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### GREEN BIOTECHNOLOGY AND FOOD SECURITY:

A joint master's programme by the University of Eastern Finland and the Kazakh National Agrarian University

# ROSEANNA AVENTO, ASSIYA SERIKBAYEVA AND ATTE VON WRIGHT

# Green Biotechnology and Food Security:

A joint master's programme by the University of Eastern Finland and the Kazakh National Agrarian University

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# Preface

This report is the outcome of a joint Master's Programme, leading to a dual-degree in Green Biotechnology and Food Security, implemented by the Kazakh National Agrarian University and the University of Eastern Finland in 2014-2016. Talks between the two Universities began in 2011 and commitment to join hands in this unique collaboration, aiming at training experts to deal with various global food security challenges, was formalized in 2012. This report gives a brief overview of the programme, its contents and final outcomes in form of the students' Master's theses abstracts. This joint effort between the two Universities has been a successful one: graduation of the students has been timely and the graduates are already working in various capacities, enhancing food security.

Kuopio, 23<sup>rd</sup> November 2016

Pertti Pasanen

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### 1. The Beginnings

A firm commitment to implementing a joint Master's level programme was formalized by the Kazakh National Agrarian University (KazNAU) and the University of Eastern Finland (UEF) in 2012, under the framework of a Team Finland delegation trade visit to Kazakhstan led by then Minister for European Affairs and Foreign Trade, Alexander Stubb. Rector Espolov Tlektes Isabaevich, representing KazNAU, and Rector Jukka Mönkkönen, representing UEF, broke bread, joined hands and signed an agreement to implement the master's degree programme "Green Biotechnology and Food Security" (GBFS).

Internationalization of education is of high importance and with this comes the development of innovative forms of learning, such as the implementation of dualmdegree programmes with foreign partner universities. In Kazakhstan, 40 universities are currently implementing dual-degree programmes. According to the strategic development plan of the Republic of Kazakhstan, by 2020, 20% of Kazakhstani universities should implement dual-degree programmes in cooperation with foreign universities. This is considered an important factor in increasing the competitiveness and strengthening of the Kazakh education system. Dual-degree programmes are an important channel for development of practical integration of the Kazakhstani higher education into the international educational space. This is also a new avenue to improve and develop the quality of education, research, competitiveness of universities in the global educational space and complies with the basic principles and provisions of the Bologna process.

The joint programme GBFS was designed to lead to a dual degree from UEF and KazNAU. The duration of study was two academic years and consisted of two phases: the first phase in Kazakhstan and the second phase in Finland. The program focused on new sustainable practices and technologies for the production and processing of agricultural products, covering the value chain from farm to table, linking topics in agriculture, including biotechnology of plants and animals, environmental health, health and welfare of farm animals, biotechnology and food safety.

The program aimed at training scientific experts who will be able to create innovative solutions and approaches to enhance food security, which suffers challenges in availability, accessibility, stability and utilization of food. Implementation of this program eventually will not only develop internationalization in the two institutions, KazNAU and UEF, but also serve to increase competitiveness and develop the food and agriculture sector.

Looking back at all this experience, we hope that the graduates keep their ambition and faith in themselves and their capabilities. We can say with confidence, that we have trained decent professionals and experts in the field of food security and that all the graduates will find a worthy place to apply their knowledge.

Professor Assiya Serikbayeva

Almaty, 23rd November 2016

### 2. Programme Description

# 2.1 MASTER'S DEGREE PROGRAM IN GREEN BIOTECHNOLOGY AND FOOD SECURITY (MSC GBFS)

The Master's Degree Program in Green Biotechnology and Food Security (120 ECTS) was a joint programme run by the University of Eastern Finland (Kuopio, Finland) and the Kazakh National Agrarian University (Almaty, Kazakhstan), leading to a double-degree from both Universities. The major was Biosciences. The duration of the studies was two academic years of full time studies, with one study phase in Almaty, Kazakhstan and the other in Kuopio, Finland. The study phase in Kazakhstan was completed in Russian and Kazakh languages, while the study phase in Finland was completed in the English language. The Master's thesis was completed in the English language and approved by both Universities.

### 2.1.1 Aim of the Master's Degree Programme

The programme focused on novel, sustainable methods and technologies for the manufacture and processing of agricultural and aquatic products for food focusing on food production chains from farm to fork. The programme aimed at providing the graduates with:

- an understanding of the fundamentals of food and nutrition security
- an understanding of the fundamentals of sustainable agricultural production
- the ability to work in the development of agrobiotechnological industry
- skills in agro-based product development
- knowhow and skills in development, innovation and research

### 2.1.2 Structure of the Master's Degree Programme

The two-year training programme consisted of compulsory and elective courses. Compulsory courses covered modules related to academic skills, practical training and advanced courses in biosciences: food and nutrition biotechnology and plant biotechnology. Elective courses comprised a module on either plant biology or farm animal behaviour and welfare and a module on food safety. Evaluation of the Master's Degree in Green Biotechnology and Food Security was based on grades from advanced studies in biotechnology and practical training, while the Master's Thesis was evaluated separately.

