314	26087	26664	21390	23378	26401	27556	29841
Milk output	8704	11344	16887	12910	15069	15369	11135	12620	14456	13846	16444
Total costs	13323	13251	18863	15694	18211	20997	20164	19761	22000	24802	25608
Variable costs	8280	8205	10679	8327	8939	10493	9721	9163	10400	11335	12388
Crop production	1047	1244	1487	1339	1331	1476	1293	1641	1900	2308	2264
Animal production	7233	6961	9193	6988	7608	9016	8428	7522	8499	9027	10124
Contribution	8793	12128	18111	12987	17149	16172	11669	14215	16001	16221	17453
Contribution ratio, %	0.52	0.60	0.63	0.61	0.66	0.61	0.55	0.61	0.61	0.59	0.58
Fixed costs	5043	5046	8183	7366	9272	10504	10443	10598	11600	13466	13220
Gross profit	3750	7082	9928	5620	7877	5668	1226	3617	4401	2755	4233
Subsidies& VAT balance	2866	4626	8778	9760	6524	7879	10790	9476	7748	9434	7761
Farm net income	6616	11708	18706	15380	14401	13547	12016	13093	12149	12189	11994
Output at BEP	9792	8460	13008	12090	14105	17320	19142	17430	19139	22877	22603
Safety margin,%	42.6	58.4	54.8	43.3	45.9	35.0	10.5	25.4	27.5	17.0	24.3

The milk farm net income were decreasing in the period 2003-2013 and it was the reason of the decreasing ratio between milk farm net income and AWU (Figure 13).

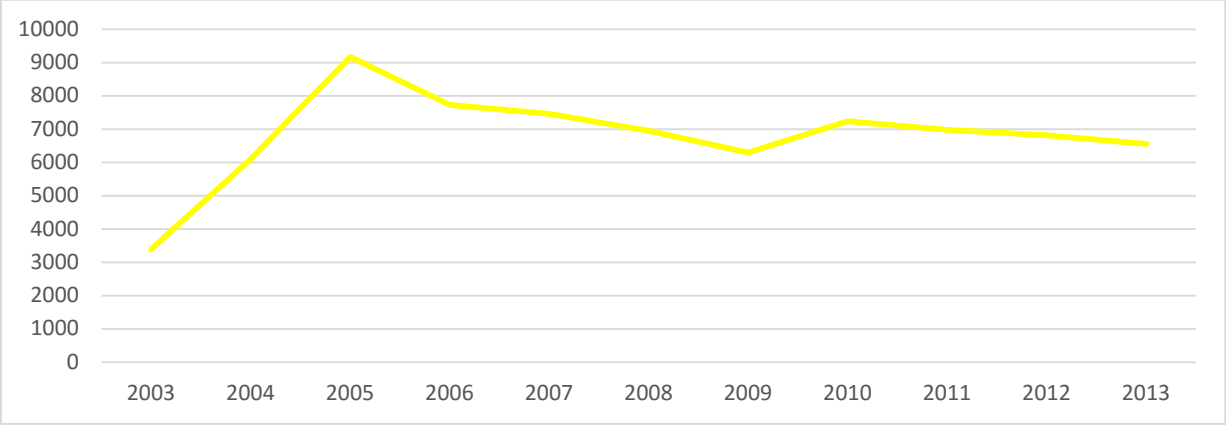


Figure 13. Milk (specialized) farm net income / AWU dynamics in Lithuania [14]

The three hypothetical scenarios were developed and compared among themselves in order to analysis the sensitivity of Lithuanian milk farms. All three scenarios were based on historical (except milk prices) milk farming income and cost structure of the year 2013 (Table 3). The three hypothetical scenarios were formulated: the first scenario comprising the output and cost structure of the year 2013 and the milk price of "New Zealand" (of the price of milk in Lithuania in May 2015; Figure 2); the second – structural data the same and the average EU milk price and; the third scenario – structural data the same and milk prices needed to sustain milk farming safety margin of 40%.

Table 3. What if… "New Zeeland's", "EU" and "status quo" scenarios compared, EUR

	2013 [14]	Scenario 1	Scenario 2	Scenario 3
Output	29841	25248	30209	34434
Milk output	16444	11851	16812	21036
Milk output, %	0.55	0.47	0.56	0.61
Total costs	25608	25608	25608	
Variable costs	12388	12388	12388	12388
Crop production	2264	2264	2264	2264
Animal production	10124	10124	10124	10124
Contribution	17453	12860	17821	22046
Contribution ratio, %	0.58	0.51	0.59	0.64
Fixed costs	13220	13220	13220	13220
Gross profit	4233	-360	4601	8826
Subsidies& VAT balance	7761	7761	7761	7761

Farm net income	11994	7401	12362	16586
Output at BEP	22603	25954	22409	20649
Safety margin,%	24.3	-2.8	25.8	40.0

The analysis revealed in the first scenario the safety margin of milk farming in Lithuania would experience negative margin, in the case of the second scenario – Lithuanian milk farms would end up with some new reality's safety margin (the safety margin statistically dominating after the 2008), and in the third scenario – milk farms would preserve their 40% safety margin in case milk prices would be 27% higher than they actually are (around 40 EUR/100 kg instead of some 21 EUR/100 kg).

Conclusions

The current milk market development raises many questions. One of them would be about the pattern of these market development. Should they be considered being permanent or temporary, sector structure changing or marginal ones? Is that new future of milk market is already here, i.e. with low milk prices and increased pressure to the small farming? The answer to the question was not analysed in depth; however, should be kept as lingering question for further milk farming analysis and forecasting. Another question that comes is on who exactly, i.e. all milk farms or just peripheral small milk farms will be those being sacrificed for the better future all milk farming sector in the light of milk market competition for the survival.However, the case of Lithuanian milk farming reveals the stubborn character of those being the smallest, structurally disadvantaged, suffering the lowest milk prices and still surviving and preserving that marginal position in the milk market.The case of Lithuanian milk farms seem to be the case to be taken into account while drawing the bottom line for the milk farming survival in the EU; however, it should not be considered as an example to follow.

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GENOMIC SELECTION IN DAIRY CATTLE – APPLICATION OF INNOVATION IN DAIRY FARMS

Kristina Morkūnienė, Lina Baltrėnaitė, Ilona Miceikienė

Lithuanian University of Health Sciences, Institute of Biology Systems and Genetics, Tilzes 18, LT - Lithuania, Kaunas

kristina.morkuniene@lsmuni.lt; lina.baltrenaite@lsmuni.lt; ilona.miceikiene@lsmuni.lt

Abstract

In a pilot study 200 dairy cows in Lithuania were selected for dairy genomic profiling including traits of productive life, somatic cell count, milk yield, fat amount, fat %, protein amount, protein %, milk proteins kappa casein, beta casein, beta lactoglobulin. The IGENITY profile calculates scores for traits using multiple DNA markers. High genomic values varying from 8 to 10 points in productive life got 23% of tested cows, in milk yield 13%, in fat kg 25.5%, in fat percent 17%, in protein kg 27%, in protein percent 41.5%. Low genomic values varying from 1 to 3 which are desirable in somatic cell count has 13% of tested animals. 40% of tested cows had AB genotype of milk protein kappa casein, 2.1% BB and 1% BE. 5.3% of cows had AB genotype of beta casein. 53.1% had AB genotype and 29.7 % of BB genotype of beta lactoglobulin.

Key words. Genomic selection, cattle, milk proteins.

Introduction

The use of genomic information in genetic evaluation has brought about revolutionary change in dairy cattle selection. In genomic selection, breeding values are estimated based on thousands of molecular markers, instead of own performance and family information. Using molecular markers, accurate breeding values can be obtained for animals of both sexes early in life, which can be used to shorten the generation interval for dairy cattle by omitting the progeny test. [1] Thus, selection decisions in dairy cattle breeding can now be made on young animals with higher accuracy than that of a parent average breeding value. This has substantial implications for the design of breeding schemes, because rather than waiting until a bull has daughters with phenotypic records, a process that typically takes 5–6 years, young bulls with no progeny can be used as sires. The development of high-throughput genotyping methods and reduced genotyping cost has made the application of genomic selection feasible. [2,3] Genomic selection could contribute to higher genetic gain without increased inbreeding, because of increased accuracy of early breeding values, which makes it possible to increase genetic gain without increasing selection intensity. [4,5]

The aim of study

A pilot study was conducted to evaluate genomic breeding value of black and white cattle bred in Lithuania by genomic profiling.

Methods

In the study blood samples from 200 dairy cows were collected. DNA was extracted by chloroform salt method. [6] Genotyping was performed by Igenity SNP panel identifying the genetic potencial for dairy

cattle traits - productive life, somatic cell count, milk yield, fat amount, fat %, protein amount, protein %, cheese important milk proteins - kappa casein, beta casein, beta lactoglobulin. The IGENITY dairy cattle profile calculates scores for traits using multiple DNA markers. The largest score idicates the best genomic value for productive life, milk yield, fat amount, fat %, protein amount, protein %, the lowest score idicates the best genomic value for somatic cell count. Combined results provide more complete picture of an animal's production potential. Genotypes were rated in Igenity dairy cattle reference group. [7]

Results and discussion

Genomic selection offers many advantages with regard to improving the rate of genetic gain in dairy cattle breeding programs. The most important factors that contribute to faster genetic gain include: a greater accuracy of predicted genetic merit for young animals; a shorter generation interval because of heavier use of young, genetically superior males and females; an increased intensity of selection because breeders can use genomic testing to screen a larger group of potentially elite animals.

By increasing the accuracy and intensity of selection and shortening the generation interval, the rate of genetic progress for economically important dairy traits can be approximately doubled.

The IGENITY profile calculates scores for milk yield, fat amount, fat percent, protein amount and protein percent using multiple DNA markers. These markers identify genetic variations that help regulate milk yield, protein and fat content, without decreasing fertility. The combined results provide a more complete picture of an animal's production potential. SCS is a profit driver for many producers as well as an indicator of potential for mastitis. Because IGENITY profiles can be used at any age, the IGENITY analysis for Somatic Cell Score (SCS) can be used to identify calves and heifers with potential for high SCS and susceptibility to mastitis before they enter the parlour. An animal which scores a 10 for SCS has the potential for higher Somatic Cell Scores and may be more susceptible to mastitis than an animal with a 1. The Productive Life (PL) analysis in the IGENITY profile uses multiple DNA markers to predict an animal's actual genetic potential for PL and it works at any age. [7]

1 Table. Summarized results of black and white dairy cattle genomic profile scores.

Genomic scores	Percent of animals which have certain genomic score acording to separate trait						
	Productive Life	Somatic Cell Score	Milk kg	Fat kg	Fat %	Protein kg	Protein %
10	2.5*	1.5	0	8.0*	6.0*	2.0*	3.5*
9	4.5	3.0	3.0*	3.5	5.5	7.0	14.0
8	16.0	9.0	10.0	14.0	5.5	18.0	24.0
7	22.5	17.0	22.0	17.5	16.5	17.0	30.5
6	24.0	18.0	27.0	19.5	18.5	22.0	22.5
5	17.0	16.0	17.5	14.5	10.5	14.0	5.0
4	8.5	19.0	14.5	10.5	19.5	12.5	0.5

3	4.5	9.0	4.5	9.0	10.0	3.0	0
2	0.5	3.0	1.5	3.5	5.0	4.0	0
1	0	4.0*	0	0	3.0	0.5	0

* indicates percent of animals, that have the best genomic score according fixed trait.

High genomic values varying from 8 to 10 points in productive life got 23% of tested cows, in milk yield 13%, in fat kg 25.5%, in fat percent 17%, in protein kg 27%, in protein percent 41.5%. Low genomic values varying from 1 to 3 which are desirable in somatic cell count has 13% of tested animals. 35% of all tested cattle had middle genomic values 5 or 6 points. (Table 1)

Average genomic values of tested dairy animals were higher than average genomic values of Igenity reference animal group especially for fat kg and fat % aproximately 1 genomic point, for milk kg was nearly same, for protein kg less than in Igenity reference animal group. Somatic Cell Score genomic value was better in our tested dairy animals than in reference animal group. (Table 2)

2 Table. Comparison of average genomic values of dairy cattle in Lithuania with Igenity reference group animals

Traits	Average genomic values of tested dairy animals	Average genomic values of Igenity reference animal group
Fat kg	6,19	5,02
Fat %	5,80	4,96
Milk kg	6,01	5,93
Productive Life	6,26	5,99
Protein kg	6,18	6,36
Protein %	7,26	6,67
Somatic Cell Score	5,38	5,55

Milk manufactoring properties important for cheese and curd yield and quality a lot depends on milk proteins variants that are inherited for individual cows. BB genotype of Kappa casein, Beta casein and Beta lactoglobulins preferred for cheese production, AB and BE genotypes intermediates for cheese production, AA, AE and EE genotypes least favourable for cheese production.

Kappa casein is a key protein in the cheese making process, as the Kappa casein variant of the milk influences the renneting time and the curd firmness. The cheese production properties of milk are the better, the shorter the renneting time is and the firmer the curd gets. The genotype of a cow determines the Kappa casein variant she produces in her milk. The percentage of Kappa casein in the milk does not only differ between breeds, but also between individual animals, depending on their genotype. There are several forms of Kappa casein – A, B and E – that are associated with milk protein and quality. The BB genotype appears to be the most favorable for cheese production. Studies have shown that the cheese yield can be improved substantially with BB milk compared to AA milk and also the renneting time is shorter. Studies have also shown that Cheddar cheese yield can be up

to 8% higher and mozzarella up to 12% higher with BB milk versus AA milk. The E variant has an adverse effect on cheese production. [8,9,10,11,12,13,14] We found that 40% of tested cows had AB genotype of kappa casein and 2.1% BB genotype of kappa casein in Lithuanian black and white cattle. (Table 3)

Like Kappa casein, there are several different forms of beta casein (A and B). Higher milk yield is associated with the A variant while higher protein and casein yields are associated with the B variant. Beta casein B is similar in effect to Kappa casein B. We found that 5.3% of cows had AB genotype of beta casein and did not found cows with BB genotype in Lithuanian black and white cattle. [15,16] (Table 3)

Beta lactoglobulin has a significant effect on casein number and cheese yield. The B variant has higher casein and cheese yields. The cheese yield does not only depend on the whole protein content of the milk, but on the casein content, which is measured by the casein number. Therefore it is also necessary to mention the Beta lactoglobulin genotypes which are determining the casein number of the milk. The casein number of the milk indicates the percentage of casein of the whole protein fraction and is the other key factor for cheese making. We found that 53.1% AB genotype of beta lactoglobulin and 29.7 % of BB genotype in Lithuanian black and white cattle. [17,18, 19] (Table 3)

3 Table. Proportion in percents of cheese important milk protein variants in dairy cattle in Lithuania

Kappa casein genotypes %						Beta casein genotypes %			Betalactoglobulin genotypes %		
AA	AB*	BB*	BE*	AE	EE	AA	AB*	BB*	AA	AB*	BB*
51.6	40.0	2.1	1.0	4.3	1.0	94.7	5.3	0	17.2	53.1	29.7

* indicates percent of animals which has genotype important to cheese makers.

Genomic selection is attractive for dairy cattle breeding, because it relaxes the need to have phenotypic measurements of close relatives of all selection candidates.Thereby it decreases generation intervals and increases genetic gain per year for all breeding goal traits. Introduction Genomic selection is revolutionising breeding programs worldwide. This new selection tool is particularly beneficial for dairy cattle breeding programs because it allows to significantly reduce generation intervals and cheaply increase selection intensity, and the accuracy of selection is only marginally lower compared with progeny testing schemes. genomic selection use a reference population to estimate effects for genome-wide single nucleotide polymorphism (SNP) that are used subsequently to predict breeding values for selection candidates. [3,5]

Conclutions

Average genomic value of dairy cattle bred in Lithuania not differ from reference group cattle which reflects genomic values of animals kept in different environments.

In Lithuania there is dairy cattle with large genomic scores for separate traits suitable for elite animal selection.

Cheese important AB genotype and B allele of milk proteins - kappa casein, beta casein, beta lactoglobulin – was found in Lithunian dairy cattle what indicates possibility for special selection of animals for cheese and curd making.

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RISK ASSESMENT IN A DAIRY FARM FOR THE DEVELOPMENT OF AN INTEGRATED SYSTEM OF SENSORS FOR COW MILK QUALITY MONITORING

A. Lombardo1, S. Amatiste1, C. Boselli1, D. Patriarca1, I. Paladini1, A. Piazza1, G. Biancalani1, C. Frazzoli2, R. Dragone3, A. De Rossi4, A. Mantovani2, G. Brajon1*

[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri". Via Appia Nuova 1411, Rome (IT); [2]Istituto Superiore di Sanità; Viale Regina Elena 299, Rome (IT); [3]Consiglio Nazionale delle Ricerche; Piazzale Aldo Moro 9, Rome (IT); [4]Cooperativa Lattepiù; Via Fontanile di Mezzaluna 401, Rome (IT);

*giovanni.brajon@izslt.it

Abstract

A preliminary risk assessment was performed in a dairy farm, within a project on a patented platform of sensors (BEST) for monitoring the milk production chain. The assessment aimed at identifying the Points Of Particular Attention (POPAs) and the parameters to be checked by BEST. The evaluation encompassed a characterization of the farm as an environment and as an animal rearing unit; six POPAs were identified and monitored: 1) well water (total bacterial count, coliforms, metals and pesticides residues), 2) feed and silage (pesticides, metals and mycotoxins residues), 3) individual faecal samples (parasitological and bacteriological analysis), 4) individual milk (mastitis bacteria, somatic cell count, milkability), 5) bulk milk (total bacterial count, somatic cell count, fat, protein and lactose content, aflatoxin M1, antimicrobial residues). The study showed no unchecked risks; monitoring three POPAs (well water, individual and bulk milk) by BEST will support the maintenance of a good farm management.

Intro and Aim

A preliminary risk assessment was performed in a dairy farm (41°54'47.94"N, 12°15'48'25"E), within the ALERT project [1,4] on the development of a patented platform of biosensors and sensors (BEST) for monitoring the cow milk production chain. The assessment aimed at identifying and evaluating the Points Of Particular Attention (POPAs) and the analytical parameters to be checked by BEST.

Methods

1) Farm characterization by the use of check lists: agricultural, fertilizing and weeding practices; general farming conditions; animal nutrition; antimicrobial usage; animal health and welfare; milking hygiene [2].

2) POPAs identification and monitoring through laboratory analysis [3] (Jan - Dec 2013), as described in Table 1.

Table 1. Analysis perfomed at 6 the identified POPAs.

POPAs	Analysis performed
Water quality (beverage and cleaning)	Total bacterial count, Coliforms, E.coli, metals and pesticides residues
Feed and silage quality	Pesticides, metals and mycotoxins residues
Gastrointestinal pathogens	Giardia duodenalis, Cryptosporidium spp. Salmonella spp., Campylobacter spp.
Udder health	Mastitis bacteria, Somatic Cell Count
Milkability and good milking practice	Flow-curves analysis
Bulk milk quality	Total Bacterial Count, Somatic Cell Count, Fat, Protein and Lactose content, Aflatoxin M_1 antimicrobials residues

Results

The analysis showed a good farm management without unchecked risks for milk safety, animal health and welfare:

Metals, pesticides and mycotoxins residues in feed and well water below the respective legal thresholds;

Good microbiological standards of well water (table 2);

Zoonotic agents not detected from faecal samples;

Low prevalence of mastitis bacteria (Graph. 1);

Good milkability profile and milking procedures (table 3);

Good bulk milk quality (graphs. 2 and 3), with no antimicrobials and Aflatoxin M1 residues.

Table 2. Water quality parameters, mean values (cleaning and beverages water)

	Cleaning water	Beverage water*
Faecal coliforms	0 MPN/100mL	0 MPN/100mL
Total coliforms	0 MPN/100mL	1 MPN/100mL
E. coli	0 MPN/100mL	0 MPN/100mL

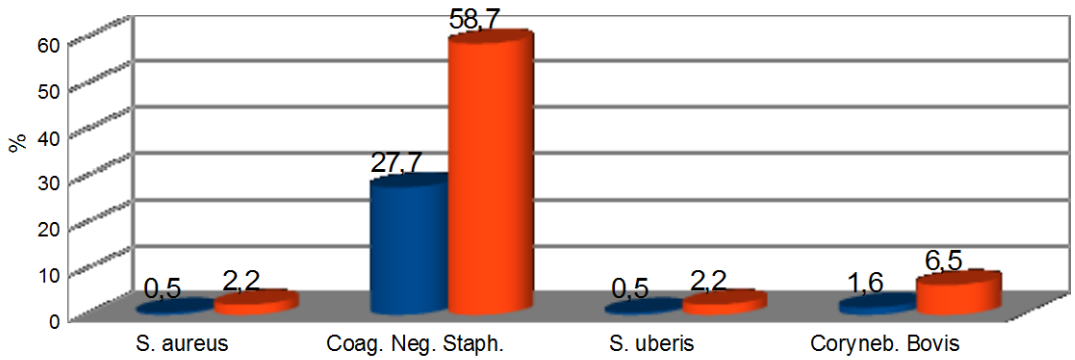
Total bacterial count (22°C)	<1 CFU/mL	23 CFU/mL
Total bacterial count (37°C)	<1 CFU/mL	<1 CFU/mL
Faecal streptococci	0 MPN/100mL	1 MPN/100mL

*Water collected from drinking throughs.

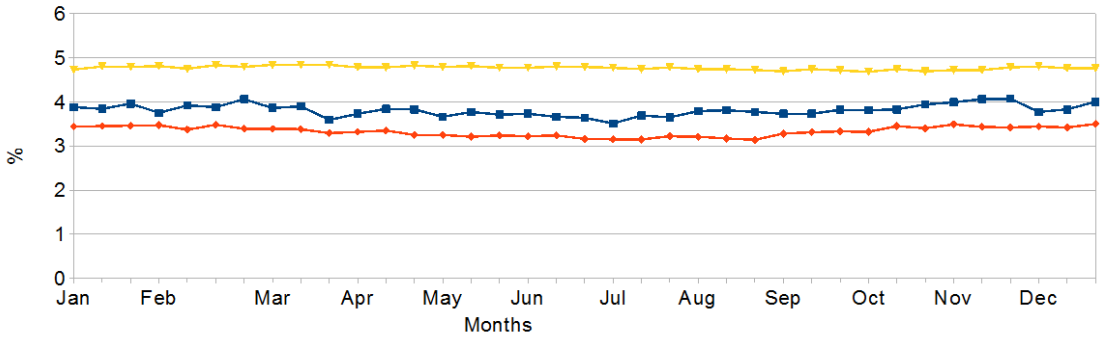
Table 3. LactoCorder analysis (mean values)

Total milking time	6,67±2,08
Ascending time	0,27±0,25
Blind milking	0,23±0,25
Bumodal curves frequency	10,00
Milk yeld/cow (kg)	12,78±5,24
Maximum flow	3,78±1,19
Average flow	2,34±0,87
Plateau/decreasing phase ratio	47,07
Electrical Conductivity	5,88±1,55

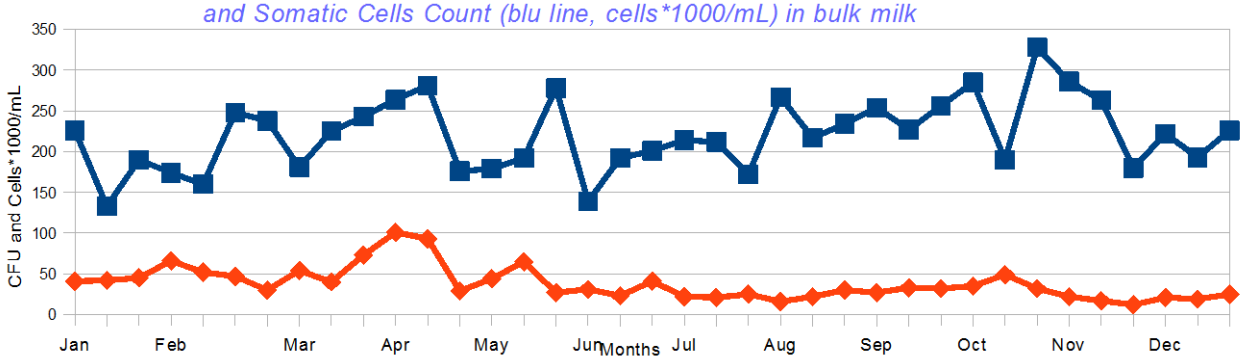
Graph. 1. Mastitis bacteria prevalences
(blu, teat samples prevalence - red, herd prevalence)



Graph. 2. Fat (blu line), Protein (red line) and Lactose (yellow line) percentages in bulk milk



*Graph. 3. Trend of Total bacterial count (red line, CFU*1000/mL) and Somatic Cells Count (blu line, cells*1000/mL) in bulk milk*



Disscussion and Conclusions

The BEST bioelectronic platform is designed as an open flexible technology, capable of updating through the integration of new probes and biomarkers. The ALERT project aims for the innovation of HACCP and self-monitoring plans, through the development of the BEST technology on-farm. This preliminary risk assessment suggests that monitoring three POPAs:

1) well water

2) individual milk

3) bulk milk

by the on-line use of BEST will support the maintenance of a good farm management and food safety by the time- and cost-effective identification of unwanted and/or unexpected.

Ackowledgements

The authors acknowledge the Consortium of the project ALERT “Integrated system of bio-sensors and sensors (BEST) for the monitoring of wholesomeness and quality, as well as for traceability in the cow milk chain” funded by the Italian Ministry of economical development under the Call Industria 2015 “Newtechnologies for Made in Italy”

“Elio Pascolini” dairy farm.

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THE INFLUENCE OF FARM SIZE ON COW'S MILK QUALITY

Ramutė Mišeikienė, SauliusTušas

Lithuanian University of Health Sciences, J. Tacas Centre of Milking Technologies, Tilžės 18, LT-47181Kaunas, Lithuania.

ramute.miseikiene@lsmuni.lt, saulius.tusas@lsmuni.lt

Abstract

The aim of this study was to analyze and compare parameters of milk quality in different-sized cows herds. Milk quality of ten dairy cows farms during one year period was analyzed. Dairy farms were divided into five groups according to number of cows in the farm (under 50 cows, 50–100 cows, 100–200 cows, 200–400 cows and more than 400 cows). Reliable linkbetweenfarmsizeandnumber ofSC in milk has not been established ($P>0.05$). However, the count of SC was significantly higher ($P<0.05$) during outdoor period in large farms (200-400 and more cows). There were no significant difference between bacteria count in milk during both – outdoor and indoor – periods ($P>0.05$). Established small number of bacteria in the milk and the maximum freezing point depression when the farms get bigger.

Keywords: cow, milk, quality, farm size.

Introduction

High somatic cell counts (SCC) present in milk are one of the main indicators of mammary gland infection caused by microorganisms. Regarding bacterial contamination, the practices and conditions under which small-scale farms and their dairy chains operate make it difficult to produce high quality milk.Increased SCC in the milk should not be underestimated as they are followed by the multidimensional negative effect related to animal health and farmer profitability [1]. High bacterial loadsnot only affect the raw milk quality but definitely pose a safety issue to consumer. The introduction of proper training and hygiene practices during milking or post-milking process to the dairy farmers was found to be efficient in reducing the bacterial load or contamination of the raw milk [2]. Season trends influence the somatic cells count [4, 5].

The aim of this study was to analyze and compare parameters of milk quality in different-sized cows herds.

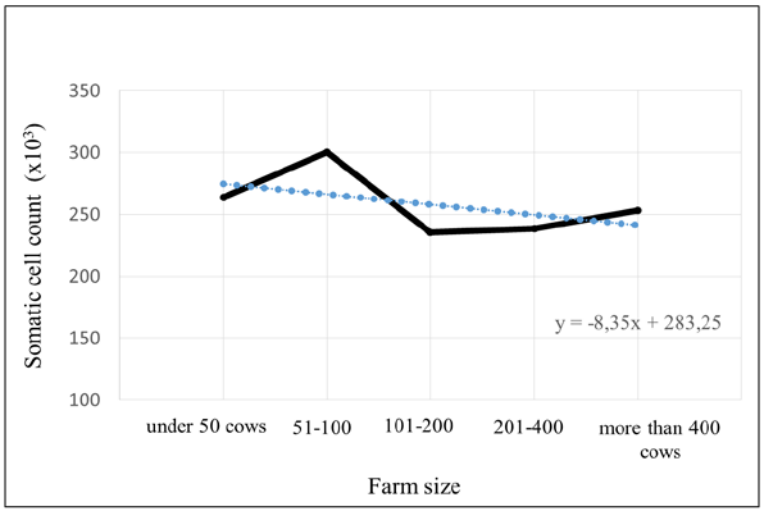
Materials and Methods

Milk quality of ten dairy cows farms during one year period was analyzed. Dairy farms were divided into five groups according to number of cows in the farm (under 50 cows (I group), 51–100 cows (II group), 101–200 cows (III group), 201–400 cows (IV group) and more than 400 cows (V group). The averages of somatic cells (SC), bacteria count in milk and milk freezing temperature were analyzed. Also these parameters of milk quality were compared during outdoor (from May to September) and indoor (from October to April) periods. Data of milk quality was taken from the SE “Pienotyrimai” database. Statistical analysis was performed with Descriptive Statistics and Independent-Samples T test procedures in SPSS 13.0 for Windows.

Results and discussion

Somatic cell count (SCC) and bacteria count are the main indicators of milk quality. Somatic cell count is usually used to detect udderinfection, especially the subclinical mastitis.The total bacteria count shows how the milk washarvested, manipulated and stored before reaching the milk processing plant [2]. Diagrame 1 showssomatic cell count in a different sized farms. The largest number of SCC was established in the smallest farms, i.e., in farms under 50 cows and 51-100 cows (respectively 264±9,19 and 300±10,24 thousand/ml).Reliable link between the smallest and largest dairy farms and farms with 101-200 and 201-400 cows and count of SC in milk has not been established (P>0.05).

Bacteriacount in milk is a reliable indicator of milk hygiene, showing a sick udder, non-hygienic manipulation (i.e., unfavorable temperature during storage, storage device, etc.) [3]. Diagrame 2 shows the microbial loud of milk in 10 analyzed dairy farms. Bacteria count had a low tendency to decrease when the number of cows on farms increased. The largest number of bacteria was determined in the farms of II group (51-100 cows). Milk produced on a small scale dairy farms can easily get contaminated by bacteria due to poor hygienic conditions maintained at “on farm” levels or due to inadequate handling, storage and transport conditions [1, 2].



