Health, Welfare and the Lameness/Reproduction Interface

PROCEEDINGS

of the

SECOND DAIRYCARE CONFERENCE 2015

Cordoba, March 3rd and 4th 2015



COST FA1308 www.dairycareaction.org







Proceedings of the Second DairyCare Conference 2015

Editor: C H Knight

Published by DairyCare COST Action FA1308 © DairyCare COST Action FA1308 2015

ISBN 978-0-9930176-1-2

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This volume comprises the Abstracts of presentations made at the Second DairyCare Conference held in Cordoba, Spain March 3rd and 4th 2015. DairyCare is a COST Action (FA1308) and the holding of the Conference was supported by COST under the Horizon 2020 programme. Further information about DairyCare can be found at www.dairycareaction.org and about COST at www.cost.eu





Proceedings

of the

Second DairyCare Conference 2015

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WELCOME TO DairyCare!

Animal wellbeing is at the start of a chain that links to farmer profitability, product quality, consumer satisfaction and environmental sustainability.

DairyCare is less than a year old, but already we have almost 400 members representing 29 (soon to be 30) different COST countries. Our first Conference Proceedings have been published, featuring Working Group scientific sessions themed on biomarkers, activity measures and systems level dairy herd management. We supported 18 Early Stage Researchers at that Conference, and will support more at this, the Second DairyCare Conference in Cordoba. The scientific theme this time is Health, Welfare and the Lameness/Reproduction Interface, and we have excellent speakers and Poster Abstracts. DairyCare has already supported 8 Short Term Scientific Missions, and there will be lots more to come. Our next Conference will be held later this year, probably in Croatia, and we have a number of focused WG meetings scheduled. Our website has been visited more than 40,000 times, and we are starting to network on LinkedIn, FaceBook and Twitter. All of this is important, but what is *most* important is YOU, your SCIENCE and the welfare of the dairy animals that your science will protect. Please be active in DairyCare, and please, enjoy the Conference!

Visit our website www.dairycareaction.org







WELCOME TO CORDOBA

This is the Second DairyCare Conference, and the first of our "postcards home". We are delighted that the University of Cordoba has agreed to host the Conference, and we are very grateful to Carlos Pérez Marin and the local organising team. We expect around 150 people to attend the Conference, and we have 70 submitted Abstracts together with Industry Platform Sessions and a Funding Workshop. So we can look forward to an exciting two days of scientific debate, networking and social interaction. Not forgetting the Dinner and Mosque/Cathedral visit!

The Conference has been organised by the DairyCare Action Steering Group:

Chris Knight, Denmark

Marcela Speranda, Croatia

Lene Munksgaard, Denmark

Gianfranco Gabai, Italy

Vivi Thorup, France

Jon Moorby, UK

Ivan Andonovic, UK Rupert Bruckmaier, Switzerland

Sigrid Agenäs, Sweden Marta Brscic, Italy

Annelies Van Nuffel, Belgium

Local organiser: Carlos Pérez Marin, Spain DairyCare Project coordinator: Sheena Knight, Denmark

We are grateful to the University of Cordoba for generous provision of Conference facilities, and of course to COST for funding.





	Programme for the Second DairyCare Conference, Cordoba
	Tuesday, 3 rd March
08:00	Registration Opens
	Health, Welfare and the Lameness/Reproduction Interface
08:45	Welcome
	Chris Knight, University of Copenhagen, Carlos Pérez Marin, University of Cordoba
09:00	Plenary Overview: Interaction between lameness, reproduction and the HPA axis
	Rob Smith, University of Liverpool
09:45	Coffee
	WG2 Invited Session: Gold standards and technologies
10.15	Chair: Vivi Thorup, France
10:15	Lameness and activity monitoring: an introduction, status and future perspectives
10:40	Vivi Thorup, INRA AgroParisTech Using activity meters to monitor cow health
10.40	Claudia Kamphuis, Wageningen University
11.20	Does lameness detection improve with a multi-sensor system
11.20	Tom Van Hertem, <i>KU Leuven</i>
12:00	Poster Introduction Themed Abstracts Session
	Chair: Carlos Pérez Marin, Spain
13:00	Lunch
14:00	Postres y Posters:
	Themed Abstracts Poster Viewing and Scientific Networking
15:00	Industry Platform: Knowing the Animal: Needs and Answers
	Chair: John Baines, Fullwood
16:00	Funding Session: the Professional's View of Obtaining EU Funding
16.45	Jeanette Mueller, Accelopment AG
16:45	Networking Mechanisms: DairyCare Website, Social Media, STSMs and ESR Forum
17:15	Chris Knight, Sigrid Agenas and Marta Brscic, DairyCare Coffee and Refreshments
17.13	Collect and Nellestifferits
17:30	DairyCare Management Committee Meeting
	,
20:00	Networking Reception and Funding Workshop
21:30	Conference Dinner
	Bodegas Campos

Programme for the Second DairyCare Conference, Cordoba								
	Wednesday, 4 th March							
	WG1 Invited Session: Biomarkers for the Lameness/Reproduction Interface Chair: Gianfranco Gabai, Italy							
08:15	Lameness, Fertility and Nutrition: Human and Animal Biomarkers Gianfranco Gabai, University of Padua							
08:40	Biomarkers of lameness in dairy animals: the challenge of translating information from in vitro systems Ali Mobasheri, University of Surrey							
09:20	Biomarkers for Bovine Pregnancy Karl Klisch, University of Zurich							
10:00	Coffee							
10:30	Invited Plenary: Lameness in Small Ruminants: What is Applicable to Larger Ones? George Stilwell, University of Lisbon							
11:10	Industry Platform: Benefitting from Knowledge of the Animal Chair: John Newbold, Cargill Animal Nutrition							
12:00	Poster Introduction Free Abstracts Session Chair: Jon Moorby, UK							
13:00	Lunch							
14:00	Postres y Posters: Free Abstracts Poster Viewing and Scientific Networking							
15:00	Developing DairyCare: the RoadMap for Future Activities Chair: Chris Knight, Copenhagen							
	WG3 Best Practice Workshop: Taking the Best of Bovine Welfare into Small Ruminants Chair: Gerardo Caja, Universitat Autonoma de Barcelona							
16:00	Animal-based welfare indicators in dairy sheep: preliminary results from on-farm monitoring Ignacia Beltrán de Heredia, Josune Arranz, Susan Richmond, Cathy Dwyer and Roberto Ruiz rruiz@neiker.net							
16:40	Effects of management practices and nutrition on the welfare and productivity of dairy sheep Anna Nudda & Antonello Cannas cannas@uniss.it							
17:20	Welfare issues related to nutrition, milking frequency and heat stress in dairy goats David R. Yañez-Ruiz, Leticia Abecia, A. Ignacio Martin-Garcia, Ahmed Salama and Gerardo Caja david.yanez@eez.csic.es							
18:00	Coffee and Close of Scientific Meeting							
19:00	Social Evening: Visit to Mosque-Cathedral and Light Show							

Thursday 5 th March March				
	Optional visit to COVAP			
	See page 16			



	Poster Session Session 1a - Themed Posters								
Health	Posters P1 to P22 are those that were submitted as Themed Posters within the topic of Health, Welfare and the Lameness/Reproduction interface. Posters P23 to P35 (Session 1b) have been added to the Themed Session to create balance between Sessions.								
P1.1	.1 Development and validation of a novel algorithm to quantify the walking beha								
	dairy cows with the aid of a three-dimensional accelerometer								
	Maher Alsaaod, Joël Niederhauser, Ladina Just, Getraud Schuepbach-Regula and								
	Adrian Steiner <u>maher.alsaaod@vetsuisse.unibe.ch</u>								
P1.2	Welfare implications when introducing dairy cows to new concrete or to rubber mat								
	flooring in a Swedish cubicle system with scraped alleys								
	Christer Bergsten, Pernilla Norberg and Gunnar Pettersson								
	<u>christer.bergsten@slu.se</u>								
P1.3	Prevalence of claw diseases in Swedish dairy herds with different grazing								
	management								
	Christer Bergsten, Jonas Carlsson and Marie Jansson Mörk christer.bergsten@slu.se								
P1.4	Biomarkers to predict severity of bovine E. coli mastitis in the periparturient period:								
	bridging the gap between genotype and phenotype								
	Christian Burvenich, Xanthippe Boulougouris, Carolien Rogiers, Catherine Delesalle,								
	Luc Duchateau, Luc Peelman, Mario Van Poucke, Gianfranco Gabai and Bart De								
	Spiegeleer <u>christian.burvenich@ugent.be</u>								
P1.5	Relationship between claw disorders, production, feet and legs type traits, somatic								
	cell and days open in Spanish dairy cattle								
	Nouredine Charfeddine and MA Perez-Cabal nouredine.Charfeddine@conafe.com								
P1.6	Validation of the CowAlert system to automatically detect lameness in dairy cattle								
	Gemma L Charlton, S Mark Rutter, Emma CL Bleach and Robert Boyce								
	gcharlton@harper-adams.ac.uk								
P1.7	IZSLER/CReNBA on-farm dairy cow welfare assessment protocol: cause-effect								
	association between management and housing factors and lameness prevalence; a								
	retrospective study in Italy								
	Francesca Fusi, Valentina Lorenzi, Alessandra Angelucci, Matteo Donati and Luigi								
24.0	Bertocchi <u>francesca.fusi@izsler.it</u>								
P1.8	Risk factors of infectious and non-infectious claw disorders in loose-housed dairy								
	herds in Finland								
D4 0	Johanna Häggman and Jarmo Juga johanna.haggman@helsinki.fi								
P1.9	Dairy Cow individual feed intake model based on monitoring feeding behaviour								
	Ilan Halachmi, Yehoshav Ben Meir, Joshua Miron and Ephraim Maltz								



The use of thermal imaging to detect digital dermatitis in dairy cattle

Stephanie Hiss-Pesch, Julia Weise and Helga Sauerwein s.hiss@uni-bonn.de

Marie J Haskell, Grace Harris-Bridge, Lesley A Smith, Marianne Farish, Colin Mason and

Adipocyte sizes in the digital fat pad and their relationship to body condition score

halachmi@volcani.agri.gov.il

Malcolm A Mitchell marie.haskell@sruc.ac.uk

P1.10

P1.11

P1.12	Geometric and non-linear parameters of heart rate variability as indicators of					
	chronic stress caused by lameness in dairy cows					
	Viktor Jurkovich, János Tőzsér, Margit Kulcsár-Huszenicza, Luca Kézér and Levente					
	Kovács <u>jurkovich.viktor@aotk.szie.hu</u>					
P1.13	Claw conformation in dairy herd management of Slovakia.					
	Radovan Kasarda <u>radovan.kasarda@uniag.sk</u>					
P1.14	Assessment of the mechanical properties of claw horn using tensile strength,					
	puncture resistance and Vickers hardness tests					
	LA Lethbridge, Jean K Margerison, RM Hooper and CW Smith					
	j.margerison@massey.ac.nz					
P1.15	A meta analysis of claw horn disease and claw horn anatomy in UK and New Zealand					
	first lactation dairy cattle					
	LA Lethbridge and Jean K Margerison <u>j.margerison@massey.ac.nz</u>					
P1.16	Improved phenotyping of dairy cow fertility traits					
	Peter Løvendahl, Erling Strandberg and Ahmed Ismael					
	Peter.Lovendahl@mbg.au.dk					
P1.17	Fiber Optic SPR biosensor for progesterone detection in raw milk as a tool to					
	improve reproduction					
	Nathalie Mariën, Devin Daems, Filip Delport, Dragana Spasic, Wouter Saeys and Jeroen					
	Lammertyn <u>devin.daems@biw.kuleuven.be</u>					
P1.18	Using Indoor Positioning System to Measure Lameness in Dairy Cows					
	Matti Pastell, Lilli Frondelius, Sari Kajava, Heli Lindeberg and Jaakko Mononen					
	Matti.pastell@luke.fi					
P1.19	New approaches for management and breeding of dairy cows in automatic milking					
	systems (AMS)					
	Olav Reksen and Adam Martin <u>olav.reksen@nmbu.no</u>					
P1.20	Incidence of claw diseases and reproductive problems in dairy cows in Serbia					
	Renata Relic, Dejan Vukovic and Jelena Miocinovic					
	rrelic@agrif.bg.ac.rs					
P1.21	Odour-based heat detection in dairy cows: a pilot laboratory study					
	Salla Ruuska, Antti Roine, Heli Lindeberg, Pekka Kumpulainen, Mikko Järvinen, Niku					
	Oksala, Jouko Vepsäläinen and Jaakko Mononen					
	salla.ruuska@uef.fi					
P1.22	Lameness research brought to a new level					
	Tim Van De Gucht, Koen Mertens, Leen Vandaele, Wouter Saeys, Jürgen Vangeyte and					
	Annelies Van Nuffel					
	tim.vandegucht@ilvo.vlaanderen.be					

	Session 1b , Themed Posters
P1.23	Use of the Lifecorder+® sensor to assess grazing time of dairy cows
	Clement Allain, J Raynal, C Beck, R Delagarde and V Brocard clement.allain@idele.fr
P1.24	A cross-sectional study of lying behaviour on 23 UK dairy farms: investigating within
	and between herd
	Sophie Collins, Dan Gammon, Jenny Gibbons, Charlotte C Burn, Jacqueline M Cardwell,
	Richard Vecqueray and Nick J. Bell scollins@rvc.ac.uk
P1.25	Effect of heat stress on daily activity patterns in lactating dairy goats
	Soufiane Hamzaoui, Ahmed A.K. Salama and Gerardo Caja
	ahmed.salama@uab.es
P1.26	Is she lying? Validation of IceQube-recorded lying in dairy cows.
	Akke Kok, Ariette van Knegsel, CE van Middelaar, H Hogeveen, B Kemp, and IJM de
	Boer <u>akke.kok@wur.nl</u>
P1.27	On-farm dairy cow welfare assessment: what's going on in Italy?
	Valentina Lorenzi, Francesca Fusi, Alessandra Angelucci, Matteo Donati and Luigi
	Bertocchi valentina.lorenzi@izsler.it
P1.28	Effect of introducing replacement heifers into the dairy herd either with or without
	adult dairy cattle and bed type on bed uptake rate, lying time and daily behaviour
	patterns of dairy cattle
	Jean K Margerison, M Aubry, M Hedley, D Horne, C Christiensen, and N Butcher j.margerison@massey.ac.nz
P1.29	Contemporaneous and lagged effects of on-farm welfare assessment on
1 1.23	reproductive parameters in dairy cattle
	Laura Molina, Carlos Pérez Marin and Estrella Aguera pv2pemac@uco.es
P1.30	Habituation of dairy heifers to the Tellington TT ouch treatment
	Marialuisa Pisani , Ute Knierim and Silvia Ivemeyer mrls90@hotmail.it
P1.31	Validation of a new monitoring system (RumiWatch®) for recording the grazing and
	rumination behaviour of dairy cows
	Markus Rombach, Andreas Münger, Karl-Heinz Südekum and Fredy Schori
	markus.rombach@agroscope.admin.ch
P1.32	The effect of high and low concentrate feeding on cow's feeding behaviour
	Maria Soonberg and David Arney
	maria.soonberg@emu.ee
P1.33	Infection-related changes in sleep and rumination time in one dairy cow - a case
	study
	Emma Ternman , Kerstin Olsson and Sigrid Agenäs Emma.Ternman@slu.se
P1.34	The effect of stocking density on drinking and feeding behavior of yearling dairy
	heifers
	Iulian Tripon, Ludovic Toma Cziszter and Evangelia N. Sossidou
D4 35	iulian_tripon@animalsci-tm.ro
P1.35	Accurate tracking of cows in a barn environment using TrackLab
	Bram van Mil, Lilli Frondelius, A Spink, Matti Pastell, Jaakko Mononen and L Noldus
	b.van.mil@noldus.nl



	Session 2 – Posters Unthemed						
P2.1	Environmental-friendly tools to detoxify maize from aflatoxin B1.						
	Alessandra Angelucci, Cristiano Bello, Massimo Reverberi, Corrado Fanelli, Annaadele Fabbri, Marzia Scarpari, Chiara Dall'Asta, Alberto Biancardi and Luigi Bertocchi						
	alessandra.angelucci@izsler.it						
P2.2	Life Cycle Inventory Analysis for a Commercial Dairy Cattle Producing Farm in Greece						
	Thomas Bartzanas, Vasileios Anestis and Constantinos Kittas						
	<u>bartzanas@ireteth.certh.gr</u>						
P2.3	Methicillin-resistant Staphylococcus aureus (MRSA) prevalence and antimicrobial						
	resistance profile in North-East Italy						
	Alessio Bortolami, Matteo Gianesella, Massimo Morgante and Michela Corro						
	<u>abortolami@izsvenezie.it</u>						
P2.4	Monitoring of drug consumption in dairy cattle farms						
	Marta Brscic, Flaviana Gottardo, Paola Prevedello, Arnaldo Azzolin and Antonio Barberio marta.brscic@unipd.it						
P2.5	Monitoring rumen pH and temperature changes by wireless sensors and logistic						
	regression in dairy goats						
	Andreia Castro-Costa, Ahmed AK Salama, Xavier Moll, Jordi Aguiló and Gerardo Caja						
	gerardo.caja@uab.es						
P2.6	Assessment of animal welfare using metabolomic condition in dairy cows						
	Irena Celeska, Danijela Kirovski, Igor Ulchar, Miroslav Radeski and Vlatko Ilieski						
	iceleska@fvm.ukim.edu.mk						
P2.7	Survey of hair cortisol concentrations in Danish cattle						
	Regitze Charmac, Alberto Prandi, Antonella Comin, Marta Montillo, Rupert						
	Bruckmaier, Josef Gross and Christopher Knight						
D2 0	regitzecc@hotmail.com						
P2.8	Detection of genetic markers associated with improved efficiency of feed utilization in Holstein calves						
	Miri Cohen-Zinder and Ariel Shabtay mirico@volcani.agri.gov.il						
P2.9	The importance of pasture in tied dairy cows						
1 2.3	Matteo Donati, Alessandra Angelucci, Francesca Fusi, Valentina Lorenzi and Luigi						
	Bertocchi matteo.donati@izsler.it						
P2.10	Can we feed the calf more milk?						
1 2.10	Kristian Ellingsen, Cecilie M. Mejdell, Nina Ottesen, Stig Larsen and Ann Margaret						
	Grøndahl Kristian.Ellingsen@vetinst.no						
P2.11	Effect of concentrate supplementation on reticular pH profile and ruminal						
	fermentation traits of cows fed herbage during the transition and early lactation						
	periods						
	Manuela Falk, Andreas Münger, Rahel S Zbinden, Josef J Gross, Rupert M Bruckmaier						
	and Frigga Dohme-Meier manuela.falk@agroscope.admin.ch						
P2.12	An experience of technological transfer: the bioelectronic platform developed in an						
	Italian dairy chain						
	Chiara Frazzoli, Alberto Mantovani and Roberto Dragone						

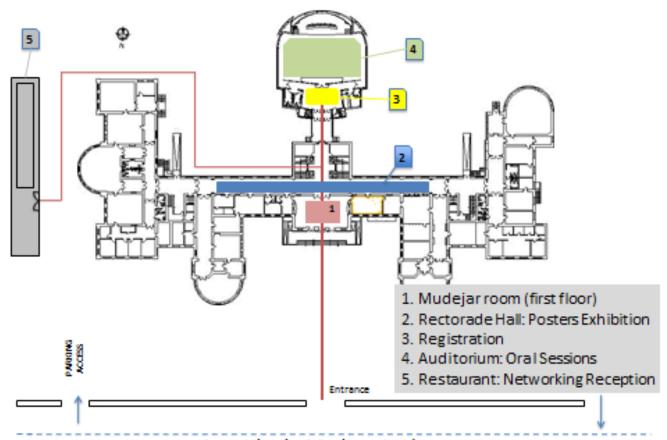
P2.13	Effect of heat stress on ketosis prevalence risk and milk production in early and mid- lactation of Holstein heifers in Croatia						
	Vesna Gantner, Krešimir Kuterovac, Marcela Šperanda and Klemen Potočnik vgantner@pfos.hr						
P2.14	Evaluation of salivary proteins as potential biomarkers of milk production in hot						
	climates						
	Ana CAPM Geraldo, Cristina Conceição, Liliana Cachucho, Flávio Silva, Catarina Matos,						
	Elsa Lamy, Fernando Capela e Silva, and Alfredo Pereira ecsl@uevora.pt						
P2.15	Oxidized protein biomarkers in the blood and milk of cows supplemented with						
	flaxseed during the dry period. Nadia Guzzo, Lucia Bailoni, Roberto Mantovani, Laura Da Dalt and Gianfranco Ga						
	gianfranco.gabai@unipd.it						
P2.16	Potential use of milk based biomarkers to assess and to select for heat tolerance in						
	dairy cattle						
	Hedi Hammami, Jérémie Vandenplas, Marie-Laure Vanrobays, Catherine Bastin, and						
	Nicolas Gengler <u>hedi.hammami@ulg.ac.be</u>						
P2.17	The effect of solid feed diet on the oral and cross-sucking behaviour of pre-weaned						
	dairy calves						
	C Hansen and Jean K Margerison j.margerison@massey.ac.nz						
P2.18	Monthly recordings of ß-hydroxybutyrate (BHB) concentrations in milk during early						
	lactation to characterize energy status						
	Julia Kesser, Christian Koch, Katharina Elsen, Christian Rietz, Helga Sauerwein and Ute						
	Müller sauerwein@uni-bonn.de						
P2.19	Interrelationship between body weight gain and insulin responsiveness in young						
	dairy calves						
	Danijela Kirovski, Ljubomir Jovanović, Ivan Vujanac, Radiša Prodanović, Miloje Đurić,						
	Snežana Stevanović-Đorđević and Mihailo Anitić dani@vet.bg.ac.rs						
P2.20	Oral vitamin D –effect on serum magnesium						
	Cecilia Kronqvist, Malin Nemeth, Elisabeth Kolp, Heini Bachmann and Annette						
	Liesegang cecilia.kronqvist@slu.se						
P2.21	Estrone sulphate and enzymatic activities in mare milk throughout gestation						
	Yu Kuai, Gisela Girmé, Mª Àngels Calvo, Anna Bassols and Mercè Olivé						
	kuainegrito@gmail.com						
P2.22	Developing Innovative Health and Welfare Management Tools for Dairy Cows from						
	Optimized Use of Milk Mid-Infrared Spectra (OptiMIR)						
	Aurélie Lainé, Catherine Bastin, Clément Grelet , Frédéric Dehareng , Xavier Massart ,						
	Carlo Bertozzi and Nicolas Gengler <u>aurelie.laine@ulg.ac.be</u>						
P2.23	How does calving in small groups with individual calving pens work?						
	Lena Lidfors, Torgunn Aslaug Skjervet and Julie Føske Johnsen						
	Lena.Lidfors@slu.se						
P2.24	Prevalence of and risk factors for Failure of Passive Transfer of immunity in newborn						
	calves in Italian dairy farms						
	Isabella Lora, Marta Brscic, Barbara Contiero, Giulio Cozzi, Flaviana Gottardo and						
	Annalisa Stefani <u>isabella.lora@studenti.unipd.it</u>						