**Course modules (**Courses marked with an \* were conducted at the KazNAU and others at UEF)

### **Compulsory Studies**

General studies (min 26 ECTS)

Compulsory general studies

History and Philosophy of Science 3 ECTS\*

Foreign Language (English) 3 ECTS\*

Psychology 3 ECTS\*

Pedagogy 3 ECTS\*

Scientific writing and journal club 2 ECTS

Design of ecological and environmental experiments 4 ECTS

### Optional general studies

Project Management 5 ECTS\*

Systems of standardization and certification 3 ECTS\*

Molecular genetics and basics of biotechnology 3 ECTS\*

Food packaging 3 ECTS\*

Conflictology 3 ECTS\*

Modelling of business solutions 5 ECTS\*

Reproduction immunology 3 ECTS\*

Veterinary sanitary 3 ECTS\*

Innovative technology for livestock products 3 ECTS\*

Modern technology for production of feed and feeding of farm animals 5 ECTS\*

### Academic skills (5 ECTS)

Orientation course in Green Biotechnology and Food Security University study skills 1 ECTS University Computing Skills lectures 1 ECTS University Computing Skills practicals 1 ECTS Practical Statistics 2 ECTS

### Advanced studies in Biosciences (61 ECTS)

Food microbiology 6 ECTS
Food and nutritional toxicology 6 ECTS
Food legislation 2 ECTS
Food Processes 6 ECTS
Industrial Biotechnology 5 ECTS

### **Thesis Work:**

Common Seminars 2 ECTS (including pro-seminar and thesis seminar)
Master's Thesis 30 ECTS
Maturity Test

### **Practical Training**

Practical Training in Biosciences 5 ECTS

### **Elective Studies (min 27 ects)**

# Choose Module 1 or Module 2 and a minimum of 12 ECTS from Module 3

Module 1: Plant Biology (17 ECTS)

Ecological Stress Physiology of Plants 6 ECTS Structural Plant Monitoring 5 ECTS Introduction to Plant Biology 3 ECTS Book examination: Plant Biology 3 ECTS

or

### Module 2: Farm Animal Behavior and Welfare (15 ECTS)

Introduction to Animal Production 3 ECTS
Animal Behavior 3 ECTS
Welfare of Farm Animals 3 ECTS
Measuring and Assessing Farm Animal Welfare 3 ECTS
Book examinations: Welfare of Farm Animals (Cattle, Pigs, Poultry, Horses) 3 ECTS

and

### Module 3: Food Safety

Development and implementation of the HACCP system for food enterprises 7 ECTS\*

Hygiene and epidemiological control 5 ECTS\*

Monitoring of environmental parameters 5 ECTS\*

### 2.1.3 Student Selection

The application process into the programme was a 2 phased process starting with a first phase in Kazakhstan (August 2014) and ending with a 2nd phase in Finland (September 2014). Prospective applicants with a BSc degree applied to KazNAU and those successful, received a Decree of Admission into a Master's programme (biology, environmental sciences, ecology, aquatic sciences, hydrobiology animal health and welfare, animal biotechnology, plant biotechnology, food safety and security, environmental health, agriculture) at KazNAU, verified by the apostille through the Ministry of Education and Science of the Republic of Kazakhstan.

33 of the new MSc students with the highest scores in the entrance examinations organized at KazNAU were, issued an invitation to the final selection process in Kuopio, Finland. The final selection process consisted of a 7-day intensive entrance examination period in Kuopio, Finland including an interview and final entrance examination.

During the selection process, the students completed a pre-task assignment, participated in orientation lectures to Green Biotechnology and Food Security (3 themes: natural resource management, introduction to animal welfare and introduction to food safety), on which they were later examined. In addition, the students had a practical orientation to laboratory work and an interview. The students were evaluated by 3 person-panels on the following four aspects: (1) pre-task assignment presentations, (2) written examination, (3) laboratory assignments and (4) interviews. The selection was made on the basis of the total score of the four components. 14 students were selected to start the programme, of whom 11 students eventually confirmed their study placements and began the programme.

### 2.1.4 Funding and Resources

KazNAU through the Government of Kazakhstan provided for the funding of the student selection process in Finland. Further, once the programme commenced, KazNAU also catered for the student stipends to cater for accommodation and living costs of the students. KazNAU and UEF catered for administrative costs of the programme, whereas KazNAU compensated UEF for the additional running costs of the programme in Finland. UEF did not charge tuition fees from the students, owing to the legislation in place in Finland during the time of implementation (2014-2016).

assigned programme was two Academic Coordinators, one Professor from each University, and an Administrative Coordinator from UEF, who was responsible for the two Universities and liaising between practical implementation of the programme. Both Universities provided teaching inputs into the programme in accordance to the planned curricula.