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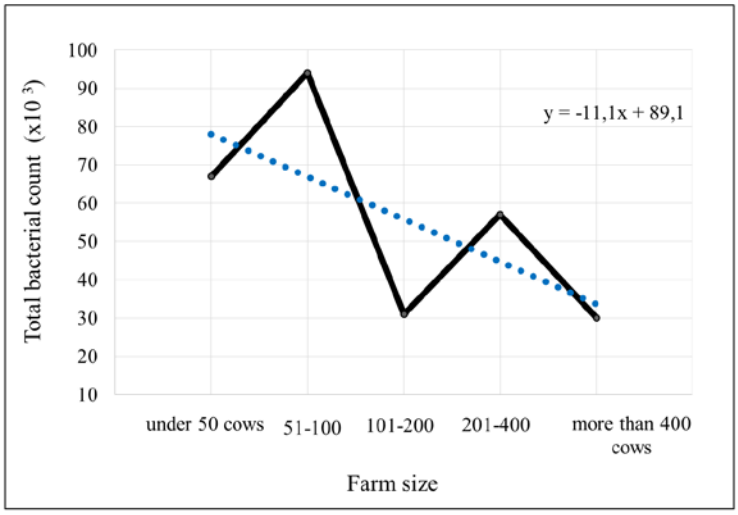
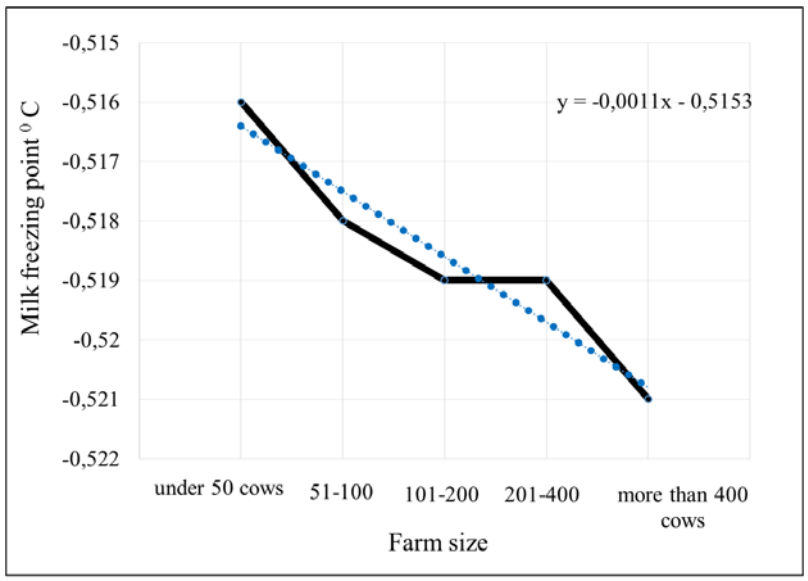
Diagrame1. Somatic cell count in milk (×10 /ml) of different sized dairy farms



Diagrame 2. Bacteria count in milk (×10 3 /ml) of different sized dairy farms

Milk bacterial contamination was 67± 5,91 thousand/ml and respectively the lowest plate count was in milk when 201-400 and more than 401 cows were considered. In the largest dairy farms bacterial contamination significantly lower than in small farms (I and II groups) (P<0.05).Statistically unreliable link established between bacteria count in milk of I and II, I and IV, III and V groups of cows.

The freezing point of milk is in important indicator of the milk quality. The freezing point of milk depends upon the concentration of water-soluble components. Freezing point of milk as a regulatory standard is really only valid for milk pooled form many cows (bulktank milk). Many factors may affect freezing pointof milk from individual cows. Were analyzed the biggest milk freezing temperature fixed in different size farms. The results are presented in Diagrame 3. With increasing the number of cows milk maximal freezing temperature decreases (significant negative trend), i. e, indicator is improving. It should be noted that in all farms milk freezing point neverexceeded requirements (-0.515 °C). The highest maximal temperature recorded in the smallest farms (-0.516 °C), while the highest - in the largest farms (-0.521 °C).



Diagrame 3. Milk freezing point in different sized dairy farms

The biggest difference between SCC in milk during the indoor and outdoor periods was established in farms with 201-400 cows (respectively 218.49 thousand/ml and 268.84 thousand/ml). However, the count of SC was significantly higher ($P<0.05$) during outdoor period in large farms (201-400 and more cows). There were no significant difference between bacteria count in milk during both – outdoor and indoor – periods ($P>0.05$). L.T. Czister et al. (2012) the highest somatic cell count and coliform bacteria count obtained during the spring and the lowest total bacteria count was obtained in winter season. There was a significant ($P<0.05$) interaction between year and season of production for all raw milk traits. The lowest freezing point in the winter and the highest density in the autumn [4].

Conclusions

The current results showed theunreliable link between farm size and the number of somatic cells in milk. Averages of SCC in milk of all intervals sized dairy farms complied with the requirements of EU Directive 854/2004 (SCC <400 thousand /ml). Microbiological quality of raw milk obtained from all farms not exceeded 100 thousand/ml. Established small number of bacteria in the milk and the maximum freezing point depression when the farms get bigger. Surveyed farms bacteria count in the milk of the season did not belong ($p <0.05$), and somatic cell count pasture period it was higher in large (200-400 and more than 400 cows) farms.

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LACTOFERRIN PRODUCTION FROM BOVINE MILK OR CHEESE WHEY

Leo De Valck[1] and Dr Jean-Paul Perraudin[2,*]

[1] Mirakal, 2 avenue Léon Champagne, 1480 Tubize, Belgium, [2] Taradon Laboratory, 4 Allée de la Recherche, 1070 Bruxelles, Belgium

leo.de.valck@mirakal.be, jp.perraudin@taradon-laboratory.com

Abstract

Today, the industrial scale production of lactoferrin is carried out in one step by extraction from bovine milk or whey. As the role of lactoferrin in the milk is to protect the liquid against the bacterial contamination binding the lipopolysaccharides (LPS) of those bacteria, it is not surprising that the lactoferrin extracted from milk is covered by bacterial LPS, losing the most part of its biological activities. It is absolutely crucial that the production of Lactoferrin consists to a two steps process. The first step consists of extracting from milk or from whey a solution that we called lactenin which contains different molecules including lactoferrin, lactoperoxidase, angiogenin and some other minor components. The second step consists of purifying the lactoferrin from the other components including the LPS. Only under such conditions, we could recuperate a high level of a pure molecule with all its biological activities as it is not done actually.

Introduction

Lactoferrin (Lf) is a single chain, iron-binding glycoprotein of the transferrin family that is expressed and secreted by glandular cells and found in the secondary granules of neutrophils from which it is released in infected tissues and blood during the inflammatory process.
Initially described as an iron-binding molecule with bacteriostatic properties, Lf is now known to be a multifunctional or multi-tasting protein. It is a major component of the innate immune system of mammals. Its protective effects range from direct anti-microbial activities, against a large panel of microorganisms including bacteria, viruses, fungi and parasites, to anti-inflammatory and anti-cancer activities. While iron chelation is central to some of the biological functions of Lf, other activities involve interactions of Lf with molecular and cellular components of both hosts and pathogens. Combined with *in vitro* and *in vivo* data, the powerful antimicrobial activities, immunomodulatory properties and prevention of septic shock, anti-carcinogenic functions and its growing importance in iron delivery and bone growth, make the Lf a very promising and fascinating molecule for health applications.
Since the first industrial production of lactoferrin and lactoperoxydase built in 1985 by the company Oléofina (Belgium) in collaboration with Dr Prieels and Dr Perraudin, we have noted that the number of research programs devote to the identification of the biological activities of the Lf have particularly increased. In fact, as it progressed with the number of industrial production units was the manufacturers and other businessmen appealed to the researchers of Universities to perform researches either by through a thesis, or by through a post-doctoral and that's how several researchers became interested in working on the Lf. That is how since 25 years, more and more scientific papers have been published, believing that each scientist wished to make a contribution about this subject. In 1993, the first International Lf Conference was created and under the control of the scientists from universities, the Congress takes place every two years, showing the synthesis of the scientific works which have been performed during the last two years.
However, in 1985, having little information about the biological properties of the Lf, it was very difficult to characterize the molecule by specifications. At that time, to establish the first specifications, Dr Prieels, Dr Perraudin (from Oléofina) and Dr Tomita (from Morinaga Milk Industry) had to limit the parameters of the specifications to the purity and to the iron content between 14.5 µg to 30 µg iron per

100 mg Lf. The parameter of purity should have to be superior to 95% in the worry to do not introduce other contaminating molecules and the parameter of iron content was based on the bacteriostatic activity of the molecule as it was described by Bullen in 1975 [1]. So, less iron contained by the Lf, more it had to be active. The other parameters were classic for such product as the protein content, ash content and moisture content and of course the bacteriological level in presence with the molecule in powder form. Although, the results of other experiments had been published in the scientific literature as the synergy between lactoferrin and lysozyme [2], the first results about the crystallography [3] and on the identification of the amino acids sequence [4], nothing allowed us to find the link to identify the molecule manufactured industrially versus the molecule produced in the laboratory by different academic institutes. Beyond, since 1985 and following all results obtained by the research programs, it is not surprising to note that there is a scientific gap which widened during all these years between the industrials and the scientists, not because the interests that they bring to the Lf but on the definition that they combine to the Lf. As we can observe in the figure 1, can we think today that the specifications defined 25 years ago correspond to the biological properties of the Lf, which have been highlighted during all these years? If the manufacturers have taken advantages of the results of the research to promote their Lf, it is surprising that they have not tried to assess one or more biological properties of their product in order to define their own specifications (figure 1).

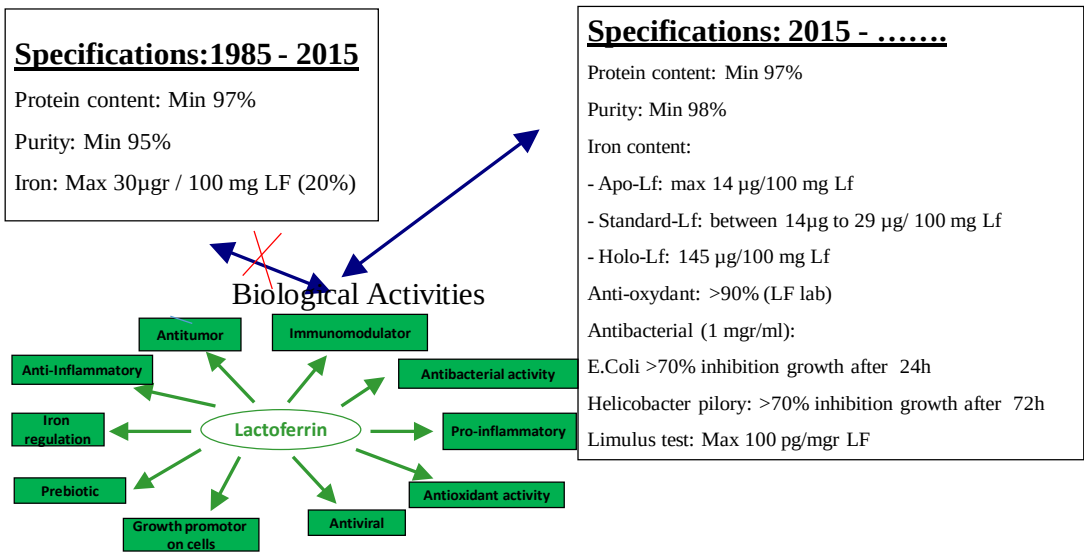

Figure 1: Relation between the biological activites of Lf and the specifications exiting actually by the manufacturers of Lf (1985 – 2009) and the specifications proposed (2010 - 2015….)

In fact, today there are several points of disagreements between the quality of the Lf produced in industry and the Lf purified and studied in the laboratory.

Industrial productions:

Taking into account that the Lf concentration in milk (+/- 150-200 mg/liter) or in whey (+/- 50-75 mgr /liter), there are mainly the milk cooperatives who present all the advantages for the industrial production of Lf from their raw material. So commercial lactoferrins are usually produced from milk or from whey by one ion-exchange chromatography, especially cation-exchange chromatography follow

by a tangential-flow membrane filtration as it is described by Tomita and his collaborators in 2002 [5] and in the figure 2 (red part).

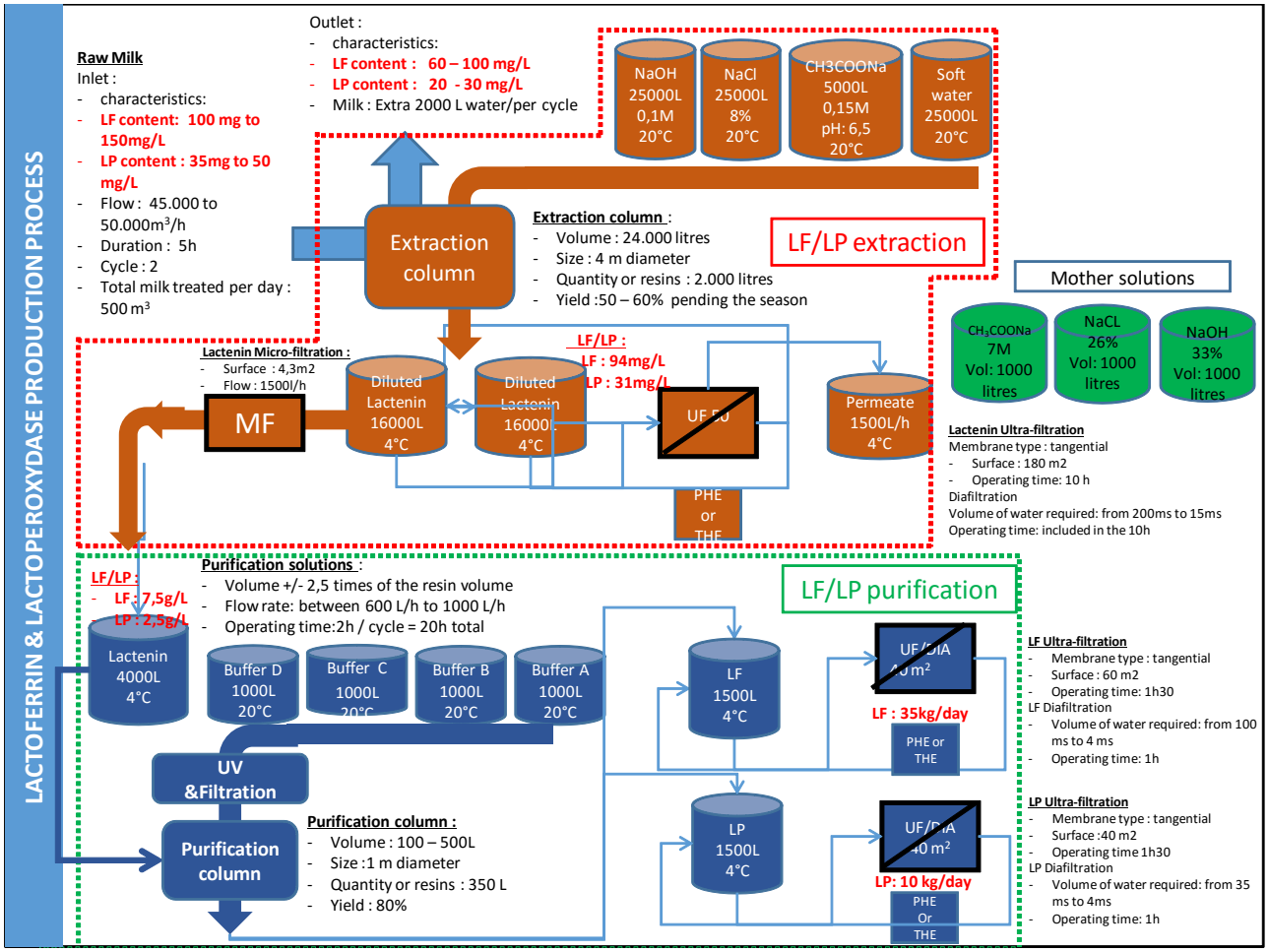

Figure 2: Scheme of Lf production process. In blue – extraction process from milk or from whey. In red – purification process of the protein mixture extracted

Since you pass the milk or the whey continuously through a chromatography column containing a cationic ion exchange resin, a mixture of milk basic proteins contained in the raw material, will bind on the resin by ion exchange effects. It is certain that the composition of this mixture will depend on the active part of the chromatographic support. Nevertheless, the part which interests all the milk manufacturers is the Lf. So as a certain quantity is bound on the resin, we will tempt to eliminate all the other components to obtain a Lf with a purity level close to 95%, then we will eliminate the salts and we will dry it.

To obtain a good quality of the molecule, the manufacturers have supposed that the Lf considered as the most basic molecule, has an isoelectric pH sufficiently different to the other basic molecules contained in milk which makes the production of Lf easier but the manufacturers did not taken care of the presence of other molecules which ,even in small concentrations, could contaminate the Lf. So only the measure of the activity of one of the biological properties could give us a general idea of the quality of the molecule if we compare the value of one of the biological activities with the value obtained by the Lf produced in the laboratory. Moreover, another parameter has been highlighted concerning the biological activity of the molecule and which consists of the interaction with the bacterial lipopolysaccharides (LPS) and whose we have to take into account for the quality and the

purity of the molecule. It is the main reason to have the extraction process followed by a purification process (blue part of the figure 2).
Finally, one of the very important parameters is also the thermal treatment that we have to take into consideration to dry the molecule which can completely perturb the biological activities of the molecule knowing that the temperature is a sensitive parameter of the biological molecules.
Although all treatments of milk necessitate, in priority, a good quality of the raw material, it is proved to be particularly true concerning the production of Lf. What we did not know, 25 years ago, became obviousness today. It is very difficult to consider that the production of Lf directly from milk or from whey, by the one step process performed by all manufacturers, is sufficient to claim a quality of product to which has to be associated all the biological activities. Details of lactoferrin purification using cation-exchange chromatography are given by several authors as Plate in 2006 [6], and van Veen in 2002 [7] but none of these processes, nor any other existing process for commercial-scale purification of lactoferrin, are able to effectively remove other minor components, to remove the bacterial lipopolysaccharides (LPS) and to treat the molecule en function of its physico-chemical characteristics affecting its stability and its activity.

Materials and Methods

Preparation of bLF-Laboratory (LF-Lab)
Lf was purified from fresh bovine milk by cation-exchange chromatography as previous reported by Mazurier & Spik in 1980 [8] and by Spik and her collaborators in 1982 [9)]. Homogeneity of the protein was checked by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The Lf was desalted on a PD10 G-25 column equilibrated in 0.01M NaCl. All buffers were prepared with pyrogen-free water. The LPS contamination of this Lf was 12pg of endotoxin/mg protein.

Preparation of bLf-NFQ
Lf was prepared from fresh bovine milk by an industrial cation-exchange chromatography (see the process in red color in the figure 2). The milk basic protein bound to the resin has been eluted with a concentrated NaCl solution. This solution containing the milk basic protein has been concentrated and desalted using an ultrafiltration membrane and has been injected in another ion exchange resin equilibrated in a buffer. For the purification, we applied stepwise buffers containing different conductivity at different pH (see the process in blue color of the figure 2). As it is described in the figure 3, we eluted:

- some minor components with the first buffer,
- the lactoperoxidase with the second buffer,
- the LPS, protease and angiogenin with the third buffer,
- the lactoferrin with the fourth buffer.

The water which has been used to prepare all the buffers, has been distillated and treated by microfiltration, ozone (O_3) and by UV 254nm. This water was pyrogen-free. The LPS contamination of this bLf was 39pg of endotoxin/mg protein. This innovative technology compatible with large-scale manufacturing practices has been developed and patented by the Taradon Laboratory company (Belgium).

Lactoferrin samples
All lactoferrin samples tested, represent different commercial lactoferrin sold on the market and which have identified by their LPS content. Lf extracted from milk: 12,000 pg endotoxin/mg protein, Lf extracted from whey: 30,000 pg endotoxin/mg protein,
Lf-A: 1650 pg endotoxin/mg protein, Lf-B: 22,000 pg endotoxin/mg protein, Lf-C: 105,000 pg endotoxin/mg protein

Endotoxin assay

Endotoxin levels were determined by *Limulus* Amebocyte Lysate Kit (QCL-1000, Biowhittaker, Walkersville, MD)

Cell culture
Human colon carcinoma Caco-2 cells were grown as semiconfluent monolayer in Dulbecco's modified Eagle's medium supplemented with 1.2 gr of $NaHCO_3$/litre, 2 mmol glutamine/litre, 100 U penicillin/ml, 0.1 mg of streptomycin/ml, and 20% heat inactivated fetal calf serum in a 5% CO_2 incubator at 37°C. Twelve hours before infection, monolayer was washed with PBS without Ca^{2+} and Mg^{2+} and then cultured in fresh media without fetal calf serum to avoid the presence of transferrin during infection.

Infection of host cells with E.coli HB101(pRI203)
The method has been described by Berlutti in 2006 [10]. Semiconfluent Caco-2 cell monolayer was been infected at multiplicity of infection 100 bacteria per cell with E.coli HB101(pRI203) either in the absence or presence of LPS free-Lf or in the presence of Lf containing different level of LPS (100 µg protein/ml). After 4 h incubation, cells were extensively washed with PBS, without Ca^{2+} and Mg^{2+}. After washing, fresh medium, containing 100µg of gentamicin/ml, was added to monolayers infected with *E. coli* HB101(pRI203) to kill extracellular bacteria, and cells were incubated for a further 2h at 37°C and washed extensively. Then the monolayers were treated with 0.3 ml trypsin-EDTA mixture (0.05% trypsin (1/250) and 0.02% EDTA) for 5 min at 37°C and lysed by the addition of 0.5 ml of 1% deoxycholic acid. Cell lysates were diluted in PBS without Ca^{2+} and Mg^{2+} and plated on agar with ampicillin (100 µg/ml) to quantify the number of viable intracellular *E.coli* HB101(pRI203).

Detection of IL-6, IL-8 and tumor necrosis factor alpha (TNF-α) in Caco-2 supernatants by ELISA
As described by Berlutti in 2006 [10], Semiconfluent Caco-2 cell monolayer was infected as described here above, either in the absence or presence of LPS free-Lf or in the presence of Lf containing different level of LPS (100 µg protein/ml). After 4h of incubation, cells were extensively washed in PBS, monolayers were added with fresh medium containing 100µg of gentamicin/ml, and cells were incubated for a further 24h at 37°C. At the end, surpernatants were collected for each cells, and the concentration of IL-6, IL-8 and TNF-α were determined using standard ELISA Quantikine kits (R&D Systems, Wiesbaden, Germany) and HBT kits (Holland Biotechnology BV, Firma Bierman, Bad Nauheim, Germany).

Antioxydant activity
The method has been described by Benzie & Strain 1999 [11]. That consists studying at low pH, the reduction of a ferric tripyridyltriazine (Fe^{3+}-TPZ) complex to the ferrous form ($Fe2^{+}$), which has an intense blue color, and which can be monitored by measuring the change in absorption at 593 nm. The Ferric Reducing/Antioxidant Power Assay (FRAP) reagent has been prepared by mixing 40ml of 0.3 acetate buffer (pH 3.6), 4 ml of 20 mM ferric chloride and 4 ml of 10mM TPTZ (2,4,6-tripyridyl-s-triazine). Different dilutions (0.1 to 10 mM) of 6-OH-2,5,7,8-tetramethyl chroman-2-carboxylic acid (CAS 53188-07-1) were used as FRAP standards. All the reagents have been brought to 37°C prior to the assay. The test has been performed in a 96-well microplate by mixing 20µl of distillated water, 10µl of Lf sample, and 150µl of FRAP reagent. In combination studies 10µl of distillated water and 20µl of Lf samples were mixed with 150 µl of FRAP reagent. After instant incubation at 37°C for 5 min for ascorbic acid and for a time lapse of 5 min to 24h for Lf samples, the absorbance of reaction mixtures was measured at 593 nm. Test compounds were given antioxidant (FRAP) values compared to the FRAP value of ascorbic acid.

Antibacterial activity
Strains :

- *Pseudomonas aeruginosa mucoïd and non mucoïd* isolated from Cystic Fibrosis patients obtained with the kindness of Bacteriology Laboratory - EA 3186 - Faculty of Besançon.

- *Burkholderia cepacia* isolated from Cystic Fibrosis patients *(strain LMG 16656 : ATCC BAA-245 from* Belgian Co-ordinated Collections of Micro-organisms)
- *Staphylococcus aureus* Resistant to methicillin and oxacillin *(strain LMG 15975 : ATCC 43300 from* Belgian Co-ordinated Collections of Micro-organisms)
- *Staphylococcus aureus strain SHY97-4320* was kindly provided by the CER (Centre d'Economie Rurale – Belgium).

All culture media were from Difco Laboratories (Detroit MI) bacteria from frozen stock were streaked onto tryptic soy or brain infusion agar plates and then incubated for 20h at 37°C.
Another method which consists of turbidometric assay has been used to measure the microbial growth. The ability of Lf samples to inhibit microbial pathogens can be measured by micro-scale optical density. 0.1 ml of broth was added to each well followed by inoculation with 0.05 ml microbial cell suspension in order to reach an optical density between 1.0 and 1.5. After inoculation, the plates were incubated at 37°C and the microbial inhibition was monitored at different times as turbidity changes in the medium by measuring optical density at 600 nm using a microplate reader.

HPLC Method

Reverse phase:

Resin: Bio-Rad Hi-pore RP-318, 250mm x 4,6mm
Solution A: Measure 50 ml of acetonitrile in a 500 ml flask and adjust the volume up to 500 ml with 0.5 NaCl water solution. Remove 0.5 ml of the solution and add 0.5 ml of trifluoroacetic acid.
Solution B: Measure 250 ml of acetonitrile in a 500 ml flask and adjust the volume up to 500 ml with 0.5 NaCl water solution. Remove 0.5 ml of the solution and add 0.5 ml of trifluoroacetic acid.
Inject 25µl of a filtered sample-solution (2mg Lf/ml).
Apply a gradient starting with A:50% and B:50% and finishing to A: 0% and B:100% with a flow rate of 2ml/min.
The purity of the lactoferrin is calculated by the percentage lactoferrin versus protein content in the powder.

Ion Exchange chromatography

Resin: Mono S (Sulfopropyl: $CH_2SO_3^-$) on a FPLC equipment from Pharmacia
Prepare a stock solution of cytochrome C (2 mgr/ml) in a 240 mM sodium acetate buffer in order to eliminate the error due to the weight and due the injections
Prepare Lf solution (10 mg/ml), diluting the powder in the cytochrome C solution.
Prepare the resin Mono S with the 240 mM sodium acetate buffer
Inject 200µl of the Lf solution
Perform the run with a NaCl gradient solution from 0.0M to 1.5M with a flow rate of 2 ml/min

Results

Lf-Lab has been produced in the laboratory and has been identified by the following parameters: LPS content, antioxidant activity, antibacterial activity, and anti-inflammatory activity. We have compared Lf-Lab with Lf-NFQ manufactured in industrial scale and other commercial Lf that we have called Lf-A, Lf-B, Lf-C, and with Lf extracted from milk and Lf extracted from whey. We have refrained from using all the commercial Lf as references dues to the diversity of their activity. In fact, some of the commercial Lf has been used to demonstrate that their production and the criteria of production are not optimal to characterize the biological functions of the Lf. All commercial Lf have different values for one of its activities and even from the same producer, the different lots of Lf manufactured have different values for the same activity.

Purity.

During the industrial process, the Lf is extracted from milk or whey in presence of other Milk Basic Proteins (MBP) such as lactoperoxidase, some immunoglobulins and other components of which the concentration is dependent of the specificity of the cationic ion exchange resin. It is an easy process that consists to extract and to purify the Lf. In fact, we have the advantage that the most part of proteins and enzymes contained in the MBP are colored. The elution of the different components bound on the resin will be performed using different solutions. Using such procedures, the industrial producers consider that a purity between 90 to 92% correspond to a Lf enough pure to be used for the different applications.
However, none of these processes, nor any other existing process for commercial-scale production of lactoferrin, are able to remove totally the minor components that affect the stability and activity of the lactoferrin.
It appears that enzymes part of the minor components are present in currently existing commercial lactoferrin preparation. These enzymes are co-purified during lactoferrin purification from milk or whey. It has been found (diagram of Lf purification described in the figure 3) that existing commercial Lf contains components responsible for protein degradation, decreasing the activity and the stability of the Lf in solution (figure 4a and 4b).

Figure 4a
Analysis at time 0:
- Markers
-LF: samples
- 1,2,3,4 and 5 from commercial Lf

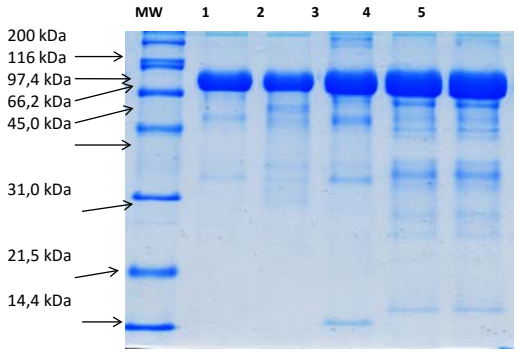

Figure 4b
Analysis after 1 month at 25°C
- Markers
- LF: samples
-1,2,3,4 and 5 from commercial Lf

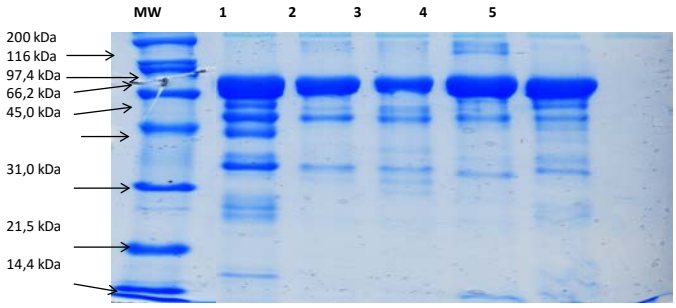

Figure 4: Electrophoresis gel representing the proteolytic degradation during the time of different commercial Lf. 4a: On the left side, there the standard markers. On the right side, from lane 1 to 5, there are the electrophoresis gels of 5 commercial samples at the time 0. 4b: Electrophoresis gels of the same samples stocked at 25°C during 1 month.

Thinking about protein degradation, we have thought to the presence of proteases and we have tested several enzyme inhibitors. Only two of them, the aspartyl protease inhibitor (10µM of Pepstatin A) and the serine protease inhibitor (1mM of AEBSF) have shown an inhibition of the degradation bands at 4°C, at room temperature and at 37°C.
Regarding the minor components, we have also found that the angiogenin can be purified during the purification of the Lf (figure 3).

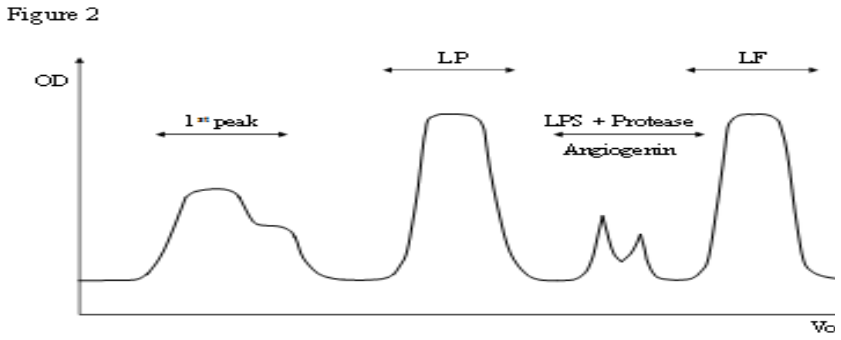

Figure 3: Chromatographic Profile of the purification process of the Lf after its extraction from milk or whey

This molecule has a molecular weight of 15 kDa and an isoelectric pH of 9.2 very close to the Lf. This molecule is responsible to the creation of the blood vessel to feed the cancer cells, neo-vascularization indispensable to the growth of tumors and to the development of the metastasis. During the purification of the Lf, this molecule has been concentrated at least 4 times what is certainly not beneficial for the health of the consumers.
Therefore, there is a great need for new purification and stabilization methods of lactoferrin preparations in order to remove minor components, the protein degradation and the LPS to enhance, the activity on bacterial growth and to preserve the protein stability, for a longer period of time.

Thermal treatment
Actually, it has been established by the producers of commercial Lf that the purity of Lf is determined by Reverse Phase HPLC using an acetonitril gradient. Analysing the purity of some commercial Lf, we observe that the proteic components represent around 8 to 9% versus the Lf peak. Nevertheless, diluting the same amount of commercial Lf, adjusted by the ash and moisture content, we have not found the same optical density at 280 nm. That means that some proteins eluting as Lf can increase the optical density. On the other hand, when we analyze the purity of the Lf by ion-exchange chromatography (Mono S resin - Sulfopropyl), we can notice that the peak of Lf is composed of two parts: peak A and peak B, very closed each other and corresponding for the peak A to the presence of one sialic acid content which give to the molecule a less basic behavior compared to the native one which does not contain sialic acid (figure 5). Anyway, we can consider that the peak A and peak B are parts of the pure Lf. We can also notice in a prominent position the presence of the peak C (shoulder), which is eluted after the Lf peak. The presence of this peak cannot be detected with the use of the Reverse Phase chromatography. To understand the presence of this peak C, we have carried out the complete absorbance spectra from 280 nm to 800 nm and we have observed a band of Soret at 410 nm (figure 5) which is independent of the iron content in the Lf because this band of Soret should have to be present at a wavelength closed to the 465 nm. Moreover, the absorbance of this peak C at 280 nm is almost double to the Lf one.
Collecting only the peaks A and B, and applying again on the Mono S resin, we can notice that only the peaks A and B are present in the chromatogram without to be contaminated by the peak C. On the other hand, if we submitted the solution containing the peaks A and B to a temperature of 72°C during 5 minutes and that we analyze this solution on the Mono S resin, we observe an important decrease of

the surface of the peak A and of the surface of the peak B compared to the original chromatogram but also an appearance of the peak C (figure 5).