P2.25	Effect of maternal dry period length and nutrition on colostrum quality, natural and						
	specific humoral immunity and development of calves						
	Novi Mayasari, Gerrit J Remmelink, Henk K Parmentier, Bas Kemp, and Ariette TM Van						
	Knegsel <u>ariette.vanknegsel@wur.nl</u>						
P2.26	The use of 1H NMR-based metabolomics for the detection of heat stress markers in						
	dairy goats						
	Nazri Nayan, Ahemd AK Salama, Alexandra Contreras-Jodar, Soufiane Hamzaoui, and						
	Gerardo Caja <u>ahmed.salama@uab.es</u>						
P2.27	NMR-detected biomarkers of tolerance to seasonal weight loss in goats: a tool for						
	breed selection						
	Mariana Palma, Lorenzo E Hernández-Castellano, Noemí Castro , Anastasio Arguëllo,						
	Juan Capote, Manolis Matzapetakis and André M de Almeida mpalma@itqb.unl.pt						
P2.28	An NGS-based gene expression profile study in the goat mammary gland: the effect						
	of undernutrition in two breeds with different levels of adaptation to Nutritional						
	stress						
	José R. Parreira, Joana R. Lérias, Lorenzo E. Hernández-Castellano, Mariana Palma,						
	Noemí Castro, Anastasio Argüello, Juan Capote, Susana S. Araújo and André M.						
	Almeida mpalma@itqb.unl.pt						
P2.29	Hair cortisol and DHEA levels in dairy cows reared on summer pastures						
	Tanja Peric, Mirco Corazzin, Antonella Comin, Stefano Bovolenta, Marta Montillo and						
	Alberto Prandi						
	tanja.peric@uniud.it						
P2.30	Effects of rumen protected choline supplementation on milk yield, plasma						
	metabolites methionine and lysine in dairy cows fed hay based diets						
	Luciano Pinotti, M Ottoboni, V Caprarulo, A Pilotto, G Invernizzi, G Savoini, and A Baldi						
	luciano.pinotti@unimi.it						
P2.31	Implementation of the project "Intramammary propolis formulation for prevention						
	and treatment of mastitis in dairy ruminants"						
	Lada Radin, Goran Bačić, Krešimir Matanović, Nino Mačešić, Tomislav Mašek, Diana						
	Brozić, Miroslav Benić, Božo Radić and Jelena Šuran <u>lada radin@vef.hr</u>						
P2.32	Proteomic analysis of differentially expressed proteins in Jafarabadi buffalo (Bubalus						
	bubalis) milk whey during mastitis						
	André M Santana , Daniela G Silva , Funmilola Thomas , Richard J S Burchmore , José J						
	Fagliari and P David Eckersall <u>andrevetms@gmail.com</u>						
P2.33	From biomarkers for oxidative stress to the development of innovative milk replacer						
	for neonatal calves						
	Vered V Sibony and Ariel A Shabtay						
	sibony.vered@gmail.com						
P2.34	Milk Recording in Dairy Cattle in Slovenia – A Valuable Aid for Herd Health and						
	Welfare Monitoring						
	Jože Starič and Jožica Ježek <u>joze.staric@vf.uni-lj.si</u>						
P2.35	Identifying biomarkers in adipose tissue by proteomic analysis that are related to						
	metabolic status, peripartum insulin resistance and body weight loss in dairy cows						
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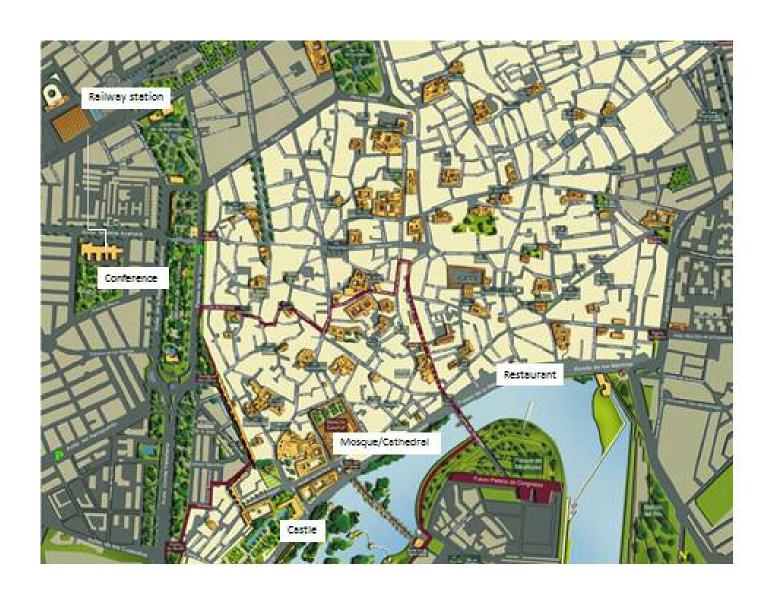


Site plan and location of rooms

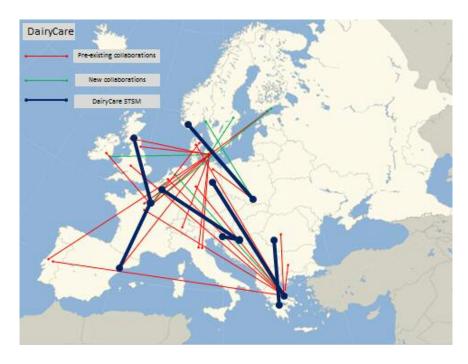


Avenida de Medina Azahara

City plan with important locations



DairyCare STSMs and other collaborations



DairyCare is currently funding 8 STSMs (Short Term Scientific Missions), visits that enable exchange of skills, techniques and expertises between participating research groups.

Applicant Host

Dr Thomas Bartzanas, Greece Prof Thomas Amon, Germany

Assessment of heat stress conditions in naturally ventilated dairy cattle buildings

Mr Tesfaye Belay, Norway Prof Maciej Kowalski, Poland

Ketosis detection based on milk spectral info

Dr Andreia Castro Costa, Spain Dr Sylvie Giger-Reverdin, France

Assessing the effects of forage:concentrate ratio on rumen indicators and lactational performance

Dr Mislav Didara

Dr Geert Bruggeman, Belgium

Development of a rumen model to measure by-pass protein and improve dairy care

Dr Vesna Gantner, Croatia

Dr Klemen Potocnik, Slovenia

Prevalence and effect of subclinical ketosis in Holstein cows in Croatia and Slovenia

Prof Zygmunt Maciej Kowalski, Poland Dr Tormod Ådnøy, Poland

DairyCare Norway-Poland

Ms Grace Smith, UK Prof Nic Friggens, France

Dry period disease indicators

Dr Iulian Tripon, Romania Dr Evangelia Sossidou, Greece

Using the effect of space allowance on feeding and resting behavior in assessing heifers welfare

Visit to COVAP Agricultural Cooperative

On Thursday 5th March it will be possible to visit the agricultural cooperative COVAP. COVAP are a major agricultural company operating in 30 countries in the dairy, beef and pork sectors. Their dairy products business includes major emphasis on sheep's and goat's milk. If you would like to visit COVAP please contact Carlos Pérez Marin, pv2pemac@uco.es The details of the visit will depend on the numbers responding. You can find COVAP at http://www.covap.es/english/



COVAP

In 2011 COVAP increased its milk production by 29% The dairy business has generated €173,4M in sales with a total of 327M units

Milk-buying { 194M bought by year end

Sheep's milk > 4.246 litres delivered by members

Goat's milk > 3.887 litres delivered by members

ORAL ABSTRACTS

1.1

Welcome to DairyCare

Chris Knight and Carlos Pérez Marin

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It is our great pleasure to welcome you to this Second DairyCare Conference, and to the warmth and beauty of Andalucia and Cordoba. We shall borrow the words of José Carlos Gómez Villamandos, Rector of the University of Cordoba (UCO):

The UCO is heir to a centuries-old historical and cultural legacy as well as a passion for knowledge, tolerance, international renown and harmony among civilizations that have made the city that houses our institution a paradigm for humanity. This equilibrium and harmony is reflected in the three areas in which the UCO undertakes its activities: Food and Agriculture and Science and Technology; Humanities and the Legal and Social Sciences; and the Health Sciences; each with its own special characteristics. This balance is also manifested in quality teaching and research, ranking it among the top academic institutions in Spain.

Cordoba is indeed a historical city, home to wonderful Moorish architecture and a rich diversity of culture. Sitting in the magnificent Rectorate building, you might be forgiven for thinking the University was founded in 1613, but you would be wrong. That is the *other* University of Cordoba, in Argentina! We are in a new University, chartered in 1972, and acutely aware of modern and future needs and research priorities. The near-by Rabanales Campus is home to Veterinary and Agriculture students, amongst others. COST is all about networking, whether it be Cordoba Andalucia, Cordoba Argentina or Cordoba Vereda, Mexico. So enjoy your visit, and if you would like to spend more time here, remember that DairyCare has funding available for STSM visits.





Interaction between lameness, reproduction and the HPA axis

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The hypothalamo-pituitary-adrenal axis (HPA) response to lameness is not as well characterised as responses to acute stimuli such as hypoglycaemia, endotoxaemia or transportation. Although basal cortisol secretion and response to a novel stimulus are similar in lame and non-lame cows, the cortisol response to a subsequent stimulus is not reduced by habituation suggesting subtle modifications to the HPA axis. Adrenal progesterone may block or delay LH surge and uncouple ovulation from oestrus behaviour in models of acute stress but its significance in lameness has yet to be determined.

Lame cows have reduced CL function and lower progesterone concentration reducing progesterone priming of oestrus behaviour. Progesterone supplementation during the previous oestrous cycle reverses the effect of lameness suggesting this may be the primary lesion.

Seventy eight percent of moderately lame animals ovulated at a synchronised oestrous. If animals additionally had a milk somatic cell count over 100,000 cell/ml, that on its own does not affect the likelihood of ovulation, only 48% ovulated. This synergistic effect of two moderate stressors increasing the detrimental impact on fertility has been noted in a further observational study on commercial farms suggesting farmers could underestimate the impact of multiple mild stressors on fertility. Lame cows have reduced mounting activity also exhibiting less secondary oestrus signs such as sniffing the vulva of other cows. Lame cows are also less likely to be detected in oestrus by automated activity systems. These systems could detect lameness early and possibly modify the optimum timing of AI in stressed animals.

Lameness is associated with reduced body condition score. The debate as to which comes first has been clarified with the finding that reduction in digital fat pad thickness in thinner cows is a risk factor for lameness and temporal relationship suggest reduced body condition leads to lameness. Fertility is reduced in animals in these studies but the relative importance of body condition score loss and lameness needs verification.

High prevalence of lameness and poor fertility are major challenges to the dairy industry. Their close association suggests that to make progress in both areas they should not be studied in isolation.

WG2 Invited Session: Gold standards and technologies

Lameness and activity monitoring: an introduction, status, and some future perspectives Vivi M Thorup INRA UMR MoSAR, AgroParisTech, 16 rue Claude Bernard, Paris, France vivi.thorup@agroparistech.fr

Lameness is the clinical manifestation of several disorders related to the legs and feet of the cow. Approximately 30% of housed cows are clinically lame. Moreover, it has been estimated that each lame cow has severe pain for 3 months, consequently, animal welfare is severely decreased by lameness. Not only does lameness pose a major problem to the health and welfare of dairy cows worldwide, lameness is also among the top three most expensive health disorders as seen from the dairy farmer's point of view, because of treatment costs, milk loss, reduced reproduction and early culling. Lameness affects the activity of the cow by altering various aspects of cow behaviour, such as the duration and frequency of feeding, ruminating, lying, standing, and oestrus related behaviours. On farm, lameness prevalence is typically underestimated be the farmer. These reasons have inspired the use of a number of technologies for monitoring different aspects of cattle activity and lameness during the last decades, however, likely due to the large number of lameness risk factors, lameness still poses a problem to most modern dairy herds. Remote monitoring of individual cow activity also opens up the possibility of assessing cow temperament and welfare, and to perform precision phenotyping to be used in genetic selection. This presentation gives an introduction to lameness, a status of the technologies used for monitoring lameness and cow activity, paying attention in particular to the research presented at the First DairyCare Conference. Furthermore, this presentation will touch on the problem regarding the lack of a gold standard for lameness detection. Finally, it will suggest some future perspectives of monitoring cow lameness and activity.

Using activity meters to monitor cow health

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In line with the ongoing trend of growing herd sizes, dairy farmers increasingly adopt automation and precision livestock farming (PLF) technologies. These technologies aim to help farmers take care of more animals without increasing labour input. From an economic point of view PLF technologies can help improve daily farm management decisions and, thus, farm profitability. From a social point of view, the knowledge that the herd is under continuous surveillance has been indicated by farmers as being highly valuable. One of the most studied and successful PLF technologies is automated heat detection on dairy farms. The majority of these detection systems use deviations in activity as indicator for an upcoming heat event. With the sensitivity and positive predictive value both being approximately 80%, these detection systems demonstrated to be fairly accurate in doing their job. Moreover, when alerting for cows in heat, the associated management action (inseminate this cow) is very clear for farmers. This combination of a good detection performance and clear management action explains why already around 20% of Dutch dairy farmers adopted this technology. Since deviations in activity have been associated with other health issues as well, investment in these activity monitoring systems may have more benefits than just aid in heat detection. Lame cows, for instance, have a significantly different activity pattern than their non-lame herd mates. A first attempt to use activity to predict lameness proved to be more difficult: only 25% of the lame cows were detected. However, adding other sensor information that was readily available (milking order and live weight) doubled this sensitivity, demonstrating that there is potential to use activity for other purposes than just heat detection. However, to make this become reality there are still challenges to overcome. These challenges include studying potential benefits of investing in activity meters for monitoring cow health, methods to use activity data in combination with other sensor information for detecting other health issues, and the development of standard operating procedures to link an activity alert with management actions to improve the utilization of these activity meters on-farm.

Does lameness detection improve with a multi-sensor system?

Tom Van Hertem, Claudia Bahr, M Steensels, S Viazzi, CEB Romanini, Kees Lokhorst, A Schlageter-Tello, Ephraim Maltz, Ilan Halachmi and Daniel Berckmans

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The objective of this study was to evaluate if a multi-sensor system (milk, activity, body posture) was a better classifier for lameness than the single-sensor-based detection models. Between September 2013 and August 2014, 3629 cow-observations were collected on a commercial dairy farm in Belgium,. Human locomotion scoring was used as reference for the model development and evaluation. Cow behaviour and performance was measured with existing sensors that were already present at the farm. A prototype of 3D-based video recording system was used to quantify automatically the back posture of a cow. For the single predictor comparisons, a receiver operating characteristics curve was made. For the multivariate detection models, logistic regression models were developed. The best lameness classification model was obtained by the multi-sensor analysis (AUC = 0.757 \pm 0.029), containing a combination of milk and milking variables, activity and gait and posture variables from videos. Second, the multivariate video based system (AUC = 0.732 \pm 0.011) performed better than the multivariate milk sensors (AUC = 0.604 \pm 0.026) and the multivariate behaviour sensors (AUC = 0.633 \pm 0.018). The video-based system performed better than the combined behaviour and performance based detection model (AUC = 0.669 \pm 0.028), indicating that it is worthwhile to use a video-based lameness detection system, regardless the presence of other existing sensors in the farm. The results suggest that Theta2 as one measure for back posture with an AUC of 0.719 is the best single predictor variable for lameness detection based on locomotion scoring). In general, this study showed that a video-based system for back posture measurements is outperforming the behaviour and performance sensing techniques measured in this farm for lameness detection based on locomotion scoring.

Acknowledgements

This study is part of the EU Marie Curie Initial Training Network BioBusiness (FP7-PEOPLE-ITN-2008) and part of the Industrial Research Fund (IOFHB/13/0136).



WG1 Invited Session: Biomarkers for the Lameness/Reproduction Interface

Lameness, fertility and nutrition. Human and animal biomarkers.

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Here we will address some activities executed and planned within WG1 "Biomarker-based Welfare Technologies", and introduce the WG1 session dealing with interactions between lameness, nutrition and fertility. WG1 aims to understand the role of biomarkers in health/welfare research, where "biomarker" means "A characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacologic response to a therapeutic intervention" (NIH Biomarkers Definition WG). In the First DairyCare conference emphasis was given to the use of proteomic and metabolomic techniques to identify novel biomarkers, especially in milk and in relation to mastitis and negative energy balance. The relevance of some circulating adipokines as potential biomarkers of metabolic health was discussed, and the potential development of robotic systems for collecting biological samples (sweat, saliva, hair) non-invasively explored. The main theme of the second DairyCare conference is more specific, targeting the interaction between lameness and fertility. Most information on how lameness decreases reproductive performance is epidemiological. It is clear that clinical lameness affects oestrous detection rates and consequently pregnancy rates. However, there is a scarcity of studies considering health status, stress and inflammation, and the mechanisms that lead to decreased conception rates in clinical or sub-clinical lame cows are still unclear. How does lameness affect the endometrium and conceptus? Is there a cause-effect relationship of lameness on embryonic loss in dairy cows? Can we identify potential 'burden of disease' biomarkers to assess the severity of the disease leading to lameness, and 'prognostic' biomarkers to predict whether a lame cow is at risk of embryonic loss? These studies require a suitable 'non-invasive' biomarker to assess embryo/early foetal viability; could the measurement of PAG (Pregnancy Associated Glycoproteins) in milk be the answer? What is the role of nutrition? How will WG1 develop after Cordoba? In the near future, attention might be given to the Hypothalamus-Pituitary-Adrenal axis and the meaning of cortisol measurement in the different biological matrixes, and subsequently we would probably like to explore the immune function in dairy animals. Should we interact more with colleagues from the human medical field? Your views and input are needed!

Biomarkers of lameness in dairy animals: the challenge of translating information from *in vitro* systems

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Biomarkers are anatomic, physiologic, biochemical, or molecular parameters that may be associated with the presence and severity of a specific disease. They are detectable and quantified using a variety of methods, including physical examination, laboratory assays, and imaging and can be used as indicators of pharmacologic responses to therapeutic interventions. The second DairyCare Conference focuses on the Health, Welfare and the Lameness/Reproduction interface in dairy animals. A topic that is of interest to members of this COST Action is the use of biomarkers to detect and assess health/welfare problems, including lameness. This presentation will provide an overview of the pathophysiological changes that occur in inflammatory and degenerative diseases of joints (i.e. osteoarthritis (OA), rheumatoid arthritis (RA) and ankylosing spondylitis (AS)) before discussing the role of biomarkers in the diagnosis, monitoring and treatment of these diseases. Biomarkers are having a significant impact on early detection and treatment in RA. However, there are currently no reliable, quantifiable and easily measured biomarkers that provide an earlier diagnosis of OA, especially during the asymptomatic and pre-radiographic stages of the disease. The current 'gold standard' for the detection of cartilage loss in human and veterinary patients with OA is radiography. Cartilage damage in OA is detected by measuring decreases in joint space width (JSW) on radiographs of patients with progressive disease. However, substantial cartilage loss must occur before any decreases in JSW can be observed. Unfortunately, by this stage significant and irreversible cartilage loss has occurred. Consequently, we need sensitive biochemical markers that can facilitate early and accurate diagnosis joint disease before the appearance of radiographic changes. This talk will provide an overview of our EU consortium's work on OA biomarkers and focus on how joint and inflammation-related biomarkers might be used to diagnose lameness in dairy animals. The availability of biomarker tools and reagents will always be a major bottleneck in the biomarker pipeline, with drastic consequences for the development of disease-modifying OA treatments. It is hoped that the presentation will lead to an open discussion about the potential for developing rapid, sensitive and affordable and biomarker assays for hoof lameness in dairy animals.



Biomarkers for bovine pregnancy

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Early and reliable pregnancy diagnosis is a prerequisite for economically successful dairy farming. An ideal test should determine pregnancy from the 3rd week onwards, should be cheap, reliable, and non invasive.

Pregnancy associated glycoproteins (PAGs) are produced by the binucleate trophoblast cells of the placenta and are secreted into the maternal organism. Serum based PAG pregnancy tests are available since many years, but were not very widely used. Recently a milk-based PAG test was developed, which can determine pregnancy from about day 30 onwards.

Interferon-tau (IFNT) is the molecule of maternal recognition of pregnancy in ruminants. IFNT induced genes are up-regulated in peripheral leucocytes, most pronounced in neutrophil granulocytes. Several of the genes were tested and maximal transcription values were found at day 14-21 of pregnancy.

Early pregnancy factor (EPF) is an immunosuppressive glycoprotein, chaperonine-10, which is produced in the female genital organs in response to a fertilised zygote. EPF can be detected by a 'rosette inhibition test' as early as 24 h after ovulation. Commercially available ELISAs were tested and were consistently identified as not reliable.

Another immunosuppressive molecule is PreImplantation Factor (PIF), which is a peptide produced by the trophoblast. An ELISA showed raised PIF-concentrations in maternal serum at days 10 and 20 post insemination, but further tests will be necessary to confirm these results and to characterise the PIF levels during later pregnancy.

Less characterised is the seed germination inhibition test. This test is based on old Egyptian reports, which were reproduced in the 1930th in Germany, that pregnant women's urine stimulated germination of wheat or barley seeds. Studies from India and Bangladesh show that in cows the opposite is true, and diluted urine from pregnant cow inhibits seed germination.

Lameness in small ruminants – what is applicable to the large ones?

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Lameness is perhaps the most important disease in farm animals because of its impact on performance and welfare. In the UK, foot-rot is responsible for losses over £24 million per annum. However, there is almost no study on the effect of lameness on small ruminants' reproductive performance. This may be due to difficulty in working with these species – seasonality, not very pronounced oestrus signs, male effect, low use of artificial insemination... – but most probably it results from the fact that the industry has not yet fully grasped the full effect of lameness on fertility. In contrast there is extensive evidence connecting lameness with reduced fertility in cattle e.g. we showed that lameness decreased the odds of pregnancy 5.1 times and of calving 3.5 times in dairy cows.

In spite of many (and important) differences, studies with small ruminants can help us understand many features of lameness in cattle. Small ruminants have some characteristics that facilitate experiments – easier to restrain, to force to walk, to flex limbs, to photograph... All being much safer for the operator.

We present some studies through which goat lameness studies may help to elucidate what is happening in cattle.

- Thermographic images of goats' limbs before and after exercise and before and after claw trimming, show an increase in temperature of the distal interphalangeal joint after 5 min. exercise and a significant difference in temperature between animals with overgrown claws and those for which natural weight bearing was regained by trimming.
- There is no gold standard to assess locomotion in ruminants. We developed a websurvey on lameness in goats that showed how numerical rating scales may fail to correctly assess the intensity of lameness and how the different scores might not be evenly spaced along the scale. We will develop now a visual analogue scale for quick lameness assessment.
- Automatic image collection of animals walking out of the milking parlour may allow identifying and quantifying locomotion by body movements and lines.
- The AWIN project is investigating the molecular basis to genetic resistance to footrot that may contribute to the study of cattle interdigital necrobacillosis.

WG3 Best Practice Workshop: Taking the Best of Bovine Welfare into Small Ruminants

Animal-based welfare indicators in dairy sheep: preliminary results from on-farm monitoring Ignacia Beltrán de Heredia¹, Josune Arranz¹, Susan Richmond², Cathy Dwyer² & Roberto Ruiz¹ *Neiker-Tecnalia, Vitoria-Gasteiz, Spain, ²SRUC, Edinburgh, Scotland, United Kingdom *rruiz@neiker.net*

The assessment of sheep welfare presents particular challenges related to the exposure to the environmental conditions, the seasonality of the breeding cycle, as well as to the management practices of the farming system. There is a certain diversity of dairy sheep production systems in Europe, from traditional farming systems in mountain areas, fitted to the seasonal utilisation of natural resources, to crop-livestock production and even landless high productive intensive systems. The development of an animal-based protocol to assess the welfare status of the sheep has to envisage this diversity. Within the EU funded AWIN project, a two-step process protocol has been designed to screen farms for welfare in stage 1, to follow up with a detailed second stage if welfare concerns are detected. The protocol takes into account the 5 freedoms and the 4 principles of good welfare outlined within the Welfare Quality (good feeding, environment, health and behaviour). This prototype has been initially tested twice a year in 9 dairy sheep commercial flocks in Spain: 2 permanently housed and high productive flocks of the Assaf breed, and 7 Latxa sheep flocks only housed during winter months. According to the preliminary results, there are certain differences in the welfare conditions of sheep depending partially on the farming system, but also on the specific management practices implemented by the farmer. In permanently housed sheep, body cleanliness is usually poorer and the number of animals requiring hoof-trimming may be particularly high. In grassland based dairy systems, the seasonality of the productive cycle conditions the evolution of certain indicators throughout the year, such as body condition score, cleanliness, dag scoring, etc. The incidence of injuries and lesions is particularly focused in the ears due either to the particularities of the breed (Assaf) or to identification practices (eartags, marks). The implementation of the protocol has allowed a first, although partial and very limited, approach to the real situation of the welfare conditions of dairy sheep in some flocks in Spain. However, a broader comparison should be faced in order to cope with a higher diversity of farming conditions.