In Finland, some courses were held solely for the Kazakh students, but there were also several courses where other UEF Finnish and international students participated, allowing for integration with other student groups.

The programme in Finland included several excursions to industry, local farms and research institutions. Internships for the students were organised not only at the various departments of UE, but also at the Natural Resources Institute Finland (Luke), taking into consideration the interests and capabilities of the students.

### 3. Pro Gradu Theses and Abstracts

All together, eleven students were enrolled in the GBFS programme. During the course of their studies, they completed practical training through internships organised at various departments at the UEF and also at the Natural Resources Institute (Luke). During their internships in the summer 2015, they gathered data for use in their MSc thesis (Table 1). The abstracts of the theses are collated in the following sections.

Table 1. Thesis topics of the GBFS programme students

Student	Thesis Topic
Anel Bekbergen	Marker assisted breeding and screening of apple scab
	resistance Vf gene) from columnar apple seedlings by PCR
Farida Baktybayeva	Unifloral honey from the boreal coniferous zone
Gaukhar Malikova	Interactions between brassicaceous plants and the effects
	on the plants' susceptibility to oviposition by pest
	Lepidoptera in an ozone enriched environment
Kalima Makhmaden	ZIP10 transporter: significance to heavy metal tolerance
	and metal accumulation in Noccaea caerulescens
Aidar Sambetbayev	Efficacy of disinfectants against Listeria monocytogenes
Gaukhartas Temirova	Antibiotic resistance and probiotic properties of lactic acid
	bacteria isolated from camel milk and shubat
Ismail Zhaboyev	Analysis of ovarian expressed genes and miRNAs in sheep
	(Ovis aries) using qPCR
Aigerim Khamzina	Copy number variations in Eurasian cattle breeds
Aigerim Suleimenova	Biochemical and sensory profile of meat from dairy and
	beef cattle
Dinara Kabylbekova	Validation of RumiWatch nose-band sensors for measuring
	nutritional behaviour of dairy cows
Nurlan Kuldybayev	Cryoconservation of Finnish Landrace chicken semen

# 3.1 ANEL BEKBERGEN: MARKER ASSISTED BREEDING AND SCREENING OF APPLE SCAB RESISTANCE *VF* GENE) FROM COLUMNAR APPLE SEEDLINGS BY PCR

Supervisors: Harri Kokko, MSc and Anna Toljamo, MSc

**Key words:** apple scab, *Venturia inaequalis*, *Vf* gene, columnar apple, DNA markers

#### ABSTRACT

Disease-free and commercially profitable apple production requires adequate cultivation and breeding processes. Apple breeding is typically a lengthy and cost consuming process. Apple cultivation is affected by the climate change and results in the introduction of pathogens and the existence of apple scab. Apple scab is an economically significant disease in apple-growing regions with high precipitation in spring and summer time that damages fruits and leaves. If uncontrolled, apple scab may result in the reduction of fruit quality and quantity and in severe infections, almost complete devastation of apple crop.

To prevent the apple scab, fungicide treatments are a requisite in commercial orchards. However, due to high costs and environmental risks, fungicides are not an optimal control method. A more effective and environmentally friendly approach to cope with apple disease is using resistant cultivars. The *Vf* resistance gene, derived from wild species *Malus floribunda* 821, has been the generally used gene in apple breeding programs against apple scab. Markers associated with resistance genes can be used to significantly fasten the selection process of resistant cultivars.

The aim of this study was to detect the Vf gene from columnar apple seedlings by using PCR. DNA extraction from the fresh leaves of apple seedlings was performed by CTAB method. PCR reaction was carried out using Vf primers followed by the electrophoresis to detect the samples with Vf genes. The Vf gene was detected from four apple seedlings. In three out of these four samples, it was observed that Vf genes were combined with the columnar growth type. Results of this study indicate that PCR reaction process is a useful way to detect the apple scab resistant Vf genes.

### 3.2 FARIDA BAKTYBAYEVA: UNIFLORAL HONEY FROM THE BOREAL CONIFEROUS ZONE

Supervisors: Anneli Salonen, PhD; Virpi Virjamo, PhD; Roseanna Avento, MSc.