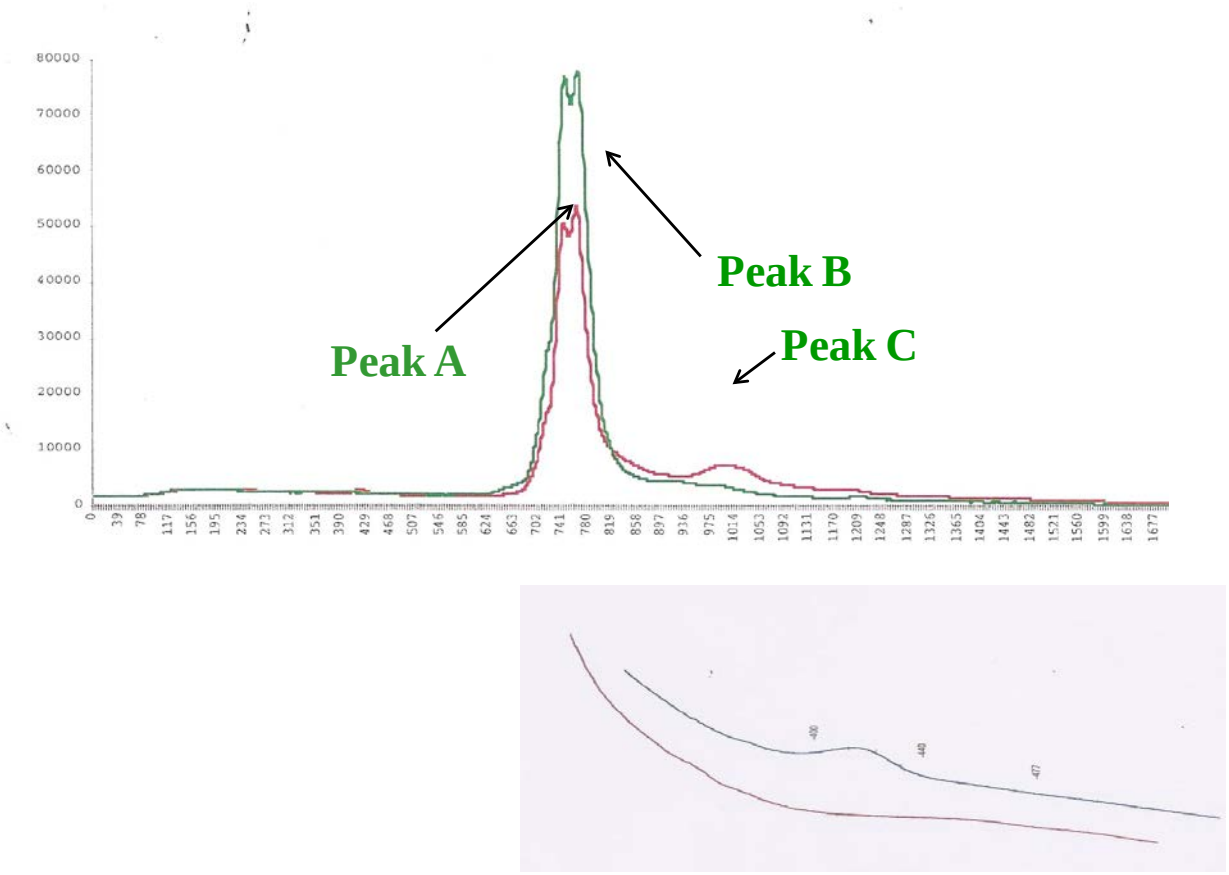

Figure 5: Chromatogram on ion-exchange HPLC chromatography of a no heat treatment native Lf represented by the peak A and peak B compared to the chromatogram of a heat treatment native Lf represented by the peak A, peak B and peak C.

The longer we submit Lf to a heat treatment, more peaks A and B will have a lower surface and more the peak C will be important.
If we compare on the Reverse Phase, the chromatogram of the Lf without heat treatment and the chromatogram of the same Lf but which has been submitted to a heat treatment (72°C) during 5 min, we can notice that the surface of the Lf without heat treatment is lower that the surface of Lf having submitted a heat treatment (figure 6).

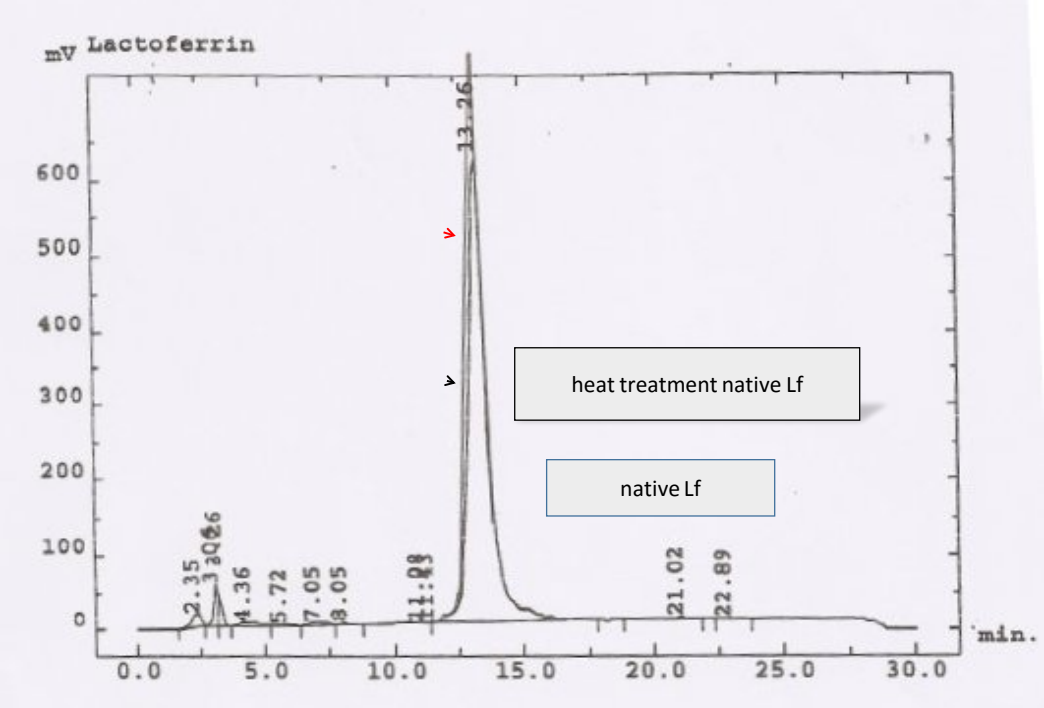

Figure 6: Chromatogram on Reverse Phase chromatography of a no heat treatment native Lf compared to a heat treatment native Lf

The peak C has been characterized as Lf polymers having a much higher absorption power. These polymers have been also observed by SDS-PAGE gels.
Automatically, when you determine the percentage of the Lf peaks compared to the minor components, the non-thermal treatment Lf has a lower purity level compared to the same Lf having been submitted to a thermal treatment and in this case, it is not surprising that the thermal treatment Lf has a much higher than 95% purity compared to the minor contaminants. That is not due to a non presence of minor components, it is due to the presence of the Lf polymers which have a higher absorbance properties than native Lf at 280 nm.

Lipopolysaccharides (LPS)

Endotoxin (lipopolysaccharides, LPS) is a predominant glycolipid in the outer membrane of Gram negative bacteria. LPS stimulates immune responses cytokine production and proinflammatory mediator secretion by monocytes, macrophages and neutrophils, which are recruited into specific host tissues by systemic LPS exposure. The response to the host to LPS is mediated by immune modulator molecules such as tumor necrosis factor α (TNF-α), members of interleukin (IL) family, reactive oxygen species, and lipids. Overproduction of those mediators induces tissue damage that precedes multiple organ failure as described by Morrison & Ryan 1987 [12]and Rietschel in 1996 [13]. Structurally, Lf contains a highly basic region (Arg) in the N-Terminal region as its derivative cationic peptide, called lactoferricin which binds to a variety of anionic biological molecules including lipid A of LPS with a high affinity (Appelmelk *et al*., 1994[14]. This LPS-binding property of Lf is considered to be part of the immunomodulatory function of Lf due to the fact that Lf inhibits LPS activity either by direct binding or by competition with lipopolysaccharide-binding-protein (LBP) for the LPS binding [15] and therefore interferes with the interaction of LPS with CD14 [16].

So it is important to analyze the anti-inflammatory effect of an industrial Lf purified from milk, and containing LPS arising from the milk bacteria. To analyze this activity of Lf, we have studied its downregulation role on the expression of pro-inflammatory cytokines in infected with E.coli HB101(pRI203) and none infected intestinal epithelial cells. For this experience, we have followed the protocol described by Berlutti [10], using Caco-2 cells and the sample Lf-A, Lf-B, and Lf-C having different LPS content and we have compared the results with Lf-NFQ. When we infected the Caco-2 cells without the presence of Lf, we observe a significant increase in the expression of the pro-inflammatory cytokines such as of Il-6, Il-8 and TNF-α compared to the non-infected cells (table 1). In the presence of Lf, the expression of the cytokines is reduced in the case of Lf-NFQ but not in the case of the other Lf samples (table 1). We can conclude that the presence of LPS on the Lf structure inhibits its activity to dowregulate the expression of cytokines by infected cells. What it was surprising, was to observe that in case of non-infected cells in the presence of Lf containing a certain amount of LPS bound on its structure, the cells are able to induce the expression of cytokines and that this expression is dependent of the concentration of the LPS bound on the Lf structure, what was not the case for the Lf-NFQ having only 39pg LPS/mg Lf (table 1).

Table 1: Uninfected Caco-2 cells or infected Caco-2 cells with E.coli HB101(pRI203) were incubated in the presence or absence of Lf-NFQ, Lf-A, Lf-B, Lf-C (100µg/ml). the concentrations of secreted cytokines was determined as described in the Material and Methods. P values < 0.01 were considered to be significant.

None infected cells

Cytokines (pg/ml)	No Lf	Lf-NFQ	Lf-A	Lf-B	Lf-C
TNF-α	44	40	105	130	165
IL-6	112	87	140	220	430
IL-8	2700	2750	3600	3650	4600

Infected cells with E.coli HB101(pRI203)

Cytokines (pg/ml)	No Lf	Lf-NFQ	Lf-A	Lf-B	Lf-C
TNF-α	160	48	154	164	160
IL-6	1200	150	1240	1350	1300
IL-8	12250	3200	10700	10800	11500

This expression could be due to the fact that it is possible that some LPS are detached from the Lf structure due to the medium used for the cell culture and play a role as pro-inflammatory agent towards the non-infected cells. This role seems more important that the downregulation role of the Lf.

Lf Activity

Antibacterial activity: We have performed an *in vitro* test regarding the inhibition of the growth of a Staphylococcus aureus strain SHY97-4320 in order to study the influence of contaminants. When we compare the minimal inhibitory concentrations of the Lf-Lab, Lf-NFQ and the different commercial Lf samples from the same suppliers as well as other suppliers available on the market, we do not observe the same activity. Clearly, the Lf-Lab and Lf-NFQ which show the same minimal inhibitory concentrations were more potent and did not lose their activity at higher concentration in the medium. The minimal inhibitory concentration was estimated at 1.2 mg per ml for the *Staphylococcus aureus* SHY-97-4320. None of the commercial Lf preparations available on the market have been able to

display a minimal inhibitory concentration. The Lf-Lab and Lf-NFQ at 6.4 mg per ml inhibited in 24h the growth of *Staphylococcus aureus* by 96% compared to control growth. On the other hand, growth inhibition of commercial Lf preparations was observed between 69 to 79%. At higher concentration than 6.4 mg per ml, these differences in favor of Lf-Lab and Lf-NFQ were even more pronounced. Knowing a part of the source of starting material from different commercial suppliers, it seems that the Lf extracted from milk or from whey shown clearly a lost of the growth inhibitory activity at high concentration. This higher re-bounding effect of commercial Lf is due mainly to the presence of a higher amount of contaminants such as the presence of angiogenin, interfering with the activity of Lf on bacterial growth.
Another in vitro tests regarding the antibacterial activity of a culture of *Pseudomonas aeruginosa* mucoïd and non mucoïd, a *Burkhoderia cepacia* and a methicillin and oxacillin resistant *Staphylococcus aureus* have been carried out to study the influence of LPS bound on their structure compared to Lf-Lab and Lf-NFQ. In these tests, we have observed a similar antibacterial profile activity between the Lf-Lab and the Lf-NFQ, corresponding to a decrease at least by 5 log CFU/ml during the first 5 hours after the contact of the microorganisms with the two Lf. In case of the commercial Lf (Lf-A, Lf-B, Lf-C), we have observed under the same conditions, a decrease between 2 to 3 log CFU/ml corresponding a better activity for the molecule having a less LPS concentration bound on its structure.

Antioxidant activity: When we tested the Lf-Lab and Lf-NFQ at a concentration of 0.1 mM as control, we obtain an antioxidant activity (FRAP units) with a value of 0.42 mM and 0.4 mM at 6h with a gradual increase to 0.990 mM and 0.875 mM respectively. In case of Lf extracted from milk containing 12,000 pg LPS/mg Lf, we obtain an antioxidant activity of 0.15 mM at 6h with a gradual value to 0.5mM at 24h representing 50% of the control. In case of Lf extracted from whey containing 30,000 pg LPS/mg Lf, we have obtain a value of 0.14mM at 6h with a gradual value of 0.39 mM at 24h representing 40% of the control.

Conclusions

We cannot say that there are several Lf, there is only one Lf which is credited with an important list of multifunctional health benefits as it is described in several scientific papers. Its purification from other ingredients contained in the milk has to require necessary several complex steps of protein engineering. On the other hand, it is important that the Lf existing in the raw material is not submitted to a heat treatment and certainly not for the Lf extracted from the raw milk. That means, we have to avoid any heat treatment such as the spray dry process and it is better to use the freeze dry or any other drying process using a low temperature treatment (<50°C). It is also preferably to do not sterilize the LF solution to eliminate the presence of bacteria and/or virus.
In 1985, when we have established the specifications, Morinaga (Dr Tomita) had insisted on the fact that it is important that the Lf had to have a purity level superior at 95%. He wanted to avoid the presence of other molecules which could be co-purified with the Lf and of which the presence could not only inhibit the biological activity of the Lf but also induce secondary effects in the newborn babies feed with baby foods containing this Lf. The presence of angiogenin, molecule having an isoelectric pH very close to the one of Lf, which has been concentrated during the Lf extraction process, is certainly not favorable to the health of the infants. Today, the commercial Lfs have a lower activity due to a too low level of purity (90 to 92%) and nobody has really characterized the identity and the concentration of the other components. The identification of the purity by the Reverse Phase HPLC is not good enough knowing that any heat treatment is going to induce the appearance of Lf polymers, increasing so the absorption power of the molecule. The best way is to combine the ion-exchange FPLC chromatography to the Reverse Phase HPLC. If we cannot detect the peak C using ion-exchange FPLC chromatography, then we could then use the Reverse Phase HPLC to determine the level of purity.

It seems quite realist to think that when Lf is extracted from milk, the molecule having used its antimicrobial activity in the milk, will be covered by bacterial LPS. If these LPS are not eliminated during the purification of the Lf, how can we think that Lf will still bind the lipid A, toxic part of the LPS and works as therapeutic agent to neutralize the effects of endotoxins. Lf could effectively reduce endotoxin influx into the bloodstream while toxins are still inside the intestinal lumen. In this process, if we ingest Lf already covered by LPS, automatically its activity may not be present in sufficient amounts to perform this function if endotoxin is continuously released in large quantities. A protective effect for Lf against lethal shock induced by intravenously administrated endotoxin has been demonstrated. Lf-mediated protection against en endotoxin challenge correlates with both resistance to induction of hypothermia and an overall increase of wellness.
Despite high purity and a low temperature treatment for its drying, the protein may harbor endotoxin contaminants which could compromise Lf functionality, so it is important to enhance the protein quality during its commercial scale production offering the highest standard quality and functional assurance to preserve its biological activities in the different applications.

Besides modulating iron homeostasis during inflammation, there is mounting evidence that Lf could directly regulate various inflammatory responses. This iron-independent mode of action is based on Lf binding to bacterial LPS, which is a major pro-inflammatory mediator during bacterial infections and septic shock. Without LPS bound on its molecular structure, the Lf could play an important role in the modulation of gastric inflammation since the protein interacts with receptors localized on gastric intestinal epithelial cells but this interaction is only possible if the molecule is free LPS. Several *in vivo* studies have demonstrated that oral administration of Lf could reduce gastritis induced by *Helicobacter pilory* and protect gut mucosal integrity during endotoxemia.
As described by Dr Ashida (Ashida *et al.*, 2004)[17], a specific receptor for Lf has been identified in the human duodenal brush-border membranes of fetal and infant intestinal. The internalization of Lf has been suggested to contribute to the cellular uptake of iron bound to Lf by intestinal cells and to the binding to a specific DNA sequences (He & Furmanski 1995)[18] activating the transcription of a specific gene (Son *et al.*, 2002)[19]. Moreover, the N-terminal of Lf, which is rich in basic amino acids, has been identified to be responsible for the ability of the cellular internalization and the nuclear localization of Lf. From then, the presence of LPS, located specifically on the N-terminal of the Lf will disturb, at the time of its ingestion, the binding on its specific receptor and also its internalization avoiding so the activity of the Lf concerning:

- The faster maturation of the gastrointestinal tract in the newborn,
- the cell generation and tissue repair of the intestinal mucosa in conditions as gastroenteritis
- The process of the increasing of the hepatic synthesis in the newborn, suggesting the anabolic function for Lf in the newborn babies.
- The process of the increasing of the iron absorption during the pregnancy and during the neonatal period of the babies.
- The cytotoxic increasing capacity of Lf
- The potential anti-tumor activity of Lf through its specific receptors on macrophages, T and B-lymphocytes and leukeumia cells.

Based on these results, we think that it is imperative today that the Lf industrial producers proceed to a 2^{nd} step which consists to the purification of the Lf extracted either from milk or from whey. As it is shown in the figure 7, the chromatogram (7a) represent a typical

Typical commercial Lactoferrin (7a)

Lactoferrin top quality LF-NFQ (7b)

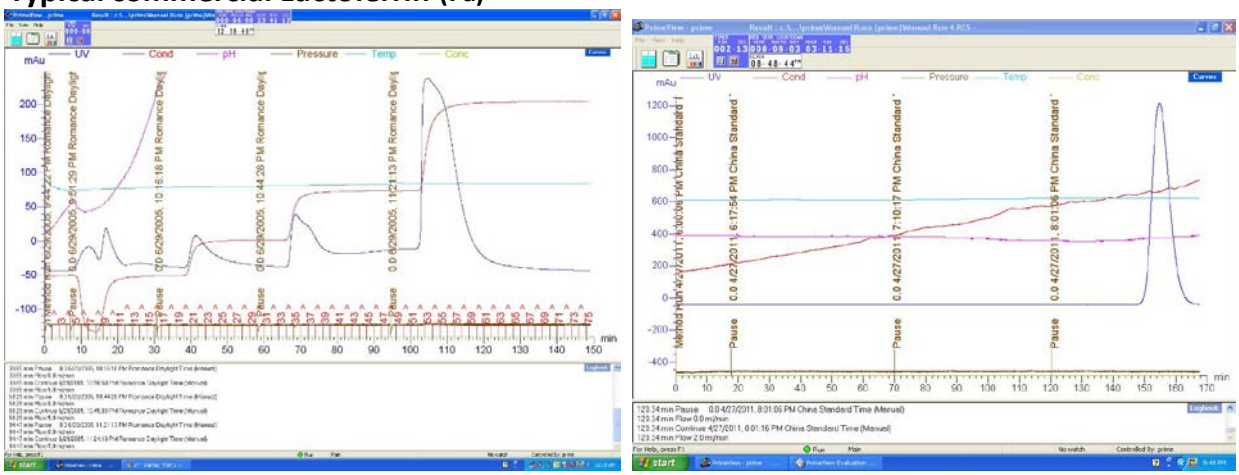

Figure 7: Chromatogram of a typical commercial Lf (7a). Re-purification of this Lf demonstrating the absence of other components including LPS that we call LF-NFQ (7b)

chromatogram of commercial Lf. Although the Lf is present only in the last peak, we have demonstrated that re-purifying this Lf (7b), the molecule is totally devoid of angiogenin and of other components including LPS. This quality is called LF-NFQ. Based on this production process (extraction + purification), it is possible to define new specifications more appropriate to the biological functions of the molecule. So, it is possible to think that the specifications (2015 ….) as it is described in the figure 1 could represent a guarantee to the manufacturers to have produced the Lf with all its biological activities.

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EFFECT OF HIGH-PRESSURE TREATMENT ON DENATURATION OF BOVINE WHEY PROTEINS WITH ANTIBACTERIAL ACTIVITY

Chafiaa MAZRI

Faculté des Sciences de la Nature et de la Vie et des Sciences de la terre, Université Akli Mohand Oulhadj – Bouira

mazrichafia@yahoo.fr

Summary

In this work the effect of high-pressure treatment on the lactoferrin, IgG and lactoperoxidase, was studied in skim milk, whey, and buffer.

The denaturation was studied by kinetic analysis. Denaturation of lactoferrin and IgG were measured by the loss of reactivity using immunochemical methods. Denaturation of lactoperoxidase was determined using a spectrophotometric technique.

Results obtained for lactoferrin and IgG when milk was treated at 500 MPa indicated higher values of D-value, of 74 x 10^2, and 123 x 10^2 s, respectively. For lactoperoxidase, no loss of activity was observed after 30 min. Thus, lactoferrine and IgG are denatured more slowly in buffer and in milk than in whey. Denaturation of these proteins follow a reaction order of n = 1.5.

The stability of lactoferrin, IgG and lactoperoxidase to pressure commonly applied to foods is an important aspect to be considered when these proteins are intended for use as bioactive components in food.

Introduction

Whey proteins are widely used in the food industry for their technological and biological properties. Lactoferrin, IgG and lactoperoxidase are whey proteins with biological properties that may provide health benefits to consumers. The antimicrobial properties of these proteins give them the potential to be used as supplements for special foods or nutraceutical products[15, 20, 21]. It is therefore important to know the effect that technological treatments have on their structure and functionality. The usual methodology to preserve milk is the application of thermal treatments. However, these treatments also have undesirable effects such as the loss of some nutritional components and the modification of sensorial properties. Likewise, heat treatment induces the denaturation of whey proteins, which could cause changes in their functional properties[12]. In order to avoid these adverse effects, alternative systems are being developed for food preservation that advantageously replaces heat treatments. These processing technologies called "non thermal preservation methods" include, among others, high hydrostatic pressure treatment.

High pressure treatment is known to induce protein denaturation by altering the equilibrium between the interactions that stabilize the folded conformation of native proteins [3]. Thus, high pressure treatment of whey protein products has shown to induce changes in proteins that modify their functional properties for different applications in the dairy industry[13]. A better understanding of the effects of high pressure on whey proteins is still required to open new perspectives in the control of their technological and biological properties, as well as to optimize treatment conditions.

This aim of this study was to determine the effect of high pressure treatment on denaturation (of minor whey proteins with antibacterial activity, lactoperoxidase, lactoferrin and IgG. The present work includes the treatment of these proteins present in skim milk and whey and as isolated proteins in buffer.

Material and methods

Materials. Bovine lactoferrin and lactoperoxidase were donated by Fina Research (Seneffe, Belgium). Lactoperoxidase substrate (ABTS), horseradish peroxidase and IgG were supplied by Sigma (Poole, UK). Fresh raw bovine milk samples were kindly supplied by Quesos Villacorona (El burgo de Ebro, Zaragoza, Spain). Recombinant chymosin was provided by Chr.Hansen (Horsholm, Denmark).

Pressure and thermal treatments. Samples of skimmed milk, whey obtained by enzymatic coagulation, and pure proteins in150 mM NaCl, 10 mM potassium phosphate buffer, pH 7.4 (PBS) were treated from 450 to 700 MPa at 20ºC in a discontinuous isostatic system from Stansted Fluid Power FPG 11500 B (Stansted, Essex, UK). After pressure treatment, samples were stored at 4ºC overnight before analysis.Samples from two independent experiments were analyzed by triplicate.

Measurement of protein concentration and enzymatic activity. Antisera against bovine lactoferrin and IgG were obtained in rabbits. Specific antibodies to lactoferrin were purified by immunoadsorption and conjugated with horseradish peroxidase using the periodate method. The concentration of IgG, was measured by radial immunodifusion [13] Lactoferrin was quantified using a sandwich ELISA [14].

Enzymatic activity of lactoperoxidase was measured by a spectrophotometric method using 2,2'-azinobis (3-ethylbenzthiazoline-sulfonic acid) (ABTS) as substrate [14].

Kinetic data analysis. Determination of D and Z values, the reaction order and the denaturation rate constants was performed by[14].

The calculation of activation volume and frequency factors were performed according to Anema, Stockmann, and Lowe (2005).

Results and discussion

We studied the effect of different high pressure treatments on the denaturation bovine whey proteins with antibacterial activity by measuring the immunoreactive concentration of lactoferrin and IgG and the residual enzymatic activity in 3 different media, skim milk, whey, and phosphate buffer at different pressures and for various holding times.

Results obtained in the pressure treatment of lactoperoxidase from 450 to 700 MPa indicated that the enzyme is highly resistant. The enzymatic activity of lactoperoxidase is stable when it is treated in milk, whey and buffer at all pressures and times assayed, even after a treatment at 700 MPa for 15 min. Our results agree with those previously reported, which indicated that lactoperoxidase shows extreme resistance to high-pressuretreatment[22, 11]. It has been also reported that the combination of high-pressure treatment and the lactoperoxidase system cause a strongly synergistic inactivation of a wide range of gram-negative and gram-positive bacteria. Therefore, lactoperoxidase may be an interesting additional technology to improve the safety of high-pressure food preservation [5].

The decrease of latoferrine and IgG concentrations, expressed as log of residual proteins concentration, versus the holding time, are shown in Figures 1 and 2. The graphs show results of individual experiment although mean values from two different experiments were used to calculate D values (Tables 1 and 2). At all pressures, immunoreactive lactoferrine and IgG decreased with time of treatment in the three media. D values decreased with the increase of pressure (Figures 3 and 4) giving Z values shown in tables 1 and 2. Lactoferrin is denatured more slowly when it is treated in buffer than in milk, and more slowly in milk than in whey. Results obtained in this work are in accordance with those reported for heat-treated lactoferrin. This fact has been attributed to changes in the calcium phosphate bound to caseins, which changes to a more amorphous state with increasing temperature. Thus, interactions of lactoferrin with caseins would be enhanced and consequently, lactoferrin heat sensitivity increased [19]. However, different behavior has been reported for IgG, which is more baroresistant in colostrum than in buffer [9] or for β-LG and α-LA. which are more pressure-resistant in milk than in whey [7]. D values obtained in this work at all pressure indicated that the baroresistance of IgG is similar when they are treated in milk and phosphate buffer, whereas a higher sensitivity is observed when treated in whey, differences among D values being lower when the pressure increased. The higher barosensitivity of IgG in rennet whey could be attributed to the presence of β-lactoglobulin, which unfolds by pressure resulting in the exposure of its free sulphydryl group. Then, unfolded β-lactoglobulin could interact with other proteins containing disulphide bonds such as κ-casein, α-lactalbumin or immunogloblins, through sulphydryl-disulphide interchange reactions [7].Therefore, the higher barosensitivity of IgG obtained in rennet whey than in milk could be

due to the absence of caseins, which results in a higher interaction of β-lactoglobulin with whey proteins. In fact, immunoglobulins have been found in disulphide-bonded aggregates formed with other whey proteins when analyzing whey protein concentrates subjected to pressure treatment using electrophoresis [16].

Pressure-induced denaturation of lactoferrin and IgG was analyzed assuming different orders of reaction. The best fit was found for the reaction order of n = 1.5, as shown in tables 3 and 4. The reaction order observed for the thermal denaturation of lactoferrin in skim milk and phosphate buffer has been reported to be n = 1 [19]. To explain differences in the order of reaction of whey proteins subjected to thermal treatment, Dannenberg and Kessler (1988) postulated intermediate and consecutive reactions that would appear as a reaction of higher order. The same could be assumed for pressure induced denaturation because the unfolding of proteins occurs under high-pressure conditions and is followed by the formation of aggregates [6, 16, 2].Differences in the reaction order of induced-denaturation of lactoferrin between thermal and pressure treatments suggests differences in the predominance of individual steps in the overall denaturation mechanism, as has been reported for other whey proteins [1, 2].

The negative values of the activation volume V_a obtained for denaturation of lactoferrin and IgG treated in the three media indicates that reactions of volume decrease are favored by high pressure[18]. Thus values are in the range reported for the pressure-induced unfolding of other whey proteins [6, 2]. Negative $V_a$values also indicate that the rate of denaturation of lactoferrin and IgG increases with pressure, as was observed in the current study.

Conclusion

The effect of high pressure treatment on denaturation of whey proteins depends on each protein, the treatment media and the pressure and time applied.Lactoperoxidase shows a high baroresistance whereas IgG and mainly lactoferrin are much more pressure sensitive proteins.

Kinetic parameters obtained in this work allow prediction of the pressure-induced denaturation of whey proteins on the basis of pressure and holding times applied.

Results obtained should be considered in the design of pressure treatments for preservation in order to maintain IgG or lactoferrin integrity, and thus their biological function when they are going to be added to special food or pharmaceutical products.

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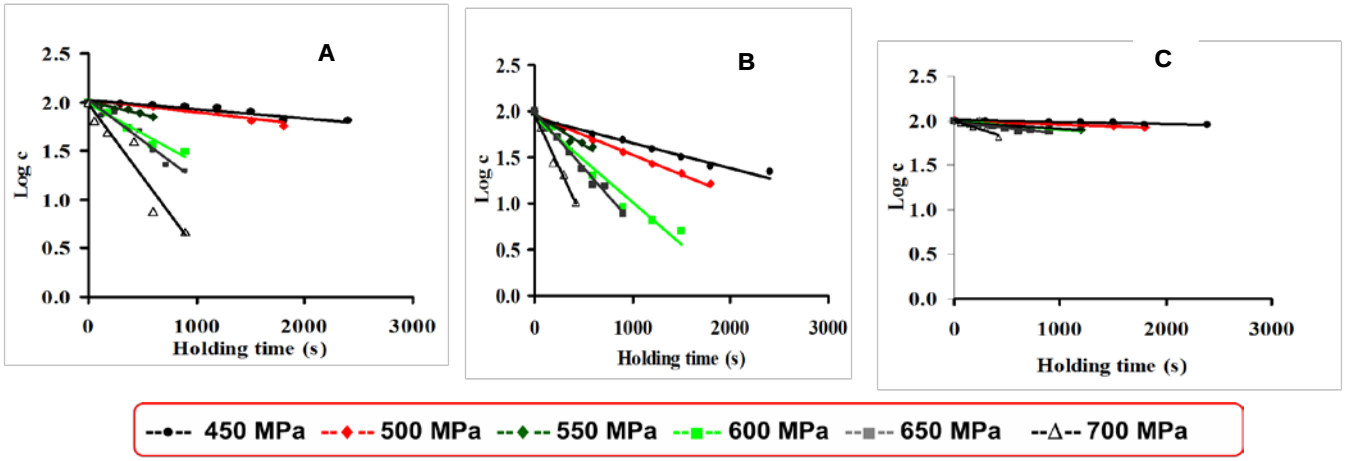

Figure 1: Reaction kinetics of pressure-induced denaturation of lactoferrin. A: skimmed milk, B: whey, C: phosphate buffer. c is the concentration of immunoreactive protein at each holding time and it is expressed as the percentage of the concentration in untreated sample (100%).