Effects of management practices and nutrition on the welfare and productivity of dairy sheep

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An increasing body of literature has shown that various management and nutritional practices can markedly impact the welfare and productivity of dairy sheep. Improper management practices or inappropriate animal-human interactions can induce negative emotional and physical stresses and thus reduce milk yield and increase somatic cell count in milk, an indirect indicator of stress. These problems are aggravated, especially during the milking period, in breeds with a nervous temperament. Other management factors, such as inadequate milking procedures, inappropriate milk equipment functioning or settings (e.g., vacuum level) can impair the welfare and impact negatively on milk production traits. Heat and cold stress, isolation or regrouping can also influence behavior, immune responses, performances and udder health conditions of ewes. Poor ventilation systems and insufficient space allowance might increase noxious gases, air ammonia, and airborne microorganisms, inducing alterations of milk yield and quality. All these factors can interact with nutritional stresses, inducing further problems. Of particular interest are the nutritional challenges during the transition from pregnancy to lactation, especially in the case of high yielding ewes. Major concerns are improper status of body reserves, unbalanced carbohydrates, proteins, mineral, and water scarcity or quality. During mid-late lactation, nutritional stresses due to energy unbalances are less likely, even though the type of carbohydrates can markedly affect productive responses, with starchy diets favouring more body reserves accumulation than milk production. Protein excess and shortages are more likely. They can be easily controlled by using milk urea and the condition of the feces as nutritional indicators. Throughout the lactation, micronutrient unbalances can induce significant increases in the milk somatic cell count, suggesting an induction of nutritional-derived stresses. Various indicators of appropriate feeding and management practices can help in assessing dairy sheep welfare and limiting possible negative effects on productivity.

Welfare issues related to nutrition, milking frequency and heat stress in dairy goats

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Goats are naturally browsers; they prefer leafy vegetation to grass, which is linked not only to the type of feed they prefer but also to the feeding behavior. Although goats adapt well to intensive dairy production systems, some concerns related to animal health and welfare arise. The production systems in which goats are permanently confined and milked once a day are currently increasing in the Mediterranean area. In Andalusia, the principal goat-farming region in Spain, 42% of goats and 47% of farms operate under this production system, which is characterized by feeding large amounts of concentrates. Diets with large amounts of rapidly fermentable carbohydrates produce the depletion of fibre degradation, which is related to acidotic conditions and may compromise milk fat levels. Data from a survey conducted on Andalusian dairy goat farms with regard to the level of concentrate used throughout lactation showed how the low levels of rumen pH are linked to depressed fibre degradation, low fat levels in milk, increased milk somatic cell counts and alteration of goat immune status. Regarding milking frequency, there are concerns that dairy goats milked with reduced frequency may suffer alterations of behaviour associated to udder distension and inflammatory responses. Nevertheless, compared to dairy cows, goats have udders with stronger teat sphincter and large cisterns that make them more tolerant to extended milking intervals with small changes in their behaviour. Dairy goats milked once daily, when compared to twice daily, spent less time eating, but plasma cortisol levels as well as the time spent drinking, standing or lying do not change. Similarly, goats are more tolerant to heat stress than dairy cows. Heat-stressed dairy goats decrease their intake and show greater haptoglobin, but normal NEFA levels, in blood. Moreover, when injected with glucose their pancreas secreted lower insulin as a way to keep their normal glucose levels in blood.

In addition, and as part of the work conducted within FP7-SOLID project (www.solidairy.eu), the results of a recently developed assessment tool for sustainability of dairy farms will be presented, including data from dairy goats farms.



POSTER ABSTRACTS SESSION 1a & 1b

Development and validation of a novel algorithm to quantify the walking behavior of dairy cows with the aid of a threedimensional accelerometer

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Change of animal behavior is one of the most important criteria to assess animal welfare and health. Traditionally, behavior research of loose-housed cows is based on direct observation or use of time-lapse video recordings. The drawbacks of both methods are that they are time consuming and labor intensive. The accelerometer system is a reliable and useful tool to detect standing and lying. So far, the previously developed accelerometers, however, are not able to detect and characterize walking patterns of cows. The objective of this study was to develop and validate an algorithm to monitor locomotor behavior based on the output from accelerometer sensors of loose-housed dairy cattle compared with video analyses (gold standard). It was hypothesized that a novel algorithm of the Rumiwatch® accelerometer (10 Hz) device can be developed that provides both standing and lying behaviour measurements with a high accuracy, similar to that of video observation. The measurements were conducted in three experiments to validate lying time, stand up, lie down and lying bouts (exp. I; n=18 cows); standing time; walking time; number of standing bouts; number of walking bouts and number of strides (exp. II; n=21 cows) and stride length (m) and stride duration (s) (exp. III; n=19). The first two experiments of this study (exp. I - II) were carried out at the experimental farm of Agroscope Reckenholz-Tänikon Research Station ART in Tänikon, Switzerland and the third experiment (exp. III) at the clinic of ruminants, Vetsuisse-faculty, University of Bern, Switzerland. Spearman correlations were calculated between accelerometer variables and respective data derived from the video recordings (gold standard). The overall difference in percent was calculated as a mean deviation between accelerometer variable and respective video recording. In all experiments, the mean difference between accelerometer data and respective gold standard was between 0% and 17% (depending on variable) and the correlation between respective data ranged vom r2 = 1 to r2 = 0.24. The results of this study show that the newly developed algorithm is able to quantify the locomotor behavior of dairy cows with an acceptable to perfect accuracy (depending on variable of locomotion), which may markedly improve automatic livestock management systems for efficient health monitoring of dairy cows. This study was generously supported by a grant of the "Fondation Sur-La-Croix", Switzerland and Kommission für Technologie und Innovation KTI, Switzerland. Key words: accelerometer, dairy cow, behavior, walking behavior

P1.2

Welfare implications when introducing dairy cows to new concrete or to rubber mat flooring in a Swedish cubicle system with scraped alleys

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Introducing dairy cows to a new barn is challenging especially regarding the floors. Newly casted concrete can damage the claws and cause lameness leading to poor welfare.

Fifty cows of Swedish Holstein (SH) and Swedish Red (SR) breeds, respectively, were introduced to either a group with concrete alleys (CA) or rubber mat alleys (RA). The animals were blocked according to breed, parity and days in milk. Each group had free access to cubicles with mattresses and they were all milked together in a common rotary milking system. The cows in each group were assessed bi-weekly for locomotion, leg lesions, and behavior during the four months study period. Claw lesions were assessed at the start and at the end of the study. Statistical analysis was carried out using Mixed procedure in SAS. Cow was treated as a random effect, and group (CA and RA), observation date, breed (SH and SR), lactation number were treated as fixed effects. The interaction between fixed effects was also included in the statistical models. Five cows were moved from CA to RA because of sore feet and were excluded.

Cows on CA impaired their locomotion both regarding prevalence and severity in comparison to RA. Raw concrete wear off the walls and the soles became sore and locomotion impaired. Claw lesions increased in both groups during the study and there was a tendency for a correlation between sole ulcer and lameness. Hair loss of hocks increased in both groups over time but were twice as common in CA cows and a tendency for more hock ulcerations was also seen in CA. Hock lesions could be a result from longer lying time due to sore feet. Social grooming behavior occurred more frequently in RA than in CA, and self-grooming was more frequent in RA but not statistically significant. It has previously been shown that rubber flooring where cows feel more comfortable also increased their activity. Preventing dairy cows from excessive exposure to abrasive and hard concrete by the use rubber floors pays off from a welfare, longevity and economical perspective.

P.1.3

Prevalence of claw diseases in Swedish dairy herds with different grazing management

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In Sweden, grazing of dairy cows is mandatory during the pasture season between May and August. Grazing is supposed to improve welfare because of better hygiene, improved claw and leg health, and less lameness. However, the legislation in Sweden is questioned because of difficulties to manage grazing in larger herds and in herds with automatic milking systems. An epidemiological study was made to evaluate different grazing management in cubicle systems. The Swedish database on claw records from maintenance claw trimmings was used to select 200 cubicle herds from north to south including two, three or four months mandatory grazing. Inclusion criteria were that the cows in the herds were trimmed and claw diseases recorded close before being grazed in spring and shortly after being housed in autumn. All herd data was retrieved from the Swedish milk recording scheme and herd related parameters, housing and grazing management, were retrieved from the herds-person by a telephone interview. The statistical analysis was based on 16,364 trimmings in 174 herds and was made by poisson regression(spring) and multilevel logistic regression (autumn).

After the housing season in spring, cows in herds with longer predicted grazing period (4 months) had a lower total prevalence of claw health remarks as well as less digital dermatitis (DD) and sole ulcers than those with a shorter grazing period. Organic herds had lower prevalence of total remarks, mild dermatitis and DD than conventional herd, and herds with Swedish Holsteins had more total remarks and sole ulcers and Swedish Reds. At autumn trimming after the grazing season only heel horn erosion had a lower prevalence than before the grazing period. Higher density of cows at pasture increased the risk of sole ulcers, mild dermatitis and DD. The most influencing risk factor for a claw health remark after the grazing season, up to 10 times, was whether the individual cow had a claw health remark at the spring trimming after the housing season. Thus suboptimal housing conditions seems to have a greater influence on the prevalence of claw diseases after grazing than a variation in grazing management itself, under Swedish conditions.

Biomarkers to predict severity of bovine E.coli mastitis in the periparturient period: bridging the gap between genotype and phenotype

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The causes of periparturient (PP) E.coli mastitis in lactating cows are complex and multifactorial (Burvenich et al., 2007). The disease is accompanied by a large variation in clinical symptoms varying from mild/moderate, to severe life-threatening sepsis. There is a small subpopulation of severely affected cows that suffer from unbalanced inflammation. It is now accepted that severity of PP E.coli mastitis is mainly determined by cow factors (Burvenich et al., 2003). Studies on isolated blood neutrophils (PMN) of healthy cows before intramammary infection with alive E.coli bacteria, showed that chemotaxis (Lohuis et al., 1990; Kremer et al., 1993) and the capacity to produce reactive oxygen species (Heyneman et al., 1990) before challenge is negatively correlated with severity and positively with pathogen elimination. At least three major issues can be discerned from these studies: (I) the role of the alteration of pre-infection PMN function in the outcome of PP E.coli mastitis. Since the nineties many studies have contributed to the understanding of the alteration of PP PMN function and viability. In vitro effects of non-esterified fatty acids, beta hydroxybutyrate, estradiol, progesterone, glucocorticoids and IGF were studied (Lamote et al., 2004; Scalia et al., 2006; Sander et al., 2011). (II) The study of potential links with other diseases during the same period. The PP period is a critical period for animal welfare and dairy economics. (III) Identification of one or more biomarkers (BM) to characterize E.coli mastitis specifically and their potential role in the management and health care of cows in general. In contrast to the afore-mentioned issues only a few studies are dealing with predictability of severity of PP diseases. This review will analyze some historical studies for potential BM discovery based on PMN function and relating gene expression to its phenotypic outcome (e.g.: CD11/CD18, alkalin phosphatase by van Werven et al., 1997, CD25-expression by Zoldan et al., 2014, and serum proteomics by Cairoli et al., 2006). It will also focus on genome- and epigenome- based tools and discusses advantages, limitations and future prospects. The potential utility of BM in experimental research and/or field studies will also be highlighted (see Figure 1).

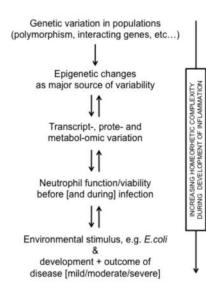


Figure 1 Hypothetical and simplified scheme illustrating the concept of severity of periparturient E.coli mastitis in lactating cows. Unidirectional and reciprocal (double arrows) interactions are shown (adapted from C. Burvenich et. al, 2003 Veterinary Research 34, 521-564; C. Burvenich et. al. 2004 Koninklijke Academie voor Geneeskunde van België, 66/2, 97-150; M. Rambeaud, 2006 PhD dissertation, University of Tennessee). Variation in genome, epigenic regulation and milieu intérieur (Claude Bernard, 1857) increases complexity. The inflammatory process is controlled by homeostatic (W. Cannon, 1926) and homeorhetic mechanisms (C. Waddington, 1957). This scheme is a compilation of considerable amount of work executed by scientists worldwide. It can be used as a working hypothesis to detect, develop and validate potential biomarkers to predict the outcome of periparturient E.coli mastitis and

other related infectious diseases.

Relationship between claw disorders, production, feet and legs type traits, somatic cell and days open in Spanish dairy cattle Nouredine Charfeddine¹ & M A Perez-Cabal²

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In 2012 a centralized electronic recording system called I-SAP for claw disorders was implemented in Spain, and genetic parameters for claw disorders were estimated for dermatitis (DE), sole ulcer (SU), white line separation (WL), interdigital hyperplasia (HP), interdigital phlegmon (PH), and chronic laminitis (CL). With the final aim of improving the Spanish selection index in the next future, the objective of the present study is to estimate genetic correlations between claw disorders and feet and legs traits, production traits, and functional traits, as somatic cell score and days open in Spanish dairy cattle. In addition to the six lesions mentioned above, a combined claw disorder trait called overall claw disorder (OCD) was also included. This trait refers to the absence or the presence of at least one of the six claw lesions. A total of 49,963 claw health records corresponding to 35,337 cows with conformation data on feet and leg traits, yield data (305-d first lactation milk, fat and protein), somatic cell score, and days open between the first and the second calving in first lactation were used. Genetic correlations were estimated using REML methodology fitting a multi-trait linear animal model. Although some type traits regarding feet and legs seemed to be more correlated to specific lesions, such as foot angle with chronic laminitis and white line disease, in general, cows with a good locomotion score are less likely to claw lesions. As expected, high yielding cows were more prone to claw disorders, especially to chronic laminitis. Our results showed a positive relationship between claw health problems and poor fertility, as well as higher somatic cell score in animals affected by sole ulcer.

P1 6

Validation of the CowAlert system to automatically detect lameness in dairy cattle

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Lameness is a chronic source of pain for dairy cows and is one of the most common welfare problems within UK dairy herds, but it can be difficult to detect, particularly at the early stages. Research has shown that dairy cow behaviour can change due to the onset of lameness; however, although use of animal monitoring technologies such as accelerometers is becoming more common within the dairy industry, there is a lack of systems which are capable of detecting lameness automatically. The objective of this three month study is to validate the use of the CowAlert system for detecting mobility changes of dairy cattle, indicative of lameness. One hundred Holstein x Friesian cows (mean ± SD; 198.5 ± 46.6 DIM) from the Harper Adams University dairy herd are currently fitted with IceQube accelerometer sensors (IceRobotics Ltd, Edinburgh, UK). These sensors record lying and standing duration, frequency and duration of lying and standing bouts and step count, together with a three-dimensional Motion Index. All animal behaviour data are stored within the IceQube in 15 minutely summaries and automatically downloaded wirelessly to the CowAlert system (IceRobotics Ltd, Edinburgh, UK). From this a daily IceScore mobility value is calculated. Statistically significant changes in individual dairy cow gait cause an increase or decrease in the IceScore, which results in a mobility alert being generated. To determine the cause of the change, cows with an alert will be assessed within 24 hours and given a mobility, injury and udder score. Additionally, cows flagged by the CowAlert system will be scheduled to see a hoof trimmer during the next fortnightly routine visit. The cows will have all four feet inspected and any problems will be mapped and recorded. If no issues are detected the cow will not be treated or trimmed, but her feet will be examined by the hoof trimmer again in two weeks' time. Throughout the study period all 100 cows will also be mobility scored and given injury and swelling scores fortnightly, and milk yield, conductivity, somatic cell count, incidents of mastitis and calving scores will be recorded. If proven to be successful, early detection of lameness could be a valuable tool for early diagnosis and treatment of developing lameness.

CowAlert, IceQube and IceScore are registered trademarks of IceRobotics Ltd.



P1.7 IZSLER/CReNBA on-farm dairy cow welfare assessment protocol: cause-effect association between management and housing factors and lameness prevalence; a retrospective study in Italy

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In 2011, the Italian National Animal Welfare Reference Centre (CReNBA) decided to develop a protocol to assess and compare the level of animal welfare and biosecurity in dairy herds, drawing a picture of the Italian situation. The protocol was applied at first to Northern Italian dairy herds; since then in 2012 it has been extended to the entire national territory. The IZSLER/CReNBA protocol comprises of 89 different indicators, both animal-based (ABMs) and non-animal-based measures (N-ABMs), divided into 5 areas: Area A - Management and personnel; Area B - Structures and equipment; Area C - ABMs; Area D - Exposure to environmental hazards; Area E - Biosecurity. The total amount of scores given to Areas A-B-C-D provides the overall level of animal welfare onfarm, ranging from 51.45 to 199.35 points; the score given to Area E (min 14.5 - max 52 points) provides the overall level of biosecurity on-farm. From 2011 to January 2015, 922 Italian loose-housed dairy herds were assessed. Herds were randomly selected in different Italian regions and assessments were carried out by 74 veterinary assessors, who all attended a specific training course in IZSLER/CReNBA in order to ensure consistent and repeatable welfare assessment outcomes. Herd size ranged from 7 to 2736 animals (259.54 on average) and from 4 to 1135 lactating cows (119 on average); average milk production was 27.8 kg per cow/day (range 10-41 kg). The overall welfare score recorded on average was 154.53 points (69.69% of the available score). The aim of this study was to identify and compare management (n=21) and housing (n=28) factors associated with the prevalence of lameness ≥ 8% (DairyCo Mobility Scoring system was used; both lactating and dry cows with a Mobility Score 2 or 3 were considered lame). The most significant (P<0.05) and strongest (OR>1.6) associations are reported in Table 1. In conclusion, the managerial factors that seem to be more associated with lameness are the number of stockpeople taking care of cows, the number of daily inspections and the overall cleanliness of the barn; while the housing factors are the lying area provided for dry cows and the type of floor.

Table 1 - Most significant (P < 0.05) and strongest (OR > 1.6) associations between management and housing factors and lameness prevalence $\geq 8\%$ (considering both lactating and dry cows).

Factors	Odds Ratio	95% CI of Odds Ratio	P-value
Area A - Management and Personnel			
Number of stockpeople	2.23	1.11 - 4.50	0.0244
Number of daily inspections of animals	31.09	3.72 - 260.18	0.0015
Diet	2.87	1.39 - 5.92	0.0045
Water availability	2.65	1.57 - 4.47	0.0002
Cleanliness of water troughs: lactating cows	2.49	1.51 - 4.10	0.0004
Cleanliness of water troughs: dry cows	1.73	1.05 - 2.85	0.0304
Cleanliness of water troughs: heifers	1.87	1.13 - 3.09	0.0143
Cleanliness of floors: lactating cows	1.70	1.13 -2.55	0.0115
Cleanliness of floors: dry cows	2.16	1.47 - 3.17	0.0001
Cleanliness of floors: heifers	1.65	1.16 - 2.37	0.0060
Bedding material management: dry cows	2.21	1.45 - 3.38	0.0002
Management of milking operations and udder hygiene	2.86	1.42 - 5.75	0.0032
Area B - Structures and Equipment			
Surface area provided for lying down: dry cows	1.74	1.12 - 2.70	0.0128
Surface area for calves housed in individual pens	2.29	1.31 - 3.99	0.0034
Type of floor: lactating cows	2.50	1.34 - 4.66	0.0038
Type of floor: dry cows	2.27	1.15 - 4.46	0.0175
Type of floor: heifers	2.23	1.21 - 4.11	0.0103
Size of drinking troughs: dry cows	1.87	1.27 - 2.74	0.0015

P1.8
Risk factors of infectious and non-infectious claw disorders in loose-housed dairy herds in Finland Johanna Häggman and Jarmo Juga
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Claw disorders reduce farm profitability, often cause lameness and are a serious welfare problem in dairy farming. Herd claw health can be improved by optimizing the housing and management of dairy herds. This research aimed to evaluate the effect of cow-level and herd-level factors on infectious and non-infectious claw disorders and to quantify the total variance of claw disorders explained by claw trimmers. The claw health data of individual cows during routine claw trimmings were collected by professional claw trimmers between 2003 and 2010. Data from 10495 Ayrshire and Holstein cows in 262 loose-housed herds were analysed using generalised linear mixed models. Statistically significant cow-level risk factors (p≤0.05) were breed, parity, trimming season, trimming year and stage of lactation. Housing type was statistically significant (p≤0.05) in both disorder groups. Cows kept in barns with solid floor had 48% lower odds of infectious and 28% lower odds of non-infectious claw disorders than cows kept in barns with slatted floor. Deep litter seemed to be a risk factor for infectious claw disorders. Bedding material was found to be significant (p≤0.05) in infectious disorder group; cows kept in barns with peat as a bedding material had 110% higher odds of having infectious claw disorders than cows in barns using shavings. Feeding system was found to be significant (p≤0.05) in both disorder groups; cows fed total mixed ration had 50% lower odds of having infectious and 39% lower odds of having non-infectious claw disorders than cows fed according to their milk yield. Annual number of maintenance trimmings were significant (p≤0.05) in non-infectious disorder group; cows trimmed once a year had 19% lower odds of having non-infectious claw disorders than cows trimmed at least twice a year. Between-claw trimmer variances and intra-class correlation coefficients (ICCs) were high, especially for infectious claw disorders, ICCs being 7.7% in the non-infectious disorder group and 20.6% in the infectious disorder group. Mixed ration feeding system and solid floor with free stalls were associated with healthy claws in both disorder groups. Standardizing the training of Finnish claw trimmers should considered because of the high inter-claw trimmer variances.

Table 1: Herd-level factors (p≤0.05) for infectious and non-infectious claw disorders, total number of observations (n), odds ratios (OR), 95% confidence intervals (CI) and p-values

		Infectious claw disorders			Non-infectious claw disorders			
Variable	Category	n	OR	CI	p-value	OR	CI	p-value
Housing	Slatted floor	7608	1			1		
type	Solid floor	3857	0.52	0.35 - 0.77	< 0.001	0.72	0.59 - 0.88	< 0.001
	Deep litter	884	2.30	1.30-4.05	0.002	0.80	0.59-1.09	n.s.
Feeding	According to yielda	9233	1			1		
system	Mixed rationb	1224	0.50	0.35 - 0.70	< 0.001	0.61	0.47 - 0.81	< 0.001
	Partial mixed ration ^b	1892	1.29	0.81-2.05	n.s.	1.18	0.94-1.49	n.s.
Bedding	Shavings	6154	1					
material	Peat	4234	2.10	1.49 - 2.98	< 0.001			
	Other	1057	0.93	0.53 - 1.62	n.s.			
	Information missing	904	1.39	0.79-2.47	n.s.			
Annual	At least twice a year	9625				1		
claw trimmings	Once a year	2724				0.81	0.68-0.96	0.013

^a concentrates and roughage fed separately

b concentrates and roughage mixed together

Dairy Cow individual feed intake model based on monitoring feeding behaviour

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The dairy cow individual voluntary feed intake is a practical measure of performance. It can be applied for: (1) nutrition design, either on group level (summing all the individuals) or on cow individual level when computerized concentrate self-feeders are stand-alone or in a milking robot. (2) given the animal production is automatically recorded (Milk Yield in dairy [MY] and Body Weight [BW] in beef cattle), the DMI allow evaluating feed efficiency on animal individual level.