**Keywords:** unifloral honey, physico-chemical analysis, antioxidant activity, pollen analysis

#### ABSTRACT

Honey is the product of bees consisting of sugars developed from nectar of plants or sweet juice of fruits in combination with substances, emitted from salivary glands of a bee. Bees process nectar, which they collect from flowers of melliferous plants into honey in a multistage process. In addition to sugars, the chemical composition of honey contains minerals, organic acids, enzymes, polyphenols and pollen, which makes it beneficial for human health. If nectar is collected mainly from one type of plant, the honey is referred to as unifloral and if the nectar of plants is collected from several types of plants, the honey is referred as polyfloral (multifloral). In the case of unifloral honey, its name depends on the dominant plant species.

The aim of this work was to investigate physico-chemical properties, antioxidant activity and pollen content of unifloral honey originating from boreal coniferous zone countries (Finland, Sweden and Norway). This work will help to make a detailed profile of the properties of these unifloral honeys and it can give more knowledge about honeys of boreal coniferous zone, which has not been done earlier.

Physico-chemical analysis included moisture content, electrical conductivity, pH, colour, antioxidant activity and pollen content. During the course of this study, altogether 12 different unifloral honeys (raspberry (*Rubus ideaus* L.), fireweed (*Epilobium angustifolium* L.), lingonberry (*Vaccinium vitis-idaea* L.), caraway (*Carum carvi*), sweet clover (*Melilotus alba* spp.), buckwheat (*Fagopyrum esculentum*), dandelion (*Taraxacum* spp), honeydew, willow (*Salix* spp), lime tree (*Tilia cordata*) and heather (*Calluna vulgaris* L.) honeys and polyfloral honey samples were studied.

Honey quality properties (moisture content and pH) of the investigated honeys were within the permitted limits (for moisture content less than 20 %; for pH between 3.5 and 5.5). Based on this study, several parameters show significant differences between the honey of different plant species origin. Thus pH, antioxidant activity, colour and electrical conductivity may be use as discrimination tool of boreal unifloral honeys. Results of this study will be useful in the future in characterization of honeys from boreal coniferous zone.

# 3.3 GAUKHAR MALIKOVA: INTERACTIONS BETWEEN BRASSICACEOUS PLANTS AND THE EFFECTS ON THE PLANTS' SUSCEPTIBILITY TO OVIPOSITION BY PEST LEPIDOPTERA IN AN OZONE ENRICHED ENVIRONMENT

Supervisors: James D. Blande, PhD, Academy Research Fellow; P. Sarai Girón-Calva, MSc Researcher

**Keywords**: Volatile organic compounds (VOCs), plant-plant signalling, tropospheric ozone, cabbage plants, *Pieris brassicae*, *Plutella xylostella* 

### **ABSTRACT**

Herbivore-damaged plants emit a blend of volatile organic compounds (VOCs), known as herbivore-induced plant volatiles (HIPVs). By emitting these compounds, plants can signal to undamaged neighbouring plants, to increase their resistance to herbivores. These mechanisms help plants to protect plants from biotic stress. In addition, HIPVs play an important role in plant-plant signalling. Tropospheric ozone is the major air pollutant that reduces VOC-mediated signalling by degrading many HIPVs. Negatively influence to the agriculture and natural ecosystems, depends on concentration of ozone.

The main aim of this research was to test if intact cabbage plants exposed to HIPVs from conspecific and/or heterospecific neighbouring plants are less susceptible to oviposition by the pest Lepidopteran *Plutella xylostella* or not. In addition, the research aimed to assess whether increased ozone levels eliminate or reduce the plant-plant signalling. In this research, the model system included cabbage and broccoli as HIPV emitting plants, and cabbage as HIPV receiving plants. Also, third-instar *Pieris brassicae* larvae were used as a plant damaging herbivore, and 4-5 day old *Plutella xylostella* adults were used in oviposition experiments.

Experiments were conducted in the laboratory and field conditions, under ambient ozone and elevated ozone concentrations and both comprised two phases: (1) exposure of undamaged plants to HIPVs from an herbivore-damaged neighbours and (2) an oviposition preference test with plants exposed to differently induced neighbours.

Plants exposed to HIPVs from cabbage plants damaged by *P. brassicae* were less susceptible of cabbage receiver plants to oviposition by *P. xylostella*, than plants exposed to undamaged plants, under ambient ozone conditions. However, this effect was impaired under elevated ozone (80ppb) conditions in the field. The findings indicate that ozone could significantly disturb the volatile mediated plant-plant signalling.