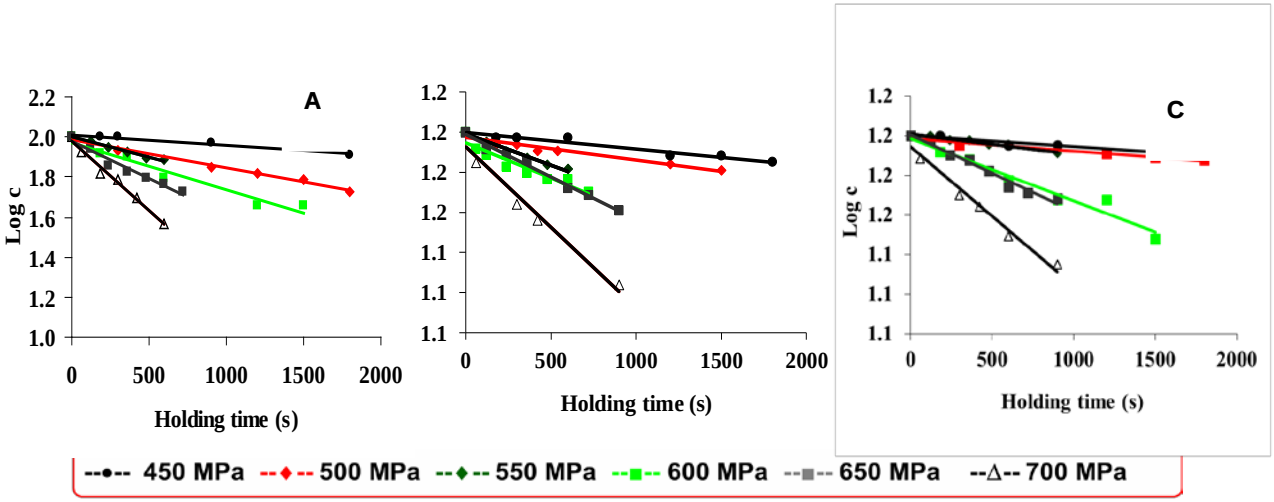

Figure 2: Reaction kinetics of pressure-induced denaturation of IgG. A: skimmed milk, B: whey, C: phosphate buffer. c is the concentration of immunoreactive protein at each holding time and it is expressed as the percentage of the concentration in untreated sample (100%)

Table 1 and figure 3.Values of D and Z for lactoferrin denaturation.D value: time in seconds required for 90% protein denaturation at constant pressure. Z-value: pressure necessary to reduce D value in one logarithmic cycle.

D value (s)	Milk	Whey	Buffer
D450	12.092	3.463	46.729
D500	7.435	2.316	25.510
D550	2.917	1.406	13.623
D600	2.068	992	12.048
D650	1.220	798	7.710
D700	682	410	3.566
Z (MPa)	200.1	283.5	243.1

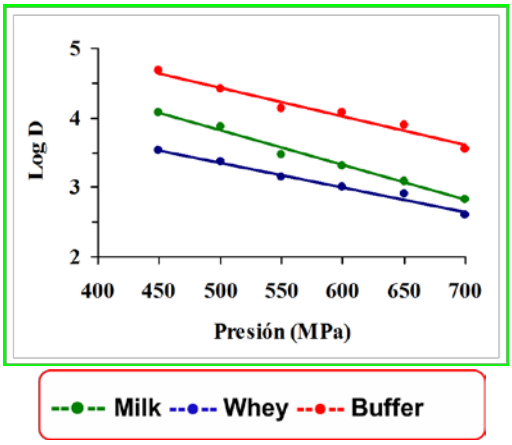

Table 2 and figure 4.Values of D and Z for IgG denaturation.D value: time in seconds required for 90% protein denaturation at constant pressure. Z-value: pressure necessary to reduce D value in one logarithmic cycle.

Parameter	Milk	Whey	Buffer
D450 (s)	27,397	12,555	24,331
D500 (s)	12,376	8,993	12,690
D550 (s)	7,215	5,376	7,353
D600 (s)	4,279	3,244	3,500
D650 (s)	2,704	1,883	2,118
D700 (s)	1,540	1,116	1,419
Z (MPa)	206.9	234.6	198.3

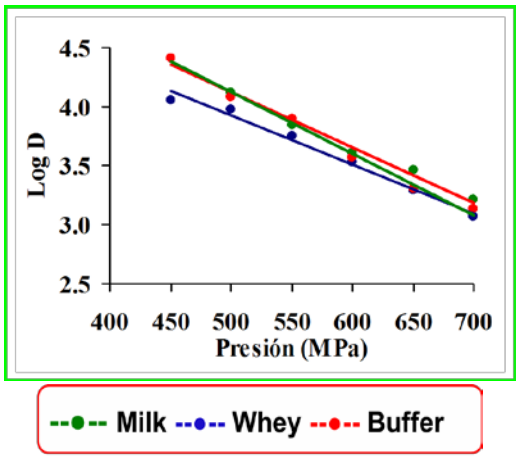

Table 3: Kinetic parameters for pressure denaturation of lactoferrin assuming a reaction order of n = 1.5, at different pressures.K, rate constant (s^{-1} x 10^4), r^2, square correlation, V_a,activation volume (ml/mol).

	Milk		Whey		Buffer	
MPa	k	r^2	k	r^2	k	r^2
450	1.06	0.943	5.26	0.992	0.25	0.877
500	1.77	0.921	8.17	0.997	0.46	0.950
550	5.54	0.921	15.26	0.954	0.91	0.951
600	8.97	0.945	24.79	0.990	1.02	0.940
650	14.59	0.935	28.94	0.972	1.61	0.896
700	40.14	0.934	73.87	0.906	3.75	0.910
Va	-35.8		-22.4		-15.5	

Table 4: Kinetic parameters for pressure denaturation of IgG assuming a reaction order of n

	Milk		Whey		Buffer	
MPa	k	R^2	k	R^2	k	R^2
450	0.34	0.893	1.02	0.940	0.49	0.860
500	1.02	0.942	1.39	0.949	0.96	0.970
550	1.77	0.977	2.50	0.967	1.71	0.976
600	3.21	0.977	4.54	0.988	3.92	0.997
650	5.24	0.989	8.40	0.970	5.88	0.982
700	10.82	0.990	17.48	0.990	12.08	0.996
Va	- 31.7		- 28.1		- 30.9	

INTERACTION OF GREEN TEA FLAVONOIDS WITH MILK PROTEINS AND THE EFFECT OF HEAT TREATMENT

Zerrin Yüksel*[1], Elif Avcı[2], Yaşar Kemal Erdem[2]

[1]Çanakkale Onsekiz Mart University, Department of Food Technology, Bayramiç, Vocational Collage, Canakkale, Turkey, [2]Hacettepe University, Department of Food Engineering, Beytepe, Ankara, Turkey,

zyuksel@comu.edu.tr*, elif_avci@hotmail.com,erdem@hacettepe.edu.tr

Abstract

In this study, the interaction of green tea flavanoids with milk proteins was studied. Reverse-phase high-performance liquid chromatography analysis revealed that the free flavonoids decreased in the presence of milk proteins and a binding ratio (%) was calculated based on this decrease. Green tea extract (GTE) was added into the milk protein system before and after heat treatment (80, 85, 90oC x 10 min). Analyses were carried out in both sodium caseinate and skimmed milk samples to observe the possible effect of serum proteins on the interaction.

The binding ratio between flavonoids and proteins was found to be higher in the samples where GTE was added before heat treatment than in the samples where GTE was added after heat treatment. The results of the PSH index and protein partition also demonstrated that heat treatment must be applied after the addition of GTE to improve the amount of GTE binding to milk proteins.

Keywords: Green tea flavanoids, Milk proteins, Interaction, Heat treatment, Hydrophobicity.

Introduction

Tea, Camellia sinensis, is the second most consumed beverage in the world, well ahead of coffee, beer, wine and carbonated soft drinks [1]. The most common flavanoids in tea are the flavan-3-ols (flavanols or flavans), which are present in relatively large amounts in tea compared to their levels in other foods. The flavan-3-ol subclasses are ranked by degree of polymerization. Tea catechins are monomers and these form 20-30% of the dry weight of green tea. The major catechins in fresh tea leaves and green tea are (-)-epigallocatechingallate (EGCG), (-)-epigallocatechin (EGC), (-)-epicatechingallate (ECG) and (-)-epicatechin (EC) [2]. The catechins represent nearly 80% of the total flavanoid content of green tea. With epidemiological and biological data supporting a potential protective role for tea and tea catechins, development of new products with tea as active ingredients has expanded [3].

Polyphenols have a significant affinity for proteins that leads to formation of soluble complexes, which can grow in size and even form sediment. Many authors have developed models to explain protein-polyphenol complexes formation and precipitation [4, 5, 6, 7, 8]. Most of these models propose that protein-polyhenol complexes are formed by multiple weak interactions (mainly hydrophobic) between amino acids side chains and polyphenol aromatic rings, indicating that the association of polyphenols with proteins is principally a surface phenomenon. Sometimes these interactions could be complemented by hydrogen bonding, which could play an important role in

reinforcing and stabilizing complexes. Additionally, each polyphenol is able to bind more than one protein, acting as a linker between two proteins [9].

Milk proteins have a common interest related with their cross-linking affinity, and its effect on the texture as well as their nutritious properties. The reactions of milk proteins with some functional groups of other compounds in food have gained increasing attention in food research. The major milk proteins, caseins, have a micellar structure formed mainly via electrostatic interactions, and that is the main result of hydrophobic characters of casein fractions. The casein fractions are αs1-, αs2-, β- and κ-caseins, of which the molar ratio is about 4:1:4:1, respectively. Caseins are hydrophobic; they have a fairly high electrostatic charge, many prolines and a few cystine residues [10]. So, caseins show a tendency to associate with other proteins and also some ligands according to the hydrophobic character of the micelle. The interactions between polyphenols and milk proteins have been reported [11]. Interaction between catechins and specific milk proteins has been studied including α-, β- and κ-casein and albumin. In general these studies conclude that ionic, hydrophobic and hydrogen bonding forces are all important factors in catechin-protein interaction. Gallated catechins such as EGCG and ECG have previously been shown to have the strongest association with milk protein [3]. There are some studies on the formation of milk β-lactoglobulin-tea polyphenol complexes and the interaction of milk α- and β-caseins with tea polyphenols [12, 13]. EGCG binds to a wide variety of proteins, especially to nonglobular extended proteins, and particularly to proteins with a high content of proline. One such protein is β-casein, the second most abundant protein in milk [14]. The importance of the interaction between green tea catechins and proteins may have resulted from possible alterations of their bioavailability and functionality [15].

Green tea flavanoids are a major subject of studies related to the functional food area and milk is well accepted as a suitable vehicle in producing novel functional foods. Therefore research on the interaction of flavanoids with milk proteins comes to the forefront in food science. The aim of this study was to contribute to the studies on the interaction of green tea flavanoids with milk proteins in terms of binding ratio by using RP-HPLC. Green tea flavanoids were added to milk protein systems before and after heat treatment to designate the addition stage of green tea flavanoids. The possible effect of whey proteins was also examined by using sodium caseinate and skimmed milk. Protein surface hydrophobicity determined by using 1- anilinonaphthalene-8-sulfonate (ANS) as a fluorescence probe was another assay to observe changes in hydrophobic character of milk proteins in the presence of green tea flavanoids.

Materials and Methods

Preparation of green tea extract and standards of green tea flavanoids

Decaffeinated green tea extracts were obtained from the GreenSelect ®, Indena (Italy). A green tea extract solution was prepared daily by dissolving green tea extracts in deionized water to concentrations of 0.1, 0.5 and 1.0 mg/ml.

Pure standards of green tea flavanoids [(+) catechin hydrate (#C1251), (-) epicatechin (#E4018), (-) epicatechingallate (#E3893), (-) epigallocatechin (#E3768), (-) catechingallate (#C0692), (-) epigallocatechingallate (#4268), (-) gallocatechingallate (#G6782)] were obtained from Sigma (Germany). All standards were dissolved in methanol and dilutions were performed with acetic acid solution in deionized water (2%) to ensure stability of green tea flavanoids [16].

Preparation of milk protein systems

Sodium caseinate (Sigma, #C8654, Germany) solution was prepared by dissolving in deionized water to a concentration of 2.8%. Raw skimmed milk samples (S) were obtained from a local dairy (Atatürk

Forest Farm Dairy Plant, Ankara, Turkey). Green tea extract solution was added to sodium caseinate at given concentrations (0.1, 0.5 and 1.0 mg/ml) and added to raw and heat-treated skimmed milk samples at given concentration (1.0 mg/ml) (Table 1).

The total protein content of the samples was determined using the Bradford method [17].

Determination of green tea flavanoids by RP-HPLC

Green tea flavanoids were determined using reverse-phase high-performance liquid chromatography (RP-HPLC) [18]. The RP-HPLC analysis of green tea flavanoids was performed on an Agilent 1100 series HPLC system consisting of a quaternary pump (Agilent, G1311A), a manual injection block (Agilent, G1328B), a variable wavelength UV-detector (Agilent, G1314A), a column thermostat (Agilent, G1316A) and a degasser (Agilent, G1379A). The equipment was controlled using Agilent ChemStation software, which controlled the solvent gradient, data acquisition and data processing. The separation was performed with a silica-based C-18 RP-HPLC column (Thermo Hypersil ODS, 250mm length x 4.6mm i.d., particle size 5µm and pore size 120oA).

Separations were performed using solvent A (2% of aqueous acetic acid in deionized water) and solvent B (acetonitrile) with a column temperature of 35°C, a flow rate of 1.0 ml/min, and a detection wavelength of 280 nm. Sample solutions (20 µl) were injected onto the column, and then subjected to a solvent gradient that started at 8% solvent B, and then increased linearly to 30% solvent B over 25 min. The solvent composition was then returned to the initial conditions in 2.0 minutes.

Carrez clarification was carried out to determine the flavanoid content of samples including protein used RP-HPLC. Carrez I solution was prepared by dissolving 15 g of potassium hexacyanoferrate in 100 ml of water and Carrez II solution was prepared by dissolving 30 g of zinc sulfate in 100 ml of water. Acetic acid solution was added to the samples at a final concentration of 2%, and then aliquots of Carrez I (100 µL) and Carrez II (100 µL) were added to 1 ml samples and mixing using vortex. The precipitate was pelleted by centrifugation at 3000 g and 20oC for 15 min. The clear supernatant was filtered through a RC filter (0.45 µm, Sartorious, GmbH, Germany) before RP-HPLC injection.

Determination of optimum interaction time between green tea flavanoids and milk proteins

Concentrations of 0.1, 0.5 and 1.0 mg/ml green tea extract were examined in sodium caseinate system to estimate optimum interaction time during 0, 1, 2, 3, 4, 5 and 10 hours and to determine suitable concentration of green tea extract in order to obtain the maximum interaction level between green tea flavanoids and milk proteins.

Significant decreases in the peak areas of the green tea flavanoid in the presence of milk proteins indicated the formation of interaction between green tea flavanoids and milk proteins. Percentage of binding (%) was calculated as follows:

Binding (%) = [(1-F(GTE+protein) / FGTE)] x 100 where F(GTE+protein) is the concentration of flavanoids in the green tea extract and caseinate mixture (ppm), FGTE is the concentration of flavanoids in the green tea extract (ppm).

Determination of the effect of heat treatment on the flavanoid-protein binding

Heat treatment was applied for 10 minutes at 80, 85 and 90oC to determine the effect of temperature on the binding between green tea flavanoids and milk proteins. Green tea extract was added to the milk protein systems before and after heat treatment to determine the most suitable application method for binding. Given heat treatment conditions were also applied to green tea extract (as such, without protein) to observe possible changes in green tea flavanoids. Green tea extract was added into raw milk and sodium caseinate which were adjusted to the same protein concentration. Green tea

flavanoids were determined using RP-HPLC, PSH measurements and protein partition analyses were carried out in the all samples.

Protein surface hydrophobicity measurements

The relative fluorescence intensity of the samples was measured using a Perkin Elmer Model LS50B spectrofluorimeter (UK) with a normal glass cell, at λex = 390 nm, λem =480 nm. 1-anilinonapthalene-8-sulfonate (ANS, Merck Cat. No. 10762, Germany) was used as the fluorescent probe. Titration of the protein solutions with increasing concentration of ANS provides information on both the hydrophobic number and affinity of the binding sites. Before ANS titration, fluorescence of the samples was measured as a blank. Milk samples or caseinate solutions do not show fluorescence or have the lowest level of fluorescence alone whereas “ANS-protein complex” has a remarkable fluorescence.

Kinetic data were obtained from ANS titration curves (not shown). All values were calculated as the average of four different kinetic approaches; Lineweaver-Burk, Hanes-Woolf, Eadie-Hofstee and Michelis-Menten [19]. PSH is protein surface hydrophobicity index and it was calculated as;

$$PSH = Fmax/(Kd * [P])$$

where Fmax denotes the number of hydrophobic sites, 1/Kd gives the binding affinity of ANS to the protein and P represents protein concentration (g/l).

Protein partition analysis

Protein partition assay was carried out according to Erdem, 2000 [19]. 9 ml sample containing milk protein was centrifuged at 10000 g and 4°C for 45 min. Precipitate was dispersed in 3 ml phosphate buffer (50 mM pH 6.8). The total protein content of the aliquots taken from supernatant and dispersed precipitate was determined using the Bradford method [17]. The proportion of protein content of precipitate to supernatant was used as protein partition values.

Statistical evaluation

Experimental data were analyzed by analysis of variance and correlations between parameters. All data were analyzed using SPSS ® 16 Windows software (IBM, SPSS Inc., Chicago, IL, USA).

Result

The concentrations of flavanoids of GTE

The concentrations of green tea flavanoids [(+) catechin hydrate (C), (-) epicatechin (EC), (-) epicatechingallate (ECG), (-) epigallocatechin (EGC), (-) catechingallate (CG), (-) epigallocatechingallate (EGCG), (-) gallocatechingallate (GCG)] determined using the peak areas in the RP-HPLC chromatograms (Figure 1a) are given in Table 2.

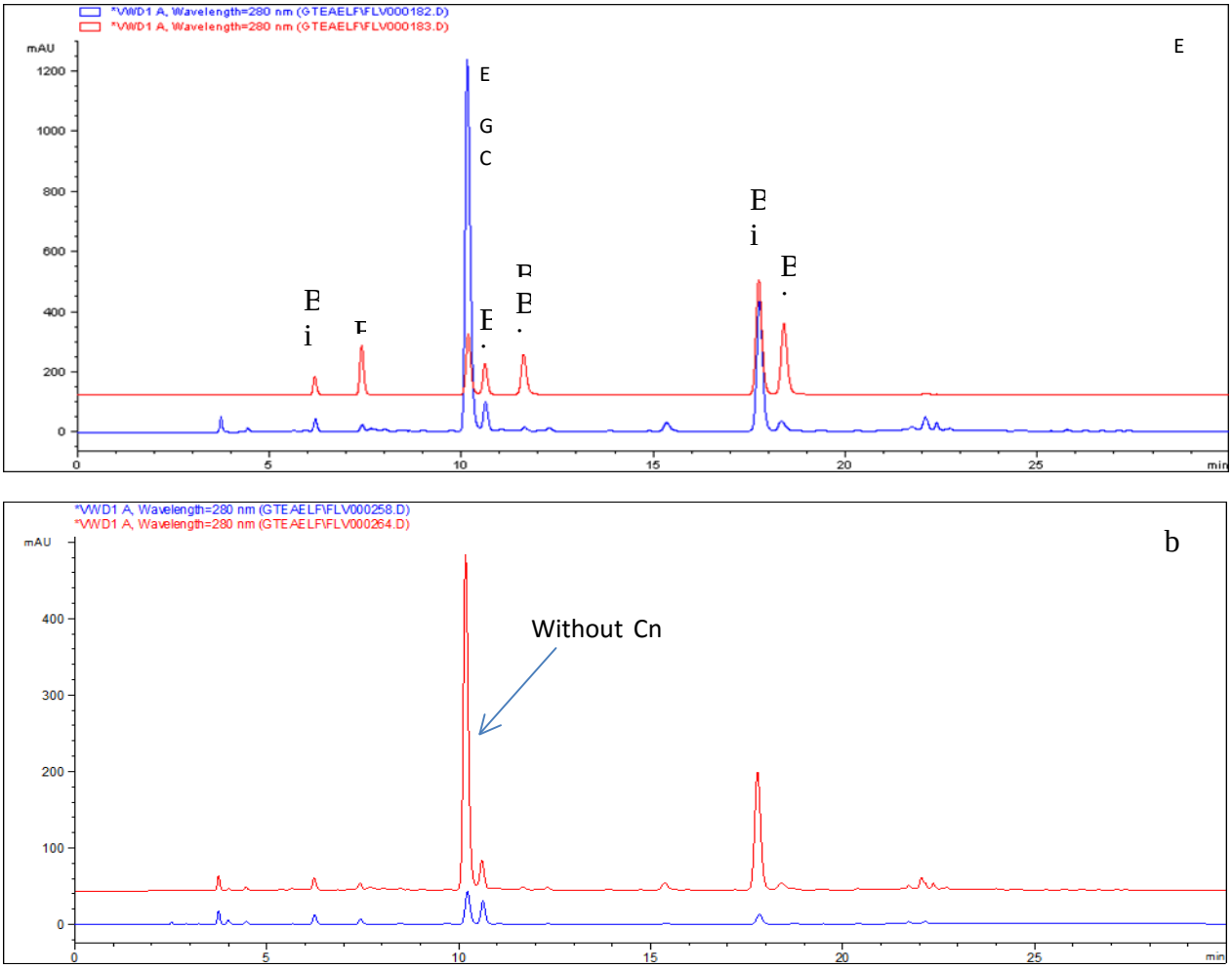

Figure 1 RP-HPLC chromatograms of green tea flavonoids standards (red) and green tea extract (blue) (a) and green tea extracts with and without sodium caseinate (Cn) (b). [Concentrations of EGC; (-) epigallocatechin, C; (+)catechin, EGCG; (-) epigallocatechingallate, EC; (-) epicatechin, GCG;(-) gallocatechingallate, ECG; (-) epicatechingallate, CG;(-) catechingallate are 100, 100, 50, 100, 50, 100 and 50 ppm, respectively in the mixture of green tea flavonoids standards].

Flavanoid-protein binding in sodium caseinate system

In order to obtain the interaction stoichiometry of green tea flavanoids with milk proteins, different concentrations of green tea extracts (0.1, 0.5 and 1.0 mg/ml) were added to sodium caseinate solution. Time dependent changes of green tea flavanoids (1mg/ml) at room temperature (21-24oC) during 10 hours are shown in Figure 2a (concentrations of green tea extracts are not shown as the same trends were observed).

Stability of green tea flavanoids at room temperature during 10 hours was determined and this finding is compatible with results of Chen et al. [20]. In brief, considering peak areas of the free flavanoids determined using RP-HPLC, it was envisaged that decreases in the peak areas of the flavanoids would result only from flavanoid-protein interaction.

Significant decreases in the peak areas of the green tea flavanoids in the presence of sodium caseinate are shown in Figure 1b ($p<0.01$). Decreases in the peak areas of green tea flavanoids indicated the formation of links between GTE and milk proteins. Binding ratio values (%) of each green tea flavanoid are shown in Figure 2b. Time dependent changes of green tea flavanoids at room temperature are also shown in Figure 2b.

Decrease in content of green tea flavonoids, especially 90% reduction in EGCG and ECG, reveals the high level interaction of green tea flavanoids and milk proteins. It was reported that the binding capacity of flavanoids increased with increasing polymerization degree [3, 12, 13]. Flavanoids containing gallate group have higher binding affinity [5]. Interaction between green tea flavanoids and milk proteins was completed initially and no significant changes were observed during 10 hours in the binding ratio values of all flavanoids especially for EGCG and ECG (Figure 2b). These results have shown that there is no need for any time period in order for green tea flavonoids and milk proteins to interact and that also the binding is stable during 10 hours.

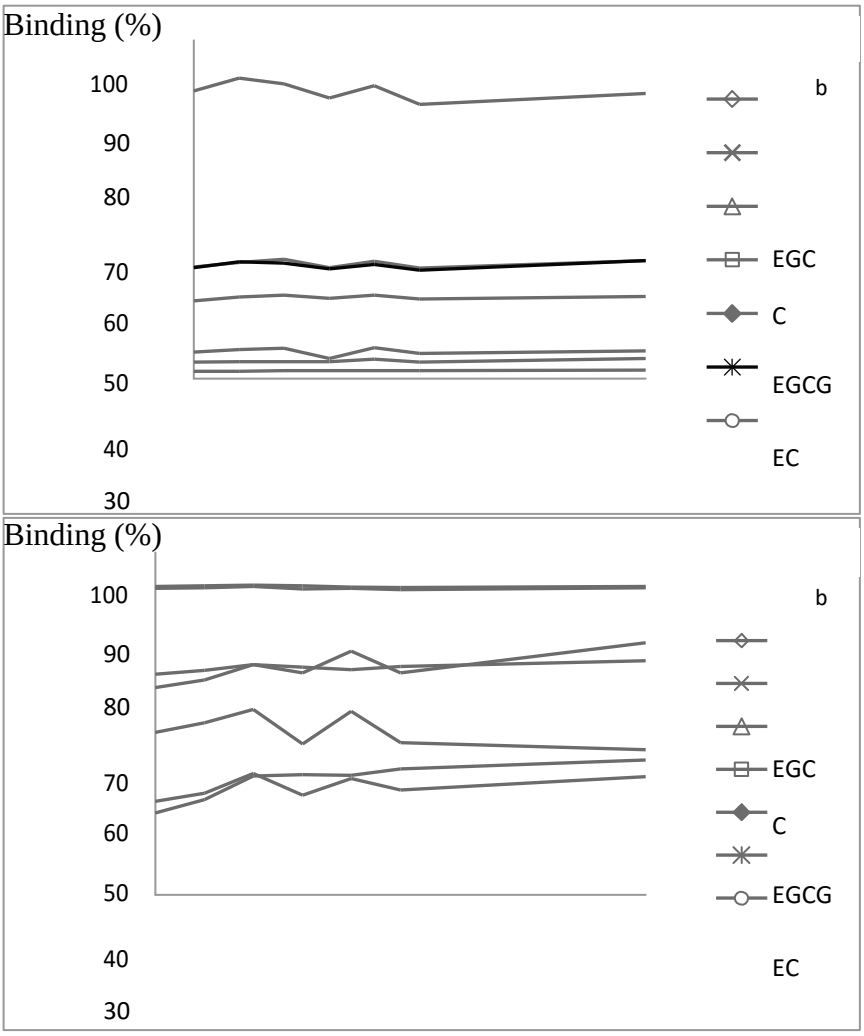

Figure 2 Variation of the concentration of free green tea flavanoids determined by RP-HPLC during the storage at room temperature (a); and the variation of binding ratios of each individual green tea flavanoids during the storage at room temperature (b).

Binding ratios of GTE flavanoids were evaluated for three different GTE concentrations at constant protein concentration (Figure 3). No significant differences were observed in the binding ratio at the concentrations of 1.0 and 0.5 mg/ml GTE ($p > 0.05$). The lowest binding ratio was obtained at the

concentration of 0.1 mg/ml GTE especially for low polymerization degree of flavanoids. The binding ratio between GTE and milk proteins is approximately 10% in the sodium caseinate solution containing 0.1 mg/ml GTE. The binding ratio ranged between 40% and 90% in the presence of 1.0 and 0.5 mg/ml GTE. The usual expectation was that the more the GTE concentration increased, the more the binding ratio would increase; however, it was exclusively seen in the results that the binding ratio for 0.5 and 1.0 mg/ml GTE was almost the same. These unexpected results for the concentration of 0.1 mg/ml GTE may be explained by the solvation effect or increasing collision distance. It is thought that the interaction between flavanoids and proteins occurs adequately in the systems that GTE concentration is over a certain value and this interaction continues between proteins bonded with flavonoids as well. However, the interaction was at its lowest level in the system containing 0.1 mg/ml of GTE and resulted in the lowest binding ratio. The lowest binding ratio could possibly be explained by excess protein content and higher distance between flavanoids and proteins. On the other hand, it couldn't be explained the same binding ratio values in the systems containing 1.0 and 0.5 mg/ml of GTE by these data. Further research needs to be carried out to discuss different binding models in this subject.

Effect of heat treatment on the flavanoid-protein binding

The addition stage of GTE to the milk protein systems was studied at three different heat treatment norms (80, 85 and 90oC x 10 minutes). At first, GTE without protein was examined at these norms to investigate the effect of heat treatment on green tea flavanoids. It was found that heat treatment had no significant effect on flavanoids ($p > 0.05$) (not shown).

GTE was added to the milk protein systems before and after heat treatment. Same experimental conditions were applied in both the sodium caseinate-GTE and skimmed milk-GTE systems to also observe the possible effects of milk serum proteins. It was observed that flavanoid-protein interaction increased significantly when GTE was added before heat treatment ($p < 0.01$) (Figure 4a and 4b). Especially for EGC and EC, the interaction increased with increasing temperature. As explained above, flavanoids containing gallate have higher binding affinity. It was found that dependence of temperature is lower in the flavanoids containing gallate than in the flavanoids without gallate.

It was determined that the addition of GTE to the milk protein system before and after heat treatment has a significant effect on binding between green tea flavanoids and milk proteins. On the other hand, the addition of GTE to the heat-treated sodium caseinate and skimmed milk systems resulted in lower binding rates (Figure 4c and 4d; total flavanoid content estimated using RP-HPLC). These lower binding rates were more significant for flavanoids without gallate although the same trend was observed for almost all flavanoids. However, flavanoid-protein interaction increased in all flavanoids especially in flavanoids without gallate when GTE was added before heat treatment. These results were possibly explained with decreasing of hydrophobic sites on the casein micelles caused by heat treatment [21]. In other words, casein micelles were transformed into a compact structure, and hydrophobic binding sites were masked.

It was thought that the presence of serum proteins would be a hint to understand the heat treatment effect. Contrary to expectations, serum proteins have no effect like masking the hydrophobic sites on casein micelles. It is thought that in an independent way from denatured serum proteins, decreasing of binding ratio due to heat treatment possibly results from more compact casein micelle structure resulting in masking of hydrophobic sites.

PSH Index and protein partition results of GTE- milk protein systems

The characterization of binding interactions between green tea flavanoids and milk proteins was examined with the PSH measurement approach in our previous study [22]. In this study, the effects of heat treatments and the addition stage of GTE were also examined using PSH and protein partition assays to put forward the alterations of the hydrophobic character of casein micelles.

PSH values are given for sodium caseinate and skimmed milk in Figure 5a and 5b, respectively. PSH index decreased in both the sodium caseinate and skimmed milk samples via interaction between the flavanoids and hydrophobic sites on the protein surface [22]. It was observed that relationship between total binding and PSH was remarkable. PSH index decreased (Figure 5a) with increasing binding ratio (Figure 4c) especially in the sodium caseinate system. The protein system turned into a more compact system and the sites that ANS could bind blocked by flavanoid binding.

Results of protein partition are given at Figure 5c. Protein content in the precipitate was increased with increasing flavanoid-protein interaction. The amount of precipitable protein in skimmed milk was lower than that in the milk samples containing GTE. This result showed that the formation of precipitable protein increased when binding between proteins and flavanoids formed. The addition of GTE to the protein system before heat treatment resulted in more protein precipitation, which in turn resulted from more flavanoid-protein binding. It was also found that the amount of precipitable protein increased with increasing heat treatment norms.

Discussion

Results of PSH index, protein partition and binding rates, which were calculated by using RP-HLC, were found to be compatible with each other. These results demonstrate that heat treatment must be applied after the addition of GTE to improve the GTE amount binding to milk proteins. This study presents a holistic approach to examine the usage of functional compounds such a green tea flavanoids in milk products. It is suggested that the binding of milk proteins by green tea flavanoids, or catechins, can be used for manufacturing of novel milk products. Therefore, interactions between green tea flavanoids and milk proteins and the effect of heat treatment on the interaction must be considered in this regard. Furthermore, the addition of green tea flavanoids to milk may affect the functionality of milk proteins. In further studies, these effects will be investigated in dairy products such as yogurt and cheese.