However, monitoring the cow individual DMI is currently feasible only under research conditions (Halachmi et al, 1998).

Indirect measurements (DMI models that are based on MY, Days In Milking [DIM] and BW) exist (NRC 2001 and others) fit group wise. DMI models on cow individual level proposed by Halachmi et al. (2004, 2012) are complex (few hundreds coefficients) and not accurate enough.

Therefore the aim of this study was to develop a DMI model for the individual animal, based on sensors data that are nowadays exist in commercial farms.

The experiment took place in the ARO research farm in Bet Dagan. 100 high yielding Holstein-Frisian cows housed in open no stall cowshed fed TMR commonly fed in Israel. The individual cows' voluntary feed intake and feeding behavior were monitored at every meal by a feed-intake monitoring system described by Halachmi et al (1998). In addition MY, DIM, and BW, sensors' data such as feeding behavior, activity (neck and steps), lying behavior, milk composition, and rumination time were recorded. The highestcorrelation parameters were calibrated into a prediction equation. Data that were used for model development were not used fir model validation.

Results. Table 1 suggests that feeding behavior improved model accuracy. Other investigated parameter (not presented in this abstract, will be presented in the lecture) contributed less.

Conclusion. The proposed model, under our farming conditions (hot climate and high-yielding cows) specified above, predicted individual DMI better (mean absolute error 2.76 vs. 6.93 kg/day per cow)). In further research, the new model should be carefully validated under other farming conditions.

Table 1. Correlation coefficients and model accuracy

Correlation coefficients on group level: DMI and potential parameters in the model,

FCM	BW^0.75	FCM/BW	feeding behavior
0.49	0.20	0.29	0.28

Model accuracy on cow individual level; NRC model vs. the feeding behavior based model

	NRC (kg/day)	The new model (kg/day)			
ME	-6.76	-0.14			
MAE	6.93	2.76			
STD error	3.69	3.60			
Model STD	1.68	2.48			
Real STD	4.25	4.25			

Where DMI=dry matter intake; FCM = Fat corrected milk 4%. BW is Body weight. ME is the mean error, MAE is the mean absolute error; STD is the standard deviation. n=100 cows. %DM of TMR=0.629

The use of thermal imaging to detect digital dermatitis in dairy cattle

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Lameness in dairy cattle is condition that is associated with pain and other welfare consequences, and loss of milk production. Early detection combined with appropriate treatment is considered to be the most effective way of reducing the impact of lameness on cow welfare and farm productivity. Thermal imaging has been used in other veterinary and medical situations to detect differentials in surface temperature associated with inflammation and altered blood flow as indicators of disease and injury, and might be used to detect causes of lameness. However, it is essential that the most appropriate area of the foot for examination is identified and that the most appropriate summary temperature parameter is determined. A FLIR SC620 high performance infra-red thermal imaging camera was used for the study. Images of the lower hind limbs of 15 lame cows with digital dermatitis or interdigital dermatitis (DD/IDD) in one foot and 22 non-lame (control) cows were captured. Images were taken of the anterior aspect of the coronary band and the posterior aspect of the heels. ThermaCAM Researcher Software and Microsoft Excel were used to extract the mean, 95th percentile (95PCT) and standard deviation (SD) of the temperature data contained within the thermal image.

Firstly, it was shown that there was no difference in any temperature measure between the non-lame feet of lame cows and the feet of control cows in any part of the foot, indicating no systemic effect of DD/IDD. When lame and non-lame feet were compared, analysis of the coronary band images showed that lame feet had significantly higher mean, 95PCT and SD measures than non-lame feet (Table 1). For the heels, lame feet had significantly higher SD than non-lame feet, and there was a tendency for lame feet to show higher 95PCT values. These findings confirm earlier work suggesting that the coronary band measures can be used to detect lameness due to DD. The results also suggest that the data from heels and other statistical summary measures can be used to detect this pathology. Further research is needed to investigate other pathologies and to determine how this procedure can be automated.

Table 1. Comparisons between lame and non-lame feet in lame and control cows in mean, 95th percentile (95PCT) and standard deviation (SD) for coronary bands and heels. Means and SEM's (in brackets) are shown. (Lame feet: n=15; non-lame feet: n=57)

Coronary	band							
Mean			95PCT			SD		
Lame	Non-lame	P-value	Lame	Non-lame	P-value	Lame	Non-lame	P-value
27.09	26.32 (0.33)	0.021	31.76	30.30 (0.34)	0.015	2.80	2.40 (0.07)	0.014
(0.61)			(0.44)			(0.18)		
Heels								
Mean			95PCT			SD		
Lame	Non-lame	P-value	Lame	Non-lame	P-value	Lame	Non-lame	P-value
26.39	25.03 (0.35)	0.156	29.96	28.36 (0.35)	0.057	2.58	2.27 (0.07)	0.034
(0.67)			(0.63)			(0.20)		

Adipocyte sizes in the digital fat pad and their relationship to body condition score

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Introduction: Lameness and body condition are closely related. Recent studies showed that cows with low body condition score (BCS) have a greater risk for developing lameness as compared to cows with higher BCS. Amongst other reasons, this relationship might be related to reduced thickness of the digital fat cushion in lean cows. The digital cushion is not a homogenous structure but consists of different fat pads and connective tissue. We hypothesized that either high or low BCS will result in concordant adipocyte sizes in the subcutaneous (sc) body fat depot and the fat pads of the digital cushion irrespective of the localization of the latter. Material and methods: The right front claws were collected from 20 Holstein Friesian cows at a local abbatoir. Cows were selected according to their BCS: 10 cows with BCS < 3 (loBCS) and 10 cows with BCS ≥ 3 (hiBCS). After dissecting the horn capsule of the lateral claw, samples (1 cm3) of the axial and abaxial fat pad were prepared for histomorphological examinations. In addition, sc fat samples were excised from the tailhead of all cows. All adipose tissue samples were stained with Hematoxylin-Eosin and adipocyte sizes (µm2) were measured. Results: The adipocyte size in sc tailhead fat was greater in hiBCS as compared to loBCS cows (6495±1819 μm2 versus 3155±1304 μm2; p < 0.05). Similar differences between the BCS groups were apparent for the adipocytes from the axial fat pad (hiBCS: $2241\pm599 \, \mu m2$; loBCS: $1378\pm282 \, \mu m2$; p < 0.05), whereby the adipocytes in tailhead fat were bigger than those in the digital cushion (p < 0.05). In contrast to the axial and the sc fat, the adipocyte size in the abaxial fat pad was similar in cows from both BCS groups (1477±495 µm2; 1598±193 µm2, p > 0.05). Conclusion: The lower adipocyte sizes in the axial fat cushion support the notion that the adipose tissue of the digital cushion participates in changes of fat mass. However, based on the differences between the axial and abaxial fat pad, these fat localisations seem to play different roles concerning their metabolic and mechanical functions.

P1.12

Geometric and non-linear parameters of heart rate variability as indicators of chronic stress caused by lameness in dairy cows Viktor Jurkovich¹, János Tőzsér², Margit Kulcsár-Huszenicza³, Luca Kézér⁴ and Levente Kovács⁴

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The effects of lameness has so far received little attention in animal stress and welfare studies. According to recent human research findings, geometric and non-linear measures of heart rate variability (HRV) are suitable for the investigation of chronic stress. We measured heart rate, HRV and faecal glucocorticoid concentrations in lame [with locomotion scores (Sprecher et al.) between 3 and 5; n = 56] and non-lame (with locomotion scores 1 and 2; n = 52) Holstein-Friesian cows. 5-min time-windows of interbeat intervals in undisturbed lying periods were used for HRV analysis.

An elevated parasympathetic tone was found in lame cows, as indicated by lower heart rate and higher vagal activity measures RMSSD, HF and SD1. Indices of the sympathovagal balance (LF/HF and SD2/SD1) were lower in lame animals. All studied geometric and non-linear HRV measures (TINN, RRtri index; Lmax, RQA, DFA1, DFA2, Correlation Dimension) were lower in lame cows compared to sound ones. Faecal glucocorticoid concentrations did not differ between groups.

Depressed heart rate of lame cows is possibly an effect of endogenous opioid secretion. The higher parasympathetic tone in lame cows requires further studies to be fully investigated. A reduction in the geometric and non-linear parameters are indicative of stress and data of lame cows are similar to that of patients with chronic illnesses and emotional distress. It appears that chronic pain does not necessarily lead to a change in faecal cortisol concentration.

Claw conformation in dairy herd management of Slovakia

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Between 2008 - 2014 claw conformation, occurrence of claw diseases and their impact on production and reproduction traits of dairy cows in Slovakia were studied. More than 1200 dairy cows (Holstein as well as dual purpose) from nucleus herds, producing in lowland as well as in highland condition were included into study. All data were from intensive, high concentration farms. It was observed that 90 % of lameness was caused by sole ulcer, heel erosion, digital dermatitis, interdigital dermatitis and chronic dermatitis. If looking on claws from view of breed, most often were claw disorders observed in Holstein cattle, where almost 43% of cow had signs of any claw disorder. Digital dermatitis (DD), interdigital dermatitis and heel erosion (IDHE) as well as sole ulcer were present similarly in 16% of cases. In case of Slovak Simmental breed it was 37%. Bit interesting is higher proportion of sole ulcer up to 21%, with DD and IDHE being present in 8% of cases. Lowest presence of claw disorders was in Slovak Pinzgau population only 33%. In this group was occurrence of sole ulcer lowest, only 7%. Presence of DD and IDHE was 13%. Visible lameness was always connected with claw disorder, where in dual purpose breeds equal to 3rd – 4th grade of disorder, whereas in Holstein even in 1st to 2nd level. Between breeds were observed significant differences also in claw formation. Claw measures were observed immediately after claw trimming. Cows with different diagnosis and level of disorder had higher age at first calving (diff. 1 – 3 months). Presence of IDHE and SU in first lactation cows had negative impact also on production of milk on first lactations. Subsequent analysis of data showed that cows with higher insemination index and therefore higher days open differed in height of heel, diagonal of the claw, height of the claw and claw width referring to the cows with only one insemination needed. It is interesting that sole ulcer was present in cows with highest production. Genetic background of healthy and ill cows was the same, because they don't differed significantly in EBV's of milk.

P1.14

Assessment of the mechanical properties of claw horn using tensile strength, puncture resistance and Vickers hardness tests

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Lameness is one of the main welfare issues of the dairy industry. Claw horn disease is one of the main causes of lameness and horn quality (mechanical properties) is known to be affected by; partition, nutrition, haemorrhaging and environmental conditions. As a consequence the mechanical properties of claw horn were assessed using, tensile test, Vickers hardness and puncture resistance. The elastic modulus (EM) was found to be significant lower in sole (P=0.009) (zone 5 of the international foot map (IFM)) horn taken from heifers at 120 dpp (days postpartum) (518.9 MPa) when compared to 30 dpp (872.4 MPa) as was the puncture resistance (PR) (P=0.001) of the white line (30 dpp zone 2: 0.9 log 10, Sole: 0.98 log 10; 120 dpp, WL: 0.63 log 10, Sole: 0.82 log10). There was a decrease in claw hardness (WL: 30dpp; 131.4 MPa, Sole; 135.3 MPa; 120 dpp: WL; 126.5 MPa, Sole; 133.4 MPa) over lactation, however this was not significantly different, but WL consistently had a significantly lower PR (P=0.005) than sole. Lesion score did not significantly affect EM or hardness, but PR decreased significantly with increasing lesion score of the WL and at a lesion score of 3 in the sole horn (P 0.014). Moisture content (MC) was shown to significantly reduce the EM of claw horn (P=0.001); at physiological MC (726.5 MPa) compared to samples which had been soaked for up to 72 hrs (299.9 MPa). However, the length of time that claw horn spent soaking in distilled water did not significantly affect EM. All three mechanical tests are valid methods to determine mechanical properties of claw horn as each one assessed different aspect of mechanical strength, but PR reflected differences in claw horn mechanical properties.

A meta analysis of claw horn disease and claw horn anatomy in UK and New Zealand first lactation dairy cattle

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In dairy cattle lameness is one of the main economic and welfare issues, which directly affects the sustanability of dairy production. However, while considerable amounts of data exist in many countries, there have been few similarly detailed studies assessing claw horn disease (CHD) and anatomical characteristics in the first lactation completed in New Zealand (NZ). This research used meta analysis to compare studies of CHD in 166 first lactation dairy heifers the UK and NZ, which were assessed by the same research methods over a 5 year period. Days postpartum (dpp) significantly affected the number, percentage and total lesion score for both sole and white line, and peak haemorrhaging in NZ occurred at 110 dpp and had declined by 160, a similar pattern where peak lesions were seen at 100 dpp and levels had reduced by 150 dpp (UK). The median locomotion score of 1 with a peak score of 2 occurred between 71 and 98 dpp (NZ), which was a little sooner than the 110 to 120 dpp found in many other studies. A similar pattern for claw lesion score (CLS) to peak approx 110 to 120 days occurs irrespective of location. However the UK based research had far lower CLS (0.3) for both sole and white line lesions than in NZ. Overall, there were no significant relationships found between claw horn growth and wear rates, CLS dpp and PR (growth = Average growth = 5.98 - 0.386 Sole PR + 0.0324 days + 0.0033 CLS Sole R-Sq(adj) = 15.4% P= 0.402 and average wear = 9.52 - 0.458 Sole PR + 0.0173 days - 0.0112 CLS Sole R-Sq(adj) = 3.4% P= 0.600, and net change Net = - 11.2 + 0.244 Sole PR + 0.0396 days + 0.0303 CLS Sole R-Sq(adj) = 12.6% P= 0.573). Monthly growth rates were generally higher in the NZ compared to the UK, while claw horn wear rates were higher in NZ compared with the UK at 100 dpp. NZ heifers maintained a shallower claw angle, shorter dorsal border and higher heel height than UK heifer resulting in a smaller more compact foot and smaller wearing surface. The dorsal borders and heel depth in UK heifers were longer and shallower than those in NZ heifers, but UK foot angle was steeper than NZ.

P1.16

Improved phenotyping of dairy cow fertility traits

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Selective breeding for improved fertility of dairy cows is hampered by low heritability of traditional fertility traits. Although fertility shows large additive genetic variation records have large unexplained residual variation. Also, in comparison to production traits, fertility traits are binomial or having censored records, which creates problems in statistical analyses. Furthermore, dairy herd managers may impose their personal judgments and decisions on when to perform AI and thereby bias records. In progeny testing schemes these difficulties are balanced through phenotypes being based on large groups of daughters. The use of DNA based genomic selection methods has to some extent improved the accuracy of breeding value estimates but further improvements can still be achieved if more accurate phenotypes become available. Indicator traits based on milk progesterone (P4) are one option; another is the use of data from activity tags (Act) already in widespread use for estrus detection. In principle, both types of indicators can deliver data free of manager-imposed bias, and both types can be automated. The key questions are how good the indicator-derived phenotypes are in terms of heritability and correlation to the "true biological" fertility traits. We have investigated these questions both on research station data and on commercial herd data. Among the fertility traits, days from calving to first estrus event (CFE) can be obtained from both traditional AI data, as days to first AI and from Act. Our findings from experimental data were that Act-CFE gave higher heritability than AI-CFE and that the two traits were closely correlated. These findings support the hypothesis that objective and automatically recorded fertility traits are useful as alternatives to the traditional traits. The findings also suggest that estrus strength and duration can be assessed using activity tags. In order to make full use of Act and P4 it must be ensured that key data are in a transferable format and that data transfer to central databases is established.



Fiber Optic SPR biosensor for progesterone detection in raw milk as a tool to improve reproduction

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In the last 15 years, the improved milk yields of dairy cows have been accompanied by a decreased fertility which is visible in the increased calving interval. Accurate estrous detection is crucial in reproductive management. The currently used methods of heat detection such as visual observation or activity sensors are not adequate due to a higher rate of false negatives. Detection of progesterone, a key hormone in the regulation of the estrous cycle, has the advantage of determining heat in combination with the ability of early diagnosis of pregnancy or abnormalities in estrous cycle.

In this work, a biosensor that quantifies progesterone in milk is developed. The bioassay is based on a fiber optic surface plasmon resonance (FO-SPR) sensor that was previously developed in our group as a cost-effective alternative for the expensive and more complex SPR systems used in research centers. Therein, the platform was evaluated for the detection of proteins and detection of single nucleotide polymorphisms (SNPs) using antibody- and DNA-based bioassays, respectively. The FO-SPR biosensor can perform fast, specific and sensitive detections.

For the detection of progesterone a competitive immunoassay is developed, which is needed for detection of low molecular weight molecules since the sensitivity of SPR sensors is determined by changes in weight near the sensor surface. In the competitive assay, a detection antibody is bound either to a progesterone molecule from a sample or its derivative that is immobilized on the sensor surface (Figure 1A). To improve the limit of detection, the signal of the bound detection antibody is amplified by use of gold nanoparticles (Au NPs) functionalized with a secondary antibody. This competitive bioassay showed a detection limit of 0.23 ng/ml in half diluted raw milk (Figure 1B) and the obtained dynamic range is compatible with the expected concentration range of 0.5-10 ng/ml in undiluted raw milk.

Future work will focus on reducing the assay time and increasing the reusability of the sensor. The final goal is to integrate this sensor with a sampling device in order to make the interaction with a milking robot and allow automated at-line measurements in a dairy farm.

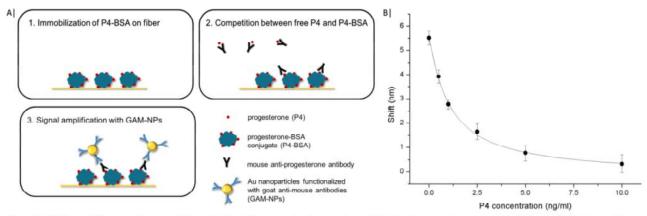


Figure 1: A | Scheme of the competitive assay for the detection of the small molecule progesterone. B | Calibration curve of progesterone detection in half diluted raw milk.

Using Indoor Positioning System to Measure Lameness in Dairy Cows

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Lameness in dairy cows causes significant economic losses to modern dairy farms and has adverse effect on animal welfare. We have studied the effect of hoof lesions on the walking and lying behavior of dairy cows.

Two experiments were performed to quantify the accuracy and the noise level of the used Ubisense indoor positioning system 1) the amount of static noise in different parts of the barn was measured using a fixed grid of 2.6x2.6m and keeping a tag stationary at each point for 60s 2) The tags were attached to the neck 32 cows and the position of the tags was recorded manually by using direct observations yielding in total of 331 reference points. These experiments were used to choose a suitable filter for the measurement system.

In order to measure the effect of lameness on the behavior of 25 dairy cows was measured over 3 weeks using Ubisense indoor positioning system and Rumiwatch pedometers in a freestall barn with slatted floors and herringbone milking parlour. Daily walking distance was calculated from the positioning data after median filtering, lying time and the number of steps were obtained from the pedometers. The cows were inspected for hoof lesions at the end of the experiment using the Finnish hoof health recording system. The effect of lesions on behavioral parameters was analysed using the Wilcoxon ranked sum test.

Hoof pathologies had clear effect on the daily walking distance and lying time of the cows. Cows with sole ulcers, white line fissures or interdigital fibroma had significantly lower daily walking distance ($-505m \pm 115m$ difference) than healthy cows. The lying time of cows with sole ulcers or interdigital fibroma was higher than for healthy cows or cows with white line fissure.

The results indicate that positioning system brings added value to lameness studies and that different hoof lesions may affect the lying behavior differently.

P1.19

New approaches for management and breeding of dairy cows in automatic milking systems (AMS)

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In Norway, we have recently received funding (2.4 mill euro) for a larger multidisciplinary project involving researchers from social sciences, animal and veterinary sciences, mathematics, physics and chemistry. We aim to expand on a long tradition for research on herd recording (NDHRS) in Norway, in that we will combine information from NDHRS with data from AMS and sensors to improve genetic evaluations of Norwegian Red, develop better feeding strategies and new systems for surveillance of energy balance (EB), health and reproduction.

Specifically, indirect assessments based on measures of fatty acids in milk by infrared (IR) spectroscopy have proven to be good indicators of EB (McParland et al., 2012) and metabolic disorders (Van Haelst et al., 2008). Our group has recently submitted results on onset of ovarian activity after calving (OLA) related to fatty acid (FA) content in milk obtained by Fourier Transform Infrared (FTIR) spectroscopy) in dry films of milk (Afseth et al., 2010), and we will expand on this work in the new "AMS project".

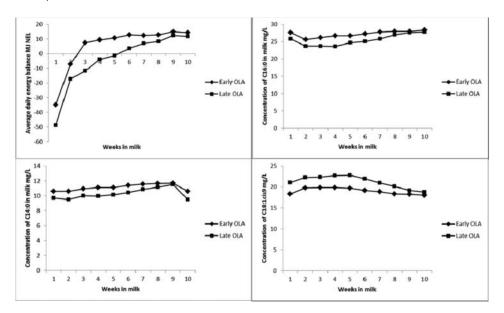
OLA was defined as the first day that milk progesterone concentrations were >3ng/ml for two successive measurements, the study population was categorised as early (n=47) or late (n=40) OLA, using the median value of 21 DIM as the cut off.

Higher concentrations of the medium chained FA C14:0 and C16:0 were found in the early than in the late OLA group from WIM 1 (Fig). Medium chained FA are synthesized de novo (Bauman et al., 2006) in an energy consuming process. In WIM 4, C16:0 concentrations predicted late OLA with 74% sensitivity and 80% specificity.

The long chain FA C18:0 and C18:1cis9 were lower in the early than in the late OLA group (Fig). FA containing more than 16 carbon atoms, are absorbed into milk from circulating blood, and originates partly from fat reserves (Stoop et al., 2009).

We concluded that dry film FTIR spectroscopy of milk can be used to provide real time information about a cow's metabolic state and OLA. Next, we will combine data from different sources to increase the predictive abilities of the system.

Figure. Graphs displaying mean values of (clockwise from top right): a) daily energy balance, b) concentration of C16:0 in milk, c) concentration of C18:1cis9, d) concentration of C14:0 in milk, by weeks in milk groups according to early or late onset of luteal activity.



P1.20 Incidence of claw diseases and reproductive problems in dairy cows in Serbia

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This paper shows data on claw diseases incidence in 2635 highly-yield Holstein-Friesian cows in tie housing system. In the analysis following data from one-year farm record were taken into account: diagnoses (Rusterholz ulcer - RH, dermatitis interdigitalis - DI, dermatitis digitalis - DD, phlegmon - PH, pododermatitis septica - PS, panaritium - PAN, and laminitis - LAM), parity of cows (1st to 5th lactation), season (1. cold season, from October to May; 2. warm season, from April to September) and month when the problem was detected. In animals with claw disease, reproductive efficiency indicators were also taken into account.

According to the results, in 37.44% of cows some claw disorders was diagnosed, and in 87.70% of these animals some indicators of impaired fertility were registered (prolonged service period, higher insemination index and/or extended interval from the first artificial insemination after parturition to the successful conception).

The highest number of cases has been registered in third lactation, and the lowest number in cows in first lactation (56.46% and 2.46% of all cases, respectively), with DD and LAM as predominant diseases (46.38% and 43.30%, respectively). The season has shown statistically significant influence on the occurrence of DD, PAN and LAM (p<0.005), and DI and PS (p<0.05), the parity on the occurrence of RH, DI, DD, PH, PAN and LAM (p \leq 0.001), and the month on the occurrence of PS (p<0.05). Statistically significant positive correlation between the season and DI (p<0.05), DD (p<0.001), PS (p<0.005), PAN (p<0.001) and LAM (p<0.005), as well as between parity and RH (p<0.001), DI (p<0.005), PH (p<0.001, PS (p<0.005) and LAM (p<0.005) were obtained.