# 3.4 KALIMA MAKHMADEN: ZIP10 TRANSPORTER: SIGNIFICANCE TO HEAVY METAL TOLERANCE AND METAL ACCUMULATION IN NOCCAEA CAERULESCENS

Supervisors: Arja Tervahauta, PhD and Pauliina Halimaa, PhD

**Keywords:** heavy metal, hyperaccumulator, *Noccaea caerulescens*, ZIP10 gene, yeast complementation

#### **ABSTRACT**

Hyperaccumulators are plants that can hyperaccumulate and tolerate excess amounts of heavy metals without any toxicity symptoms and through metal transporter genes they can transport metals from roots to the leaves. They can be also used in the phytoremediation of soils. One of these hyperaccumulator plants is *Noccaea caerulescens*. In this study, four contrasting *N. caerulescens* accessions were used: La Calamine (LC) from Belgium, Ganges (GA) from France, Monte Prinzera (MP) from Italy and Lellingen (LE) from Luxemburg. The accessions show tremendous differences in their capacity to tolerate and accumulate metals.

The aim of this study was to characterize the function of the *ZIP10* transporter gene from *Noccaea caerulescens* and its closest homolog from *Arabidopsis thaliana*. The *ZIP10* gene was chosen because it is differently expressed among *N. caerulescens* accessions, showing highest expression in accession MP. The ZIP10 protein function also was characterized by comparing ZIP10 sequences between *Noccaea* accessions.

The ZIP10 transporter genes from four Noccaea accessions were transferred into yeast and they were grown in metal containing plates to determine the effect of the metal transporter to yeast metal tolerance. Also A.thaliana RNAi line plants, which show silencing of the A. thaliana NcZIP10 homolog metal transporter gene expression, were exposed to different metal concentrations and were analyzed for their capacity tolerate heavy metals. The root lengths of RNAi and wild-type plants were measured and compared to each other to determine the effects of ZIP10 gene on plant metal tolerance. Statistical analyses were done by using two-way ANOVA.

All *ZIP10* yeast transformants transport Co and Ni into cells except LC1 *ZIP10* transformant because of its shortest protein sequence, which most likely results in a nonfunctional protein. The silenced

ZIP10 RNAi lines support a role in transportation of Zn and Cd. The ZIP10 metal transporter gene may have a role in hyperaccumulation. In this study only a few metals were analyzed, and in further studies the ZIP10 gene function on other metals should be characterized. The ZIP10 gene from different *N. caerulescens* accessions could be overexpressed in *A. thaliana*.

## 3.5 AIDAR SAMBETBAYEV: EFFICACY OF DISINFECTANTS AGAINST LISTERIA MONOCYTOGENES

Supervisors: Jenni Korhonen, PhD; Roseanna Avento, MSc

 $\textbf{Keywords}: \textit{Listeria monocytogenes,} \ \text{listeriosis,} \ \text{disinfectant}$ 

### **ABSTRACT**

Listeria monocytogenes is a human pathogenic bacterium which can cause serious disease, and nowadays the regulation of the bacterium in the food processing environment has become a primary issue in the assurance of food safety. Listeriosis is mostly related to the ingestion of contaminated food, with 99% of human cases of listeriosis connected to food sources. However, the epidemic and sporadic incidences of listeriosis are relatively low compared to other foodborne diseases, for instance campylobacteriosis and salmonellosis, but the mortality from listeriosis is significantly higher. This makes listeria one of the most important pathogenic bacteria.

Various studies substantiate the ability of *L. monocytogenes* to contaminate food processing environments. The main target for food enterprises is to achieve a high level of hygiene, which can be achieved by adequate sanitation, especially disinfection practices with disinfection agents that are effective.

The aim of this study was to determine the efficacy of five commercial disinfectants against five *L. monocytogenes* strains. Disinfectants were tested in accordance with BS-EN1276 quantitative suspension test for the evaluation of the bactericidal activity of chemical disinfectants. The experimental part was undertaken during the period of May/June 2015.

According to European Standard BS-EN 1276:2009 disinfectants must achieve five log reduction for assessing the ability of disinfectant to eliminate or inhibit bacterial activity. The reduction was calculated by subtracting the log of control from sample (after disinfection) group. In this study, during laboratory tests all five disinfectants achieved at least 6.96 log reduction, the majority of results exceed 7 log reduction. Based on the results, it can be assumed that disinfectants used in this study are effective against *L.monocytogenes* bacterium.

# 3.6 GAUKHARTAS TEMIROVA: ANTIBIOTIC RESISTANCE AND PROBIOTIC PROPERTIES OF LACTIC ACID BACTERIA ISOLATED FROM CAMEL MILK AND SHUBAT

Supervisors: Atte von Wright, PhD, Professor and Jenni Korhonen, PhD.

**Key words**: lactic acid bacteria, probiotic properties, antibiotic resistance, medium, Lactobacillus

### **ABSTRACT**

The aim of the study was to identify the lactic acid bacteria (LAB) isolated from camel milk and shubat, to assess their antibiotic resistance profiles and to characterize some of their probiotic properties. The LAB were tested against a set of antibiotics by broth microdilution method, according to standard operating procedures (SOP), and compared to recommendations of the European Food Safety Authority (EFSA). The probiotic properties, such as acid tolerance, bile tolerance and pancreatic enzyme tolerance and the capability to ferment milk, were subjected to testing.