Acknowledgements

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IDENTIFICATION OF ANTIOXIDANT ACTIVITY OF PEPTIDES OF BEYAZ CHEESE PRODUCED FROM OVINE MILK

Zübeyde ÖNER, Ayşe Mine SARIDAĞ

Süleyman Demirel University, Engineering Faculty, Food Engineering Department,

Isparta-TURKEY
zubeydeoner@sdu.edu.tr, a.mine_srdg@outlook.com.tr

Abstract

Cheese is one of the most important dairy products worldwide. It contains various major and minor milk proteins and numerous peptides that are originated from casein breakdown during production and ripening period. The objective of the study was to determine proteolytic activity and peptides and their antioxidant effect, from Beyaz cheese made using ovine milk. Turkish Beyaz cheese was manufactured according to traditional procedures. The cheese was ripened at 5–6 C for 9.th mth. Cheese samples were taken on 1d, 3, 6, 9 months of ripening. Proteolysis was monitored with Water –soluble nitrogen (WSN), 12% trichloroacetic acid soluble nitrogen (TCA–SN), and 5% phosphotungstic acid–soluble (PTA–SN) of the cheese. Peptide fractions were determined by Reversed phase-high-performance liquid chromatography (RP-HPLC). The antioxidant activities of cheese samples and HPLC fractions were determined by 2,2′-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) method. Scavenging activity ranged from 1.79 to 2,32 mM trolox/g in 1.d and 9 mth in Beyaz ovine cheese respectively.

Keywords: peptides, antioxidantactivity, RP_HPLC

Introduction

Over the past few years, there has been lots of research work that investigated the properties of food protein-derived peptides because of the potential health benefits that could result from their biological activities. The role of free radicals and active oxygen species in various diseases, including aging, cancer, inflammation and the toxicity of numerous compounds has been well documented. The search for natural antioxidants as alternatives to synthetic ones is a subject of great interest nowadays. Several food proteins such as milk caseins and maize ze in have been found to possess antioxidant properties [1, 2]. The aim of the work was to study antioxidant activities of an Ovine Beyaz cheese and their peptides. The peptides from most active fractions collected by reverse phase high performance liquid chromatography (RP-HPLC).

Materials and Methods

Materials: Three Beyaz cheese samples were purchased from farmer in Isparta-Turkey.

Methods: Water-soluble extracts, 12% (v/v) trichloroacetic acid-soluble nitrogen (TCA-SN) and 5% (v/v) phosphotungstic acid-soluble nitrogen (PTA-SN); Kjeldahl method [3].

Measurement of antioxidant activity: Antioxidant activity was measured using ABTS method of Re et.al [4]with some modifications. Trolox was used for the standard reference data .

Separation of peptides by RP-HPLC: Semi preparative RP-HPLC was performed on a Shimadzu LC-20 AT HPLC system equipped combination with an automatic fraction collector. The peptides were

eluted with a linear gradient program. The absorbance of the eluent was monitored at 214 nm. The fractions were collected in time periods of 10min. The fractions were lyophilizedand the dried sample was dissolved in % 0,1 TFA in water for antioxidant activity analysis [5].

Results and Discussion

The levels of nitrogenous compounds soluble in water or various precipitants such as 12% (v/v) TCA or 5% (v/v) PTA give some information on the extent of cheese proteolysis which used an indication of cheese maturity (Wallace &Fox, 1998). Inthe 1-day-old cheeses, the percent WSN contents as a percent of TN ranged from 0,609±0,001 and reached 0,83±0,006 at 270 days (9.mth) old samples (Fig.1). The changes in TCA-SN contents of cheese during ripening were shown in Fig.1. The 12% (v/v) TCA-SN fractions contain small peptides which were between two to twenty amino acid residues and free amino acids, ammonia and other minor compounds. The 5% (v/v) PTA-SN indicate the levels of small peptides(<600-700Da (di-, tri- andtetra- peptides) and free amino acids. TCA_SN and PTA-SN contents had the highest levels in the 9.mth during ripening (Fig.2), indicating the levels of small peptides andfree amino acids [6] .

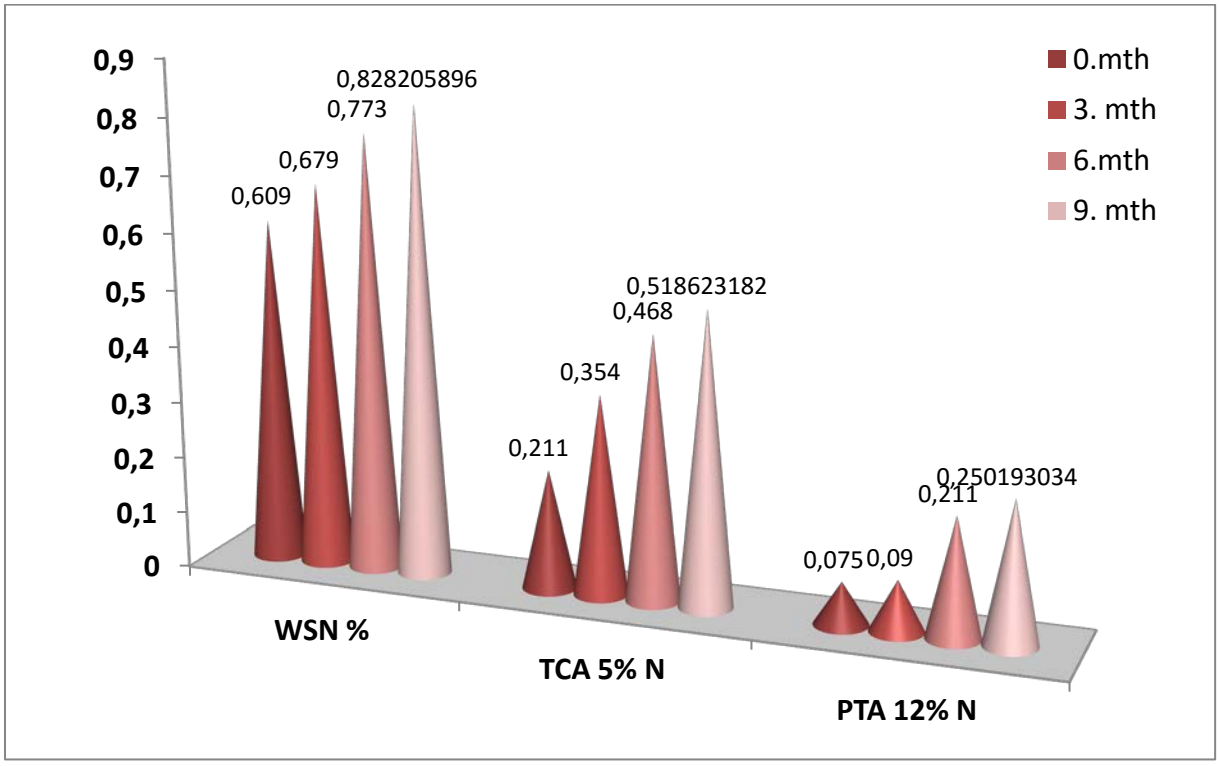

Fig 1. WSN% ; TCA 12% ; PTA 5% of cheese samples. WSN; Water soluble nitrogen, TCA-SN; trichloro acetic acid soluble nitrogen, PTA SN;phosphotungstic acid-soluble nitrogen , RI: ripening index.

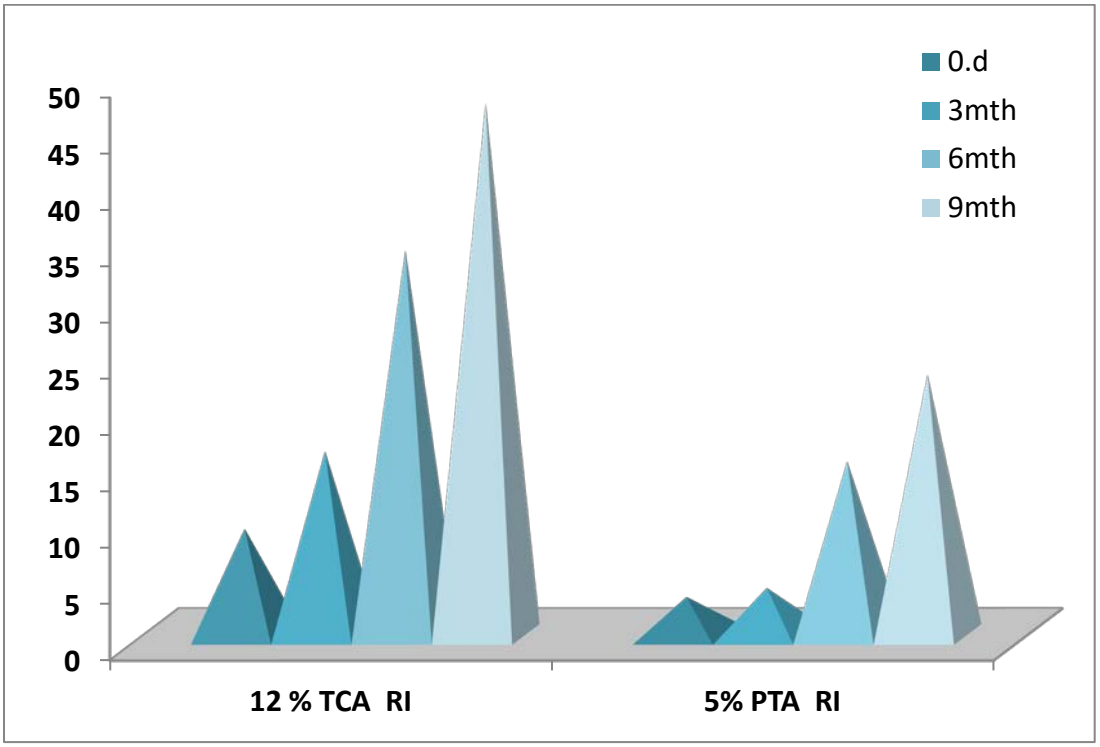

Fig 2. Ripering index of cheesesamples

RP-HPLC analysis revealed that fraction in 6.mth, in 9.mth had higher inhibition rate the other fractions (Fig. 3, 4). Antioxidant activity of cheese and fractions showed Table 1, 2 respectively. Antioxidant activity was lower than that found for the whole F7 fraction ovine beyaz cheese in 9.mth. Antioxidative properties were attributed to the presence of amino acids such as histidine, methionine, cysteine, tyrosine and phenylalanine as well as otherhydrophobic amino acids, all of which have antioxidant properties [7, 8, 9]. Antioxidant activity showed a good correlation with per cent (v/v) WSN,PTA-SNand TCA-SN ($p<0.01$).

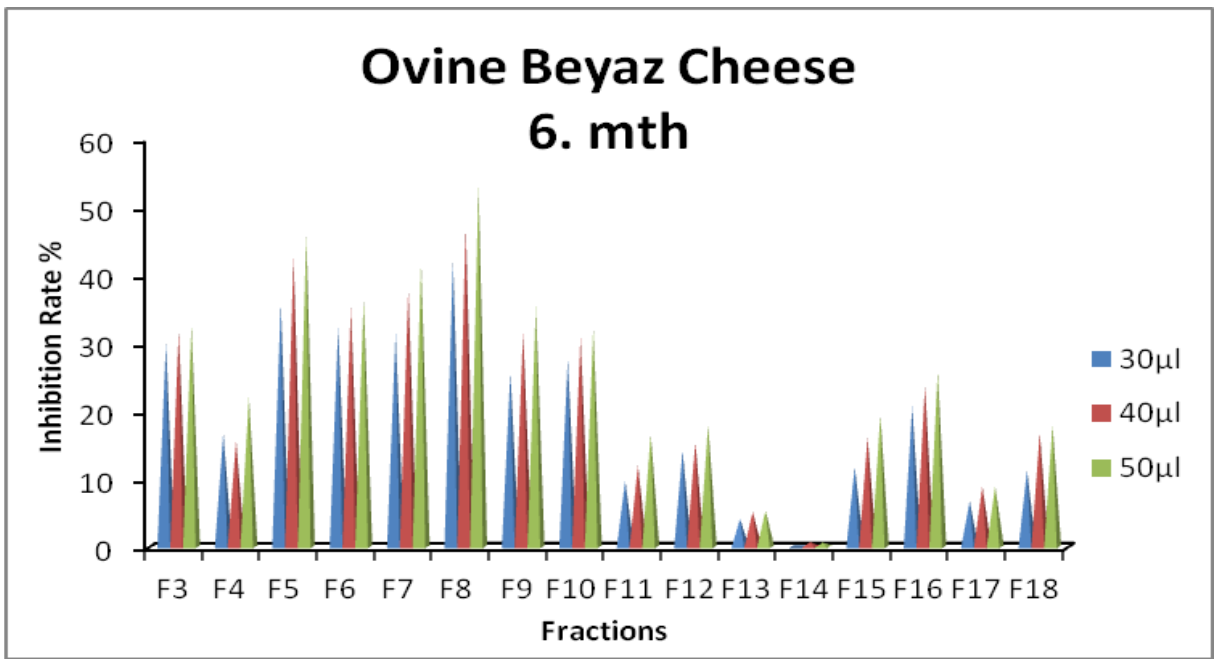

Fig 3. Inhibition rate % of ovine beyaz cheesefractions in 6.mth

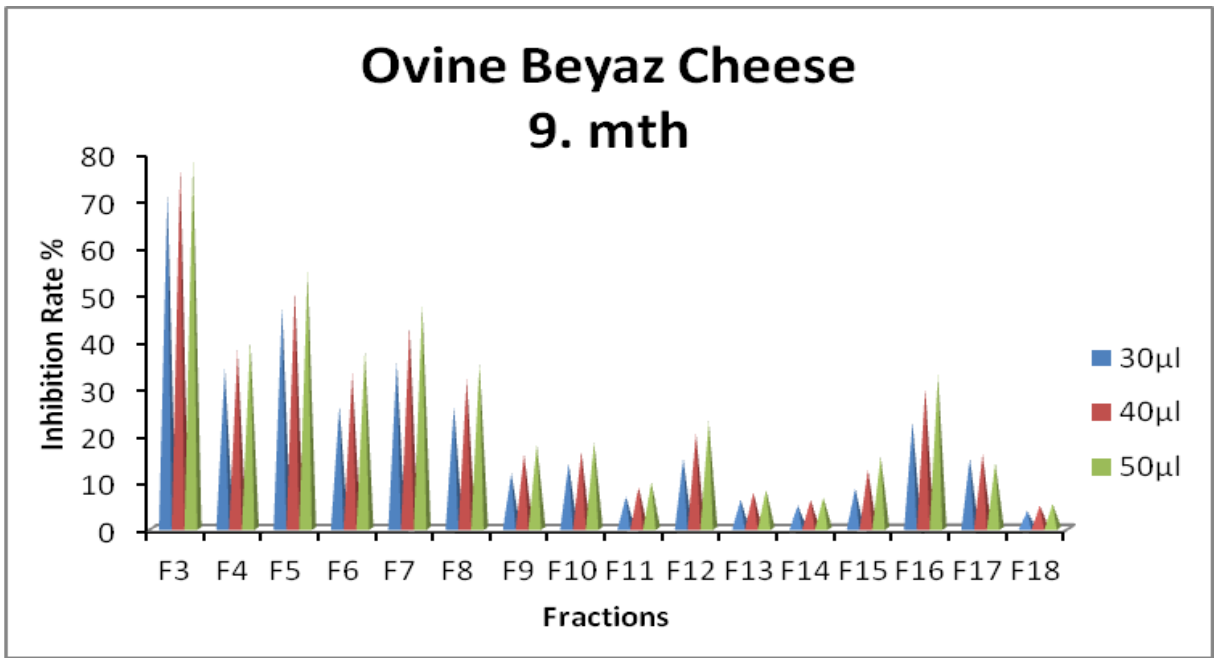

Fig 4. Inhibition rate % of ovine beyaz cheese fractions in 9.mth

Table 1. Antioxidant activity of cheese

6. mth	InhibitionSlope %	mMTrolox/g	9.mth	InhibitionSlope %	mMTrolox/g
F5	0,8646	940,70	F3	2.0003	2176,37
F7	0,6310	686,54	F5	1,0284	1118,92
F8	0,3177	345,66	F7	0,4854	528,12

Table 2. Antioxidant activity of fractions

6. mth	InhibitionSlope %	mMTrolox/g	9.mth	InhibitionSlope %	mMTrolox/g
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Acknowledgements

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MICROBIOLOGICAL AND PHYSICAL PROPERTIES OF TURKISH WHITE CHEESE

Zübeyde ÖNER Ayşe Mine SARIDAĞ

Süleyman Demirel University Engineering Faculty Food Engineering Department

Isparta-TURKEY

zubeydeoner@sdu.edu.tr, a.mine_srdg@outlook.com.tr

Abstract

The objective of this study is to determine microbiological and biochemical changes of Turkish White cheese from manufacture to the end of the ripening period. Total solid, fat in solid, titratable acidity, pH, salt in total solid, total nitrogen, water-soluble nitrogen, ripening index were determined. Also, the counts of lactic acid bacteria, total mesophilic aerobic bacteria, yeast, moulds and coliforms were done. Total mesophilic aeropbacteria counts were found 6,85-6,99 logcfu/g, and they were not change significant during the ripening ($p>0.05$). Lactic acid bacteria (i.e. lactococci and lactobacilli) were quantitatively the dominant groupsandchange of their viable numbers was significant throughout the ripening period ($p<0.05$). Numbers of microorganisms indicative of the hygienic quality, such as coliforms, enterococci and staphylococci were present in cheese at relatively high levels. Numbers of staphylococci and yeast and mould were not reduced significantly ($P>0.05$), while numbers of coliforms were also reduced significantly depending on the ripening time ($P<0.05$).

Keywords: white cheese, microbiological change, biochemical change

Introduction

Turkey has a very long tradition in producing a great variety of fermented dairy products and white cheese is one of the most significant among them. The average annual consumption of white cheese was estimated to be around 600.266 tons in 2013 [1]. Turkish White cheese is usually manufactured from raw milk in small plants. Starter culture is not added to the milk during cheese-making. Microbial composition of the cheese changes during ripening. Lactic acid bacteria predominate over the other microbial groups during ripening and thus, may regulate the ripening characteristics. The flavour profile of a given food product is generally the most important criterion for consumers' preference. Development of unique aromas in cheeses is the result of a complex set of interdependent reactions. Microorganisms in milk have an important role on the formation of aromas in cheese. The typical flavour of white cheese depends on the manufacturing operations and the presence of ripening agents, such as native milk enzymes, and the enzyme systems of the non starter microflora. However, the type of milk used in cheese-making influences the quality and organoleptic characteristics of the cheese.

Material and Methods

Cheese-Making: Artisanal Turkish White cheeses were manufactured according to traditional procedures. The cheese was ripened in the cans at 5–6 ^{o}Cf or 270 days. Cheese manufacturing was performed in triplicate. Cheese samples were taken after 0, 90, 180, 270 days of ripening.

Physico-Chemical Analysis: The percentage of total solids (TS) and lactic acid, fat and percentage of NaCl were determined according to the IDF Standards [2, 3,4]. Total nitrogen (TN) and water-soluble nitrogen (WSN) contents were determined by the Kjeldahl method [5]. The ripening index (RI) was calculated from the ratio of soluble nitrogen to TN.The pH values of the cheeses were measured with a pHmeter (HANNA). Allphysico-chemical analyses were performed in duplicate.

Microbiological Analysis: For microbiological analyses, 10 g of cheese sample was taken and decimal dilutions were prepared with the Ringer solution. Total mesophilic aerobic bacteria (TMAB) was counted by plating the appropriate dilution of cheese sample on Plate Count Agar (Merck, Germany) and incubated, at 30^{o} C for 48 h. Yeasts and moulds were cultivated on Potato Dextrose Agar (Merck, Germany) (pH3.5) at 24 ^{o} C for 4 days. Coliform bacteria were determined on Violet Red Bile Agar (Merck, Germany) at 37^{o} C for 24 h and Staphylococci on Baird Parker Agar (Merck, Germany) at 37 ^{o} C for 24–48 h. Lactobacilli were counted on MRS agar, lactococci on M17 agar, at 28 ^{o} C for 48 h. [6-8]

Statistical Analysis: Statistical analysis was performed by using the SPSS System for Windows.

Results and Discussion

The physico-chemical characteristics of the cheeses during ripening period are shown in Table 1. The procedure of ripening period of the cheese involves changes in the physico-chemical properties of the cheese accompanied by the development of a characteristic flavour. The levels of TS, pH, fat and FT were found to be in agreement with the Turkish Standard 591(Table 2). According to TS 591 total solid in the cheese not to exceed 60 g/100 g, fat in solid should be minimum 45 g/ 100 g and pH is required to be higher than 4.5. While the acidity and TS decreased, salt, WSN content and RI values of the ovine Beyaz cheese increased during storage. The pH values showed decrease during the ripening. Ripening index was 59%, 90,11 % at 180 days, 270 days respectively (Table 3). However, some fluctuations were observed in moisture,

S/S, and total protein contents of the cheeses during ripening may be due to salt absorption and/or water diffusion of some soluble components between brine and cheese mass [9,10, 11].

Table 1. Chemical Analysis of Beyaz Cheese

Beyaz Cheese	TS (%)	FS (%)	SS (%)
0 Day	50,99 ± 1,6^{a*}	48.18± 1,2^{a}	6,42 ±0,2^{c}
90 Day	50,78 ±1,7^{a}	48.24± 1.1^{ab}	6,27 ±0,2^{c}
180 Day	39,87 ± 1,1^{b}	58.03± 1,3^{b}	14,9 ±1,0^{a}
270 Day	40,48±0,07^{b}	70.52± 1.7^{c}	14,04±0,14^{b}

TS: Total solid, FS: Fat in solid, SS: Salt in solid ^{a,b}Means in the same column having different letters are significantly different *(P<0.05)*

Table 2. Acidity of Beyaz Cheese

Beyaz Cheese	Lactic Acid %	SH	pH
0.Day	1.35 ± 0.03[b*]	60,43 ± 2,6[b]	5,73 ± 0,02
90. Day	1.50 ± 0.04[a]	68 ±3,1[a]	5,7 ± 0,02
180. Day	1.39 ± 0.13[b]	61,50 ± 3,2[b]	4,94 ± 0,04
270. Day	1.32 ± 0.05[b]	59,75 ± 3,7[b]	4,98 ± 0,01

[a,b]Means in the same column having different letters are significantly different *(P<0.05)*

Table 3. Protein of Beyaz Cheese

Beyaz Cheese	Protein %	Protein in water %	Ripeningindex
0.Day	14,334 ±0,25[a]	1,419±0,12[d]	9,77
90. Day	13,869 ±0,43[b]	1,973 ±0,07[c]	14,23
180. Day	8,767 ±0,87[c]	5,172 ±0,18[b]	59
270. Day	6,925±0,39[d]	6,245±0,08[a]	90,11

[a,b]Means in the same column having different letters are significantly different *(P<0.05)*

Microbiological analysis

The evolution of the microbial groups was examined throughout ripening. As shown in Figure 1, all groups continued their presence in cheese. All of the groups except for coliforms were at maximum levels at the first day. Numbers of lactococci were slightly higher than those of lactobacilli and TMAB. In other studies on raw milk cheeses, the predominance of lactococci during the early stages of ripening has also been reported [12, 13]. Lactic acid bacteria (i.e. lactococci, lactobacilli) were quantitatively the dominant groups, and change of their viable numbers were significant (P<0.05) throughout the 90 days. Numbers of microorganisms indicative of the hygienic quality, such as coliforms, enterococci and staphylococci were present in cheese at relatively high levels. These counts suggest that contamination was very high in raw milk. Numbers of coliforms, staphylococci and enterococci were reduced significantly (P<0.05), depending on the ripening time, but they remained alive. This can be explained by the pH levels and the quantity of lactic acid.

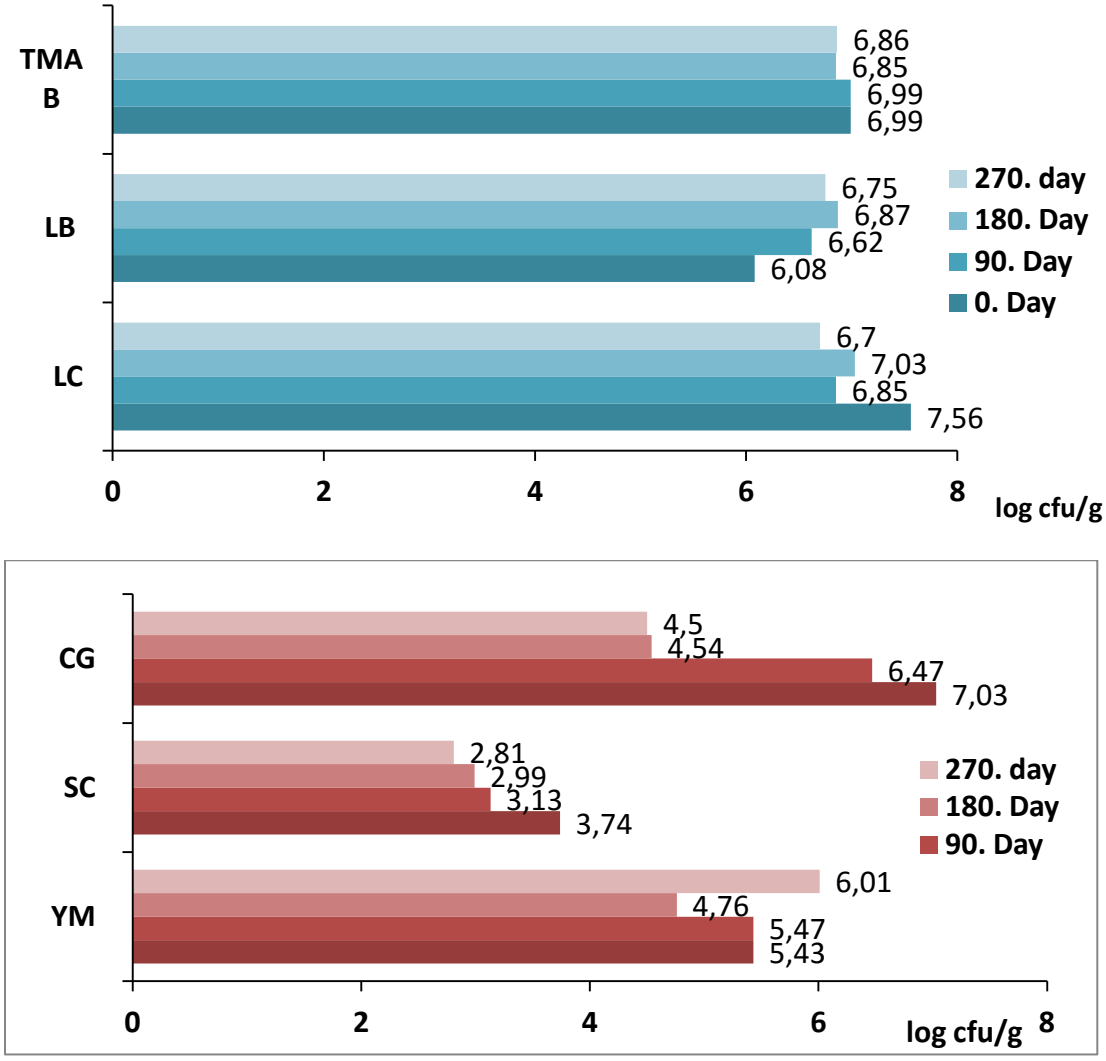

Fig 1. Mean counts (log cfu/g) of microorganisms in cheeses during ripening

LC: lactococci; LB, lactobacilli; EC: enterococci; TMAB: total mesophilicaerobicbacteria; SC:staphylococci; CG: coliform group; YM: yeasts and molds

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EFFECT OF SALT REDUCTION IN INDUSTRIALLY MANUFACTURED HARD AND SEMI-HARD CHEESE (FOIL RIPENED GOUDA AND EMMENTAL TYPE)

Jan POMMER*, Veronika DIEKER, Anne BUCHHOLZ, Dirk EUWENS, Ralf ZINK

Corporate R&D, Group Cheese-Development

(DMK Deutsches Milchkontor GmbH, Bremen, Germany)

jan.pommer@dmk.de

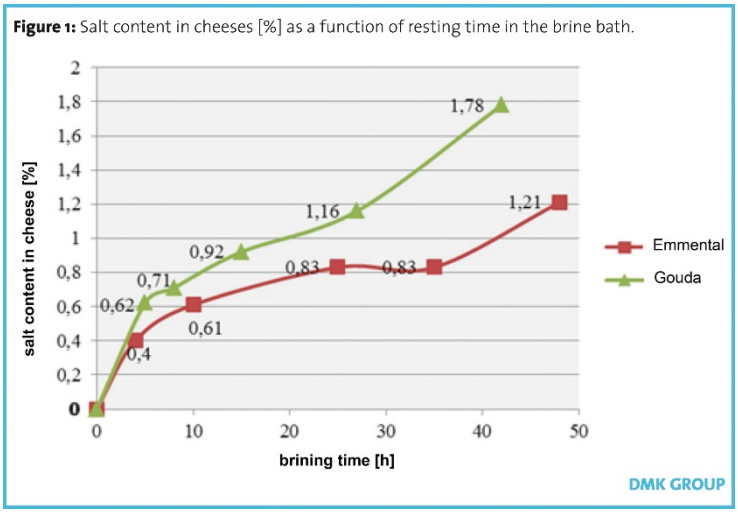
Introduction

From a nutritional point of view, a maximum intake of no more than 6g sodium salt per day is recommended. However, the daily consumption in Europe is more than double of the recommended amount. This situation significantly increases the mid- to long-term risk of cardiovascular diseases [1, 2]. Alongside bread, cheese is still one of the major sources of sodium in food systems, even though it is only consumed in small amounts. The objective of these studies was to ascertain the feasibility of a significant salt reduction in different cheese systems with constant and demanded high-level sensory properties and overall product quality [3].

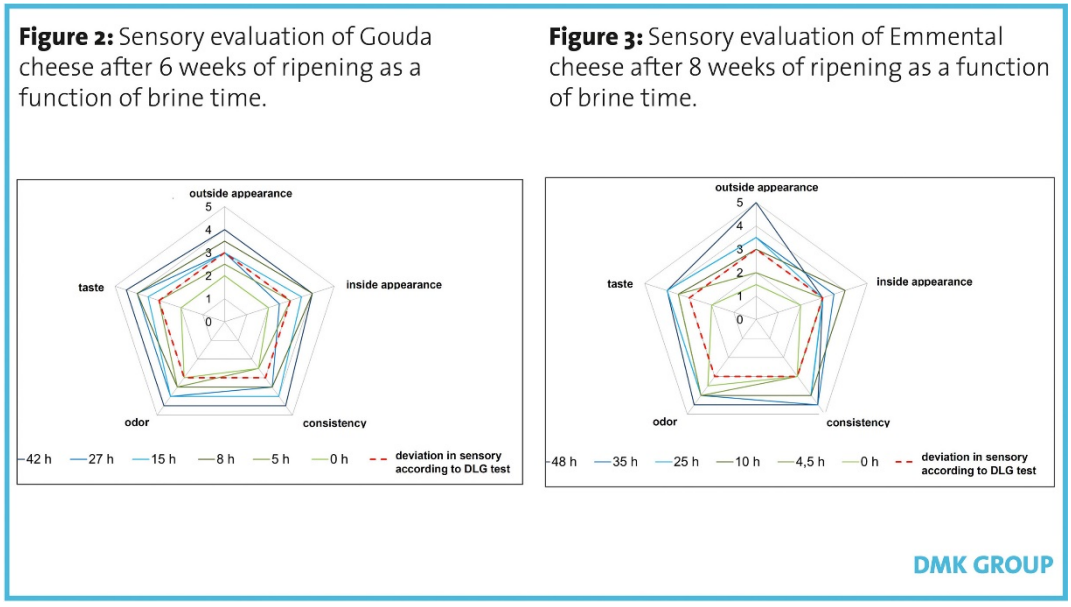
Results and Discussion

Different cheese systems and salinities were varied and modified during the process work flow at the MIC (Milk-Innovation-Center) in Edewecht (Germany). Various Gouda (48% f.i.d.m.) and Emmental (45% f.i.d.m.) cheese-types were investigated. Pressed curd was placed in the brine bath for different resting times (Gouda: 5h, 8h, 15h, 27h, 42h; Emmental: 4,5h, 10h, 25h, 35h, 48h) or already directly packed after pressing. After ripening, cheeses were analyzed regarding their intrinsic salinity levels. The resulting effects on product quality, sensory characteristics and thermo-physical properties as a function of the various maturation phases were additionally investigated.