At large capacity farms, considering the number of heads and the time required for hoof trimmers to inspect all animals, a number of cases certainly remains undetected and their treatment is delayed. Inter alia, the health and welfare of cows from these farms can be improved by constant education and stimulation of personnel, and by appliance of better methods of diagnosis, control and recording of cows' claw diseases and other health disorders

Odour-based heat detection in dairy cows: a pilot laboratory study

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Current automated heat detection methods for dairy cattle are based on activity meters or milk progesterone analyses. The profile of volatile pheromone compounds varies during the oestrous cycle. An eNose is a device developed for analysis of gas mixtures and could potentially be used to detect heat, as well as health problems such as sub-clinical ketosis, in cattle. In the present pilot laboratory study, we investigated whether eNose could discriminate odour samples (vaginal fluid) between oestrous and nonoestrous dairy cows. The odour samples (OS) were collected from 13 dairy cows by rotating a cotton tipped applicator in the vagina (minimum depth of 7.5 cm) through a complete revolution in each direction before withdrawing. During the odour sampling, the cows were either in dioestrus (between Day -14 and Day -8, 6 OSs), in oestrus (Day 0, 28 OSs), or pregnant (Day 42, 26 OSs). Oestrus was defined as the day of standing heat (a cow allowed other herd mates to mount her while she remained standing). Dioestrus and pregnancy were defined by rectal palpation of ovaries and uterus confirmed with ultrasound examination. The OSs were kept in a freezer (-20 °C) and thawed on the day of the laboratory analyses. Each OS was analyzed using ChemPro 100i eNose (Environics Inc., Mikkeli, Finland) which yields a smell print with 18 variables. A Sammon projection (Figure 1) shows that the smell prints of the cows in oestrus are in one cluster and the smell prints of the cows in dioestrus or pregnant in another cluster. Logistic regression was used to identify a classifier that discriminates the former cluster from the latter cluster. Leave-one-outcross-validation was used to validate the results. After cross-validation, the classifier correctly classified 90% of the samples, resulting in a sensitivity of 88% and specificity of 93%. The results show that eNose has considerable potential for heat detection. However, further biological validation with a larger data set is needed. Future studies should focus also on development of less invasive sampling such as analysis of milk headspace or development of environmental sensor that simply sniffs the animal.

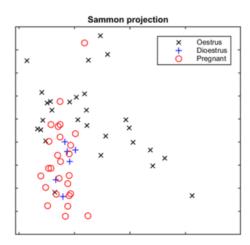


Figure 1. A Sammon projection visualizing the distribution of odour samples (N = 60). Pregnant cows and cows in dioestrus overlap significantly. Cows in oestrus form a distinct cluster, suggesting that odour-based detection of heat is feasible.

Lameness research brought to a new level

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Lameness research in dairy cows has reached a whole new level at ILVO with the new dairy stable and its accompanying gait monitoring setup. The gait of up to 144 cows will be monitored daily using a wide range of technologies. This data will be used for the development and fine-tuning of lameness detection systems. The sensor systems used are placed in an outdoor alley where they measure the walking pattern of every individual cow while returning to the barn after milking. Besides two Gaitwise systems, also the StepMetrix and antennas to collect the data of different accelerometers are implemented in the setup. In addition, extra space is foreseen in order to add other technology to the setup in the near future. A selection gate at the beginning of the alley allows cows to walk over the setup one at a time and allows to identify the cows. Using a bridge that allows the surface of all systems to be at the same level and a similar rubber flooring, cows walk undisturbedly without noticing the different type of sensors in the flooring. Additional lightning and the presence of a roof top ensure normal cow walk during dark weather and at night. A selection gate at the end of the setup guides cows to go back to the stable, to the pasture or into the separation box. Simultaneous video recordings are stored in order to link the aberrant data to gait scores based on visual observations of the video recordings of the considered measurement. Gait scoring will be performed by experts once a week on a scale from 1 to 3 (non lame, slightly lame and severely lame) using different lameness indicators. Claw health records will be stored to assess hoof and lameness problems. The gathered data will be used to improve automatic lameness detection results and will be combined with other available cow data like milk production, lactation stage and parity.

P1.23

Use of the Lifecorder+® sensor to assess grazing time of dairy cows

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The Lifecorder+® is a uniaxial neck mounted activitymeter. It was tested to assess grazing time in two French experimental AMS farms (20 cows equipped in Derval farm, 14 cows equipped in Trévarez farm). The Lifecorder+ raw signal (from 0 to 9) was converted into a grazing yes/no information over a certain threshold. The data from the sensors were compared with visual observations as reference: trained observers recorded activity with a scanning every 10 minutes in the pastures. The recorded activities were the following: grazing / ruminating and standing / lying / walking. Observation sessions were performed in Derval and Trévarez farms. Finally, 20 recordings were available for Derval farm (121 h of cumulated observation time in pasture) and 91 for Trévarez farm (336 h of cumulated observation time in pasture). The results show a high correlation of grazing time between the visual observations of activity and the information from the sensor (R2 = 0.93 in Derval farm and 0.82 in Trévarez farm) with a mean prediction error (MPE) of 18 min (9%) in Derval farm and of 29 min (20%) in Trévarez farm. Some slight biases related to the recording of walking in the pathways were noticed. Lifecorder+® appears to be a possible cheap, easy and precise tool to record grazing time at pasture.

A cross-sectional study of lying behaviour on 23 UK dairy farms: investigating within and between herd

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Dairy cow lying behaviour is sensitive to changes in farm management and individual cow characteristics such as health status, and is therefore a promising tool for the assessment of cow comfort and wider welfare status. There is currently a drive to provide evidence based consultancy services to UK dairy farmers on issues such as cow comfort. To date, however, most research into lying behaviour on commercial farms has been conducted on cubicle systems in North America. To inform improvements in UK farm management and consultancy services, we aimed to describe variation in daily lying time, lying bout frequency and bout duration at the day, cow and herd level in a cross-section of UK farms; and investigated the effect of individual cow and farm environment factors on lying. Lying behaviour was recorded using accelerometers over a three or four day period on 23 UK dairy farms representing a range of housing systems. Large individual differences in lying behaviour were observed with much greater variation occurring within herds than between herds. Whereas mean lying time ranged from 7 to 12 hours/day between herds, ranges of up to 12 hours (e.g., 5 to 17 hours/day) were observed within herds. The level of variation across different recording days varied substantially between cows, with within cow range in daily lying time ranging between 0.2 and 11 hours. Linear mixed effects modelling was used to determine the potential causes of these individual differences, with separate analyses undertaken for cows housed in cubicles and straw yards. In cubicle cows, there were significant effects of stage of lactation, parity, mobility, cubicle length, cubicle surface and recording day; with, for example, late lactation, severe lameness and deep bedded sand associated with increased lying time. Results support previous findings of an association between lameness and increased lying, reiterate the importance of soft lying surfaces in cow comfort, and highlight differences in lying behaviour across different days of the week (possibly a result of differences in farm daily routine on different days). The effect of these and other factors on the lying behaviour of straw yard cows will also be discussed.

P1.25

Effect of heat stress on daily activity patterns in lactating dairy goats

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Heat stress (HS) induces hormonal and behavioral changes, but little is known about these changes in dairy goats. Eight multiparous Murciano-Granadina dairy goats ($43.3 \pm 1.6 \text{ kg BW}$; $2 \pm 0.04 \text{ L/d}$; $81 \pm 3 \text{ DIM}$) were kept in metabolic cages and randomly assigned to 2 climatic treatments according to a crossover design (two 28-d periods). Treatments were:1) thermal neutral (TN; 15 to 20°C, 40 to 45% humidity, THI = 59 to 65), and 2) heat stress (HS, 12 h/d at 37°C and 40%, and 12 h/d at 30°C and 40%, THI = 86 and 77, respectively). Eight video cameras with infrared illuminator were installed on the top of each cage. Changing the position bouts, duration of remaining standing, as well as eating and drinking bouts and duration were measured. Changing the position (from lying down to standing and vice versa) during daylight and night was much greater (P < 0.01) in HS (18.9 times as daily average) than TN goats (7.1 times as daily average). The increment in the frequency of position changing indicates that HS goats were uncomfortable and had extra-movements. The TN and HS goats had similar total daily eating bouts (40.7), but the duration of each bout was shorter (P < 0.05) in HS (4.8 min) than in TN (8.4 min). When temperature decreased from 37 to 30°C during the night, eating bouts were greater (P < 0.05) in HS (16.4) than TN (8.8) goats. For the water drinking, HS had greater number of drinking bouts (30.4 vs. 12.8) with no change in drinking bout durations between both groups (0.48 min on average). In conclusion, the typical reduction of feed intake in HS is due to the shorter time of eating bouts rather than the number of bouts. On the other hand, the greater water consumption is explained by the increment in the drinking bouts with no change in the duration of each drinking action.

Is she lying? Validation of IceQube-recorded lying in dairy cows.

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Lying behaviour is a relevant indicator for the evaluation of cow welfare. Lying can be recorded automatically by data loggers. A threshold is required, however, to discard false lying records caused by horizontal leg movements (e.g. scratching). No such threshold exists for the relatively new IceQube sensors. We aimed to 1) validate lying periods recorded by the IceQube sensor and 2) determine a threshold for lying duration in dairy cows. It was assumed that true lying can always be recorded on both hind legs. False lying, on the other hand, was assumed to occur in one hind leg at a time. Therefore, both hind legs were equipped with an IceQube sensor and used as each other's validation. This two-sensor approach was applied to 28 cows for a period of six days. Lying records that occurred on both hind legs were classified as true, while lying records that occurred on one leg only were classified as false. False lying constituted 4 percent of the records. The durations of false and true lying records overlapped. Based on maximum accuracy, a minimum lying duration of 33 seconds was determined. This threshold filtered 98 percent of the false and retained 99 percent of the true lying records. Applying the threshold hardly affected estimates of total lying time, but especially improved estimates of the average lying duration and lying frequency per cow. Next to sensor validation, the two-sensor method is potentially valuable for automated welfare monitoring on dairy farms: false records could be related to grooming activities and impaired cow mobility, which may be indicative of floor slipperiness and lameness.

P1.27

On-farm dairy cow welfare assessment: what's going on in Italy?

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The Italian Reference Centre for Animal Welfare (CReNBA) has set-up a system to assess loosely housed dairy cows welfare. CReNBA method fundamentals are the European research projects (WQ®), publications (EFSA) and laws, and the scientific literature.

CReNBA on-farm assessment system, developed in 2011, includes both animal based measures (ABMs) and non-animal based measures (N-ABMs) and examines all the animals present within the farm (lactating and dry cows, young animals). The system is organized in four areas: A, B, C and D (see Figure). The method also includes the analysis of biosecurity parameters (Area E 15 indicators). Within a specific area, each single indicator is weighed basing on its importance in relation to welfare or biosecurity. The final outcome reports the scores of the five areas (A, B, C, D, E). The sum of the scores obtained in areas A, B, C and D gives the farm level of welfare. Legislative non-compliances are also reported.

Before the on-field application, the system was validated for inter-observers agreement. From 2011 to January 2015, 922 dairy farms (size: 7 2736 total animals; milk production: 10 41 kg) were assessed by trained veterinarians. System on-farm application took maximum half a day. The examined farms covered the entire National territory. Since 2013, the method has included also biosecurity assessment, which has been applied to 527 out of 922 currently examined herds. Analysis of the collected data (922 farms) reveals a good level of welfare in the majority of the evaluated farms: 881 farms (95.6%) showed a welfare level over the achievable mean score. Despite this result, in 527 farms (57.2%) legislative non-compliances were recorded. The most frequent non-compliances were related to calves lack of contact in individual pens (36.8%) and disbudding over three weeks of age (24.3%). Concerning biosecurity assessment 362 out of 527 (68.7%) evaluated farms resulted under the achievable mean score.

Relationship between the scores obtained in N-ABMs areas (A, B, D) and the score achieved in Area C (ABMs) was analysed by multivariate linear regression (results in the Table). The analysis demonstrated that improvements in areas A and B can positively affect Area C result.

Area A Farm management and personnel 21 indicators		Area B Facilities and equipment 28 indicators		
		FARE VEL	Area D	
Area C Animal based measures 18 indicators		Inspection of microclimatic environmental conditions and alarm systems 7 indicators		

Variables	Estimated coefficients	Standard error	t value	P value
Intercept	11.310	2.933	3.856	< 0.001
Area A	0.708	0.074	9.615	< 0.001
Area B	0.358	0.055	6.507	< 0.001
Area D	0.057	0.212	0.270	0.787
R ² =0.26				

Effect of introducing replacement heifers into the dairy herd either with or without adult dairy cattle and bed type on bed uptake rate, lying time and daily behaviour patterns of dairy cattle

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This research aimed to compare Effect of introducing replacement heifers into the dairy herd either with or without adult dairy cattle and bed type on bed uptake rate, lying time and daily behaviour patterns of dairy cattle. A total of 75 animals were divided into four groups of 18 dairy cattle, which were of similar age, live weight and condition score were either; offered access to two types free stall bed surface (rubber wingflex (R) or foam matrass (M)) either with heifers only (Solo) or with mixed age adult cows (Cows) and dairy heifers. All animals were introduced to beds with activity meters attached and were observed for 24 h each day, for a period of 5 days. The data was found not to be normally distributed was transformed and analysed using mixed Proc GLM in SAS using diet as a fixed effect and calf as a random effect in the model. Data was presented as means with standard errors for each observation according to diet group. The introduction of heifers solo resulted in complete uptake (100 % of heifers) of beds in less than 3 (1.41) days, whereas heifers mixed with cows resulted in heifers remaining standing for up to 3 d continuously and 3 to 4 % of cows not lying in free stall beds. Freestall bed, foam matrass and wingflex rubber matts) surface had no effect on the rate of bed uptake. The introduction of heifers to freestalls as a group of heifers without adult dairy cattle is recommended as the better option, rather than with adult dairy cattle. This can be achived when heifers return to the dairy unit, following teat sealing and prior to calving.

Contemporaneous and lagged effects of on-farm welfare assessment on reproductive parameters in dairy cattle.

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Stress is an altered homeostasis of the animal with welfare loss. It affects negatively on reproduction, being the alteration in GnRH's secretion the most important effect. Likewise, stress results in an increase in plasma of cortisol, resulting in a decreased capacity of the immune response of animals. Therefore if stress is low, there will be more healthy animals and farms with better reproductive performance. The aim of this work was studying if welfare indicators (WI) measured at a given time will affect reproductive performance (R) at that moment or 6 and 18 months later. This would allow us to advise the farmer on preventing problems in the future. Six dairy farms were studied from 2012 to 2014. Data collected were: body condition score, size of troughs, dirt of lower legs, hindquarters and udders, cleanliness of the animal, time needed to lie down, lameness, eye and nose discharge, diarrhoea, downer cows, dystocia, calving intervals, open days, fertility, calving-1stservice interval, services per conception, heat detection rate, days in milk and milk production. When contemporaneous data were analyzed we observed that housing factors were the most strongly correlated with reproductive performance; feeding factors were also correlated with reproduction, whereas few health parameters show correlation. Regarding to asynchronous data lagged for 6 months, the tendency was the same, although dirt of udder was the housing factor most significantly correlated with different reproductive indexes. When the interval between WI and R collection was lagged for 18 months, few correlations were detected. To sump up, dirt of lower legs, hindquarters and udders showed a strong correlation with some reproductive indexes at the synchronous and lagged (6 and 18 months) time of data collection. These results reaffirm the importance of comfort around the resting and highlight the impact of dirtiness of dairy cows on the on-farm reproductive data.

P1.30

Habituation of dairy heifers to the TellingtonTTouch treatment

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The Tellington TTouch is a positive handling procedure, which is deemed able to improve human animal relationship. The aim of the present study was to assess the habituation of 10 heifers to this treatment. In this study the treatment consisted of circular or stroking movements conducted on the left side in two body regions, hind leg + udder and hind quarter + tail. Animals received 4 treatment sessions in two days about a week before parturition. For each animal and each session the treatment was terminated when the heifer accepted to be touched without showing adverse responses. The mean duration of each session was 9,4 ±2,4minutes (mean ± S.E). During preliminary observations performed in vivo, repetitive behaviours were identified, which were deemed indicative of adverse reactions(e.g. number of kicks, steps, and escape attempts, duration of moving around in the box). Three possible responses were identified and qualitatively assessed: "calm" (no overt adversereaction), "low response" (one or two adversereactions), and "nervous" (more than two overt adversereactions). The evaluation method was developed during the present study; therefore, it was necessary to verify its reliability. Forty videos (4 sessions x10 heifers)concerning experimental animals were used to assess inter- (two observers were used) and intra-observer reliability (each video was observed twice). The evaluation of the videos was performed using the software "The Observer XT". Both inter- and intra-observer reliability were high (r > 0.7, P<0.001). The duration of treatment without adverse reactions increased with the TTouch session (P<0.01). The response "calm" also increased from the first to the fourth treatment, albeit not significantly. These results indicate that heifers tend to habituate to the Tellington TTouch treatment. The lack of a marked effect of the TTouch method may be attributed to the fact that the treatment was either too short (only two applications x two days) or too weak.

Validation of a new monitoring system (RumiWatch®) for recording the grazing and rumination behaviour of dairy cows Markus Rombach ^{1,2}, Andreas Münger¹, Karl-Heinz Südekum² and Fredy Schori¹

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The RumiWatch system, consisting of a noseband sensor and a pedometer, is promoted as a measurement system for automatic health monitoring by detecting jaw movements. Currently, several studies are undertaken using this system, which investigate assessment of animal welfare and early disease detection. Until now the system has not been validated under grazing conditions. The aim of this study was to compare the RumiWatch records to direct visual observation with grazing dairy cows. Eighteen lactating Holstein cows were kept on pasture during 18 hours per day and 12 of them were supplemented in the barn with maize silage or maize silage plus a protein concentrate. Cows were equipped with the RumiWatch halter over three two-week periods. During these periods the grazing and rumination behaviour were also visually observed on pasture, each cow 30 times over 600 sec. The visual data were compared to the concurrently recorded data of the RumiWatch. RumiWatch detected the jaw movements reliably, but classification errors occurred and 120 out of 540 files got lost. The main causes for loosing files were, cable break or failure of the noseband sensor. Finally 420 files could be used for the validation. In 80 out of these 420 files the jaw movements were (totally) incorrectly assigned to grazing or rumination. The error rate is expressed as average percentaged deviation of the RumiWatch records compared to the visual observations for three major parameters. These are chews during grazing, -19%, 605 (SD 279) observed chews / 10 min, chews during ruminating -11%, 580 (SD 206) observed chews / 10 min, and rumination boli 1%, 10 (SD 4) boli / 10 min. In the beginning a matter of concern were injuries caused by wearing the RumiWatch halter 3.0 over longer periods but, with a new halter design, this problem could be solved. The results indicate, that there is a potential to detect characteristics of dairy cow eating behaviour with the RumiWatch system. To achieve this, the analysis algorithms need to be refined for a better differentiation of detected jaw movements.

P1.32 The effect of high and low concentrate feeding to cow's feeding behaviour Maria Soonberg and David Arney Estonian University of Life Sciences maria.soonberg@emu.ee

In robotic milker dairy systems, where ration concentrate and forage components are offered to the cows separately, lack of control over intakes can result in problems balancing the forage and concentrate portions of the diets, leading particularly to problems associated with high concentrate intakes and concomitant low forage intakes, such as acidisosis, effects on milk components and overcondition at the end of lactation. In order to check this as a problem on a dairy unit, the feeding behaviour of a sample of cows were observed by video recording and automatic positional data estimation (trackacow*). In particular, the number of daily visits to the feed barrier, the duration of these visits and actual feeding, of high and low yielding cows were recorded. As a pilot study, four cows (two at the highest yield and two at the lowest yield of the milk production range) were selected from a commercial herd of sixty lactating robotic-milked cows. The cows, all Estonian Holsteins, were robot-milked and fed a ration comprising, separately, concentrate feed from a robot and a feeder, and a grass/clover silage mix forage at the feed barrier. With the low number of samples the results are indicative and descriptive, but it appears from the raw data that individual variation in visiting times and times spent at the feed barrier are greater than the effect of level of production. Moreover, that cows spend a significant portion of their time idling at the feed barrier, not actively feeding. Assumptions that, because a cow is positioned at the feed barrier it must be feeding, are likely to be erroneous. It is concluded that care should be taken to presume behaviour from positional data, and there is no evidence that cows with higher and lower milk yields are differentially motivated to feed from a forage source.

Infection-related changes in sleep and rumination time in one dairy cow - a case study

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As part of a study on sleep in dairy cows registrations were done during three consecutive days to evaluate sleep pattern. However, one cow became ill on the first recording day, with a high rectal temperature, clotted milk and reduced milk yield. The cow received analgesic and antibiotics and E-coli mastitis was diagnosed. Since observations of sleep do not seem to have been reported during acute infections in dairy cows we hereby present our findings in this cow as a case study.

Non-invasive electrophysiological techniques were used to register brain activity (EEG), neck muscle activity (EMG) and eye movements (EOG) to determine state of sleep. Standing and lying was automatically recorded. Data had been collected for the same lactation week (21) for this particular cow in her previous lactation.

Lying time during the first day of illness was 16 hours compared to 14 hours in the previous lactation. Lying time during the second and third day was 13 and 14 hours, respectively. NREM sleep time was 104, 148, and 101 minutes during the first, second and third day of illness, respectively, whereas mean NREM sleep time per 24 hour in the previous lactation was 92 min. REM sleep time was reduced compared to the previous lactation: 16 minutes during the first day compared with a mean of 54 minutes during previous lactation. Drowsing time was longer during the first two days than on the third day (130, 123 and 80 minutes, respectively) but still longer than that observed in the previous lactation (66 minutes). Rumination time was only 20 minutes on the first day ill, but increased to 3.4 hours during the second and 9.6 hours during the third day. Rumination time recorded in the previous lactation was 7.4 hours.

These results show how much a cow may sleep and rest if given the possibility during an attack of fever and mastitis. Further studies on the effect of sub-clinical conditions on rest, sleep, feed intake and rumination could contribute to better management for preventing sub-clinical conditions turning clinical.

P1.34

The effect of stocking density on drinking and feeding behavior of yearling dairy heifers

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The aim of this paper was to measure the effect of different feeding and drinking space allowances on drinking and feeding behavior in twelve months old heifers. Trial was carried on a number of twenty five twelve months old heifers. Heifers were housed in 25 m2 pens five per pen and fed using 0.4, 0.6 and 0.84 m feeding space per heifer. Water was provided using water troughs of two dimensions with 0.20 and 0.25 m2 water surface. During the experiments the behavior of heifers was video recorded with the help of a surveillance system and the following feeding and drinking behavior aspects were determined: number of feeding, rumination and drinking periods, the length of one feeding, rumination and drinking period and the total length of feeding, rumination and drinking periods. Heifers spent feeding per day 10 hours and 20 minutes when 0.4 m of feeding space per heifer was available, almost 10 hours when 0.6 m of feeding space per heifer was available and 8 hours and 25 minutes when 0.84 m of feeding space per heifer was available. There was a significant difference for the time spent feeding in 24 hours (p≤0.05) between the experimental variant with maximum feeding space and the variants when the space was reduced. Heifers spent ruminating per day 8 hours and 23 minutes when 0.4 m of feeding space per heifer was available, almost 8 hours when 0.6 m of feeding space per heifer was available and 7 hours and 46 minutes when 0.84 m of feeding space per heifer was available. Heifers spent drinking, on average, 13.2 minutes per day when the area of water troughs was 0.04 m2 per heifer and 10.8 minutes when the area of water troughs was 0.05 m2 per heifer. It was concluded that the different stocking densities have an effect on twelve months old heifers' feeding and drinking behavior. A reduction on feeding and drinking space increased feeding and drinking times. These results will be used for future research on heifer's growth rate and welfare to identify what stocking density is best for them.



