

SABRE Placement Report: Dr. Ivan Dimitrov Ivanov

On the short term placement (12 weeks) of Dr. Ivan Dimitrov Ivanov at the Laboratory of Neurogenetics and Stress, INRA, University of Bordeaux "Victor Segalen", France, Director Dr. Pierre Mormède.

I would like to express my gratitude to the SABRE Integrated Project for financing my short term placement in Bordeaux to visit Pierre Mormède at INRA. I would like, also, to address my deep gratitude to Pierre Mormède for the acceptance at the laboratory directed by him, giving me the chance to learn the methods of evaluating glucocorticoids, catecholamines and creatinine in urine of sheep, developing in this way our further collaboration on stress, genetics and welfare assessment.

The aims of this placement were: 1) to learn the methods of evaluating glucocorticoids, catecholamines and creatinine in urine, and evaluate the feasibility of measuring these hormones in sheep; 2) to work on publications concerning the relationships between levels of cortisol, some parameters of natural immunity and individual emotional reactivity (positive and stress) in terms of welfare of dairy animals; 3) to make more precise statistical analysis of the data on the indicators above; consulting about and refining the methods and approaches used up to now and in the future; 4) to discuss some genetic parameters (heritability) of predispositions to stress and positive emotions in terms of welfare.

Methods of evaluating glucocorticoids, catecholamines and creatinine in urine of sheep

Hormonal consequences of stress and positive emotions with the rise of cortisol and prolactin in plasma in dairy animals at the Research Institute of Agricultural Science, Stara Zagora, Bulgaria, have been studied. In terms of welfare measuring and monitoring it is important to diversify the methods used by non invasive ones. Measuring catecholamines and glucocorticoids in the urine of dairy animals would be a supporting approach for assessing their welfare. These methods are less invasive for animals and secretion of such hormones in urine sums up over several hours, and thus their measure can be considered as more integrative than in plasma.

Urinary catecholamines (CA) - adrenaline and noradrenaline, were assayed using an ion-exchange purification procedure followed by liquid chromatography with electrochemical detection. Urine samples were loaded on cationic columns and CA were eluted with boric acid. Eluates were then assayed by high-pressure liquid chromatography (HPLC) with electrochemical detection, using an oxidizing potential of +0,65V.

Urinary cortisol and cortisone were assayed using a solid phase extraction procedure followed by HPLC with UV absorbance detection (254 nm). Filtered urine samples were loaded onto C18 cartridges mounted on a vacuum processing station. Corticosteroids were eluted using absolute ethanol. After evaporation of ethanol, the dried residues containing corticosteroids were redissolved in mobile phase and injected in the HPLC system. Creatinine levels were determined using a colorimetric quantitative reaction (Procedure 500, Sigma diagnostics, Saint-Quentin-Fallavier, France). This method is based on the bleaching of the color derived from the reaction between creatinine and alkaline picrate (Jaffe's reaction) when the mixture is acidified. Thus, the difference in color intensity measured at 500 nm before and after acidification of the mixture is proportional to creatinine concentration. Catecholamines and glucocorticoid levels are expressed as ng/mg of creatinine to take into account urine dilution.

Publications

"Cortisol, natural immunity, stress and positive emotional reactivity in dairy sheep of different temperaments"

This publication was a collective work with a purpose to examine stress, positive emotional reactivity, and endocrine and natural immunity characteristics in dairy sheep of different temperaments submitted to acute and chronic stress situations during the normal farm rearing practice. The SABRE Project will be acknowledged.

The lack of stress is critical for the proper functioning of the milk ejection reflex. Predisposition to some emotional states and moods (positive and negative) is a good premise for investigating individual differences and personality in animals.

Signs of pleasure and fear have been used to assess the temperament in both approaches – tests where the animals are not dissociated (in the flock) and through dissociated tests.

The main temperament estimation was done through a Complex Score (CS), reflecting the behavioural traits of each animal (not dissociated approach) during machine milking in a milking parlour in four consecutive observations. A factor analysis for behavioural traits produced four factors: 1) Activity towards neighbours; 2) Feed reaction towards forage offered by hand; 3) Reaction towards positioning teat cups; 4) Taking position into the milking parlour; Each trait was described by four degrees. On the base of the CS varying between 24 and 96 [4x(6x4)], two temperaments distinguishing significantly ($p < 0.001$) in the behavioural traits, were established: Calm (C) – 135 ewes and Nervous (N) – 60 ewes. In order to confirm the temperaments, emotions were additionally assessed by a system of methods (in 54 animals, dissociated approach): fear inducing, open field and learning tests (Pavlovian conditioning - food motivation - learning).

For the purpose of stress and endocrine measurements, the most typical representatives of the temperaments were chosen among them, C – 20 ewes, N – 20. The animals were of the same age and date of lambing (± 10 days). They were submitted to the following tests: machine milking (chronic stress for N); shearing (acute stress for both, C and N) and 48h after shearing, after morning machine milking (hypothermia stress, C and N); plasma cortisol, lysozyme and complement levels were assessed immediately after machine milking and shearing.

It was established that plasma cortisol levels between C and N were valuable indicators of chronic stress in N (machine milking: C, 1,31 ng/ml; N, 7,68 ng/ml; $P < 0,05$) and acute stress in N and C (shearing: C, 6,72 ng/ml; N, 13,79 ng/ml; $P < 0,05$) in terms of improving dairy sheep welfare. The different plasma lysozyme and complement levels between C and N animals, even not so contrasting, were also considered as supporting stress indicators (shearing -lysozyme: C, 0,102 $\mu\text{g/ml}$; N, 0,065 $\mu\text{g/ml}$; $P < 0,05$). Over 25% of the animals (mainly N) in the dairy flock had higher emotional sensitivity, revealing on this base different problems concerning the milk let down, level of milk production, reproduction and technology of milking and rearing;

During the period of short term placement in Bordeaux we started two packages of analysis, important for the further development of our collaboration:

- Precise statistical analysis of the data with a view to refine methods and approaches used up to now;
- Calculating some genetic parameters (heritability) of predispositions to stress and positive emotions in terms of welfare.

This will finally result in publications where the SABRE Integrated Project will be acknowledged, as well.

Ivan Dimitrov Ivanov

Research Institute of Agricultural Science,

Stara Zagora, Bulgaria

e-mail: iv.behav@dir.bg

tel. +359 (42) 638337

fax. +359 (42) 607048

Summary Report

On the short term placement (12 weeks) of Dr. Ivan Dimitrov Ivanov from Research Institute of Agricultural Science, Stara Zagora, Bulgaria at Laboratory of Neurogenetics and Stress, INRA, University of Bordeaux "Victor Segalen", France, Director Dr. Pierre Mormède.

Hormonal consequences of stress and positive emotions with the rise of cortisol and prolactin in plasma in dairy animals at the Research Institute of Agricultural Science, Stara Zagora, Bulgaria, have been studied. For the purpose of welfare measuring and monitoring in dairy animals, it is important to diversify the methods used by non invasive ones. The main task of this placement was to learn the methods of evaluating glucocorticoids, catecholamines and creatinine in urine. Another, very important aim of this placement was to work on publications concerning the relationships between levels of cortisol, some parameters of natural immunity and individual emotional reactivity (positive and stress) in terms of welfare of dairy animals. Making more precise statistical analysis of the data on the indicators above, consulting and refining the methods and approaches used up to now and in the future, were considered, as well. We discussed, also, the possibility to work on some genetic parameters (heritability) of predispositions to stress and positive emotions in terms of welfare, which is an essential part of the modern breeding strategies.