Figure 1 shows the salt content in cheeses [%] as a function of resting time in the brine bath. For both cheese types, salinity increased rapidly and mostly at the beginning of the cheese-making process. This was attributed to the higher initial concentration gradient between the brine and the lower concentrated moisture phase in the inside of the cheeses. With increasing salt content in cheeses this gradient decreased and the rate of salt diffusion declined. Compared to Emmental cheese, Gouda cheese absorbed a significantly overall higher amount of salt and the uptake took place more rapidly in a shorter timeframe. Emmental cheese is characterized by a higher dry matter which interferes with the salt diffusion process.



In Gouda cheese, sensory evaluation resulted in minor deviations compared to the used reference already at a brining time of 27 hours (Figure 2). When Gouda was exposed to the brine bath for equal or less than 8 hours, significant differences were detectable. In Emmental cheese, differences in sensory were apparent after a brining time of 10 hours or less (Figure 3).



Cheese firmness was also influenced by overall extrinsic and intrinsic salinity. With decreasing salt content within the cheese, the resulting firmness declined. Additionally, longer ripening times resulted in softer cheeses. This was caused by microbiological and enzymatical degradation of proteins in cheese systems.

The thermo-physical characteristics (cheese browning, melting behaviour) as well as the functional properties (e.g. slicing and shredding capability, clotting) still decreased with reducing the salt content and progressive ripening time. Nevertheless, additional work in this context indicated that an improvement in the characteristics is feasible.

Conclusions

- A selective and defined possibility to lower overall salt contents could be demonstrated. These process-related-results could have a direct contribution towards lowering the overall daily amount of salt intake within a balanced diet containing dairy products.
- Salt reduction in industrially manufactured Gouda cheese is possible to an overall certain extent without resulting in negative impacts on desired sensory and technological requirements. It was observed that a brining time of 15 hours, which corresponds with a salt content of 0,9 %, represents the lowest limit for producing Gouda with appropriate thermo-physical and functional properties.
- In Emmental cheese, the reduction of salt content had a lower influence on cheese characteristics because of its different chemical composition, especially with regard to its higher dry matter.

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A NEW TECHNOLOGY TO PRODUCE A WHEY INGREDIENT LOW IN BACTERIAL COUNTS

Sven-Rainer DEORING*, Ralf ZINK

Corporate R&D, Group Dairy Process-Development

(DMK DeutschesMilchkontor GmbH, Bremen, Germany)

svenrainer.doering@dmk.de

Introduction

Whey ingredients like whey protein concentrates (WPC) up to 60-85% play an important role in the entire food industry in various food applications. In this segment quality demands rise constantly year by year. One important quality issue is the thermophilic bacterial count [1,2]. These bacteria have the nature to grow at temperatures above 40-45°C and will only be inactivated by temperatures above 121°C. This kind of bacteria is undesired in the range of dry blended products, as its presence can negatively influence shelf-life and functionality of ingredients as well as final products dramatically [4].

The aim of this new technology is to supply the dairy industry with the opportunity to remove these bacteria without applying any high heat treatment. Therefore, the main step to remove thermophilic bacteria is a cold microfiltration step, which will be described further below.

Initial Situation

In a first step the source of the thermophilic bacteria was overall analyzed in detail. Figure 1 shows the amount of thermophilic bacteria in a standard whey from an average continental cheese production monitored over a period of 20 hours. In average this whey resulted in thermophilic bacterial counts of at least 40 CFU/g. This observation indicates that the problem with any thermophilic bacteria is mainly caused by the sweet raw whey in its origin. Processing of such a sweet whey - containing bacterial counts as stated above - to WPC with up to 80% protein in dry matter via ultrafiltration (UF) results in average in thermophilic bacterial counts of approximately 1.200 CFU/g as outlined in Figure 2. Usually during the whey processing steps to WPC products, the overall protein is concentrated by a factor of 10 to 40 times depending on the type of WPC produced [3,5]. In principal the thermophilic bacterial counts develop in a linear manner along the same concentration factor (cf) from raw whey to resulting WPC products. All experimental work was performed at the MIC (Milk-Innovation-Center) in Zeven (Germany).

Figure 1: Thermophilic bacterial counts in a standard sweet whey.

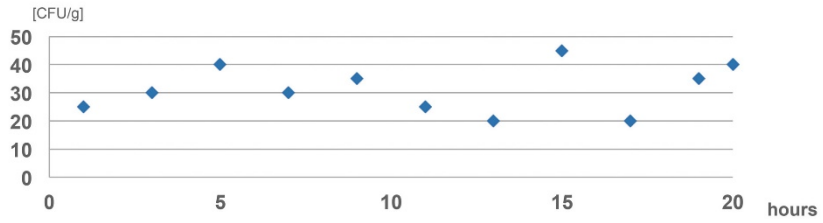
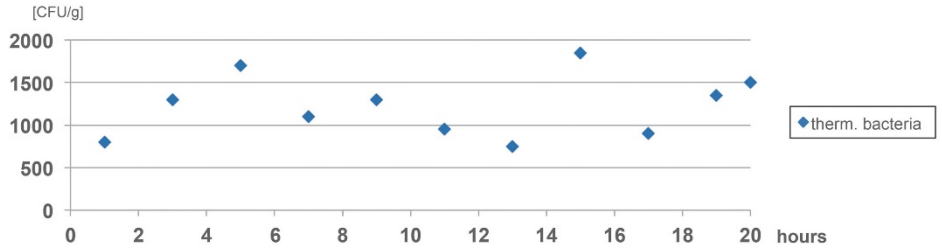


Figure 2: Thermophilic bacteria in a standard whey protein concentrate.



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Results and Discussion

By using this cold MF (Figure 3) it is possible to obtain a whey ingredient having an overall total thermophilic bacterial count of less than 100 CFU/g as shown in Figure 4 over a time-span of 20 hours.

Combining this MF step with various UF-lines it is possible to produce a wide range of whey protein concentrates with highly significant low thermophilic bacterial counts [1,5]. This product range offers the instant advantage to enable a direct access to new markets and future applications. Additional functionalities and applications of these products will be further examined.

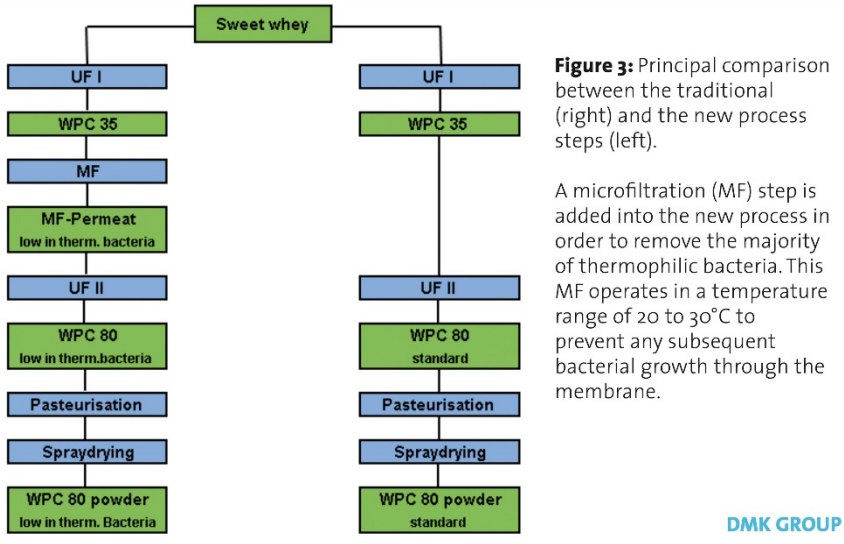

Figure 3: Principal comparison between the traditional (right) and the new process steps (left).

A microfiltration (MF) step is added into the new process in order to remove the majority of thermophilic bacteria. This MF operates in a temperature range of 20 to 30°C to prevent any subsequent bacterial growth through the membrane.

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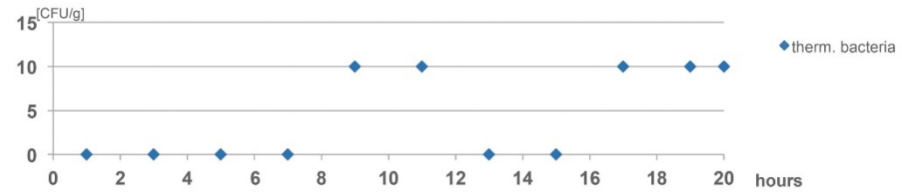
Figure 4: Thermophilic bacteria in microfiltrated UF-concentrate.



Conclusions

- It is possible to produce whey protein concentrates with low thermophilic bacterial counts.
- The applied microfiltration shows a highly stable performance over a period of 20 hours and even more when operated in a cold temperature range of approximately 20 to 30°C.
- The load of mesophilic bacteria that may grow through the membrane is directly inactivated by the heat treatment applied before drying the liquid protein concentrate.
- This new technology development (patent pending) paves the way for a wide variety of new whey ingredients and their subsequent application.

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INFLUENCE OF PACKAGING ON LONG-TERM STABILITY OF EVAPORATED MILK CUPS

Mareike HUNOLD*, Markus STEING, Markus SCHACHT, Wilhelm BEIDENHAUSER, Sven-Rainer DOERING, Ralf ZINK

Corporate R&D, Group Dairy Process-Development and Group Packaging-Development

(DMK Deutsches Milchkontor GmbH, Bremen, Germany)

Mareike.hunold@dmk.de

Introduction

Long-term stability tests of evaporated milk cups showed thickening and phase separation rising over time. It can be assumed that these

Instabilities were induced by water vapour permeation

through the plastic cups into the storage environment (Figure 1). This results in product concentration and an imbalance within the product. Barrier properties of different composite films as well as storage conditions were tested under realistic, comparable conditions (deep-drawn cups with food contact) at the MIC (Milk-Innovation-Center) in Zeven (Germany).

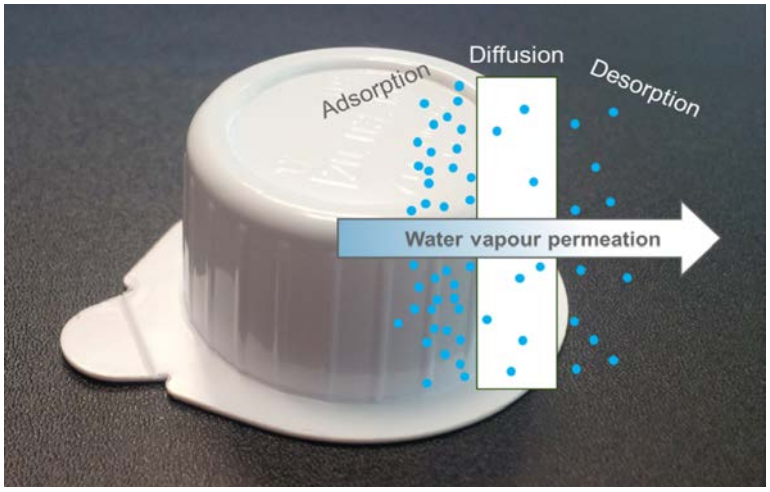

Figure 1**:** General mechanism of water vapour permeation through a plastic cup driven by a concentration gradient [1].

Objective

Selection of an appropriate polymer packaging with improved water vapour barrier in order to minimize moisture loss and to maintain product stability of evaporated milk cups over 9 months.

Approach

Evaporated milk cups (7.5 g) were stored without additional barrier and in a sealed environment (PE-Inliner inside carton). Weight loss and stability of cups made of different barrier polymers (Table 1) were determined and evaluated over a time period of 9 months.

Table 1**:** Overview of tested films, their main barrier layer and characteristic [2,3].

Barrier layer	**Film composition**	**Characteristic**
Polyethylene (PE)	PS-PE-PS	Common water vapour barrier
Ethylene-vinyl alcohol (EVOH)	PS-PE-EVOH-PE-PS	Oxygen barrier, water sensitive
High-density polyethylene (HDPE)	PS-HDPE-PS	High water vapour barrier
Polystyrene(PS)	PS-mono (competitor product)	Low water vapour barrier

Results & Discussion

The PS-mono cups of a competitor had the highest weight loss among the tested films. The cups with EVOH barrier showed a higher water vapour permeation than the cups with the common PS-PE-PS composition. The EVOH polymer is in its dry state a good oxygen barrier but not effective against water molecule diffusion [2]. These two films are not suitable for an evaporated milk packaging system as clumping and phase separation are already pronounced in the first months and long-term stability cannot be ensured (Figure 2). Taking PS-PE-PS cups as a basis the water vapour permeability was lowered by about half by using a film with PS-HDPE-PS composition. This HDPE polymer acts as an excellent water vapour barrier due to its low degree of branching, close packing and resulting high density so that water vapour diffusion is prevented [3]. The packaging of the cups into aPE-Inliner, which provides an additional barrier, gives another opportunity to reduce moisture permeation. The sensory analysis showed that the cups made of these three films differ just slightly from each other in overall quality with regard to water vapour permeability (Figure 2).

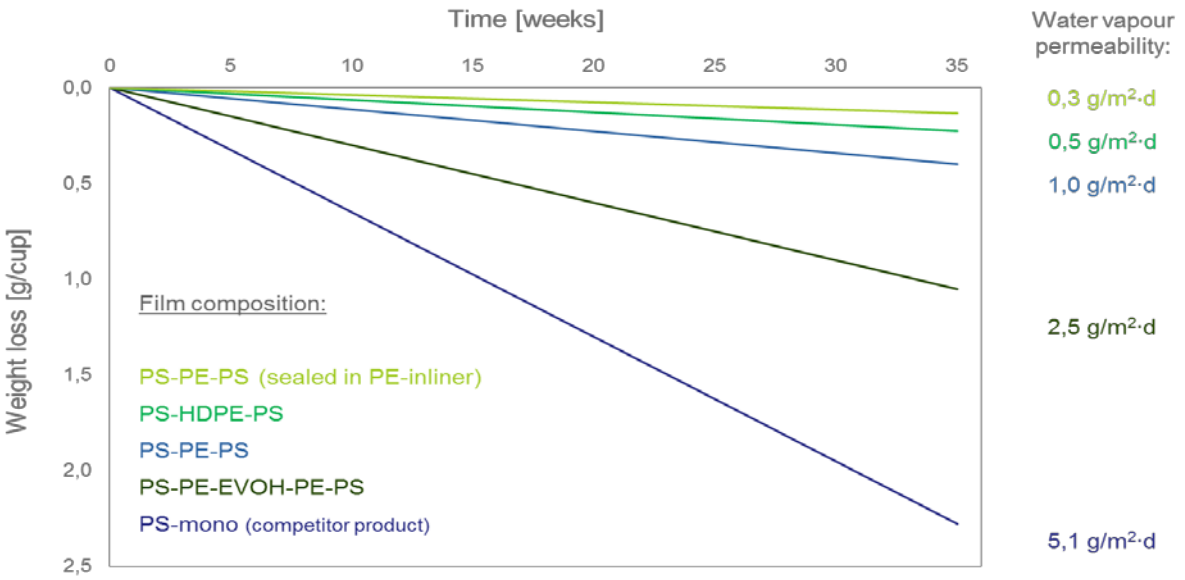

Figure 2:Linearized weight loss of evaporated milk cups with different film compositions measured over 9 months and corresponding water vapour permeability.

Conclusions

- Instabilities like thickening and phase separation were more pronounced in evaporated milk cups with an overall high weight loss. Thus, an improvement of appropriate barrier properties will contribute to maintain overall product stability within its shelf life.
- Two highly effective packaging options for evaporated milk cups against a water vapour permeability were determined:
    - Improved water vapour barrier by HDPE layer
    - PE-Inliner inside carton as additional barrier

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ACIDIC WHEY – A BASIC RAW MATERIAL FOR VARIOUS FOOD PRODUCTS

Katja BORCHERDING*, Matthias HOCH, Ralf ZINK

Corporate R&D, Group Dairy Product Development

(DMK Deutsches Milchkontor GmbH, Bremen, Germany)

katja.borcherding@dmk.de

Introduction

Most acidic whey is considered as a by-product, usually going to waste, partly because of its poor taste. Nevertheless, this whey contains vitamins, mineral salts, lactose and proteins and therefore has varying emulsifying potential[1]. At the MIC (Milk-Innovation-Center) in Zeven (Germany), a specific formulation and adapted process were generated. The aim was to develop a basic dairy recipe for the preparation of industrial emulsion products like dressings or delicatessen sauces.

Does acidic whey have an emulsifying potential?

- Comparison of different acidic whey contents in emulsified products
- Analysis of various additional components

Which process is suitable for developing acidic whey products?

- Develop an adapted yoghurt process
- Application trials in dressing in the MIC (Milk-Innovation-Center)

Results and Discussion

Recipe & Process

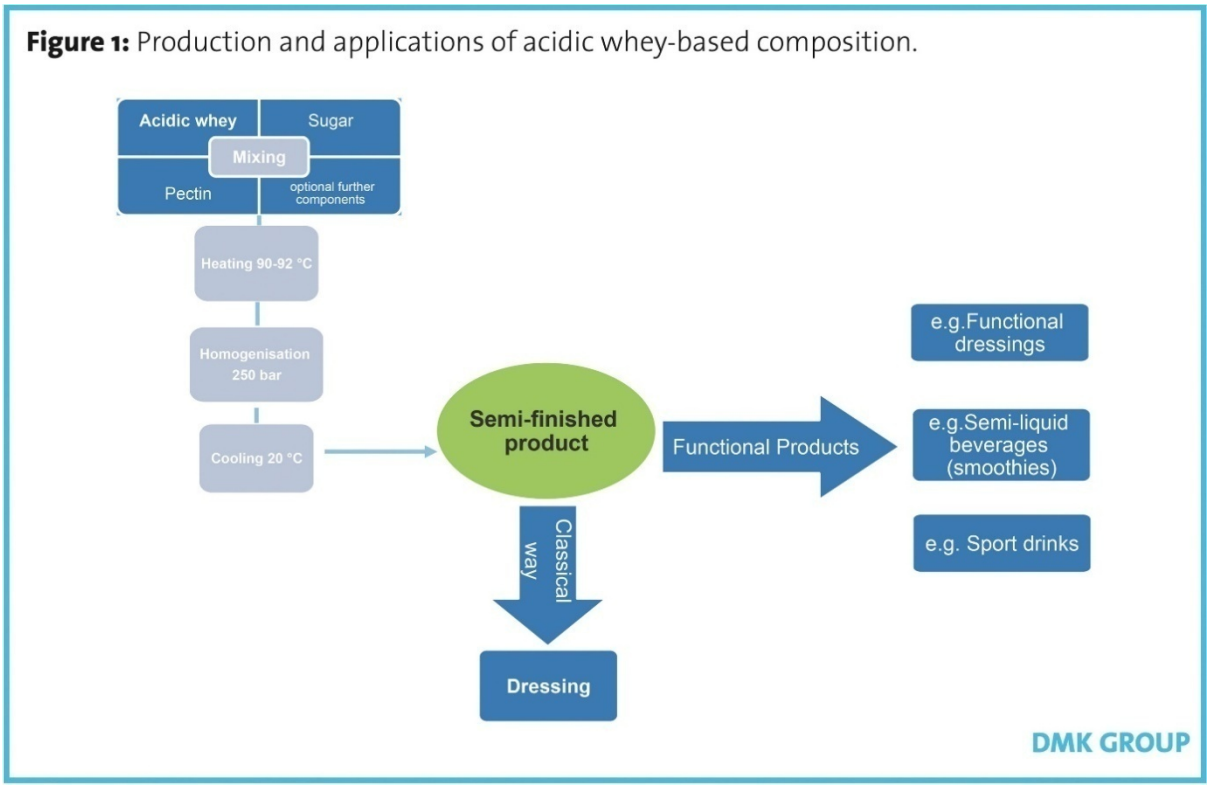
The basic composition consisted of acidic whey, pectin and sugar. Different pectins and varied dosages were tested. The stability and organoleptic properties of all compositions were assessed (Table 1). Dosage of 1% pectin or more led to the best results regarding stability and taste.

The influence of further components like yoghurt, butter milk powder, whey protein powder, egg yolk or egg yolk fractions were additionally tested. For manufacturing the acidic whey semi-finished product a standard yoghurt process was adapted (Figure 1).

Table 1: Semi-finished products with different pectin contents.

	Semi-finished product					Assessment	
Mixture	Acidic whey [g]	Yoghurt [g]	Pectin [g]	Sugar [g]	Egg yolk [g]	Visuell stability	Organoleptic assessment
A	67	33	0.42	-	-	stable	inadequate
B	67	33	0.42	5.43	-	less stable	inadequate
C	67	33	0.8	5.43	-	fairly stable	good
D	67	33	1.1	5.43	-	stable	good
E	67	33	1.1	5.43	0.84	stable	adequate
F	67	33	1.0	5.43		stable	good

Figure 1: Production and applications of acidic whey-based composition.



Application trials in dressing

For testing the emulsifying potential of the semi-finished product a common dressing process was used. The test series were performed with an IKA equipment in the MIC in Zeven. The stability was determined by particle size distribution measurements in x(50 %) µm (HELOS laser diffraction particle analysis, Sympatec GmbH).

The evaluation scale is shown in the following:

< 10 µm = very good

< 20 µm = good

< 30 µm = adequate

> 30 µm = inadequate (i.e. unstable)

The results demonstrated that combinations of acidic whey, pectin and sugar have respective emulsifying properties [2,3]. An adequate emulsion stability with a median value lower than 30 µm was observed in the final emulsion (Table 2). The addition of further surface active components like egg yolk could improve the emulsifying properties of the acid whey-based composition and dressing stability. However the sensory acceptance for these samples was lower (Table 2).

Table 2: Particle distribution of semi-finished products in dressings (20 % fat content) with and without egg yolk.

Mixture	Acidic whey [g]	Yoghurt [g]	Pectin [g]	Sugar [g]	Egg yolk [g]	Particle distribution in dressing x (50 %) µm
I	67	33	1.0	5.43		29.0
J	67	33	1.0	5.43	0.84	6.6
M	100	-	1.0	5.43		20.5
N	100	-	1.0	5.43	0.84	5.7

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All trial results demonstrated clearly that acidic whey based semi-finished products offer various possibilities for new developments, for example egg-free dressings [4]. Additionally, it offers the possibility of cost-improvements and rendering an additional health-benefit to a given product range.

Patents:

• EP 288945 (A1) – Compositions based on acidic whey (DMK Deutsches Milchkontor GmbH)

• EP 288946 (A1) – Use of pectin for improving the sensorial properties of compositions based on acidic whey (DMK Deutsches Milchkontor GmbH)

Conclusions

- Acidic whey has an emulsifying potential in various food applications.
- It is possible to create an emulsion on the basis of acidic whey without further surface active components (Texturizing benefit).
- Based on its content of vitamins, mineral salts, proteins and lactose, this could facilitate and improve the development of functional product concepts with additional health or wellness benefits (Nutritional benefits).

- Therefore, acidic whey has unique double functional properties (i.e. textural & nutritional) for various food applications.

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INFLUENCE OF COLD MICROFILTRATION FOR GERM REDUCTION ON THE COMPOSITION AND FUNCTIONAL PROPERTIES OF SKIM MILK POWDER

Anja KAPPLER*, Sven-Rainer DEORING, Ralf ZINK

Corporate R&D, Group Dairy Process-Development

(DMK DeutschesMilchkontor GmbH, Bremen, Germany)

anja.kappler@dmk.de

Introduction

A newly developed, practically germ-free skim milk- powder (SMP) is subjected to a microfiltration (MF) step at a temperature below 30°C prior to concentration and spray drying. The cold microfiltration is expected to have influence on SMP composition, physico-chemical properties and functionality. Aim was to identify these influences and to characterize the new SMP.

Does pre-drying microfiltration have an influenceon the chemical and physical properties of SMP?

- Comparison of a standard- and a microfiltrated SMP
- Analysis of various chemical and physical parameters during the process and of the final powder

How does the microfiltrated SMP perform when usedas an ingredient in different dairy applications?

- Comparison of a standard- and a microfiltrated SMP
- Application trials with reconstituted milk, yoghurt, ice cream and cream cheese

  in the Milk-Innovation-Center (MIC) in Zeven (Germany)

Results and Discussion

Statistical data evaluation and sensory

Statistical evaluation of differences between the MF- and standard SMP was carried out (two-tailed student‘s t-tests; statistics software by Minitab Inc., USA) and a $p<0.05$ was statistically significant. Sensory evaluation (triangle testing) of the application trials was done with a trained panel (n=20) [1].

Implications of the microfiltration on the milk during the spray drying process

The microfiltration membrane retains not only bacteria, but also a small amount of casein and fat (Table 1). Concentrated and spray dried permeate was lower in fat and casein (of total protein) than the raw milk.

Table 1: Retention capacity of a microfiltration membrane with 1.4µm pore diameter.

SAMPLE	RAW MILK FEED	PERMEATE	RETENTATE
FAT (%)	0.06 (±0)	0.04 (±0)	0.97 (±0)
PROTEIN (%)	3.385 (±0.092)	3.410 (±0.058)	4.835 (±0.021)

Physico-chemical properties of microfiltrated SMP

The MF removes some fat and casein from the milk[2].Upon evaporation concentration, the permeate has a lower viscosity, resulting in a finer dispersion and an increased incorporation of air during spray drying. Drying efficiency is increased and less humidity remains, resulting in a finer grained, less dense SMP with improved flowability, being less sticky and slightly less dispersible in water (to be be taken into account when handling the SMP for further processing). From a nutritional standpoint, the SMP‘s higher WPNI and lower fat content do not imply its nutritional quality (Table 2, Figure 1, Figure 2).

Methods (except for stickiness, which has been measured with an own method)were obtained from GEA NIRO A/S (2014) [3].

Table 2: Composition of microfiltrated and standard SMP. Statistically significant differences ($p<0.05$) are marked with a*.

Skim milk Powder	MF (Ø, (SD))	Standard (Ø, (SD))
Humidity (%)	2.82 (±0.11)*	3.69 (±0.11)*
Fat content (%)	0.36 (±0.01)*	0.75 (±0.11)*
Protein content total (%)	33.05 (±0.07)	32.8 (±0)
Casein total (%)	24.4	24.7
Whey protein total (%)	6.6	6
WPNI (mg/g)	1.77 (±0.07)*	1.5 (±0.07)*

Figure 1: Particle characteristics of microfiltrated and standard SMP.

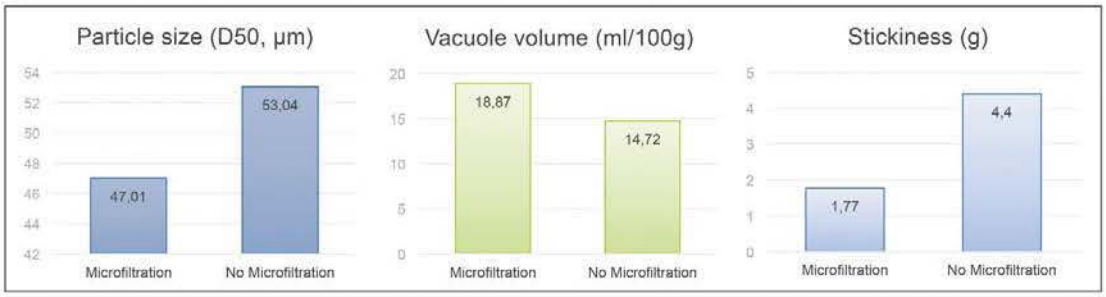

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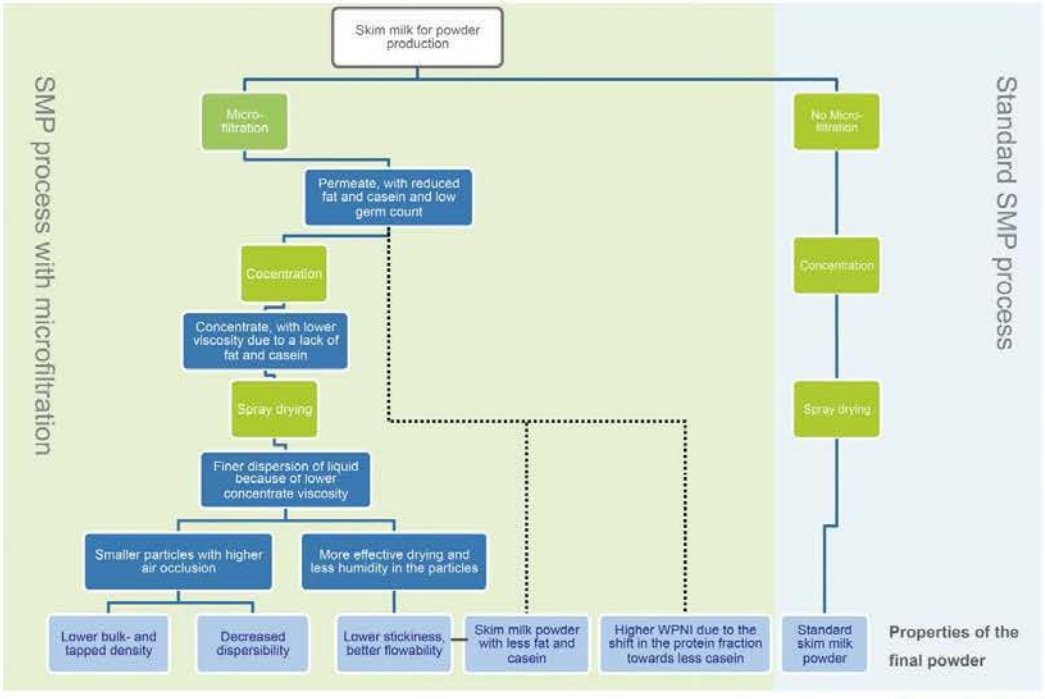
Figure 2: Implications of the MF on powder properties.



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Application trials

The microfiltrated SMP was suitable for all dairy applications. No sensory differences were found between dairy products made from standard- and microfiltrated SMP. All powders had a good heat stability. The new SMP might be of special interest for the baby food industry, as it can deliver a high nutritional and functional quality with a superior microbiological quality.

Conclusions

- With cold microfiltration prior to spray drying, a germ-reduced, still functional, high-quality SMP could be produced.
- The new SMP has slightly different properties than a standard SMP, but neither nutritional nor functional quality are negatively implicated.
- Upon further processing, the new SMP can be handled like a standard SMP.

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DEVELOPMENT OF NEW TECHNOLOGY TO PRODUCE SKIM MILK POWDER WITH LOW THERMOPHILIC BACTERIA COUNT, LOW SPORE COUNT AND HIGH PROTEIN NATIVITY

Torben WIEGERS*, Sven-Rainer DOERING, Ralf ZINK

Corporate R&D, Group Dairy Process-Development

(DMK Deutsches Milchkontor GmbH, Bremen, Germany)

Torben.wiegers@dmk.de

Introduction

Today skim milk powder is available in various qualities. They mainly differ in the heating process effecting the functionalities of the proteins by denaturation as well as the total bacterial and spore count in the final product [1]. Figure 1 shows the conflicting interests of these two parameters and the challenge this development contains.