Accurate tracking of cows in a barn environment using TrackLab

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Tracking the location and movement of cows in a barn gives insight into the behavior, health and welfare of cows. That information can be used for oestrus detection, lameness detection and allows for social relations in a herd to be studied. In order to carry out those studies, a system for recognition and analysis of spatial behavior (TrackLab) has been developed. Combined with Ultra Wide Band (UWB) sensors and collar-mounted tags, TrackLab accurately tracks cows in real-time. The data can be visualized, processed and analyzed. The system automatically quantifies parameters like time spent in cubicles or at the feeding station, the average speed of the animals whilst moving, etc.

A validation study was conducted in a real barn environment at the Natural Resources Institute Finland. A TrackLab system with UWB sensors and tags in their research facility to track the position and movement of 50 cows was installed. For the validation study two tests were used: a single measurement point test for 20 tags and an accuracy test for 2 tags.

The validation test showed that TrackLab with UWB sensors and tags formed an accurate tracking system for tracking cows in real barn environments, with an accuracy of 30cm or better and that the analysis gave valuable insights into the cows' behavior.

SESSION 2 UNTHEMED POSTER ABSTRACTS

Environmental-friendly tools to detoxify maize from aflatoxin B1.

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Aflatoxins, secondary metabolites with a cytotoxic, mutagenic and carcinogenic activity in both man and animals, represent one of the most important issue in food quality and safety, being maize one of the most significant target of contamination by these toxins. Maize is also amongst the most used raw materials for livestock feeding. Current state of the art indicates that, nevertheless the effort done by maize breeders, agrochemists and food process management, the tenure of aflatoxins in food is still growing. Over recent years some methods for forecasting and preventing aflatoxin contamination in field has been well established, otherwise no or few environmental-friendly approaches have been settled for preventing A. flavus development, seed cleaning and detoxification. This study has focused its attention on selecting bioactive compounds, exo-polysaccharides and laccase enzymes, from edible mushrooms Trametes versicolor, for preventing and detoxifying aflatoxins in maize. The two low cost formulations proposed, were able to inhibit the biosynthesis of aflatoxin B1 from A. flavus, and to degrade up to 40% of aflatoxin B1 content in contaminated maize (fig. 1). Artificial rumen experiments, confirms the effectiveness of T. versicolor laccase enzymes, at degrading aflatoxin B1.

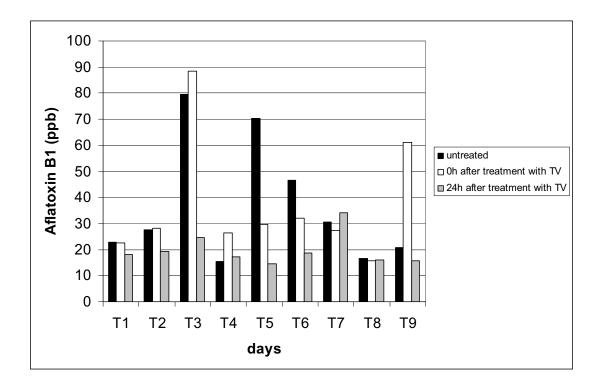


Figure 1. Effect of culture filtrate of T. versicolor (TV) on the degradation of aflatoxin B1 in contaminated maize samples. Aflatoxin B1 was determined after 0 and 24 hours after treatment.

Life Cycle Inventory Analysis for a Commercial Dairy Cattle Producing Farm in Greece

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During the last decade, the environmental impact of livestock production processes has received considerable attention by the scientific community. An integrated assessment tool that allows for environmental impact optimization by taking into account the full life cycle of a livestock product is the Life Cycle Assessment (LCA) methodology.

The objective of this paper is to present a "cradle-to-farm-gate" Life Cycle Inventory (LCI) based on farm-specific data for a commercial dairy cattle producing farm in the region of Thessaly, Greece. Special attention is given to the estimation methods of inputs and outputs and to the co-product handling for the processes where this is needed. The functional unit was set as 1kg of Fat and Protein Corrected Milk (FPCM) leaving the farm gate. The estimated on-farm emission flows include Greenhouse gas (GHG), Nitrogen and Phosphorus losses. The estimated off-farm emission flows include those associated with the production of all the purchased ration ingredients (including processing of raw materials and transport to the farm) as well as the production of diesel and electricity.

With the compilation of this inventory it is intended to gain knowledge on the "status quo" environmental impact (attributional modeling) of the produced milk's partial life cycle in order to further identify the system's environmental weaknesses.

Methicillin-resistant Staphylococcus aureus (MRSA) prevalence and antimicrobial resistance profile in North-East Italy

Alessio Bortolami ^{1,2}, Matteo Gianesella¹, Massimo Morgante¹ and Michela Corro'²

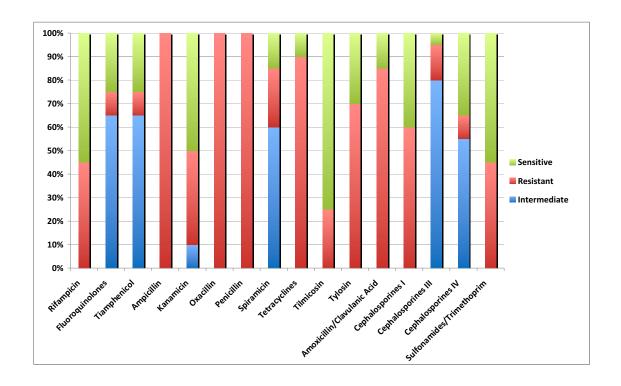
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Methicillin-Resistant Staphylococcus aureus(MRSA) intramammary infection is a serious threat for dairy health because it adds a characteristic multidrug resistance pattern to the problems of reduction of productivity and milk quality, typical of S. aureus infections. Moreover MRSA infection is a serious welfare issue in the dairy sector because it reduces the possibilities, already low for Methicillin-Susceptible S. aureus infections, of a successful treatment thus increasing the involuntary culling rate. The aim of this study is to investigate the prevalence of MRSA in dairy farms of North-East Italy (Veneto, Trentino Alto Adige (TAA) and Friuli Venezia Giulia (FVG) Regions) and to evaluate MRSA isolates antimicrobial resistance characteristics.

224 farms have been sampled. In each farm 30 composite milk samples and 1 bulk milk sample were randomly collected in farms with more than 50 lactating animals, in smaller farms only the bulk milk sample was collected. A specific research for MRSA was conducted on milk samples with selective media and antimicrobial susceptibility testing was performed on the MRSA strains.

8 farms were positive for MRSA with differences in prevalence in the three regions: 8.33% in Veneto, 1.70% in FVG and 0% in TAA. Susceptibility testing to antimicrobial agents showed multidrug resistance in 75% MRSA isolates with high rates of resistance to Tetracyclines (90%), Spiramycin (70%), Kanamicin (50%), Sulfonamides+Thrimetoprim (45%) and Rifampicin (45%) in addiction to constitutive resistance to all the β -Lactams, including Cephalosporin of common Veterinary use. Tilmicosin had the highest in vitro susceptibility rate (75%).

The results of this study highlight the differences in MRSA prevalence between the three Regions. These differences are likely connected to different size of farms, management and antimicrobial usage. Since most of the antibiotics approved in Italy for intramammary use are β -Lactam and Cephalosporin are ineffective against these strains, more difficulties of treatment and management of MRSA positive cows. Further studies are needed to better understand the impact of MRSA on dairy health and welfare.



Monitoring of drug consumption in dairy cattle farms

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Veterinarians are increasingly responsible to guarantee better safety of animal source food because pharmaceutical treatments use in livestock is often a consequence of deficiencies in animal husbandry and management. This study aimed at assessing the use of drugs in 7 dairy cattle farms in the Veneto region, Italy and to identify the main relationships between drug consumption and milk yield and quality because increasing interests are nowadays oriented towards controlled use of drugs in livestock farming, antimicrobials in particular. Drug consumption data were gathered using a specific software that allows electronic management of the veterinarians' drugs dispensary and registered treatments records (GeFaVet® Dr. Aldrovandi Franco, Vignola (MO), Italy). Data of the registered treatments were referred to one year from October 2013 to October 2014 and were expressed, per each farm, as ratio between the number of days of treatment over the average number of dairy cattle present. Data regarding milk yield and quality were obtained from National Milk Records. Results showed the largest use for antibacterials for systemic use; followed by antidiarrheals, intestinal anti-inflammatory and anti-infective agents; nonsteroidal anti-inflammatory drugs; and antibacterials for intramammary use (31, 25, 14, and 11 days/cow/year, respectively). Two farms (2 and 6) showed the largest use of drugs especially antibacterials for systemic use and antidiarrheals, intestinal anti-inflammatory and anti-infective agents. Association of these data to milk production and quality revealed that the highest number of treatments per kg of milk produced was recorded in farm 2 but this farm fall within those with the lowest somatic cell counts, which might point out a large scale preventive use of the drugs. Results regarding antibacterials for intramammary use revealed that farms 7, 3 and 5 had the lowest consumption (1.1, 1.3, and 1.4 days/cow/year, respectively) while farms 1 and 3 had the highest percentage of cows with mastitis (13.9 and 11.2%, respectively). These results might be seen as an effective use of drugs in farms 7 and 5 and could be a warning for farm 1. The monitoring of drug consumption in all farms is essential allowing to highlight potential abnormalities in use of pharmaceutical products. It can also help in assisting farmers towards the implementation of effective preventive management measures.

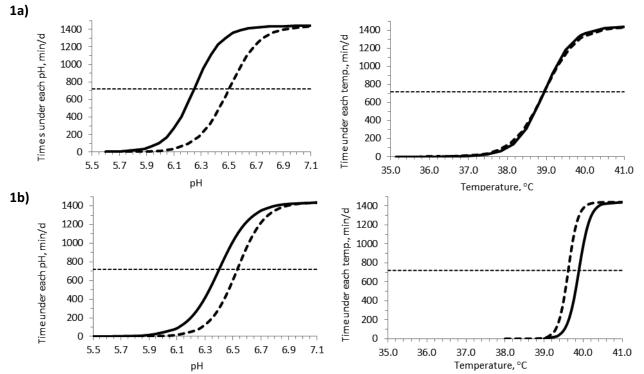
Monitoring rumen pH and temperature changes by wireless sensors and logistic regression in dairy goats

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Non-lactating dairy goats (Murciano-Granadina, n = 16, 41.3 ± 1.5 kg BW), provided with wireless rumen sensors for pH and temperature measurements and fed once-daily at maintenance, were used to assess the rumen function changes produced by dietary (Exp. 1) and climatic (Exp. 2) conditions. Rumen sensors (KB1001, Kahne, Auckland, NZ) were inserted surgically and programmed to collect data every 30 min. Obtained values were modeled by logistic regressions. Rectal temperature was recorded 3 times daily. In Exp. 1, 2 groups of 4 goats each were fed high forage (HF, 70:30) or low forage (LF, 30:70) to concentrate diets according to a crossover design. Goats were fed for 4-h and tap water (4 L, 9.8 ± 0.4°C) offered for only 30 min at 6-h. Rumen pH fell after feeding, reaching different nadir according to diet (HF, 6.35 ± 0.07 at 11-h; LF, 6.07 ± 0.07 at 6-h post-feeding, respectively) and on average were greater ($\pm 0.31 \pm 0.06$) in HF than LF (Fig. 1a). No diet effect was detected in rectal ($\pm 0.1^{\circ}$ C) and ruminal (38.9 \pm 0.1°C) temperatures which positively correlated (R² = 0.83). Rumen temperature (fasting value, 38.2 \pm 0.1°C) dramatically changed by feeding (+1.4 ± 0.1°C) and drinking (-3.4 ± 0.1°C) in both groups. In Exp. 2, 2 groups of 4 goats fed a 50:50 diet were exposed to thermal neutral (TN, 20 to 23°C day-night) and heat stress (HS, 12-h day at 37°C and 12-h night at 30°C) conditions following a crossover design. Humidity (40%) and photoperiod (light-dark, 12-12 h) were similar and water was freely available at ambient temperature. Despite no differing in dry matter intake, rumen pH was lower in HS than in TN goats (-0.12 ± 0.04). On the contrary, rumen temperature (+0.30 ± 0.01°C), rectal temperature (+0.4 ± 0.01°C), respiratory rate (+77 ± 5 breathes/min) and water intake (+3.2 ± 0.7 L/d) were greater in HS than TN, indicating an altered rumen fermentation (Fig. 1b). In conclusion, wireless bolus sensors and logistic models proved to be useful for monitoring rumen function.

Figure 1. Logistic models of rumen pH and temperature daily changes in dry goats (A = 1,440; P < 0.001): 1a (Exp. 1). pH and temperature of goats fed HF (; y = A/[1+e^{57.90-8.903x}], R² = 0.99; and y = A/[1+e^{96.47-2.477x}], R² = 0.99) or LF (; y = A/[1+e^{61.65-9.867x}], R² = 0.98; and y = A/[1+e^{108.54-2.787x}], R² = 0.99) diets, respectively. 1b (Exp.2). pH and temperature of goats under TN (; y = A/[1+e^{66.46-10.174x}], R² = 0.97; and y = A/[1+e^{316.41-7.987x}], R² = 0.95) or HS (; y = A/[1+e^{57.45-8.974x}], R² = 0.99; and y = A/[1+e^{277.83-6.965x}], R² = 0.96), respectively.



Assessment of animal welfare using metabolomic condition in dairy cows

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Dairy cows have high genetic merit for producing milk. During the transition period, tremendous changes in metabolic pathways can cause metabolic distress. Metabolic requirements for galactogenesis are the main reason for overloading physiological capacity of homeostatic metabolism. Dairy cows have genetic selection for producing high quantities of milk and onset of lactation is period of homeoeretic mechanisms. In order to assess metabolimic condition in dairy cows, examination included 317 cows from different dairy farms in Macedonia. Blood samples were taken from jugular vein. Cows were divided in two groups: first group involved cows in dry period, divided in two subgroups: "far off" (n=64) and "close up" (n=62). Second group comprise cows in early lactation, divided in three subgroups: immediately after calving (n=66); 60 days after calving (n=63) and 100 days after calving (n=62). After serum separation, standard biochemical analyses were performed: glucose, NEFA, BHBA, triglycerides and cholesterol, as well as thyroid status tT3, tT4, fT3 and fT4 with chemiluminescence (Immulate) method. Descriptive statistics of biochemical parameters of metabolomic condition, reveals that negative energy balance in dairy cows started so early with prolongated duration, but with not expressed intensity. Thus the cows were adapted in the appropriate stage of lactation, but didn't realize genetic potential. Thyroid status completely followed metabolomic energy balance in the different stages of lactation according hormonal regulatory mechanisms in different stages of lactation. Regulation between biochemical parameters and thyroid status function independently in all stages in lactation, but follow the trend of energy metabolism. As a general conclusion, metabolimic condition in dairy cows showed hypoglycemia and early lipomobilisation during the dry period. Welfare problems of animals in different breeding condition are related to adaptation. From our investigation we can conclude that dairy cows posses adaptation ability in different stages of lactation. Realizing of genetic potential for productive and reproductive performances depending of different (inappropriate) multifactorial system of breeding management. Continuous assessment of the metabolomic condition of dairy cows could be seen as a potential indicator of the welfare animal's state.

P2.7

Survey of hair cortisol concentrations in Danish cattle

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Cortisol is an important component of the hypothalamic-pituitary-adrenal (HPA) "stress" axis and a metabolic regulatory hormone. Plasma cortisol is subject to minute-to-minute variation, whereas cortisol accumulates in hair gradually over prolonged periods. Measurement of hair cortisol may, therefore, give a better impression of long-term stress. We used the Danish national cattle registry to contact 232 beef cattle farmers from 7 postcode areas throughout Denmark. From 35 positive responses we were able to visit 24 farms to obtain hair samples, comprising approximately 2cm of forelock hair taken close to the skin. Samples were carefully washed, extracted and analysed for cortisol following a standardized procedure. A total of 16 breeds were represented in the overall sample of 306 cattle, comprising 97 heifers, 142 cows and 71 young bulls. Data are reported as mean ± SE pg/mg. Since this was a survey and the data were not balanced, single factor ANOVAR analyses (Minitab release 11) were used as preliminary tests for significance of various effects prior to a more rigorous analysis. The overall mean hair cortisol value was 2.69±0.07. Cortisol concentrations varied between herds (1.36±0.36 to 4.68±0.27), between breeds (Scottish Highland having the lowest value: 1.61±0.19) and with gender (F<M). Surprisingly, pregnant cattle had lower cortisol (2.36±0.10) than either lactating (2.61±0.14) or young females (2.79±0.12). Age was a significant factor in the full dataset, and analysis restricted to males (to remove the effects of physiological state) confirmed that younger animals had higher cortisol concentration. In a subset of farms where each had some permanently housed and others allowed access to an outdoor paddock, the latter had lower cortisol concentration. The majority of cows were healthy and free from clinically evident disease at the time of sampling. There was no evident effect of recent health status as reported by the farmer. The data are complex and final interpretation will require care to exclude compounding factors. Nevertheless, the data suggest that hair cortisol may be of value in assessing stress and point to several factors that are worthy of more rigorous investigation.



Detection of genetic markers associated with improved efficiency of feed utilization in Holstein calves

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Improving efficiency of feed utilization (IEFU) in livestock is one of the most challenging tasks facing the agricultural community in the 21st century. Genomic selection for animals that metabolize feed more efficiently is of particular significance from economic and environmental points of view, as it impacts crop utilization and greenhouse gas emission, respectively.

Genomic DNA samples of sixteen intact Holstein calves, phenotypically classified as low and high in RFI performances, were used for a targeted sequence capture of specific chromosomal regions. These chromosomal regions harbour functional candidate genes associated with metabolic-related traits, including energy metabolism, muscle activity, thermoregulation and mitochondrial respiration. These were previously positioned in known QTL regions associated with feed utilization and growth performances. It has been revealed that under various nutritional conditions, different chromosomal regions exposed distinctive DNA markers for IEFU. While under optimal nutritional conditions, genes from the lipid metabolism cascade were significantly highlighted, maintenance regime exposed markers related to muscle activity.

In summary, a series of technical approaches was used to identify DNA polymorphisms in highly small groups of individuals. The last are representing two extreme phenotypes of RFI performances in the Holstein breed, eliminating the necessity of large sampled populations. A similar approach might be implemented on other traits of interest within different cattle breeds, and more specifically can be used: 1) on early prediction of IEFU. 2) On a broader basis, to model additional aspects of cattle husbandry and management, such as stress relief and welfare.

P2.9

The importance of pasture in tied dairy cows

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Tie-stalls have still an important role for dairy cattle breeding, in both Europe and Italy1. This kind of breeding is one of the most controversial for the scientific community and for public opinion. European legislator has tried to regulate tie-stall breeding but to date no indication has been given.

With these assumptions, the aim of our research is to find variables which improve animal welfare for dairy cows housed in tiestalls.

We have conceived and validated (Fleiss Kappa Index and free-marginal Kappa) a method for assessing welfare of tied dairy cows. This method is based on the studies made by EFSA2 and Welfare Quality®3. It is composed by 77 multiple choice questions, divided in 5 areas (A: Management and Staff – 16 items, B: Structures and Equipment - 18 items, C: Animal Based Measures – 21 items, D: Control of environmental conditions and alarm systems – 7 items, E: Biosecurity – 15 items).

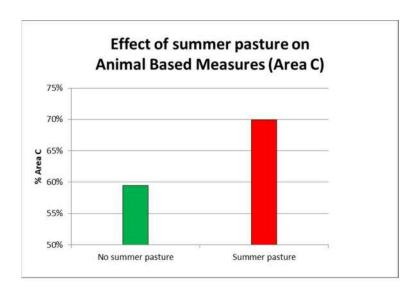
To date, we have visited and assessed animal welfare in 53 livestock with tie-stalls situated in Northern Italy. The average number of animals per farm is 66, with the mean of 34 lactating cows and 23 kg milk/cow/day.

The first important data is that only 16 (30.18%) of these livestock go on summer pasture with all the group of animals. Secondly we have noticed that cows which go to summer pasture have overall less adverse effects on Animal Based Measures (avoidance test, lesions, cleanliness, BCS, SCC, lameness, mortality) then those which are housed in tie—stalls all over the years (Pearson Index = 0.35, P value = 0.017) (Graphic 1).

Although the number of data is small to draw conclusions, these preliminary results give the direction for further investigations.

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P2.10
Can we feed the calf more milk?

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During the first weeks of life, calves are functionally monogastric and milk is the primary source of nutrition. It is commonly believed that young calves should not be fed more than 2-3 liters of milk per meal. If calves are fed beyond this volume, it is said that the capacity of the abomasum may be exceeded and that milk could leak into the rumen. This can disturb the microbial flora/fauna of the rumen and enhance the risk of indigestion, diarrhea and reduced growth. During the first weeks of life the calves are unable to compensate for the low amounts of milk given to them by eating more grain and concentrates. The calves may therefore be unable to meet their daily energy requirements, leading to chronic hunger. The aim of this study was to determine the abomasal capacity of Norwegian Red Cattle calves. Six calves (19-23 days of age at the beginning of the experiment) were fed two liters of warm whole milk by teat bottle three times per day, a recommended Norwegian feeding regime. During three morning feeding sessions, each separated by 48 hours, all calves were offered larger meals. The offered amounts were calculated according to the within patient, 3-level Response Surface Pathway (RSP) design. The milk given on the three test days contained contrast medium, barium sulphate (BaSO4), and the animals were radiographed before, during and immediately after intake. The highest voluntary intake was 6.8 liters in one meal (13% of BW). Abdominal radiographs showed that the abomasum has great capacity for expansion. Leakage into the rumen was not observed in any of the calves, regardless of intake. Behavior of the calves was observed for two hours after the meal. No signs of abdominal pain were recorded regardless of intake. The results are of great practical importance as they show that when milk is administered from a teat bottle, farmers can safely increase the amount of milk they offer their calves per meal without risk of leakage into the rumen. Hence, farmers who want to feed their calves more milk can do so by increasing meal sizes.



Effect of concentrate supplementation on reticular pH profile and ruminal fermentation traits of cows fed herbage during the transition and early lactation periods

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The effect of concentrate supplementation on reticular pH profile and ruminal fermentation in cows fed only herbage was investigated during the transition and early lactation periods. Twenty-four multiparous Holstein cows, subdivided into a high (H, 8959±984 kg previous lactation milk yield) and a low production subgroup (L, 6204±1000 kg) were allocated to 2 treatment groups and received a diet either consisting of only herbage (C-; n=11) or herbage and concentrate according to their predicted nutrient requirements (C+; n=13). The experiment started individually for each cow 2 wk before predicted calving date (LW-2) and lasted until wk 8 of lactation (LW8). Dry matter intake and milk yield were recorded daily and reticular pH was measured continuously by a telemetric pH bolus (eBolus, eCow Ltd, Exeter, UK) throughout the experiment. In LW-2, 2, 4, 6 and 8 ruminal fluid was collected with a stomach tube and analyzed for ammonia and volatile fatty acids (VFA). Milk yield was higher in C+ compared to C- cows (P<0.001) and in H compared to L cows (P<0.05). Although supplementation of concentrate lowered (P<0.001) herbage intake, total DM intake was higher (P<0.001) in C+ compared to C- cows. The milk yield potential had no effect (P>0.05) on intake. Within subgroups the intake difference between C+ and C- cows was bigger in H compared to L cows (treatment × potential interaction (P<0.05)). This might be explained by 2 cows falling ill in group C-H between LW2 and LW6. Mean, maximum, and minimum reticular pH were neither affected by treatment nor potential for milk production but decreased (P<0.001) from LW-2 to LW6. Ruminal ammonia was lower (P<0.05) in C+ compared to C- cows and decreased (P<0.001) from LW-2 to LW4. Supplementation caused a numerical increase (P=0.11) in total VFA and an increase (P<0.001) in the proportion of propionate at the expense of acetate with progressing lactation (P<0.001). In conclusion, ruminal fermentation was affected by supplementation as expected. The lack of differences in pH might partially be explained by the reticular environment responding less to different diets than the ruminal milieu.