Thirteen strains were isolated from camel milk (n=8) and shubat (n=5). Antibiotic resistance profiling method was performed with two types of medium (LSM and MRS). The results turned out to depend on the medium used. MRS broth supported well the growth of LAB, but LSM broth produced more reliable results, particularly in the case of amoniglycoside antibiotics (kanamycin and streptomycin).

To assess the probiotic properties, the strains were subjected to simulated intestinal stress factors (low pH, bile acids, pancreatic enzymes). After a 24 h incubation the viability (as CFU/ml) was assessed by plating. One *Lactobacillus paracasei* and two *L. casei* (018k-3 µ 010k) showed high tolerance in these tests. Accordingly, three *Lactobacillus* strains could be used as potential antimicrobial probiotic strains against human pathogens and also as starter cultures.

# 3.7 ISMAIL ZHABOYEV: ANALYSIS OF OVARIAN EXPRESSED GENES AND MIRNAS IN SHEEP (OVIS ARIES) USING QPCR

Supervisors: Juha Kantanen, PhD Professor; Kisun Pokharel, MSc; Roseanna Avento, MSc.

**Keywords**: mRNA, Ovary, miRNA, Finnsheep, Texel **ABSTRACT** 

From the times when people started to breed animals, they understood that the traits of the offspring are dependent on the traits of the parents. By selecting and crossbreeding the best individuals, people for decades were creating new and better animal breeds of the higher grade, which also led to the higher interest of heredity phenomenon. In sheep breeding, one of the main objectives is to increase the productivity of the animals.

One of the most important organs in fertility is the ovary. By investigating genes that are expressed in ovaries, one may be able to find genes influencing fertility traits such as ovulation rate and litter size. It has also been shown that environmental factors such as diet play important role in sheep reproduction.

In this study, ovarian transcriptome mRNA profiling of two pure breeds of sheep namely: Finnsheep and Texel (in total 12 ewes) was studied. The 12 ewes were kept in flushing diet (additional nutrition) to study the effect of diet in gene expression. Finnsheep, a native breed of Finland is characterized as a high fecundity breed, while the Texel breed whose origins lie in the Texel island of Netherlands, is characterized as a low fecundity breed. Ovarian expressed mRNAs have been detected using RNA sequencing technology and bioinformatics tools.

RNA sequencing revealed that altogether 179 genes were significantly differentially expressed between Finnsheep and Texel, kept in flushing diet. This study aimed at validating the RNA sequencing data using qPCR and focused on five randomly selected genes (SLCO2A1, GJA2, ABLM3, BMX and CNTN4). The qPCR results were analyzed using the comparative CT Method and compared with the results obtained from NGS and computational methods.

Validation using qPCR approach was inconclusive in determining that the gene expression pattern of four out of the five candidate genes (ABLIM3, SLCO2A1, GJA5, BMX) was similar to that of computational analysis, as indicated by the high standard deviation in qPCR results. Errors may have occurred due to ovarian sampling where the whole ovary was utilized, rather than focusing on specific cell types, or due to technical errors in either or both RNA sequencing and qPCR.

## 3.8 AIGERIM KHAMZINA: COPY NUMBER VARIATIONS IN EURASIAN CATTLE BREEDS

Supervisors: Juha Kantanen, PhD Professor; Melak Weldenegodguad, MSc; Roseanna Avento, MSc.

**Keywords**: Copy number variation (CNV), cattle breeds, DNA, gene, Single nucleotide polymorphism (SNP)

### **ABSTRACT**

Cattle have been important domestic animals for human culture and society especially for milk, meat and transportation. Genetic variation within and between cattle breeds has been investigated using blood groups, microsatellites and mitochondrial DNA. One recently used marker for genetic characterization of cattle breeds is Copy Number Variation (CNV). CNV refers to chromosomal structural variation and is defined as chromosomal regions which are repeated and the number of repeats can vary between individuals and breeds. Studying the cattle genome and CNV's will improve knowledge about the CNV influence on phenotypic attributes.

In this research work, CNVs from 10 Eurasian cattle breeds have been identified using data of approximately 50 000 SNP-markers (Single Nucleotide Polymorphisms) The breeds studied were Northern Finncattle, Western Finncattle, Eastern Finncattle, Finnish Ayrshire, Yaroslavskaya cattle, Kalmykian cattle, Yakutian cattle, Ukrainian Grey, Podolian cattle and Busha cattle. The CNVs were detected using PennCNV and CNVRuler programmes to detect gain or loss of CNVs, while the Ensemble biomart programme was used for identification of genes showing CNVs, and the AgriGO programme for gene ontology the genes.