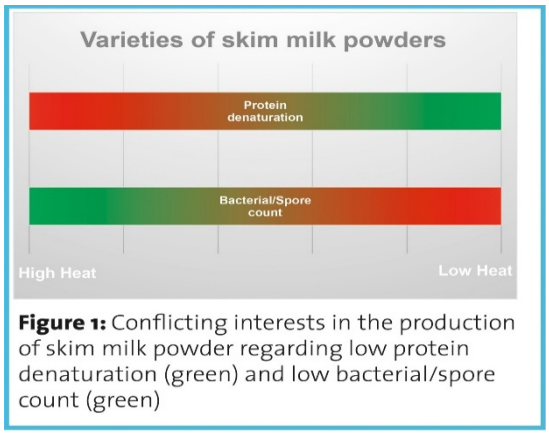

Figure 1: Conflicting interests in the production of skim milk powder regarding low protein denaturation (green) and low bacterial/spore count (green)

Aim

Key objective for this technology-development is to obtain a skim milk powder which meets market demands for high microbiological standards (as in high heat powder). Additionally, the valuable functionalities of proteins present in skim milk (SM) have to be maintained (as in low heat powder).

Specific Goals:

- Integration into the existing powder process

- Powder needs to keep its high protein functionalities which will been sured by a high whey protein nitrogen index (WPNI>6)
- A low mesophilic and thermophilic bacterial/spore count (<500 cfu/g)

All experimental work was performed at the MIC (Milk-Innovation-Center) in Zeven (Germany).

Results and Discussion

By integrating the microfiltration unit into the existing process additional new powders can now be produced meeting risen costumer standards regarding functional properties (High WPNI) and low bacterial/spore count (Patent: EP 2679098).

The final process is a variation of the ESL-Milk technology (Figure 2) [3]. Normally performed at a temperature of 55°C [2], the process of microfiltration has various negative impacts during the production cycle. By a reduction of the filtration temperature from 55°C to 25°C, thermophilic bacterial growth through the membrane is prevented. After a defined production time mesophilic bacteria may grow through the membrane, but they will not survive the evaporation process and hence will not cause a recontamination of the final powder product (Figure 3).

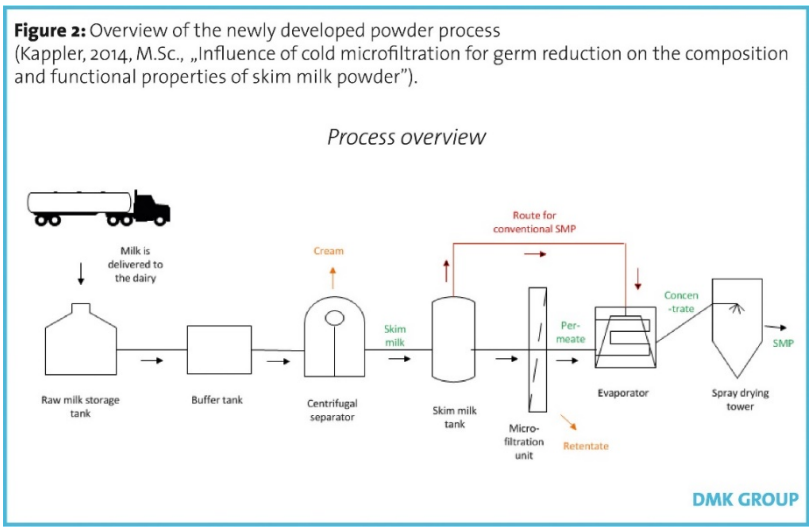

Figure 2: Overview of the newly developed powder process (Kappler, 2014, M.Sc., „Influence of cold microfiltration for germ reduction on the composition and functional properties of skim milk powder").

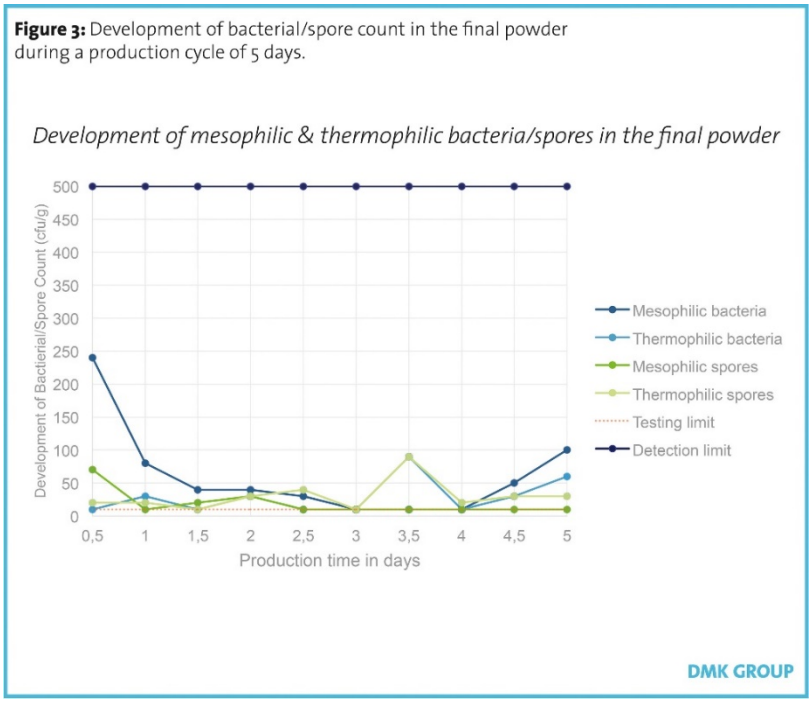

Figure 3: Development of bacterial/spore count in the final powder during a production cycle of 5 days.

Conclusions

- A number of new powder products could be introduced by the technology of cold microfiltration resulting in the preservation of the functional properties of proteins.
- By performing the process with a microfiltration unit it is possible to keep the process on a low temperature level resulting in a final powder product with a low protein denaturation in combination with a low bacterial/spore count.
- Due to the stability of the process an introduction to even higher microbiological standards is planned (<100 cfu/g).
- Further development involves the introduction of a whole milk powder based on this process (Patent: EP 2679098).

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INETGRATION OF DENATURATED WHY PROTEINS INTO THE CHEESE MATRIX BY MENAS OF HIGH-TEMPERATURE HEATING IN A PARTIAL FLOW METHOD

Jan POMMER*, Veronika DIEKER, Henrike ZIETZ, Melanie PIEPER, Dirk EUWENS, Ralf ZINK

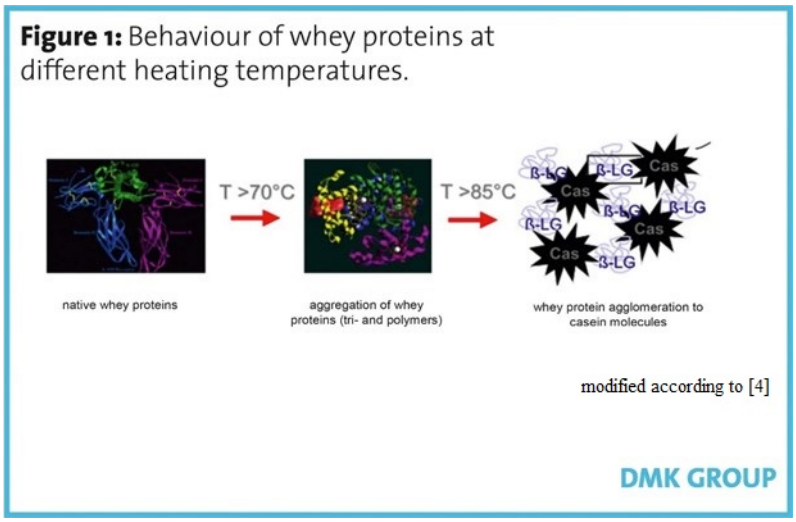
Corporate R&D, Group Cheese-Development

(DMK DeutschesMilchkontor GmbH, Bremen, Germany)

*e-mail: jan.pommer@dmk.de

Introduction

It is essential to optimize raw material utilization (e.g. for sustainability reasons) in the cheese production process to build an ideal product matrix and consider various economical factors. Technological actions are necessary to increase the cheese yield[1,2, 3]. The target of this study was to create an additional denaturation level of whey proteins through defined high-temperature treatment in a partial flow method and thereby incorporate these denatured proteins into the cheese matrix (Figure 1). Furthermore, the impact of this high-temperature treatment on thermo-physical functionalities and sensory properties was investigated.

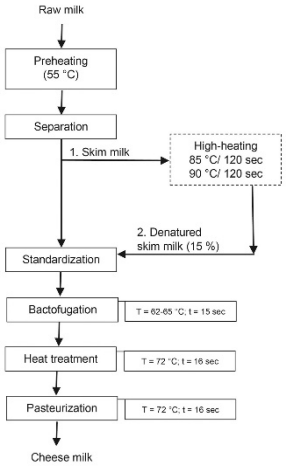


Results and Discussion

Three different types of cheese (Goudacheese 48 % f.i.d.m., Edam cheese 30 %

f.i.d.m., German „Butterkäse“ 45 %f.i.d.m.) were produced in the DMK pilot plant facilities at the MIC (Milk-Innovation-Center) in Edewecht (Germany). For each type of cheese, two different testing conditions were selected besides the standard process conditions: A partial flow of skim milk (15 % of the total milk quantity) was heated at 85 °C or 90 °C for 120 sec. For the reference samples, no heating of a partial flow was performed. The processing of cheese milk preparation is shown in Figure 2. Cheeses were analyzed after a maturation time of 4 weeks in 2-week intervals with regard to functional, thermo-physical and sensory properties.

Figure 2: Processing of cheese milk preparation.



High-temperature heating of a partial flow method enhances whey protein to total protein ratio in cheese (Figure 3). Due to high-temperature heating of a partial skim milk flow, more whey proteins were incorporated into the cheese matrix. The cheese yield increased significantly (Figure 4). While a temperature treatment of a partial skim milk flow at 85 °C had only a minor effect on cheese yield, the impact was significantly stronger at 90 °C. The output for Edam cheese and Gouda cheese increased by relatively 2.9% for Gouda and 3.9% for Edam compared to the reference cheese values, which is highly significant in a large-scale, industrial context. Regarding analysed cheese properties cheese firmness, cheese color as well as thermo-physical functions (melting behaviour, oiling-off property, cheese browning) slightly differ from the particular reference cheese. The sensory evaluation confirmed that there were no differences in elasticity, cooked flavor, mouth feeling and bitterness between the reference and the modified cheeses.

Figure 3: Whey protein to total protein ratio [%] in examined cheeses.

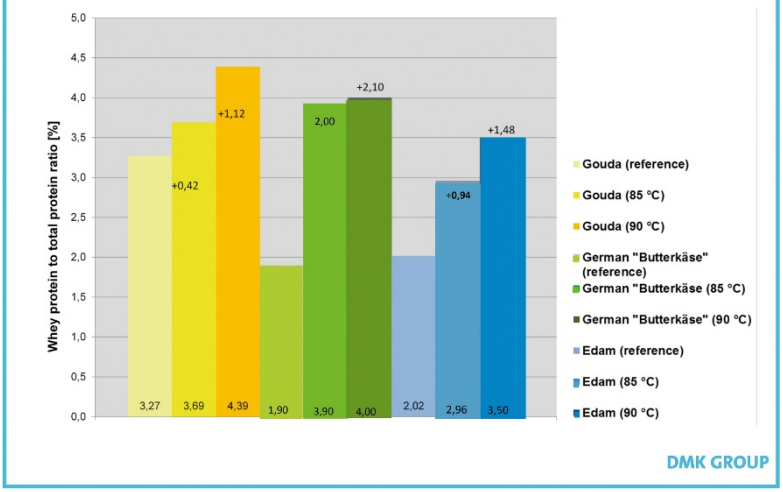
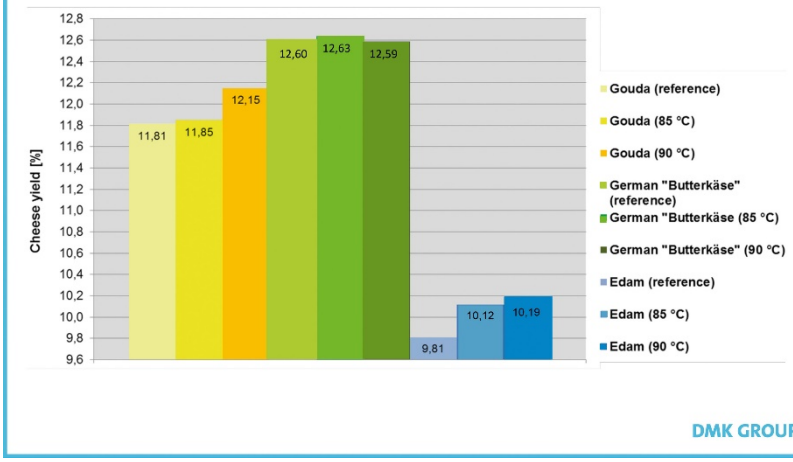


Figure 4: Cheese yield [%] of modified cheeses in comparison to reference cheese.



Conclusions

Temperature-induced high-heating in a partial flow method resulted in the integration of denatured whey proteins into the cheese matrix and in higher cheese yield.

From a sustainability perspective and an economic point of view, a temperature of 90 °C is preferred to obtain the best protein transition and an overall higher cheese yield.

The high-heating of a partial skim milk flow resulted only in slight differences. Cheeses produced with a high-heated partial flow of skim milk met customer demands and defined specifications.

Temperature high-heating in a partial flow method provided significant economical advantages in the context of sustainability and economy by reduction of raw materials.

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DETERMINATION OF LACTOSE REDUCTION DURING CHEESE PRODUCTION PROCESS AND MATURATION, AND IMPLEMENTATION OF AN ENZYMATIC PROCEDURE FOR LACTOSE ANALYSIS

Jan POMMER*, Veronika DIEKER, Melanie PIEPER, Dirk EUWENS, Ralf ZINK

Corporate R&D, Group Cheese-Development

(DMK Deutsches Milchkontor GmbH, Bremen, Germany)

jan.pommer@dmk.de

Introduction

Lactose intolerance and sensitivity require a precise knowledge of the actual lactose content of our food as well as a mastery of the tools to build a meaningful monitoring of the actual lactose content in different foods and dairy systems [1, 2]. In Germany cheese is allowed to be labelled as „lactose-free“ if the content of lactose is below 0,1 %. There is no further legal requirement for the label „lactose-free“. This limit value depends on the detection limit of lactose for enzymatic determination. Spain already made in 2006 a proposal for lowering the limit value for lactose free products from 0,1 % to 0,01 % demanding the knowledge of lactose content in cheese even more important. The target of this study was to develop an experimental design for the determination of lactose contents in real cheese production conditions during the entire production and maturation process investigated in the pilot plant facilities of the MIC (Milk-Innovation-Center) in Edewecht (Germany). The identification of relevant influential factors as well as the implementation of a trustworthy lactose analytic monitoring system were aimed.

Results and Discussion

Conventional types of cheese (Edam cheese 40 % f.i.d.m.; Maasdam cheese 45 % f.i.d.m.) were investigated. Several samples were taken from standard cheese production considering the existing process conditions (e.g. activity of cultures and possibly used direct starter set, microbiological impact during production, pH-value and temperature of the brine bath, process parameters, process interruptions).

The lactose content of the cheese samples was analysed before and after the brine bath as well as at different maturation levels by means of enzymatic determination [3] and high performance liquid chromatography (HPLC). The results of both methods were subsequently analysed and compared.

In Edam cheese proportionally the highest lactose degradation took place in the brine bath (Figure 1). Process times of the heaters were considered to investigate the impact of an undesired acidification because of accompanying bacterial microflora. Thereby, differences in lactose content of various cheese vats were observed before the brine bath. The desired limit value of less than 0,01 % lactose

was not reached after 12 weeks of maturation, although maturation time of cheese is usually lower (4-6 weeks). After a maturation time of 12 weeks 20 % of the analyzed cheese samples still had a lactose content above 0,01 %. The analysis by means of enzymatic determination correlated very well with the determination by HPLC (Figure 1).

Figure 1: Determination of lactose content (HPLC) in Edam cheese 40 % f.i.d.m.

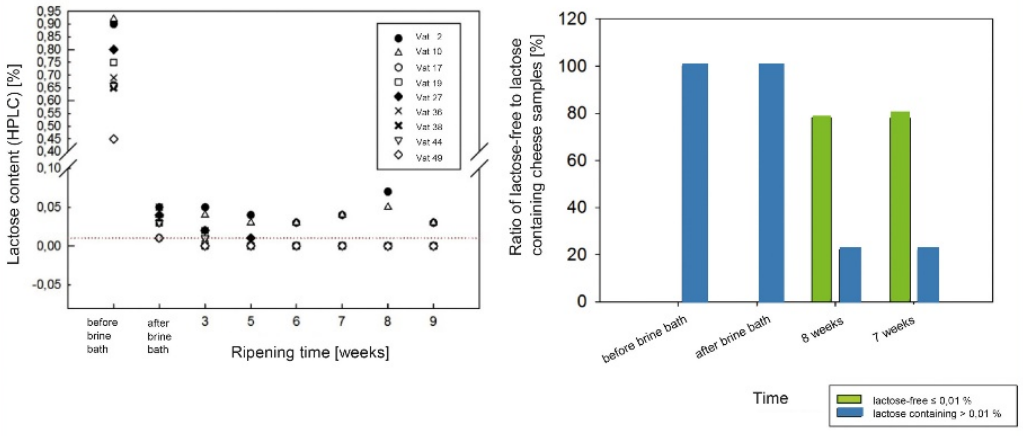
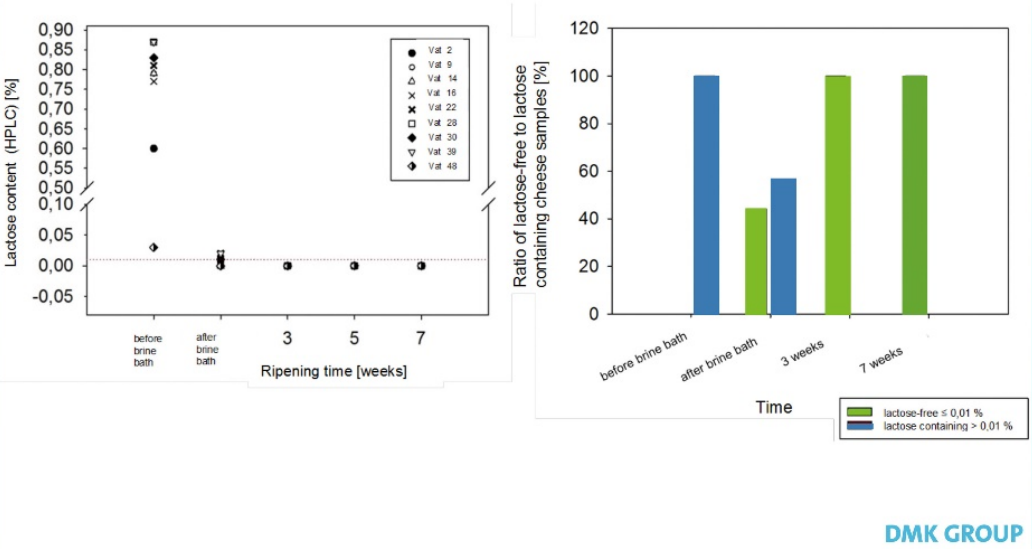


Figure 2: Determination of lactose content (HPLC) in Maasdam cheese 45 % f.i.d.m.



In Maasdam cheese lactose was also degraded mainly during resting in the brine bath (Figure 2). Already after 3 weeks of ripening, the lactose content in Maasdam cheese was already below the limit

of 0,01 %. The low lactose content could be confirmed after further ripening. The reason for this could be identified in the additional starter cultures, which metabolize lactose, as well as in different ripening conditions of the cheese (e.g. ripening temperatures). Comparing the results of enzymatic determination and HPLC, it was confirmed that there were no significant differences between the results obtained with these two analysis methods (Figure 2).

Conclusions

- In Maasdam cheese, the lactose content rapidly declined under the value of 0,01 % within a maturation time of only 3 weeks. However, the lactose content in Edam cheese did not always decrease under this level (even after longer ripening times).
- Principally, most of the respective lactose was degraded during resting in the brine bath. It could be determined that cheeses lost up to 98 % of their lactose content in the brine bath.
- Regarding the analysis methods of lactose in cheese, the enzymatic determination correlated very well with results achieved by HPLC, thus enabling a rapid, cost-effective and reliable detection-system.

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STUDIES ON MOZZARELLA PRODUCTION: CAN IMPORTANT FINAL PRODUCT CHARACTERISTICS SRUVIVE AN ECONOMIC STARTER CULTURE CHANGE AND REDUCED PRODUCTION COSTS?

Eddy VON THADEN*, Dirk EUWENS, Ralf ZINK

Corporate R&D, Group Cheese-Development

(DMK Deutsches Milchkontor GmbH, Bremen, Germany)

* eddyvonthaden@dmk.de

Introduction

There is diverse and growing economic pressure resulting from increased storage and logistical costs, as well as very different requirements from final consumers or industrial partners towards quality and final application properties [1]. This requires greater economic efficiency on the part of the manufacturer to offer products with clearly defined, recognizable, and consistent qualities. This study was completed using experimental approaches at the MIC (Milk-Innovation-Center) in Edewecht (Germany).

Aim

The aim of this study was to investigate the influences of using two different thermophilic starter culture systems (direct set cultures versus bulk starter) in Low Moisture Mozzarella Cheese (LMMC) production. More specifically, the influences on process technology and thermo-physical behavior were investigated [2, 3] with the possibility to reduce production costs in the background.

Is it possible to create a stable production process by using a thermophilic bulk starter instead of direct set cultures?

- Evaluation of the optimal parameters for thermophilic bulk starter
- Acidification curves
- Phage check

How does the use of bulk starter influence the chemical and thermophysical properties of LMMC?

- Cheese composition

- Analysis of melting [4], browning and stretching properties
- Strength measurement and texture analysis

Is there a positive economic effect with regard to manufacturing costs?

- Cost calculation

Results and Discussion

Production process

Acidification curves show slight differences in acidification rapidity between direct set cultures and bulk starter. This may be caused by variations in lag-phases. In two daily productions (40 vats), bulk starter reached target-pH before cooking and stretching the curd (Figure 1). Phage sensitivity-tests indicated negative results.

Table 1: Average composition of LMMC produced with direct set or bulk starter.

	DIRECT SET S1	DIRECT SET S2	BULK SET 1	BULK SET 1
MOISTURE [%]	46,99	46,95	47,19	46,72
FAT [%]	21,83	21,78	21,49	21,65
PROTEIN [%]	26,37	26,26	26,32	26,62
NACL [%]	1,08	1,27	1,26	1,22

Chemical and thermo-physical properties

Table 1 shows only small differences in cheese composition between thermophilic direct set starter and thermophilic bulk starter. Only small process adjustments in the mozzarella production process were necessary to obtain comparable chemical cheese characteristics. Melting, browning and stretching tests provided almost identical results. Little browning, similar stretching and melting properties were detected in mozzarella cheese produced with direct set cultures as well as in bulk starter trials. Firmness in cheese dough and cheese rind was carried out by a Texture Analyser Strength Measurement (TASM). Results show overall comparable strength properties in cheese dough and rind (Figure 2).

Figure 1: pH in cheese curd before cooking and stretching.

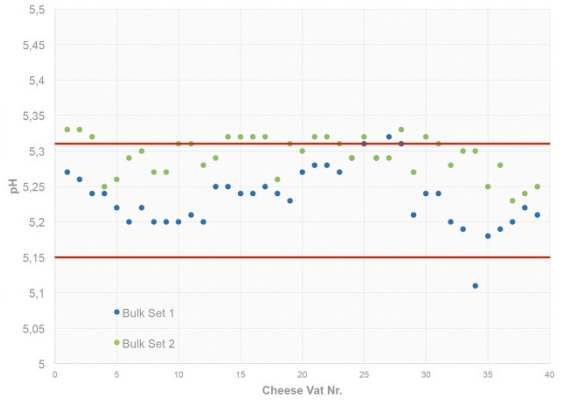
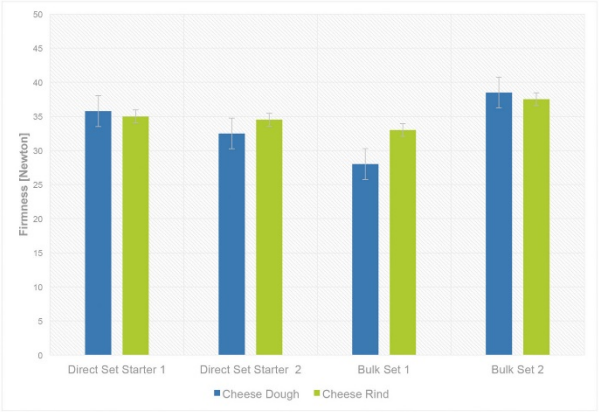


Figure 2: Firmness of LMMC, 7 days storage time (4°C).



DMK GROUP

Cost calculation

Cost calculation was done by comparison of the procurement costs for direct set starter and the procurement costs, as well as additional and related costs for breeding a bulk starter. Usage of bulk starter decreased costs by up to 54% compared to procurement costs for direct set cultures.

Conclusions

- The acidification performance of direct set cultures and bulk starter in mozzarella production process are overall comparable.
- Nevertheless, the usage of bulk starter resulted in a significant reduction in costs compared to direct set cultures.
- It was possible to implement the bulk starter system into the existing production process without major modifications.
- By using a bulk starter, no adverse effects on overall product quality and sensory parameters was observed.

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STATISTICAL ANALYZES FOR PROCESS IMPROVEMENTS MUST HAVE AN IMPACT ON CUSTOMER SATISFACTION (A SIX SIGMA APPROACH ON ITS PRACTICAL USE)

Ralf Mankiewitz* [1], Wilfried Weber[2], Dirk Euwens[1], Ralf Zink[1]
[1]Corporate R&D, Group Cheese-Development (DMK DeutschesMilchkontor GmbH, Bremen, Germany,) [2]Pro-Process GmbH, Kempten, Germany

e-mail: ralf.mankiewitz@dmk.de

Introduction

This example describes a process improvement and its impact on customer satisfaction. An interdisciplinary team of R&D, quality assurance, production processes, leading management and external support was formed. Our goal was to achieve an „Operational Excellence“ (OE) by applying new process improvement techniques for this complex task. A Six Sigma methodology was introduced [1, 2]. This practical examples increased knowledge of the Six Sigma methodology including the useage of powerful statistical tools. By implementing a statistical software for analysis support, a solid base for this methodology could be ensured.

Initial situation

Selected products can show varying types of defaults when evaluated by the customer. While the feature “taste“ was assessed as being excellent, optimization potential regarding product appearance and consistency occurred [3]. We were able to define the problem, but it was extremely difficult to quantify it, as the problem occurred only at the customers site. Retention samples did not identify the problem, visual inspection by customer random sampling confirmed the challenging task . Quality problems arose with different products and at varying frequency. No solution could be directly found, as the project was highly complex.

Approach

In order to ensure an effective and efficient approach the Six Sigma improvement model [1, 2] in a well-structured approach, including a shared vision, with the following DMAIC phases (D = Define, M = Measure, A = Analyze, I = Improve, C = Control) and highly detailed as outlined in Figure 1.

Materials and Methods

The following methods were individually applied:

- The 5-Whys to analyze the problem
- SMART Method

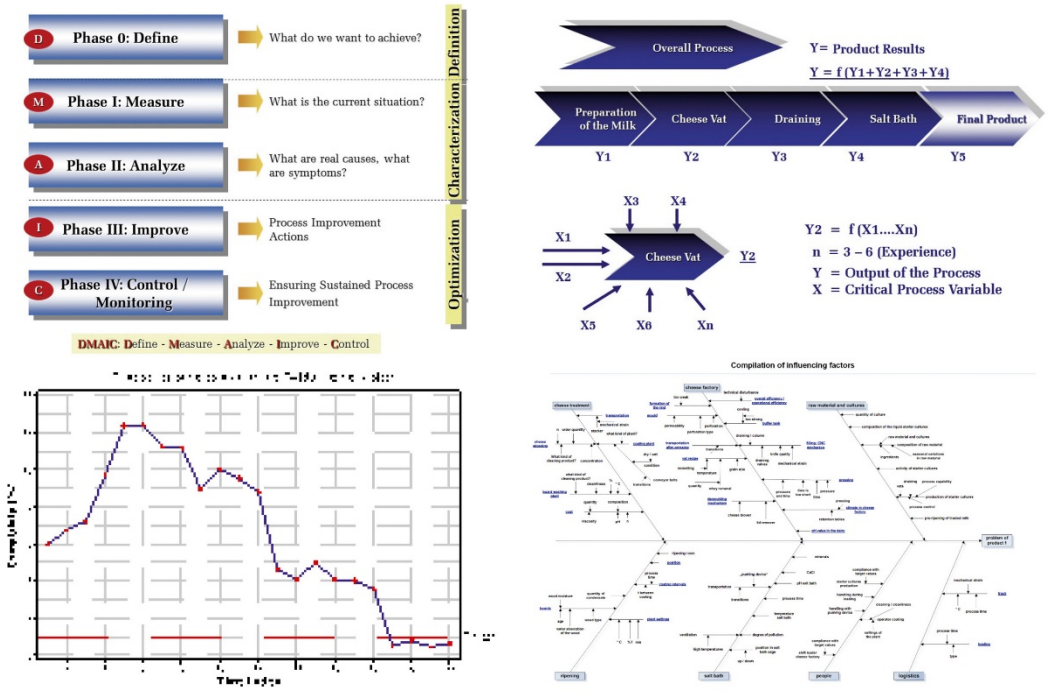
- Cause-effect Analysis
- Pairwise Comparison
- SIPOC Analysis
- Process Mapping
- Value Stream Mapping
- Short-term Capability tests
- Correlation Analyses
- Analyses of the Process Capability and Process Control
- ANOVA
- Various graphical techniques (Histograms, Probability Plots, Error Bar Charts, Time Series Graphs, etc.)

Results and Discussion

The specific tools were used in each phase of the overall improvement process.

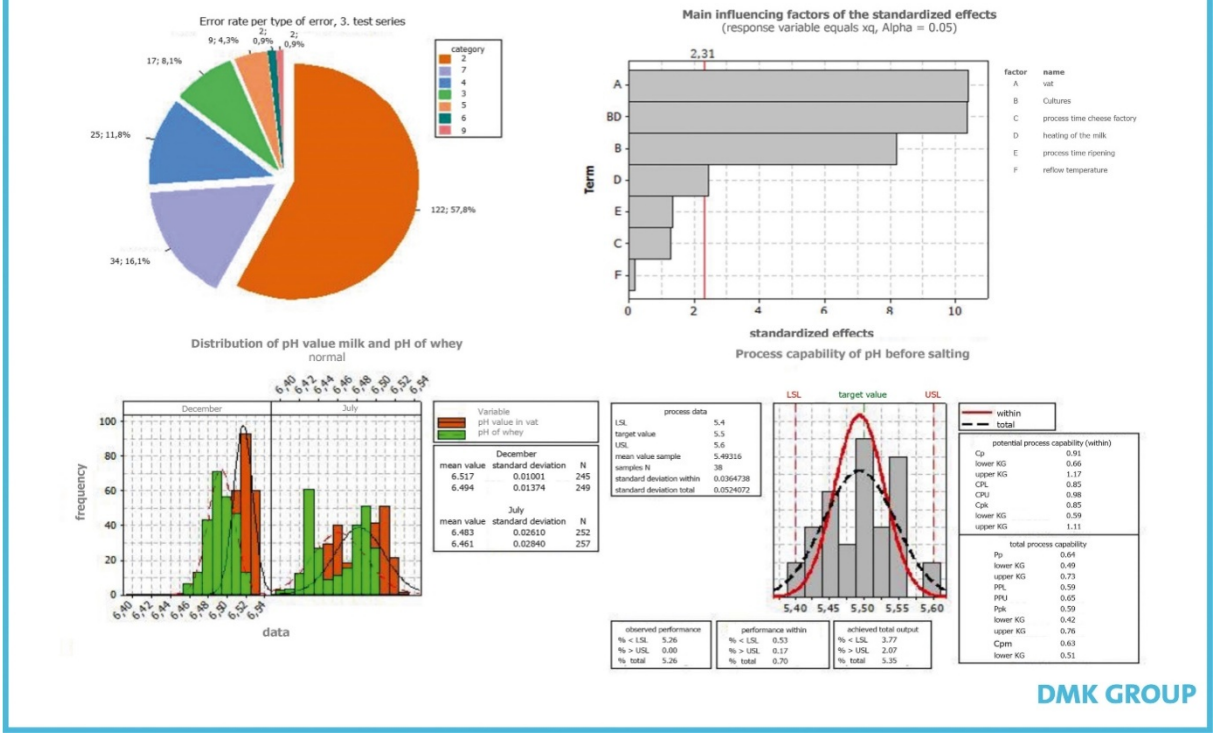
Measurable goals were set and achieved [4]. This target figure was regularly reviewed with a monitoring system in order to identify the progress in the course of the project. Financial quantification of the improvement potential (OE) was established to emphasize its urgency and significant impact [3, 4]. The customer was informed about the fact that a tailor-made improvement project was recommended to achieve the overall necessary changes for quality improvement. During the improvement process all actions were fact-, data- and figure-based. Results and analyses were jointly interpreted by the respective project group and the appropriate actions were rapidly identified and realized (Figure 2).