P2.12

An experience of technological transfer: the bioelectronic platform developed in an Italian dairy chain

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Basing on a multidisciplinary team (agronomists, veterinarians, biologists, chemists, engineers) and a public-private Consortium, the project ALERT (www.alert2015.it) funded by the Italian Ministry for economical development under the Call Industria 2015, develops and transfers a automated technological platform (BEST) based on sensors and biosensors to an Italian dairy chain (Lattepiù-Centrale del Latte di Roma/Parmalat). Such probes are currently based on electrochemical and optical sensors and enzymes and whole cells as biological media in biosensors. The BEST bioelectronic platform is designed as an open field technology, which is capable of update through the integration of new probes and/or biomarkers. In order to survey the probes developed as field transferable tools by the scientific community (universities, research institutes, companies, etc), the consortium ALERT has launched a census web tool (http://www.alert2015.it/piattaforma/area-riservata/autocensimento).

Thanks to remote (Wi-Fi) technical control and data assessment, the project has the purpose of identifying and characterising innovative metabolomic-based biomarkers for early warnings and self-monitoring purposes, designing modern HACCP plans including the toxicological risks, and establish a long-term dialogue between producers and research bodies to foster effective innovation.

In fact, the European strategy for food safety requires that the Official control is increasingly integrated by renewed systems for self-monitoring by food business operators, that are subjects ethically and legally responsible for the safety of their products. The two-lane (top-down and bottom-up) system for food safety requires the development of field biomarkers as measurable biochemical or molecular indicators. (Bio)sensors arrays find particularly important application in primary productions, where environmental conditions and poor resources stress both chances of contaminations and challenges for prevention. A particularly interesting food matrix is milk that is highly consumed by infants, highly vulnerable to toxic contaminants, suited sentinel matrix for monitoring purposes, and business core of a particularly precious and suffering group of food business operator like farmers.

Effect of heat stress on ketosis prevalence risk and milk production in early and mid-lactation of Holstein heifers in Croatia

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Aiming evaluation of heat stress effect on ketosis prevalence risk and cow's yielding 180,355 test-day records from 50,574 cows reared on 4,638 farms in period 01/2003 - 12/2013 provided by Croatian Agricultural Agency were used. Milk recording was performed according to A/BT4 method. At each recording, temperature (Ta) and relative humidity (RH) in the barns were measured. Logical control of milk data was performed according to ICAR standards (2003). Records with missing or nonsense values were deleted from dataset. Regarding the lactation stage, heifers were divided into nine groups (by 20 days). Regarding the recording date four season were formed. Daily temperature-humidity index was calculated using equation by Kibler (1964): THI = $1.8 \times Ta - (1 - RH) \times (Ta - 14.3) + 32$. According to daily THI, heat stress classes were defined: HS (stressful environment) when THI ≥ 72 , HN when THI < 72. The ketosis prevalence (KPR) risk was indicated in cows in with $F/P \geq 1.5$. and calculated as frequency of cows indicated with risk in total number in regard to heat stress and lactation stage classes during the summer season. For cows in KPR the significance of differences in yielding between the heat stressed and normal in summer were tested by Scheffe's method using mixed model (SAS/STAT).

Heat stress conditions occurred in summer with average THI ≥ 72 observed from mid-June till the end of August. The highest KPR was observed in first 60 days with similar prevalence regardless the environmental conditions. Higher KPR in stressed animals was determined from 80th till 120th lactation day after what it was similar regardless the environmental conditions. Regarding the milk production in KPR the significant differences between the heat stressed and normal during summer were not found. The research results points out that test-day records and environmental measurements collected in milk recording could be used as tool for dairy herd monitoring enabling the early detection of unfavourable environment condition and the subclinical ketosis. Since environmental conditions significantly affects daily milk yield and components further research with purpose of detailed formulation of metabolic disorders risk and environmental conditions relation is needed.

Key words: test day records, temperature-humidity index, prevalence risk, ketosis

P2.14

Evaluation of salivary proteins as potential biomarkers of milk production in hot climates

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Dairy cattle are particularly sensitive to high temperatures, lowering milk output as well as milk quality (fat, solid, lactose and protein). Given the trend of global warming, the joint selection for productivity and adaptability is an objective, i.e. greater heat loss without lowering the metabolism. So, to search and validate the existence of non-invasive biomarkers that could allow selection of the animals best adapted is crucial to achieve such an objective. The interest of saliva as a source of biomarkers starts to increase in animal production. Salivary cortisol has been used in some studies, but it presents some limitations for thermal stress, which can be circumvent by salivary proteins.

The hypothesis that cows presenting high potential for milk production under adverse thermal conditions also present differences in salivary protein profiles comparatively to the ones less productive, is actually being tested at ICAAM-University of Évora, Portugal, by our research team.

The experiment was design to identify salivary proteins whose expression levels can be related to the potential for milk production under unfavourable thermal conditions. Twelve animals (6 high and 6 low milk potential production) were analysed for salivary protein composition at three different acclimation time points: first, during summer, under hot temperatures; second, under thermoneutrality; and third, during winter, under low temperatures. Whole saliva was collected directly from cow mouth, using cotton rolls (salivettes). At each time point, collection was performed in two different days, to avoid bias due to particular variations due to environment. One and two-dimensional electrophoresis are being performed to compare salivary proteomes from animals with different milk production potential, and to assess how differences between these groups are maintained through the different acclimation periods.

Oxidized protein biomarkers in the blood and milk of cows supplemented with flaxseed during the dry period.

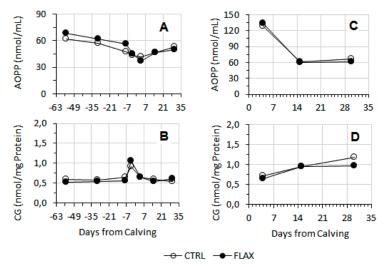
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Oxidative stress (OS) is the result of an imbalance between reactive oxygen species (ROS) production and neutralizing capacity of antioxidant mechanisms. A state of OS is observed in the periparturient cow, when a great amount of ROS is formed in response to copious milk yield and mammary gland involution. Flaxseed is an excellent source of alpha-linolenic acid, and many studies have considered the supplementation of flaxseed in diets for lactating cows. Conversely, few studies have dealt with flaxseed administration during the dry period. Here, we investigated whether flaxseed administration during the dry period can affect the cows' OS response around calving. Because of OS biomarkers in colostrum and milk may reflect the OS status of the mammary gland, we also investigated if biomarkers of oxidized protein can be measured in colostrum and milk. At the beginning of the dry period, high yielding Holstein cows in the same parity and production level and with a close date of drying off were randomly assigned to a control (CTRL; n=35), or to an experimental flaxseed supplement dry-off diet (FLAX; n=38; 200 g/head/day). Blood samples were taken at d 55, 30, 8, and 3 before the expected calving. Blood and colostrum/milk samples were collected at d 4, 15 and 30 after calving. The OS was monitored by measuring reactive oxygen metabolites (ROM), advanced oxidation protein products (AOPP) and carbonyl groups (CG). Somatic cell count (SCC) was also recorded. Variables were analysed using a hierarchical mixed model for repeated measures (PROC MIXED, SAS). Flaxseed administration did not affect any of the biomarkers measured in plasma and colostrum/milk. The day of sample significantly affected all measured parameters (P<0.001). Weak but significant correlations were found in milk between AOPP and SCC (r=0.305; P<0.01), AOPP and ROM (r=0.333; P<0.01), AOPP and CG (r=-0.309; P<0.01). Plasma and milk AOPP were negatively correlated (r=-0.220; P<0.01). Around calving, AOPP and CG showed an opposite trend both in plasma and in milk, which deserves further investigations. It is possible that different radicals can produce different alteration in the protein structure and, thus, a panel of parameters may better characterize the OS status.



Advanced Oxidation Protein Products (AOPP) and Carbonyl Groups (CG) measured in plasma (A, B) and whey (C, D) of dairy cows. Animals in the FLAX group received 0.2 kg/head/day flaxseed during the dry period.

Supported by the Italian Ministry of Education, University and Research (PRIN 2010)

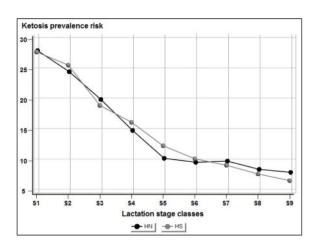
Potential use of milk based biomarkers to assess and to select for heat tolerance in dairy cattle

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Heat stress (HS) has major impact on milk production, fertility, health, and welfare of dairy cows. Comfortable cows exhibiting minimal stress will consume, produce, and reproduce better. Therefore, controlling HS is important for animal welfare. Genetic selection has been proven as an effective tool to improve traits of interest and therefore potentially also heat tolerance, by inducing in a cost effective manner permanent and cumulative effects. Yet, direct measures of HS in routine remain complex and difficult. High-throughput analytic methods are increasingly available from milk recording schemes allowing routine quantification of several potential biomarkers in milk. Therefore, the main goals of this study were i) to estimate HS impacts on milk production, udder health, and milk fatty acids (FA) and ii) to assess the potential of milk FA as biomarkers of HS for dairy cattle. A total of 202,733 test-day records for milk, fat, and protein yields, fat and protein contents, somatic cell score, but also 7 groups of FA and 10 individual milk FA contents predicted by mid-infrared spectroscopy were collected from 34,468 Holstein cows in first lactation between 2007 and 2010 in 862 herds in the Walloon Region of Belgium. Test-day records were merged with a daily temperature humidity index (THI) measured at the nearest meteorological station to each herd. Reaction norm models without fixing priors for thresholds were used to estimate HS effects. Results show that when THI values increased, most milk and FA traits showed negative phenotypic and genetic trends. Moreover, genetic correlations between low and high THI values for these traits were relatively high (>0.80) indicating moderated influence of HS on genetic expression of traits. Conversely, somatic cell score, fat yield, unsaturated FA and specifically C18:1 cis-9 content in milk increased with THI. These traits showed lower genetic correlations (<0.68) between extreme THI values. Content of C18:1 cis-9 in milk was the most sensitive trait to hot conditions. Since this trait is known to reflect body reserve mobilisation, using its changes under hot conditions could be a very affordable milk biomarker of HS for dairy cattle expressing the equilibrium between intake and mobilization under HS.



Heat		Lactation stage classes							
stress status	S1	S2	S3	S4	S 5	S6	S 7	S8	S9
HN	22.17	22.35	22.66	22.81	21.73	22.30	21.85	22.22	22.08
HS	22.09	22.51	22.49	23.24	21.96	22.60	21.66	21.74	21.87

Figure 1: Effect of heat stress on ketosis prevalence risk and milk production according to lactation stage classes

The effect of solid feed diet on the oral and cross-sucking behaviour of pre-weaned dairy calves

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In the dairy industry calves are most frequently artificially reared in groups, which create a greater opportunity for solid feed consumption and cross-sucking behaviour. This study aimed to compare the effect of differing solid feed diets on the pre- and post-weaning feed intake, growth rate and oral behaviour of calves reared artificially in groups. This experiment was a randomised block design with the treatments diets allocated at random, in blocks. Research was completed at Massey University's dairy calf unit #4 and involved 108 Friesian and Jersey x Friesian dairy calves that were allocated to one of three treatment diets: lower forage (LF) alfalfa total mixed ration (TMR); a higher forage alfalfa (HF) TMR; and perennial ryegrass hay along with a pelleted starter (HPS). Calves were reared in 36 groups of three calves per group and monitored until 12 weeks of age and behaviour was monitored using activity meters and manual observation. The data was transformed and analysed using mixed Proc GLM in SAS using diet as a fixed effect and calf as a random effect in the model. Data was presented as means with standard errors for each observation according to diet treatment. Calves fed HPS had the greatest dry matter intake (LF: 0.80 (0.012), HF: 0.95 (0.012), HPS: 1.70 (0.011) kg/DM/d), live weight at 40 d of age (LF: 60.3 (1.41), HF: 63.8 (1.41), HPS: 67.1 (1.38) kg) compared with TMRs. These calves also spent the most time eating (LF: 129.1 (0.14), HF: 163.7 (0.14), HPS: 154.1 (0.14) mins/d), and spent the least amount of time engaged in non-nutritive pen sucking (LF: 13.4 (0.16), HF: 11.2 (0.17), HPS: 10.3 (0.16) mins/d). It was concluded that, while cross-sucking was not entirely eliminated, providing perennial ryegrass hay along with a pelleted starter resulted in the least non-nutritive sucking behaviour, along with the greatest feed intake and growth rates compared with low and high forage alfalfa based total mixed rations.

P2.18

Monthly recordings of ß-hydroxybutyrate (BHB) concentrations in milk during early lactation to characterize energy status Julia Kesser¹, Christian Koch², Katharina Elsen¹, Christian Rietz³ Helga Sauerwein¹ and Ute Müller¹

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Milk recording organisations (MRO) use the Fourier Transform Infrared Spectroscopy to measure milk ingredients. The analysis of BHB in milk using the Middle Infrared area (MIR-BHB) can be routinely included in the monthly milk recordings of the MRO. Our study aims to evaluate the informative value of the MIR-BHB content in milk during the first weeks post partum (p.p.) with regard of identifying cows at risk for ketotic health disturbances.

Milk and blood samples were collected weekly from 65 German Holstein cows from one experimental farm during the first 9 weeks p.p. Besides MIR-BHB analyses, milk yield and composition as well as animal specific data e.g. body weight, feed intake, days in milk (DIM), previous performance and body condition were recorded in weekly intervals. All data were combined and statistical analyses were done with SPSS. The linear regression had, when compared to the logistic regression, the highest coefficient of determination to predict the BHB serum concentration in the first 5 weeks p.p. ($R^2 = 0.48$) and the energy balance in the 9 weeks p.p. ($R^2 = 0.61$). The milk MIR-BHB concentration was, next to fat to protein ratio, DIM and previous performance, a significant predictor of the BHB serum concentrations in the regression model (p < 0.001). However, for predicting the energy balance, the regression model included, next to the fat:protein ratio and DIM, milk yield and the fat:lactose ratio, whereas MIR-BHB was not included.

The results demonstrate that in the context of the monthly milk recordings, the model to predict BHB serum concentrations can support the farmer to screen cows at risk of high BHB serum concentrations during the first 5 weeks p.p. as a basis for taking further animal-individual steps to attenuate the risk for disease. Predicting energy balance after parturition can be achieved using the "classical" fat:protein ratio combined with other animal specific data and supports the farmer in monitoring the energy intake of the fresh cows.

Interrelationship between body weight gain and insulin responsiveness in young dairy calves

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Metabolic changes early in life may exert long-term effects on the metabolic function of calves due to the link between the plane of nutrition and growth in early life and future animal performance. The objective of this experiment was to determine if the body weight gain of young calves before weaning affect their insulin responsiveness during intravenous glucose tolerance test (GTT). One month old Holstein calves (n= 25) were divided in two group that were on different dietary regiment for the next 30 days. First group (n=12) was fed by low protein/adequate energy diet (18.0% CP and 5.0% fat), blended on farm. Second group (n=13) was fed by high protein/adequate energy diet (20.0% CP and 5.0% fat, Pre-calfy) formulated and blended by Patent Co. Consumption was recorded on a pen basis. Calves from second group ingested significantly higher amount of feed than calves from first group. First group gained 16.89 ± 1.53 kg during experimental period (lower weight gain group – LWG), while second group gained 18.23 ± 1.21 kg during the same period (higher weight gain group – HWG). Intravenous glucose tolerance tests (GTT) were performed in selected calves from each group at the end of experiment. Blood samples were obtained at 0, 30, 60, 90 and 120 min relative to glucose infusion. During the GTT, similar glycemic levels were measured in both groups. Nevertheless, LWG calves tend to produce more insulin to maintain similar glycemic levels than those found in HWG calves. Insulin to glucose ratio (I/G) was calculated as indicator on insulin responsiveness. I/G tend to be higher in LWG than HWG at minutes 30 (7.42 ±0.83 in LWG and 6.03 ± 3.06 in HWG), 60 $(9.90 \pm 1.55 \text{ in LWG})$ and $6.30 \pm 2.72 \text{ in HWG}$, $90 (10.52 \pm 1.40 \text{ in LWG})$ and $6.82 \pm 2.38 \text{ in HWG}$ and $120 (8.95 \pm 1.87 \text{ in LWG})$ and $120 (8.95 \pm 1.87 \text{ in LWG})$ and $120 (8.95 \pm 1.87 \text{ in LWG})$ 5.56 ± 0.88 in HWG). More research is needed to assess whether the impaired insulin responsiveness observed in LWG may be long lasting and may have negative effect on productive and reproductive performances in older animal.

Research was funded by Ministry of Education, Science and Technical Development of the Republic of Serbia Project of No III 46002

P2.20

Oral vitamin D -effect on serum magnesium

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To prevent hypocalcemia different methods are used to improve the calcium (Ca) regulating mechanisms in dairy animals. Two examples are increased magnesium (Mg) level in the diet and addition of vitamin D compounds close to parturition. There are indications that addition of vitamin D may have a negative effect on the magnesium status of the animal. The aim of this experiment was to examine the effect of two different dietary vitamin D compounds, cholecalciferol and calcitriol-glycosides, on serum Mg in goats. Twelve Saanen goats (10 adults and 2 one-year old) were assigned one of three treatments; control, Solbone (10 mg/kg BW Solbone A-cws, a Solanum glaucophyllum extract containing calcitriol-glycosides), and vitamin D3 (0.5 g per adult and 0.25 g per young goat of a mixture with 0.125 g cholecalciferol/kg). Treatment compounds were mixed with water and given orally once daily. Treatments were given in two periods, and each goat got two different treatments during the trial. Serum was sampled before the first treatment, and on day 5 of the first and second treatment period, and analyzed for Mg, Ca, calcidiol and calcitriol. Statistical analysis was made using mixed models, with goat as a random factor and treatment, period and their interaction as fixed factors. The baseline values for each variable were used as covariates. Calcitriol concentration in serum was 28±7.0, 36±7.1 and 188±6.9 pg/ml for goats given control, vitamin D3 and Solbone, respectively (least squares means ± SEM). Serum calcidiol was 7.6±3.1, 16.6±3.1, and 20.5±3.1 nM in control, vitamin D3 and Solbone goats, respectively. Calcium concentration in serum was 2.8±0.1; 2.8±0.1, and 3.8±0.1 mM in control, vitamin D3 and Solbone goats, respectively. Magnesium concentration in serum was 1.11±0.04, 1.09 ± 0.04, and 1.25 ±0.04 mM in control, vitamin D3 and Solbone goats, respectively. The measured serum variables were significantly higher in the Solbone goats compared to control goats, and calcidiol was elevated also in vitamin D3 goats compared to control goats. In conclusion, the used dose of calcitriol-glycosides increased serum magnesium, as well as serum calcium in goats, while addition of cholecalciferol did not alter the levels of these minerals.

Estrone sulphate and enzymatic activities in mare milk throughout gestation

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Introduction: Conjugated estrogen can be used for treatment in menopausal symptoms. Concentration of conjugated estrogen has been found in cow milk which provides a positive influence for menopausal milk consumers, but high concentrations may be harmful for patients with estrogen-responsive breast cancer. Retrospective studies reported the concentration of estrone sulphate (E3S, a type of conjugated estrogen) in cow milk, cow plasma, and alpaca saliva. Mare milk is supposed to have healthy properties due to the extensive and ecological system of production, but its composition is largely unknown. We assumed that mare milk also contains E3S and its concentration differs in different physiological periods in mare. To date, it has only been studied in detail cow's milk enzymes; information on other species such as goat, sheep or buffalo comes from limited studies. Extending the range of investigated species would bring interesting results, considering the large interspecific differences in the levels and activities of enzymes found in these studies. Objectives: The aims of this study were to describe the evolution of the E3S from mare milk along the pregnancy, and to define its constitutive enzyme activities. Materials and methods: Raw milks were obtained from two mares from Saldes (Barcelona). Samples were collected in non-gestate period, 4, 5, 7, 8, 9 and 10 months of pregnancy. The E3S concentrations were determined using enzyme linked immunosorbent assay (ELISA, Demeditec, Germany). The enzymatic profiles were determined by API ZYM® (Ref: 25200, bioMérieux SA, France). Results: The concentration of E3S remains under 10 ng/mL. In non-gestate period and significantly increases from the 4th month of pregnancy. The E3S concentration reaches its peak in the 9th month of gestation and slopes down in the 10 month. The results of the enzymatic activities show peaks in the 5th, 9th and 10th month of pregnancy, reaching the lowest levels in 3rd and 8th month. The lowest enzymatic activity was found in non-gestate period. Conclusion: The mare milk contains E3S and an intense enzymatic activity. Both concentrations vary depending on its different physiological periods.

P2.22

Developing Innovative Health and Welfare Management Tools for Dairy Cows from Optimized Use of Milk Mid-Infrared Spectra (OptiMIR)

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Mid-infrared (MIR) spectra of milk already showed a high potential for predicting many different milk components including recognized biomarkers such as fatty acid profiles. The MIR technology is non-invasive; it is already used in milk analysis labs to determine fat and protein contents as well as other major milk components; and it allows for a large scale sampling, especially through milk recording organizations. Until recently, direct MIR data were not available and therefore biomarkers of animal status were estimated using black-box prediction equations thereby accumulating potential prediction errors. Overcoming this bottleneck was warranted in order to develop second generation tools. OptiMIR is a European project co-funded by the INTERREG IVB program developed as a transnational cooperation between 5 research institutions, 11 milk recording organizations and 1 milk lab from Belgium, France, Germany, Ireland, Luxembourg and United Kingdom. Based on a transnational research database established within the project, MIR spectra was directly associated to reference values of traits related to management decision allowing involved research institutions to have access to relevant data. Major concerns of the dairy sector being health and welfare of dairy cattle, a large part of the project is dedicated to the development of advisory tools for health and thereby animal welfare. Innovative MIR modeling methodologies are under development in order to optimize the extraction of information contained directly in the shape of the MIR spectra in order to detect change in physiological cows' status. Spectral signatures for metabolic disorders such as ketosis, acidosis, but also negative energy balance, mastitis and fertility related traits can be identified within the MIR spectra. Through the close cooperation with OptiMIR industry partners, this research enables the use of the monthly milk analysis as a basis for monitoring tools at the individual cow level and, by extension, at the herd and even population levels, enhancing significantly the usefulness and opportunities linked to milk performance recording.

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How does calving in small groups with individual calving pens work?

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The aim was to evaluate cow-calf behaviour up to 24 h after calving in a new type of group calving area with individual calving pens. The study was conducted at a private dairy farm with 86 cows of Norwegian Red Cattle kept in cubicle housing with two milking robots. A calving area (8.8×6 m) was built by separating part of the cubicle stall. It had two calving pens (3×2.4 m each), four cubicles and a feeding area. Silage and water was supplied in the feeding area, and 3-5 cows were kept in the calving area. Cows were moved to the calving area at least one day prior to calving, and were locked into the calving pen at signs of contractions, and left there for the first 4 h after calving. Cow-calf pairs were filmed continuously during 24 h with two surveillance cameras. The films were scored continuously from hour 4-24 in periods of two hours. Out of 12 cow-calf pairs video filmed 7 were used for descriptive data analysis. All dams left the calving pen on average 12 times (min 5, max 33), and during a total duration of 1 h and 37 minutes (min 30 min, max 3 h 13 min). Each time they were away during a mean of 38 minutes (min 10 min, max 1 h 54 min). Six of the cows left the calf while it was lying down, whereas one cow left the calf when it was active in the pen. One calf followed the dam when she backed out of the pen while the calf was suckling. Three of the calves left the calving pen during the observations. Of these, one spent < 6 min in the cubicle area, while one spent < 2 h in the feeding area. All three dams followed the calves out of the pen. One cow only followed the calf 4 out of 6 times, but the calf was never outside her reach. One calf left the calving pen approximately half an hour before the observations started. Contacts between the calf and other cows were recorded for all calves, but 5 of the 7 dams acted aggressively towards other cows. Other cows entered the calving pen of all cow-calf pairs, but the highest score was 5 times. Once a stronger cow entered the calving pen and forced the dam out of the pen for <3 h. In conclusion this new type of calving area functioned well for the calving cows, but future research on a larger number of cows from different dairy breeds is important.