In the SNP data, only 46 out of 225 cattle passed the standard filtering samples and most of the samples showed a low quality samples when applying default parameters of PennCNV. A total of 337 CNVs in 46 samples were detected. After gene ontology analysis, CNV variations were found in genes having the following functions: phagocytic vesicle membrane, MHC protein binding, immune system process, immune response, defense response, and also olfactory receptor activity, ion channel activity.

### 3.9 AIGERIM SULEIMENOVA: BIOCHEMICAL AND SENSORY PROFILE OF MEAT FROM DAIRY AND BEEF CATTLE

Supervisors: Roseanna Avento, MSc and Heli Lindeberg, PhD

**Key words**: Beef, cattle, *longissimus dorsi* muscle, fatty acids profile, Warner-Bratzler shear force test, meat pH, colour, sensory characteristics, and fat content.

#### **ABSTRACT**

Meat is one of the most important foods in the diet of the vast majority of people. In order to produce high quality meat, it is necessary to understand the characteristics of meat quality traits and factors that affect it. Quality of meat is influenced by a variety of factors, for example, animal (breed, sex, age), meat production activities (feeding, transporting and slaughtering condition), and processing (storing time/temperature condition).

This thesis aimed to evaluate quality of meat, focusing on the biochemical composition and sensory characteristics, of meat from the commercial breeds Holstein, Ayrshire and Aberdeen Angus and the native Finnish breeds Eastern Finncattle, Western Finncattle and Northern Finncattle. This study evaluated the fatty acid composition, pH, colour, intramuscular fat content, tenderness and juiciness of meat.

Between the breeds analysed the highest levels of intramuscular fat were prevalent among Aberdeen Angus bulls and the meat was redder. Sensory panellists were unable to detect significant differences in juiciness among the breeds. The least tender meat was determined as being from Northern Finncattle, the oldest animals of the research.

The ratio of PUFA:SFA (P:S) is one of the important nutritional indices used to describe the fatty acid composition of foods, where values of 0.4 are considered optimum. According to the results P:S ratios did not differ significantly among the breeds, and were around 0.2, except for some Northern Finncattle bulls having a relatively high value,0.69. The ratio of the n-6:n-3 fatty acids is the second important index, where values of less than 4 are considered optimum. According to the results optimum values of n-6:n-3 ratio were found in meat from Aberdeen Angus, Northern Finncattle, Eastern Finncattle and Ayrshire breeds.

From the preliminary data, it can be concluded that age of the cattle had an effect on the meat quality. Activities on the farm such as feeding practices and type of feed, as well as transportation between farm and slaughterhouse can also affect meat quality. There is a need for further studies to determine exact effects of these factors on meat quality of cattle.

# 3.10 DINARA KABYLBEKOVA: VALIDATION OF RUMIWATCH NOSE-BAND SENSORS FOR MEASURING NUTRITIONAL BEHAVIOUR OF DAIRY COWS

Supervisors: Jaakko Mononen PhD, Professor and Salla Ruuska, MSc.

**Key words**: dairy cattle, automated monitoring, feeding, rumination, drinking, validation

#### **ABSTRACT**

Changes in rumination and feeding behaviour may be signs of health problems in cattle. Automated systems for monitoring behaviour can be an effective tool in the prevention and diagnosis of the disease in cattle in the early stages. The objective of the thesis was to evaluate the reliability of the functioning of the RumiWatch System (RWS) – a sensor-based device that measures ruminating, feeding and drinking behaviour in dairy cattle. The device registers the cow's jaw movements through a pressure sensor.

RumiWatch noseband sensors were attached to five non-lactating dairy cows of the breeds Nordic Red and Holstein. Video recording observation was used to validate the system by using a confusion matrix method. In terms of the performance indicators, rumination behaviour accuracy was 89%, and RWS was especially good in differentiating "not ruminating" from "ruminating" (specificity 93%). However, it was slightly worse in recognizing all true rumination cases (sensitivity 78%), and classified some of the other behaviours erroneously as "rumination" (precision 79%).

For eating behaviour, accuracy was much lower (67%) than for ruminating, resulting mainly from poor precision (51%) and to some extent from lower specificity (82%). Instead, RWS's sensitivity was slightly higher for eating (82%) than ruminating. In the case of drinking, accuracy (98%) and specificity (99%) were very high but precision (6%) and sensitivity (7%) were extremely low, which reflected the accuracy paradox of the imbalanced data for drinking.

In conclusion, the RWS in this study proved to be a relatively useful device for measuring ruminating and eating but not drinking. RWS might become a valuable tool for researchers and farmers in the future, but further validation is desirable. The confusion matrix approach is useful in the validation of RWS, as well as other devices, since it reveals the types of the misclassifications a classifier makes, which helps to adjust the classifying algorithm.