Figure 1: DMAIC PHASES (Define, measure, analyse, improve and control)



This project was at customer´s satisfaction successfully completed , as the number of complaints declined rapidly. The well-structured approach using Six Sigma was effective and provided a clear guideline. At every point project team members had a solid and transparent base how to deal with figures, data and facts, as well as how to take necessary decisions. Individual perspectives were consolidated or refuted by statistical analyses. Interpreting and analysing facts and figures had priority to approaches exclusively based on individual knowledge (Figure 2).

Figure 2: Error rate per type via the interaction process.



Conclusions

The key success factors for this successful project realization were

measured and quantified with the Six-Sigma approach:

- A system to monitor the progress was established
- Overall team focus was on process modification
- Parameters not to be measured, cannot be improved (Facts & Figures)
- Realization of the to be improved targets could be clearly demonstrated

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A QR-CODE LABELLING SYSTEM GUARANTEES MILK ORIGIN IN THE 'PECORINO' PRODUCTION CHAIN IN TUSCANY (ITALY)

A. Lombardo1, S. Busti2, F. Corzani1, M. Lovicario1, M. Ceniccola1, S. Cecchini1, G. Fantini1, M. De Pietro1, G. Brajon1*.

[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana 'M. Aleandri'. Via Appia Nuova 1411 Rome (IT), [2]Caseificio Busti – Via G. Marconi 13 A/B – Acciaiolo di Fauglia, Pisa (IT)

*giovanni.brajon@izslt.it

Abstract

The origin of two types of "pecorino" produced in Tuscany is guaranteed by a certified traceability system implemented in a cheese factory involving five sheep farms, using a QR code-based labeling system. The QR code can transfer to consumers all the traceability data for each cheese wheel (dairy farms involved, date of milk collection, transport and transformation steps). Such data are recorded by: 1) an automated system for milk collection installed on the collection truck; 2) the cheese factory software, which attributes a lot number that links the milk and its traceability and transformation data with the cheese daily production. The software creates also a web page available for the consumer by reading the QR code on the cheese wheels.

Aim

The aims of this project are:

- tracing the origin of the following two types of 'Pecorino' cheese produced in Tuscany, with a certified process in accordance with UNI EN ISO 22005/2008 [1].
- transferring to consumers all the traceability data by mean of a QR code labelling system.

System description

1) Milk collection in 6 farms:

An automated and human independent informative system installed on the collection truck [2], permits:

1. identification of dairy farms by GPS;
2. date and time of milk collection recording;
3. automatic measurements of collected milk [3];
4. pH and temperature evaluation;

5. collection of representative milk samples for further qualitative analysis.

2) Cheese factory

Data are transferred wi-fi from the truck to a specific software of the factory computer, while the milk is discharged from the truck to the factory's tank.

3) The software:

- attributes a lot number that links the milk and its traceability data with the cheese daily production.
- records also date and time of each transformation step (pasteurisation, curding, cutting of the curd, cooking, salting, ageing and packaging).
- creates a QR code label for each cheese wheel.

4) The QR code label on the cheese wheels can transfer to consumers all the traceability data for each cheese wheel (dairy farms involved, date of milk collection, transport and transformation steps) on a web page available for the consumer (www.toscopecora.it)

Results and conclusions

The implemented system has been certified in accordance with UNI EN ISO 22005/2008 and represents and important tool for guaranteeing the typical origin and quality of 'Pecorino' cheese produced in Tuscany.

Acknowledgements

The authors acknowledge "Caseificio Busti" cheese factory and "Regione Toscana" local authorities.

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HEALTH INVESTIGATION AND MILK QUALITY EVALUATION IN NATIVE AMIATA DONKEYS (EQUUS ASINUS)

G.Ragona[1], M.Martini[2], M.Benedetti[1], F.Salari[2], I.Altomonte[2], I.Paladini[1], A.Piazza[1], E. De Rienzo[1], S.Casini[1], G.Brajon[1]*

[1]Istituto Zooprofilattico Sperimentale del Lazio e della Toscana "M. Aleandri", Via di Castelpulci 43, 50010 Scandicci, Firenze (Italy); [2]University of Pisa, Department of Veterinary Science, Viale delle Piagge 2, 56122 Pisa (Italy).

giovanni.brajon@izslt.it

Abstract

The growing interest toward donkey's milk as food for children with cow's milk protein intolerance implies strong hygiene, health and nutritional requirements. Aim of this study was to collect data about donkey health and nutritional, hygienic and health characteristics of donkey milk. Thirtyone Amiata jennies, a local breed from Tuscany (Central Italy), were examined. Animal health status was monitored by collecting biological samples for infectious agents. From each jenny were collected milk samples for hygienical and nutritional analysis. Coprological analysis showed prevalence of 96.77% strongyles, 19.35% *Dyctiocaulus arnfieldi*, 3.22% *Oxiuris equi. Staphylococcus aureus* was detected in 1 milk sample. The serological investigation showed no significant risks for animal health. Amiata donkey milk showed the following average composition (g/100 mL): 9.38 dry matter, 1.57 protein, 0.40 fat, 7.21 lactose, and 0.36 ash. The unsaturated: saturated fatty acids ratio in milk was close to 1. The donkey MFGs showed an average diameter of 2.05 µm.

Introduction

The growing interest toward donkey's milk as food for children with cow's milk protein intolerance and for the prevention of obesity and dyslipidemia implies strong hygiene, health and nutritional requirements.

Aim

Aim of this study was to collect data about donkey health and nutritional, hygienic and health characteristics of donkey milk.

Materials

31 Amiata jennies (Fig.1), a local breed from Tuscany (Fig.2), were examined for Body Condition Score, temperature, heart and respiratory rate.Animal health status and milk quality were monitored by collecting:

- 62 milk samples for mastitis agents (2 samples from each jenny);

- 31 faecal samples for parasites;

- 31 cervical swabs for the detection of reproductive disorders' bacteria;

- 31 sera in order to estimate the prevalence of the following infectious agents: Brucella spp., Leptospira spp., Salmonella abortus equi, Equine Arteritis Virus, Equine Herpesvirus I and Toxoplasma gondii;

- 31 milk samples for hygienic and nutritional analysis: Somatic cell count, Lysozyme content and activity, Dry matter, Fat, Lactose, Proteins, Caseins, Ashes, morphometric analysis of Milk Fat Globules (MFG) and Fatty Acid Profile.

Figure 1. Amiata donkey

Figure 2. Farm position

Results

All 31 donkeys were clinically healthy. *Staphylococcus aureus* was detected in 1 milk sample, while *Streptococcus equi zooepidemicus* in 2 samples. No mastitis symptoms were detected.

Coprological analysis showed prevalence of 96.77% strongyles, 19.35% *Dyctiocaulus arnfieldi*, 3.22% Oxiuris equi. Bacteria potentially causing infertility and endometritis were detected without any disease symptoms: *Streptococcus equi zooepidemicus* and *Klebsiella pneumoniae* respectively in 3 and in 2 jennies. The serological investigation showed no significant risks for animal health (table 1).

Table 1. Results of animal health monitoring

SAMPLES			RESULTS
CERVICAL SWABS	Reproductive disorders' bacteria	*Taylorella equigenitalis*	0%
		Klebsiella pneumoniae	6.45%
		Streptococcus equi zooepidemicus	9.68%
		Pseudomonas aeruginosa	0%
FAECES	Parasites	*Gastrointestinal strongyles*	96.77%
		Oxiuris equi	3.22%
		Parascaris equorum	0%
		Dyctiocaulus arnfieldi	19.35%
		Fasciola epatica	0%
MILK	Mastitis agents	*Staphylococcus aureus*	1.61%
		Streptococcus equi zooepidemicus	3.22%
SERA	*Brucella* spp.		0%
	Leptospira spp. (*L.interrogans serovar bratislava*, *icterohaemorrhagiae, pomona, saxkoebing*)		0%
	Salmonella abortus equi		0%
	Equine Arteritis Virus		0%
	Equine Herpesvirus type 1		0%
	Toxoplasma gondii		0%

The gross and fatty acid composition of Amiata donkey milk (table 2) showed similarities with the milk from other Italian donkey breeds. The average morphometric characteristics of milk fat globules highlighted a smaller diameter than in species that are typically used to produce milk for human consumption.

Table 2. Amiata donkey milk composition

GROSS COMPOSITION			MILK FATTY ACID (FA) CLASSES AND GLOBULES DIAMETER		
		Mean±SD			Mean±SD
Protein	g/100 mL	1.57±0.248	Saturated FAs	g/100g of total FAs	56.65±8.304
Fat		0.40±0.196	Monounsaturated FAs		22.17±8.184
Lactose		7.21±0.243	Poliunsaturated FAs		21.18±4.051
Ash		0.36±0.061	Unsaturated:saturated ratio		0.80±0.347
Dry matter		9.38±0.546	Diameter of the Milk Fat Globules	µm	2.05±0.932

LYSOZYME		
		Mean±SD
Content	% of the milk protein	9.52±2.778
Activity	U/mL of milk	3986.21±277.80

SOMATIC CELL COUNT		
		Mean±SD
Number of cell per mL		$9.06*10^{3} \pm 3.67*10^{3}$

Conclusions

This preliminary study suggests good standards for animal health and milk quality.

A HACCP and self-monitoring plan based on this data will support the maintenaince of good farm management and food safety, especially for particular categories of consumers.

PREVALENCE OF AFLATOXIN M_1 IN RAW MILK IN LITHUANIA

Jurgita Jovaišienė, Bronius Bakutis, Violeta Baliukonienė, Lina Skredėnaitė

Department of Food Safety and Quality,Veterinary Academy of Lithuanian University of Health Sciences, Kaunas, Lithuania

Jurgita.Jovaisiene@lsmuni.lt

Abstract

Aflatoxins are toxic fungal metabolites found in foods and feeds. During 2008-2014 were investigated 150 ensiled feed samples which include maize silage, grass-legume silage, hay and ensiled crimped maize, 48 samples of concentrate feeds and 147 raw materials of concentrate feeds samples (maize, barley, wheat, oats, rye, triticale cereals, oil meals and cakes) made in Lithuania. In this investigation aflatoxin B_1 (AFB_1) was found in 85% ensiled feed samples at average levels ranging from – 1.7 µg/kg to 7.0 µg/kg and in 81% concentrate feeds samples at levels ranging from 0.1 µg/kg to 15.0 µg/kg and in 58% raw materials of concentrate feeds samples at average levels ranging from 1.0 to 8.0 µg/kg. Leading of this study were monitored aflatoxin M_1 levels in the raw milk samples.

The aim of the present study was to estimate raw milk contamination with aflatoxin M_1 in Lithuanian dairy farms in winter and summer 2014.

During the summer months in the 20 milk samples AFM_1 was detected in 4 samples (20%). The mean concentration of AFM_1 in contaminated raw milk samples was 0.18±0.08 µg/L, and the minimum and maximum levels ranged 0.065 µg/L and 0.425 µg/L, respectively. Aflatoxin M_1 in all raw milk samples (n=20) during winter months was found below detection limit.

The level of AFM_1 contamination in winter raw milk was lower than in summer period. The occurrence, of AFM_1 in raw milk was low.

Keywords: Aflatoxin M_1, Aflatoxin B_1, raw milk, dairy farms

Introduction

Aflatoxins are toxic fungal metabolites found in foods and feed. When ruminants eat aflatoxin B_1 feedstuffs, they metabolise the toxin and excrete aflatoxin M_1 (AFM_1) in milk. AFM_1 in milk are a public health concern and have to be regularly monitored [7, 2].

During the climate change the increase of mycotoxins including aflatoxins find in the forages in Lithuania. Certain contents aflatoxins accumulate forages for dairy cows and is transformed into aflatoxin M_1 and subsequently partially excreted into milk [5, 3]. From aflatoxins (B1, B2, G1 and G2) AFB_1 is the most prominent mycotoxin and toxic, human and animal carcinogen. AFB_1 that escapes rumen degradation is partially converted by hepatic metabolism into AFM_1 (the 4-hydroxymetabolite of AFB_1) [5], which is excreted with dairy milk at a transfer rate that varies between 1% and 6%. AFL (total) intoxication in dairy cattle is characterized by liver cell injury, a fatty liver syndrome (pale livers), poor feed conversion, and a significant reduction in milk yield [3]. Based on a literature review, there is little information on the occurrence of aflatoxins during the summer and winter periods.

The aim of the current study was to estimate the occurrence of AFM_1 in raw milk during the summer and winter seasons in two Lithuanian dairy farms in 2014.

Materials and methods

Milk sampling. Raw milk samples (n=40) were collected twice in April and in September of 2014, from two randomly selected farms. Milk samples were taken from each farm's in winter and in summer periods, from the same cows (n=10), of 3–4 lactations. The samples were refrigerated immediately after collection and taken to the laboratory where they were stored at – 18°C till the time of analysis.

Aflatoxin M_1

The quantitative analyses of aflatoxin M1 (AFM1) in milk were performed with the enzyme linked immunosorbent assays: with test kit RIDASCREEN®Aflatoxin M1 (R-Biopharm AG, Germany). Absorbance was determined using the microwell strip reader at 450 nm. The mean lower detection limit of the RIDASCREEN®Aflatoxin M1 is 0.01 µg/L.

Aflatoxin B_1

Dietary contents of AFB_1 was analyzed by TLC and described by Romer Labs Inc.® Method (Code: a/z-tl-01-00.2).

Results

The Commission has set a limit for AFB_1 of 5.00 µg/kg for supplementary feedstuffs for lactating dairy cattle. However this tolerance level is difficult to observe because the average daily individual intake in a herd should be limited to 40.00 µg AFB_1 per cow, in order to produce milk with less than 50.00 ng AFM_1 per kg [7].

In summer, diet was based on pasture grass and cereals and in winter – grass haylage, hay, maize silage, grain. The feedstuffs in the diets were naturally contaminated with mycotoxins (Table 1). In the both farms feedstuffs aflatoxin B_1 concentration was higher during summer period. The higher AFB_1 concentration – 8.0 µg/kg was found in pasture grass in the Y dairy farm. During summer period the higher mycotoxins concentrations were detected in the ground barley.

Tab 1. Aflatoxin B_1 concentration (µg/kg) in feedstuffs during trial

Feedstuffs	Aflatoxin B_1 concentration, µg/kg	
	Summer	
	X dairy farm	Y dairy farm
Pasture grass	3.00	8.00
Ground barley	6.00	5.00
Total daily intake (mg/animal)	0.252	0.590
Haylage	<1.00*	7.00
Maize silage	<1.00*	<1.00*

Hay	<1.00*	6.00
Ground barley	3.00	3.00
Total daily intake (mg/animal)	0.042	0.442

*limit of detection

During summer months in the 20 milk samples (Fig. 1) AFM_1 was detected in 4 samples (20%). The mean concentration of AFM_1 in contaminated samples was 0.18±0.08 µg/L, and the minimum and maximum levels were 0.065 µg/L and 0.425 µg/L, respectively.

AFM_1 in all raw milk samples (n=20) during winter months was found below European Union detection limit.

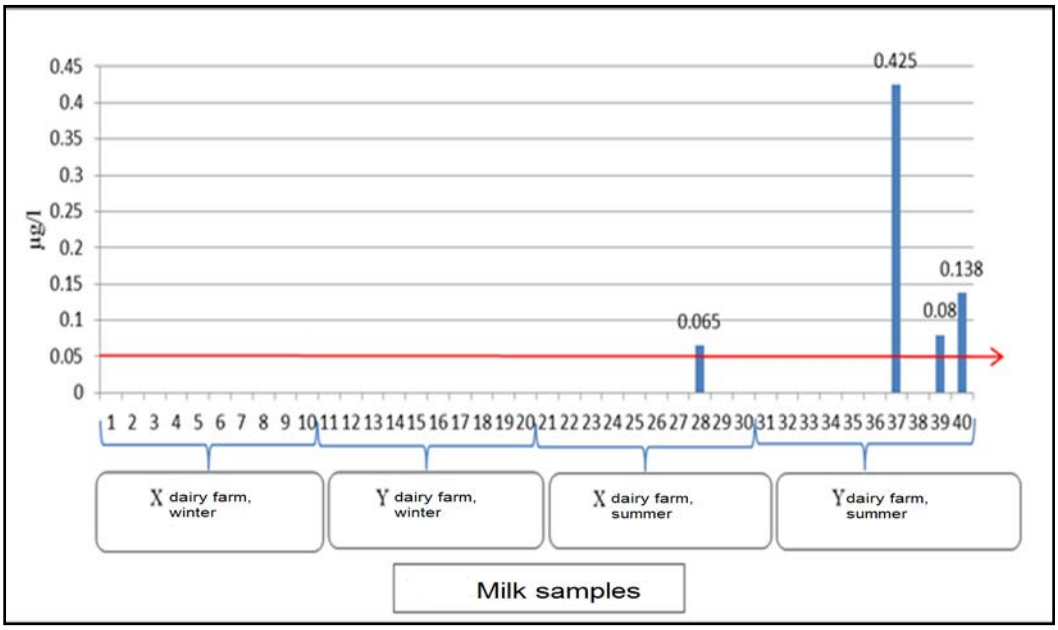

Fig1. Occurrence of aflatoxin M_1 (µg/L) in Lithuanian raw milk samples in 2014

Discussion

The results of present study indicated, that contamination levels of aflatoxin B_1 in feedstuffs harvested in Lithuania are low and occurrence of average aflatoxin M_1 concentration in raw milk samples is low too. This latter result confirms results of an earlier survey of mycotoxin contamination of feed and milk in Lithuania. In that survey, which was conducted during 2008 and 2009, the highest mean levels of aflatoxins (total) 21.2±3.9 µg/kg was detected in ryegrass mixture silage from bales, compared silage produced according different technologies the contamination with AFL (total) was detected 14% ($P>0.05$) higher in silage from bales.

When in ensiled forages of dairy cows were detected AFL (total) from 0.0 to 27.0 µg/kg, the average concentration AFM_1 was 0.019±0.01 µg/L in these cow's milk [1]. In the study of Jasutiene & Garmiene (2007) contamination level of aflatoxin M_1 was below 0.005 µg/kg in the 82 raw milk samples.

In 2014 year the contamination during the summer season was higher. These differences are due to quality of feed given to cows. In summer animals fed with pasture grass and ground barley which were naturally contaminated with the higher mycotoxins levels than in winter period.

These results are in contrast with survey of Marnissi et al. (2012), where the highest incidence and highest level of AFM_1 contamination were observed in autumn (58%) with an average concentration of 31 ng/L, while no positive samples were detected in spring. These differences are probably due to geographical regions, quality of feed given to animals.

Conclusion

In the current study during 2014 occurrence of AFM_1 in raw milk samples from Lithuanian dairy farms was low and in winter AFM_1 concentration in raw milk was lower than in summer period. However, these data are insufficient to speculate on presence, it is necessary to take care of feed and raw milk contamination with mycotoxins and to prevent aflatoxins outbreaks.

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HARMONIZED ANALYTICAL WORKFLOW FOR MILK QUALITY CONTROL IN MUTIPLE MARKETS

Lucie Racault, Karin Kraehenbuehl

Nestlé Research Center, PO box 44, CH-1000, Lausanne 26, Switzerland

Introduction

Quality control of raw milk and processed dairy ingredients sourced from multiple geographical areas is a challenge to ensure that the related finished products are compliant with international trade standards and of course of the highest possible nutritional quality. To achieve this goal, an integrated approach encompassing all entities involvedin the production chain is needed. This includes agricultural services on the field, regulatory and quality management at local and global levels as well as analytical experts that develop and implement the harmonized analytical tools. The analytical strategy includes qualitative screening tests for release parameters at milk collection points and/or at factory reception and confirmatory, quantitative techniques for surveillance purposes of semi-processed and finished products in dedicated laboratories equipped with sophisticated instrumentation.

Monitoring of the milk from farm to factory

Milk monitoring from farm to factory can differ from one market to another one andtypical workflows and processes vary depending on local infrastructure and logistics.

In some geographical areas the acceptance or rejection of the milk is done at collection center. Such decision is taken by using simple tests to check for sensory, temperature, freshness (alcohol test) and density. Other controls for neutralizer and anti-microbial agents can also be performed based on a risk approach.

In other locations or markets part of the analyses is conducted by local authorities. At factory reception, milk coming from milk collection center or from direct supplying farm or from third party can be controlled for: sensory, dirt test, temperature, elementary composition (e.g. fat, protein, total Solid Non Fat). Other quality parameters are checked as well such as pH, acidity, freezing point, alcohol test, boiling test, density.For adulteration, targeted methods for major adulterants are considered based on past incidents, e.g. melamine crisis in 2008. Finally, microbiological (e.g. aerobic mesophilic germs, somatic cells count) and contaminants (e.g. residues of veterinary drugs) analyses are also directly performed at factory reception.

Potential contaminants in milk

The potential contaminants in milk can be divided into two main classes:

1. Veterinary drug residues such as antimicrobials, non-steroidal anti-inflammatory drugs and corticosteroids.
2. Environmental contaminants such as pesticides, mycotoxins, heavy metals and polychlorobiphenyl compounds (PCBs).

The case study of veterinary drug residues has been chosen to illustrate the example of a harmonized analytical workflow for milk quality control in multiple markets.

Analytical testing of veterinary drug residues is a challenge from a global perspective as shownin Figure 1 considering the many regulatory limits appliedin different countries for ampicillin, sulfadiazine, ampicillin and neomycin. Meeting the local regulation requirement is mandatory for import and export activities.

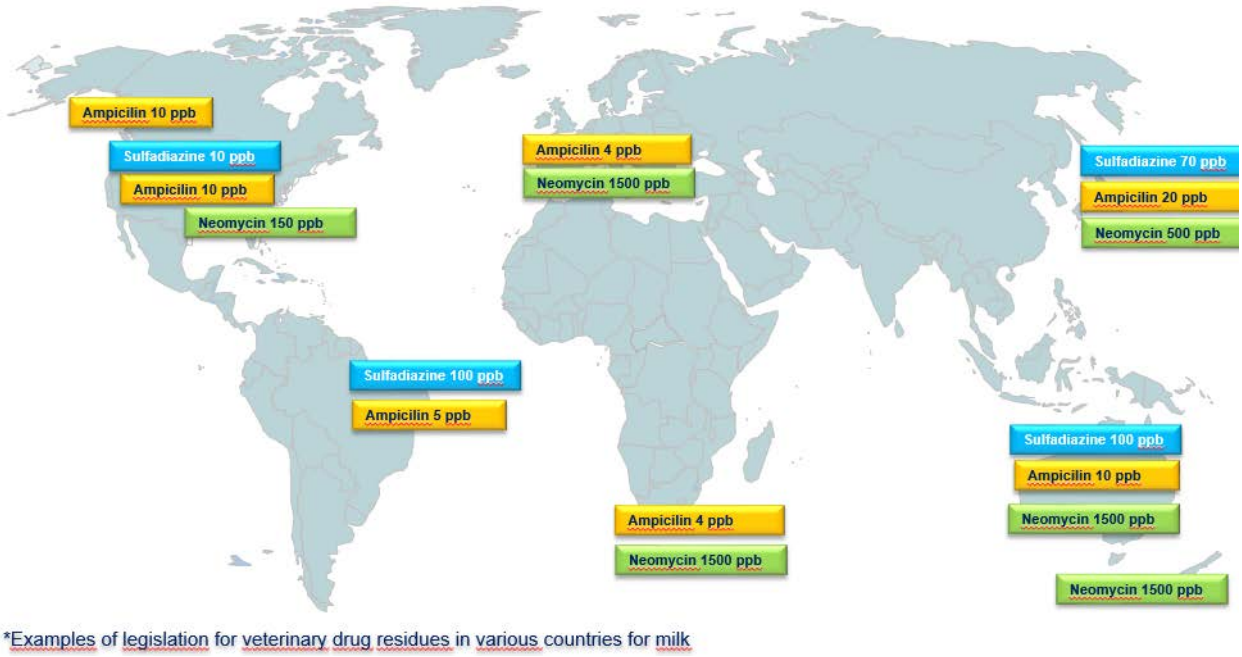

Figure 1: Import/export requirements for some drugs to evidence analytical testing challenges

Integrated approach for analytical development

To support quality control worldwide, an integrated approach for method development is necessary. A team of experts defines a harmonized action plan to be deployed globally. Several inputs are needed as illustratedin Figure 2.

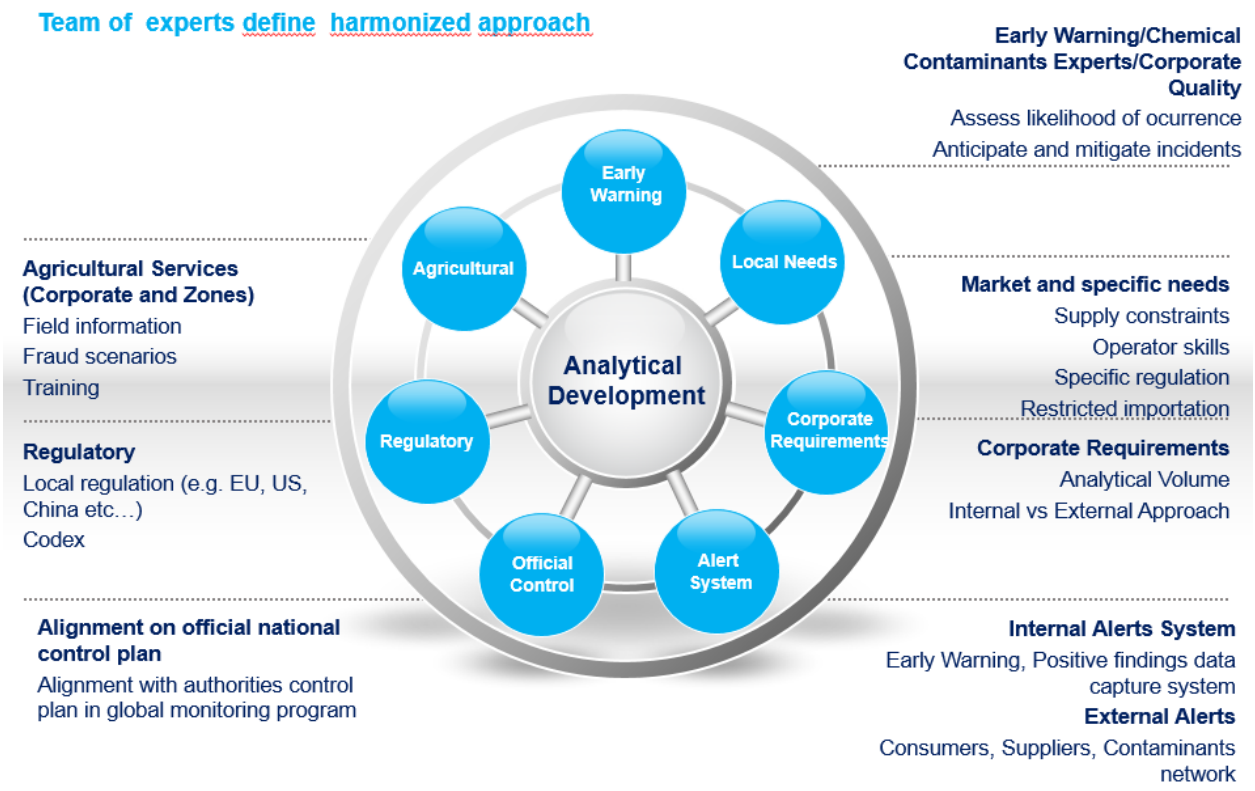

Figure 2: Inputs needed and transversal team work to initiate and focus analytical method development

Agricultural services at corporate and regional levels capture field informationand provide local trainingor advices to farmers. Regulatory experts evaluate the challenges locally and globally e.g. through international standardization bodiessuch as Codex. The alignment with official national testing plans is reviewed by a team of experts. Trends and alerts are collected through internal positive findings database and external information from suppliers, contaminants network orauthorities databases. Corporate requirements guide the team in the definition and implementation of an analytical strategy and subsequent surveillance plan. Early warning and contaminants experts assess likelihood of occurrence of contaminants, anticipate and mitigate incidents and may trigger new internal analytical developments to fill gaps. Market specific parameters are investigated prior to decision making e.g. supplierregional support, laboratory set up, operator skills, specific regulation or any trade/import restrictions.

Dairy raw materials can be analyzed at different stages along the food value chain which requires the appropriate methods at the right spot for example at farm, supplier level, factory reception, or finished product release. Contaminants residues must be detected as early as possible in the production chain to minimize costs of finished products rejection, destruction or withdrawal. For the analysis of veterinary drug residues, a combination of on-site rapid testing and confirmatory methods are implemented. Rapid methods such as dipstick tests, ELISA tests, Inhibitory tests are used from farm to factory reception. Their scope is mainly dedicated to the analysis of antibiotics and banned drugs and they are applicable to fresh milk and milk derivatives (skimmed milk, whey powder, lactose, etc). Confirmatory methods can be either qualitative or quantitative. They are mostly mass spectrometry (MS) based with a large scope of residues such as antibiotics, growth promoters, antiparasites and banned drugs. They are applicableto fresh milk, milk-based finished products and other food ingredients of animal origin. Due to the sophistication of the analytical instruments, those analyses are mainly performed in central laboratories.

Rapid screening methods - current status and future needs

Rapid screening methods must be adapted to the factory environment, meaning that they have to be robust, easy to use for a high throughput application,properly validated by suppliers and should cover the right scope of analytes at relevant detection levels.For generic tests thetarget analyte vs.sensitivity profiles are not always optimal for matchinglocal compliance needs. More flexibility in testing format using one single platform would be an added value.Given the large analytical volumes for rapid testing the consumable costs need to be negotiable and the supplier should be globally present worldwide to ensure fast distribution and technical support.

Confirmatory methods - current status and future needs

Analytical methods can be qualitative or quantitative using LC-MS/MS or high resolution MS (HRMS). Reaching a good sensitivity for a large range of food matrices is often a hurdle when monitoring simultaneouslya large number of targets. Systematic equivalence of instrumentation capabilities is a must for allowing transfer of methods from one central laboratory to another with identical performance. With the increase of large multi-residue methods the rate limiting step of analytical workflows today is data treatment. Therefore there is a need to develop more “user-friendly and fully integrated” data treatment software driven by routine end-users’ input and applicable for high throughput data production. Official standards for the validation of large multi-residue methods by HRMSwould also be an added value for harmonization purposes. Those methods have a great potential for investigation and anticipation of new issues knowing their capacity to measure a large number of targets in a large number of matrices (e.g. milk, meat, fish, honey, finished products).

Conclusions and Perspectives

Multiple daily challenges are faced for contaminants quality control highlighting the importance of developing an integrated approach. Rapid Multiplexing platform could answer some of the needs at factory reception such as broader analytical coverage, flexibility and rapidity. Confirmatory targeted qualitative or quantitative methods need to be more robust and user friendly. Data-treatment and equivalence of instrument performance are currently the limiting factors. The screening and surveillance of unknowns (non targeted screening) can be achieved using HRMS. For routine application, this technology will require to reach a good sensitivity, reproducibility, and the creation of customized databases.