P2.24

Prevalence of and risk factors for Failure of Passive Transfer of immunity in newborn calves in Italian dairy farms

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The aim of this study was to determine the prevalence of failure of passive transfer of immunity (FPT) in newborn calves in Northeast Italy and to identify the potential risk factors associated to it at farm level considering that an adequate transfer of immunoglobulins (IgG) from colostrum is the first relevant aspect affecting health of calves and heifers. Blood samples were taken from 222 calves (2-5 days old) born from Holstein cows in 19 dairy farms of the Veneto region from late March to early August 2014. Farm size ranged from 64 to 142 cows. Farmers were asked to collect and freeze a sample of the first colostrum meal of each calf and to provide information about the newborn calf and its management. Serum and colostrum samples were analyzed by electrophoresis to determine IgG concentration. Results showed that the prevalence of calves having FPT (serum IgG <10 g/L) was 43.7%. An adequate passive transfer of immunity (serum IgG 10-15.9 g/L) was observed in 32.9% of the calves, and only 23.4% of them had an optimal one (serum IgG ≥16 g/L). Independent variables (farm, sex, breed, dystocia, time and quantity of first colostrum meal, and colostrum quality) were first submitted to univariate logistic regression analysis to test whether they were significantly associated to FPT and if not significant, they were excluded from further analysis. The multivariate regression model showed that FPT resulted affected by time from birth to first colostrum meal and colostrum quality. Calves fed within 6 hours from birth showed higher risk for FPT (OR = 3.2, 95% CI = 1.41-7.23; P<0.05) compared to those fed after 6 hours. Calves fed low quality colostrum (IgG <50 g/L) showed higher risk for FPT (OR = 3.8, 95% CI = 1.82-7.96; P<0.001) compared to those fed high quality colostrum (IgG ≥50 g/L). Despite the wide knowledge on passive transfer of immunity in calves and risk factors for FPT, results of this study show evidence of poor awareness and attention by farmers towards an effective management of newborn calves and of their colostrum feeding.

Effect of maternal dry period length and nutrition on colostrum quality, natural and specific humoral immunity and development of calves

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The objective was to study the effect of maternal dry period length and dietary energy source on colostrum quality and humoral immune responses, birth weight and development of calves. Holstein-Friesian dairy cows (N=167) were randomly assigned to three dry period lengths (0, 30 or 60 days, respectively) and two early lactation diets (either glucogenic or lipogenic). In colostrum total IgG and IgM were measured. Body weight was measured from birth till 12 weeks of age. Female calves were immunized with KLH (keyhole limpet hemocyanin) and HuSA (human albumin serum) at week 6 and 10 of age. Natural antibody (NAbs) binding to KLH and HuSA were determined in plasma of female calves from week 0 till 6 of age. Primary and secondary antibody responses were determined between week 6 and 12 of age. Colostral IgG and IgM concentrations were lower for cows with no dry period compared with a 30-d or 60-d dry period (P<0.01). Calves from cows with no dry period were born 2 days earlier (P=0.01) and had a lower birth weight (P=0.04) compared with calves from cows with a 30-d or 60-d dry period. Birth weight of calves from cows with a 30-d or 60-d dry period was similar. Growth and body weight at week 12 of age of female calves did not differ between dry period lengths or diets. Natural antibodies (IgG, IgM and Total Ig) levels binding KLH and HuSA in plasma were lower for calves from cows with no dry period compared with calves from cows with a 30-d or 60-d dry period. Specific antibody responses of the calves were not different between dry period lengths. Diet did not affect levels of antibodies in colostrum and antibodies in plasma of calves. The results of this study demonstrate that despite omission of the dry period which resulted in lower colostrum antibody levels, lower birth weight, shorter pregnancy length and lower levels of natural antibodies in plasma of calves during the first 6 weeks after birth, specific immune responses after 6 weeks of age and development of the calves were similar between dry period lengths.

P2.26

The use of 1H NMR-based metabolomics for the detection of heat stress markers in dairy goats

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Murciano-Granadina dairy goats (n = 8; 42.8 ± 1.3 kg body weight) kept in metabolic cages were used in a replicated 4×4 Latin square design with 4 periods; 19 d each (14 d adaptation, 5 d for measurements). Goats were allocated to one of 4 treatments in a 2 x 2 factorial arrangement. Factors were no feed supplementation (C) or 4% of soybean oil supplementation (S), and thermal neutral (TN; 15 to 20° C) or heat stress (HS; 12 h/d at 37° C and 12 h/d at 30° C) conditions. This resulted in 4 treatment combinations: TN-C, TN-S, HS-C, and HS-S. Urine samples were collected and analyzed with 1H NMR spectroscopy for a qualitative metabolomic study. Principal component analysis (PCA) and partial least square—discriminant analysis (PLS-DA) were used to identify possible metabolite markers for the effects of heat stress and soybean oil supplementation. Several metabolites involved in various physiological response of animals to HS were identified, such as increased harmful gut microbiota activity (hippurate); increased catecholamines and neurotransmitter activities (L-phenylalanine, glycine); and decreased degradation of energy-related metabolites (acetate, isoleucine and glutamate). No significant regression model was found for the effects of soybean oil supplementation. In conclusion, urine metabolomics provide a deeper understanding of responses to heat stress, which could help in the establishment of new strategies to alleviate its effects.

NMR-detected biomarkers of tolerance to seasonal weight loss in goats: a tool for breed selection

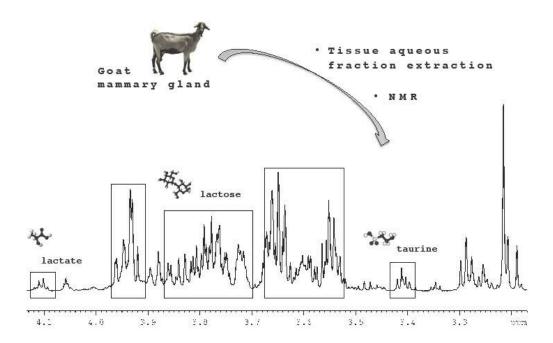
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Goat milk and dairy products are valuable nutritional resources with significant economic importance, especially in the Mediterranean. Milk production varies throughout the year due to the scarcity of pastures during the dry season, leading to seasonal weight loss (SWL), one of the most important setbacks for animal production in the tropics. This study aims the identification of physiological markers of SWL tolerance, at the level of the mammary gland and in two dairy goat breeds with different levels of SWL tolerance. Results will be used to define breed selection strategies in drought prone regions.

The metabolome of aqueous extract of mammary glands from two dairy goat breeds were compared: one tolerant to dry environment (Majorera) and another susceptible (Palmera). Ten Palmera and 9 Majorera dairy goats in mid lactation were used in this study. Breed groups were further divided into two nutritional groups: a control and a group fed with low nutritional quality food (loss of 15 - 20 % of their initial live weight), resulting in 4 experimental groups. Mammary gland biopsies were collected at the end of the trial (day 23). Aqueous fractions were obtained by tissue aqueous/organic extraction using Bligh and Dyer method with modifications. 1H NMR spectra (1D-NOESY and CPMG) were used to assess the metabolome profile of each sample.

Profiling analysis has so far, led to the identification of 37 metabolites in the aqueous fraction of mammary glands, being the most concentrated: lactose, glutamate, glycine, lactate and glucose. Preliminary statistical analysis using Principal Component Analysis (PCA) showed differences between control and restricted fed groups in the PCA2, although no differences between breeds were observed. Particularly, the PCA of the underfed animals exhibited much wider distribution than the control ones, suggesting a wide diversity in the responses to underfeeding. Complete analysis and statistical evaluation of the differences within and between groups is currently in progress.



An NGS-based gene expression profile study in the goat mammary gland: the effect of undernutrition in two breeds with different levels of adaptation to Nutritional stress

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Domestic goat (Capra hircus) milk has a high relevance worldwide, particularly in Tropical and Mediterranean countries where goats represent the main livestock. Since they are a source of income to farmers, their milk productivity performance is very important for animal production and the dairy industry. Undernutrition during the dry season causes Seasonal Weight Loss (SWL), being a relevant constraint for animal production. Knowledge on the genetic factors regulating milk production is limited and the impact of feed restriction, in this mechanism has been so far poorly addressed. A Systems Biology approach can provide a new and comprehensive insight on the molecular mechanisms underlying milk production, under feed restriction, as well as the regulation of the several biological processes involved. With such strategy, the identification of specific biomarkers and metabolic pathways involved could be used to develop new strategies to overcome this productivity constraint. Herein, it is reported a preliminary study of a quantitative differential analysis of mammary glands transcriptome using the Illumina high-throughput transcriptome sequencing (RNA-Seq). Two Canary Island (Spain) goat breeds were used; Majorera (feed-restriction tolerant) and Palmera (feedrestriction susceptible), with different productivity performances. The aim of this study was to study the impact of nutritional restriction on the transcriptome of mammary gland secretory tissue. The results show that a concerted re-programming of genes expression occurs as result of the stress imposed, irrespective to the breed studied. For instance, serine dehydratase (SDS) and adrenoreceptor beta 2 (ADRB2) were differentially expressed between treatments. These results also suggest a different behaviour of both breeds in response to the treatment applied. Moreover, an enrichment analysis of the differentially expressed genes provided some insight into what biological processes are related with the response to SWL during lactation. Finally, in the near future, a validation of the RNA-Seq assay using RT-qPCR with validated reference genes will be performed. The knowledge obtained through this project will be useful to release new genomic resources as well as shed some light into the genes and regulatory networks underlying milk production under nutritional stress.

P2.29

Hair cortisol and DHEA levels in dairy cows reared on summer pastures

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Cortisol and DHEA are involved in adaptive and constructive coping strategies response to environmental changes. The aim of this study was to examine changes in hair cortisol and DHEA concentrations (and consequently their ratio) produced in response to a change from indoor winter to summer grazing conditions in dairy cows. The study population comprised 15 dairy cows from one single herd. Hair samples were obtained using electric razor from the animal's forehead at the time points: 0 (ST1), 30 (ST2) and 60 days (ST3) after the start of summer grazing. Hair cortisol concentrations increase during the first month of grazing from ST1 to ST2 (1.29 \pm 0.083 pg/mg vs. 1.64 \pm 0.120 pg/mg; P < 0.05) and then remained constant from ST2 to ST3 (1.64 \pm 0.120 pg/mg vs. 1.40 \pm 0.066 pg/mg; P > 0.05). The hair DHEA concentrations were higher during grazing, ST2, than in stall, ST1 (342.06 \pm 17.594 pg/mg vs. 200.40 \pm 9.989 pg/mg; P < 0.05) and then remained constant from ST2 to ST3 (342.06 \pm 17.594 pg/mg vs. 303.67 \pm 18.819 pg/mg; P > 0.05). The cortisol to DHEA ratio decreased during the first month of grazing (6.58 \pm 0.480 pg/mg vs. 5.04 \pm 0.578 pg/mg; P < 0.05) remaining then constant from P2 to P3 (5.04 \pm 0.578 pg/mg vs. 4.77 \pm 0.254 pg/mg; P > 0.05). During transition from winter housing to high mountain conditions variations in hair cortisol and DHEA levels are produced that suggest that the HPA axis reacts to environmental changes such as changes in social groups, diet, housing, and transport. Data suggest that even if animals undergo important environmental change moving from winter housing to summer highland grazing maintain the resilience. A beneficial impact of summer grazing on the health of dairy cows may be evaluated using hair matrix.

Effects of rumen protected choline supplementation on milk yield, plasma metabolites methionine and lysine in dairy cows fed hav based diets

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The aim of the study was to evaluate the effect of RPC supplementation in early lactating dairy cow receiving hay-based diet. For this purpose 12 Italian Holstein multiparous cows, in the first month of lactation (28 DIM), were divided into two experimental groups: control group (CTR) receiving no choline, and RPC group receiving 20 g/day of choline in rumen protected form (Balchem, US). Diets contained 50% hay (alfa alfa and Meadow Hay), and 50% concentrates. Cows were housed at the experimental farm of the Università degli Studi di Milano, fed in a Roughage Intake Control feeding system, and milked twice a day. The experiment lasted 9 weeks. Dry matter intake and milk yield were measured daily. Plasma was collected on week 1, 2, 3, 5, and 9 of the experimental period and analyzed for glucose, cholesterol, triglycerides, nonesterified fatty acids, betaidrossibutirrate, and urea N. On week 1, 2, 3, and 4 plasma collected from three animal per group methionine and lysine have been measured. Before statistical analysis, daily measurements for DMI and milk yield were condensed to weekly means. Data were analyzed as a completely randomized design by Proc MIXED procedure of SAS using treatment and time as fixed factors and cow within treatment as a random factor. The REPEATED statement was used for variables measured over time. Through the 9 weeks, treatment did not affect DMI (22.7 vs. 23.5 kg/d in CTR and RPC respectively) and milk yield (28.2 vs. 29.4 kg/d). Dietary treatment did not affect the metabolic profile of experimental cows, except for plasma glucose concentrations that tended (P=.010) to be higher in RPC cows than those of CRT (61.08 vs. 56.75 mg/dL). Plasma lysine content were not influenced by the treatment. By contrast methionine tended (P=0.10) to be higher in RPC cows than those of CTR (18.21 vs. 24.53 µmol/mL). In this study RPC supplementation did not affect milk yield and plasma metabolites in dairy cow receiving a hay-based diet, even though an exhaustive comparison with silage-based diet, merit further investigations.

P2.31

Implementation of the project "Intramammary propolis formulation for prevention and treatment of mastitis in dairy ruminants"

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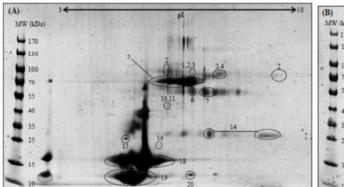
The project "Intramammary propolis formulation for prevention and treatment of mastitis in dairy ruminants" is a part of a collaboration between Faculty of Veterinary Medicine Zagreb and a small enterprise Hedera d.o.o. from Split. It is financed by European Regional Development Fund and EU Structural Funds. Its implementation started in October 2014 and will last until February 2016. The main goal of the project is the research and development of the innovative concept of intramammary formulation of propolis as an alternative to existing prevention and treatment of mastitis (with antibiotics). The development of such original product is aimed at strengthening of milk farmers through the reduction of their therapy and prevention costs. It also represents a shift in meeting the standards of ecological animal husbandry. The formulation development is conducted parallel with the research in dairy cows and goats together with veterinarians and livestock owners. Main activities of the project are: analysis of the formulation composition, in vitro study of its efficacy against common mastitis pathogens, as well as clinical trials on Holstein cows and Saanen goats during dry period and lactation. Health and productivity of animals, the incidence of subclinical and clinical mastitis as well as milk quality will be monitored through clinical observation and sampling of blood, milk and saliva. Samples obtained in a non-invasive way (milk and saliva) will be validated for measuring oxidative stress and metabolic biomarkers in comparison to blood. During preliminary screening of total 4 dairy herds, 800 milk samples per farm were analyzed microbiologically, for somatic cell count and chemical composition. Clinical safety trials revealed different tolerability of cow vs. goat udder towards propolis formulation. This finding lead to the modifications in the composition of the initial formulation. In vitro analysis has shown high efficacy of propolis formulation in preventing the growth of more than 20 strains of common mastitis pathogens. The opinion of veterinarians and breeders of the practicality and usefulness of this propolis formulation will be of great importance for the development of the product. The project will seek to directly respond to the needs of its end-users.

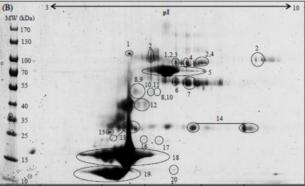
Proteomic analysis of differentially expressed proteins in Jafarabadi buffalo (*Bubalus bubalis*) milk whey during mastitis

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Reliable detection of mastitis is necessary for controlling disease and monitoring milk quality. Indicators of inflammation can be used to screen quarters for intramammary inflammation and be useful as diagnostic and prognostic parameters. In this context, acute phase proteins (APP) can be used as a powerful diagnostic tool. Therefore, this study investigated, using 2-DE approach, modifications of the milk whey proteome profile in Jafarabadi buffaloes with mastitis, in order to identify possible biomarkers for this disease. Milk samples were collected from mammary quarters of healthy (G1, n=82: negative bacteriology and CMT, SCC<100,000 cells/mL) and mastitic (G2, n=97: positive bacteriology, SCC>100,000 cells/mL) Jafarabadi buffaloes on farms located in Sao Paulo State, Brazil. Whey samples were obtained by addition of renin solution followed by centrifugation. Whey samples with low (n=5, G1) and high (n=9, G2) haptoglobin concentrations where selected for separation of proteins by 2-DE. Before performing 2-DE, samples were submitted to acetone precipitation. Protein concentration was measured by Bradford assay and 2-DE was accomplished by loading 200 µg of total protein into 11 cm, pH 3-10 IPG strips, followed by SDS-PAGE on 4-15% polyacrylamide gels, stained with Coomassie blue. Protein spots were excised and subjected to tryptic in-gel digestion and analysed by LC - MS/MS. Protein identifications were assigned using the Mascot search engine to interrogate protein sequences in the NCBI databases. Haptoglobin concentrations ranged from 0.10-0.37 µg/mL (G1) and 5.24-10.04 µg/mL (G2). 20 different proteins were identified, with haptoglobin, a major APP in bovines, known to be increased during bovine and buffalo mastitis, being the only protein identified only in G2. From the 20 identified proteins, 13 were visually increased in G2 (Figure 1), all of which have been reported as increased in bovine milk whey during mastitis. In conclusion, 2-DE separated buffalo milk whey into its constituent proteins, allowing a comparison of protein spots between healthy and mastitic buffaloes. The protein profile showed that 13 identified proteins were visually increased in mastitis milk whey and could be potential biomarkers of mastitis in buffaloes. This project received financial support from FAPESP (2013/26498-5) and CNPq.





Protein no.	Protein name	Protein no. Protein name		Protein no.	Protein name	
1	IgM	8	CD14	15	Ig J Chain	
2	Lactoferrin	9	Endopin 2B	16	Apolipoprotein AI	
3	α2-microglobulin	10	Complement C3	17	Haptoglobin	
4	Serotransferrin	11	Cathepsin B	18	β-Lactoglobulin	
5	Serum Albumin	12	Clusterin	19	Alfa – Lactalbumin	
6	IgG1 Heavy Chain	13	Beta-casein	20	Fatty acid binding	
7	Lactadherin	14	Ig Light Chain	20	protein 3	

Figure 1. 2-DE of whey sample of healthy (G1) (A) and mastitic (G2) (B) buffaloes with identification of protein spots analysed by LC – MS/MS. *In bold, proteins increased during mastitis (Proteins 1, 2, 3, 4, 6, 7, 8, 9, 10, 11, 12, 14 and 17).

From biomarkers for oxidative stress to the development of innovative milk replacer for neonatal calves

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Bovine respiratory disease complex (BRD) represents multifactorial etiology disease model with major economic impact on bovine industry. Holstein calves are considered more sensitive to BRD due to selection for high production level, which is not compatible with their pulmonary capacity. Although BRD is the most studied feedlot cattle disease, to date there is no effective tool for its early prediction and thus for its prevention. Previous work in our research group revealed successful prediction of transportation stress related BRD episodes by oxidative stress biomarkers at early life stage. Moreover, healthy claves were characterized by higher plasma reduction capacity pre-transportation (p=0.002), that was correlated with lower levels of lipid peroxidation post-transportation (p=0.007). These results imply that controlling plasma reduction capacity may be a key element to reducing transportation stress related morbidity. This may be achieved by designing the composition of milk supplemented to neonatal calves.

With the intention to generate a research infrastructure that will enable the development of designated prebiotic milk replacer that will improve the health status of suckling calves, we will initially characterize the metabolomic profile of relevant bio-active compounds of milk from different time points during lactation, and examine their effectiveness on growth performance and prevention of intestine and pulmonary diseases.

P2.34

Milk Recording in Dairy Cattle in Slovenia - A Valuable Aid for Herd Health and Welfare Monitoring

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Dairy cattle in Slovenia are voluntarily monitored with monthly milk recordings, which are run by the Agricultural institute of Slovenia and can be followed also electronically at http://www.govedo.si/. The method used is predominantly an AT4, performed according to ICAR recommendations. About 80% of dairy cows (almost 100 000 animals) are included in milk recording in Slovenia. In the recordings milk yield, percentage of milk fat (F), milk protein (P), lactose, content of urea (optional) and somatic cell count (SCC) are followed. Besides some reproductive parameters (days open, number of inseminations per conception, days between inseminations, days between calvings, gravidity), longevity and breed structure, including pedigree of cows are also recorded. Information obtained from milk recordings are very valuable tool for monitoring of dairy herd health. The milk fat content is valuable for screening of subclinical ruminal acidosis and subclinical ketosis or negative energy balance (NEB), milk protein content and ratio between F/P are also valuable for NEB detection. Lactose and SCC are valuable indicators of udder health. Milk urea concentration exhibit us short term protein supply as well as non-protein nitrogen. Following milk recordings in context of days in milk for individual animals and groups of animals with included reproductive performance and longevity at a dairy cattle operation give us an overview of heard health and welfare status and opportunity to focus our clinical investigation. Dairy cattle breeders find milk recordings very valuable for heard health management and decide in very high share to enter it even though there are some costs for participation. In the future additional parameters can be added to the panel, like metabolites (for example beta hydroxy butyrate) or antibodies for infectious diseases to even better use the obtained individual milk samples.

Identifying biomarkers in adipose tissue by proteomic analysis that are related to metabolic status, peripartum insulin resistance and body weight loss in dairy cows

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Adipose tissue is a central regulator of metabolism in dairy cows. Yet, little is known about the involvement of various proteins in the development of insulin resistant (IR) adipose and the association to metabolic status in peripartum dairy cows. Therefore, the objective of this work was to identify biomarkers in adipose tissue that are linked to IR and to the metabolic state of dairy cows. Adipose tissue biopsies were obtained from 8 multiparous cows at 260 d of pregnancy and at 4 d postpartum. Proteins were analyzed by intensity based, label-free quantitative shotgun proteomics (nanoLC-MS/MS). Cows were divided to subgroups that exhibited either IR or insulin sensitive (IS) adipose. Cows with IR adipose lost more body weight (BW) postpartum and produced less milk (100d) than cows with IS adipose. Moreover, cows with IR adipose lost more BW postpartum during all lactations (number 1-5) than cows with IS adipose, which indicates that the degree of BW loss at early lactation could have a genetic basis. Proteomic analysis revealed 586 proteins in adipose tissues. Comparing IR to IS adipose revealed that 18.9% of proteins were differentially expressed; among them, proteins related to lipid metabolism (monoglyceride lipase, fatty acid synthase, hormone sensitive lipase, perilipin) were increased in IR vs. IS adipose. Elevated lipolysis is expected in IR adipose as insulin is an anabolic hormone. Potentially, these proteins could serve as biomarkers in adipose tissue that are correlated to IR, BW loss and metabolic status of the cows. Taken together, we postulate that cows with IR adipose represent a sub-population of dairy cows, and that these traits might have a genetic basis. The differential protein expression in adipose tissues of a sub-group that exhibited IR adipose further supports the hypothesis that these adipose tissue biomarkers might be used to characterize dairy cows better adapted to metabolic stress within the herd; however this subject requires further investigation.

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ISBN 978-0-9930176-1-2