# 3.11 NURLAN KULDYBAYEV: CRYOCONSERVATION OF FINNISH LANDRACE CHICKEN SEMEN

Supervisors: Juha Kantanen PhD, Professor; Roseanna Avento MSc

Keywords: Finnish Landrace chicken, lineages, in vitro, semen cryoconservation, phenotypic

data, morphology parameters, cryobank

### ABSTRACT

The main objective for conservation of indigenous breeds of domestic animal species utilized in agriculture is the conservation of genetic resources for various future agricultural and societal needs. Finnish Landrace chicken is an old, native breed of Finland which was endangered. However, owing to voluntary activities of hobbybreeders, the conservation program and the support of the MTT (currently Luke, Natural Resources Institute Finland) the population size of Landrace breed has increased.

In the present MSc thesis, the in vitro - cryoconservation of Finnish Landrace chicken semen is described. This is the first time it has been carried out for conservation purposes in Finland. There are eleven lineages of Finnish Landrace chicken, and the present research focused on four: Hornio, Kiuruvesi, Savitaipale and Piikkiö. A semen pool (deep-frozen doses) and phenotypic data (measurements) belonging to these four lineages were collected from 11 Landrace chicken flocks. Sperm morphology parameters, such as sperm concentration, normal and dead spermatozoa as well as body, neck and tail abnormalities were evaluated by microscope in laboratory conditions. The donor roosters were photographed and their phenotypic characters recorded including body parameters, such as length of ulna (radius), shank length, shank thickness, keel length and weight.

Semen collection based on the 16 semen doses of four Finnish Landrace chicken lineages were cryopreserved and kept in Natural Resources Institute Finland for long-term cryobank storage. It was unrealistic and impossible to collect the recommended number of doses of semen from each rooster in each lineage, and thus it is recommended that a review and revision of number of doses of semen set under the Finnish National Strategy for Farm Animal Genetic Resources is conducted.

Sperm morphology evaluation indicated that such traits as sperm concentration, dead spermatozoa and head defects of spermatozoa are highly different between lineages. Also, the results of body phenotypic observation demonstrate differences in weight, shank length and thickness between Piikkiö and Kiuruvesi lineages.

Dissimilarities among the lineages in sperm quantity and quality and in phenotypic traits can be explained by differences in selection activities of breeders and effects of inbreeding. Cryopreserved biomaterial (semen) of the Landrace chicken breed should be used for forming of a centralized cryo bank of this native breed according to the National Action Plan for Farm Animal Genetic Resources.

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### 5. Epilogue

In 2012, when the first talks between the Kazakh National Agrarian University (KazNAU) and the University of Eastern Finland (UEF) were started on a joint MSC program, it was very clear for both parties that something rather unique in the history of both institutions was being planned. While the difficulties were not underestimated, a shared enthusiasm and willingness to take even certain risks prevailed.

Quite from the beginning certain principles behind the program were self-evident: the academic standards of either Kazakhstan or Finland should not be compromised, and the specific study modules should provide the students both a sound basic understanding of the field and give room for specialization according to each student's individual interest and plans for the future. Particularly the topics of the MSC thesis were selected taking into account their relevance to the students' career prospects, either in academia or other walks of life.

The one-year study period at UEF and the subsequent finalization of the MSC thesis in Kazakhstan had their challenges both for the students and their teachers. The study program and schedule were tight, the culture shocks inevitable, teaching in English a challenge both to students and teachers for instance English versions of many courses had to be developed at short notice. Much of the thesis writing was done by "remote control" without the usual personal interaction between the student and the supervisors.

At this point, it is a pleasure to say simply: "We did it". There were no drop outs. The enthusiasm and commitment of both the students and the teachers carried the day. The MSc thesis produced are of high quality and have already resulted in scientific publications, and more of these will undoubtedly be produced in the near future.

As the responsible Professor of the program in the UEF it is my pleasant duty to thank the students and my colleagues both in Finland and in Kazakhstan for the present success. Particularly the tireless efforts of Roseanna Avento, the programme coordinator, in solving the innumerable practical problems during the ups and downs of the program are gratefully acknowledged.

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Since the finalization of this Joint Degree Program coincides with my retirement, it has almost a kind of symbolic personal meaning for me.

Ars longa, vita brevis.

Professor (Emeritus) Atte von Wright

Kuopio, 23<sup>rd</sup> November, 2016

### ROSEANNA AVENTO, ASSIYA SERIKBAYEVA AND ATTE VON WRIGHT

The Master's Degree Programme in Green Biotechnology and Food Security was designed and implemented by the University of Eastern Finland and the Kazakh National Agrarian University, aiming at training scientists and practitioners that will create innovative and novel solutions to enhance food security by addressing the challenges of availability, access, utilization and stability in food value chains from farm to fork.



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