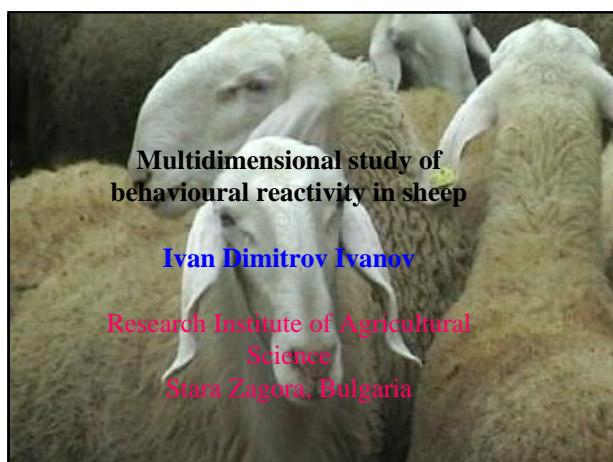


Multidimensional study of behavioural reactivity in sheep
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Introduction

- General aim - Understanding biological responses in sheep of different behavioural reactivity (temperament) related to welfare and productivity for a sustainable breeding – in a flock of 250 dairy ewes, since 1989.
- Investigation of biological responses – behavioural, physiological, hormonal, immunological, pathological and productive traits.
- Temperament can be defined as the characteristics of individuals that describe and account for consistent patterns of feeling and behaving (Pervin et al., 1997).
- To provide understanding of biological systems central to sustainability - a need of studies on animals predisposed to positive and negative emotions (methods for distinguishing).
- Genetic background (AI selected couples), behavioural consistency (tests), experience (behavioural development), distribution of individuals (Calm, Nervous, Intermediate).
- Two PhD Studies and over 60 publications.

Methodological approaches currently used to assess temperament and welfare in dairy sheep

Measurements of the biological responses during the five most important periods within the year – insemination, lambing, lactation, shearing and grazing:

- 1) Assessment of temperament and biological responses in dairy ewes in a milking parlour:
 - since 1989; first results – ISAE – 93, Berlin; ISAE -94, Foulum;
 - reliable measures;
 - time-consuming;
 - validity – other tests.
- 2) Evaluation of the maternal behaviour and mother-young bond, affiliative behaviours and vocalisations, male sexual activity in terms of emotional sensitivity.
- 3) Assessment of temperament in sheep by a system of fear-inducing and learning tests.
- 4) Assessment of behavioural reactivity by other tests – dominance, sexual activity, docility, behaviour on the pasture etc.

Methods - Animals

- Experimental flock of Synthetic Bulgarian Dairy Population
- Artificially inseminated by rams of the same breed and temperament as themselves
- Milked by machine twice a day – “Alfa Laval Ltd.” 2x24, milked in 6 groups.
- 20 Calm and 20 Nervous ewes selected for blood sampling

Experimental design: Temperament assessment

- Four behavioural traits:

 - 1) Feeding activity towards neighbours
 - 2) Feeding reaction towards forage offered by hand (of a stranger)
 - 3) Reaction to positioning teatcups
 - 4) Spontaneity and persistency of taking place in the milking parlour

Observation were carried out on four consecutive days during morning machine milking

One month later the same procedure was repeated by a different observer.

Experimental design: Temperament assessment Complex Score (CS)

1. Activity toward neighbours:
 - passive - 1 point (p)
 - passive/active (alternating) - 2 p.;
 - active, occasional inactivity - 3 p.;
 - active toward neighbours - 4 p.;
2. Feeding reactions toward forage offered by hand (of a stranger):
 - no feeding - 1 p.
 - rarely feeding - 2 p.;
 - cautiously feeding - 3 p.;
 - feeding - 4 p.

Activity - dominating neighbours and eating their forage.

Passiveness - not eating forage.

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Experimental design: Temperament assessment

Complex Score (CS)

3. Reactions to positioning teatcups:
- violently kicking and dropping teatcups - 1 p.;
 - moving and stepping throughout entire milking or kicking without dropping teatcups - 2 p.;
 - moving only during positioning teatcups - 3 p.;
 - standing quietly - 4 p.
4. Taking position in the milking parlour:
- not spontaneity and persistency - 1 p
 - not spontaneity and not persistency - 2 p
 - spontaneity and not persistency - 3 p
 - spontaneity and persistency - 4 p
- Spontaneity* - voluntarily entering into and taking position in the milking parlour without any assistance from the milker.
- Persistency* - persistency in taking group, subgroup and position (\pm 3 positions) in the milking parlour in over 75% of the milking events.

Experimental design: Temperament assessment

Complex Score (CS)

Based upon a PCA of the four behavioural traits, 3 factors were defined: 1) Feeding; 2) Milking; 3) Interaction. To simplify the procedure, the behavioural trait Feeding by hand (F) was eliminated because of it had a similar loading to the other trait (FA) contributing to the factor "Feeding". Therefore, 3 behavioural traits were used for calculation of an individual's complex score (CS) - FA, PT and TP.

Complex scores were calculated as the sum of scores for the three behaviours over the four observation periods. For example, if a ewe had the highest score (4 p.), for each of the three behavioural traits, during each of the four periods, it would have a CS of 48, (4 points x 3 behaviours x 4 periods). Temperament groups were defined according to CS as follows: **Nervous** ewes - CS between 12 and 24; **Intermediate** ewes - CS between 25 and 36 ; **Calm** ewes - CS between 37 and 48.

Methods: Procedure

- Three stress-inducing situations were used to assess the physiological and immune reactions of ewes of different temperaments: 1) Machine milking; 2) Shearing; 3) Machine milking 24 hours after shearing.
- Blood sampling - *Jugular vein*

For treatments 1 and 3 - immediately after machine milking.
 For shearing - 5-6 min after the start of shearing

Methods: Measurement plasma cortisol, lysozyme and complement activity concentrations

- Automated Chemiluminescence System ACS:180 Plus, Bayer Health Care measures plasma cortisol concentrations up to 2069 nmol/l with a minimal detectable concentration of 5,5 nmol/l.
- Plasma lysozyme concentrations were determined according to the method of Lie (1985).
- Activation of the alternative pathway of complement (APCA) was studied using the method of Sotirov (1991).

Results: Mean values of the Complex Score in different temperaments

Temperament	N	Mean	Std. Dev.
Nervous(N)	68	20	3.9
Calm(C)	71	38	3.5
Intermediate(I)	80	30	4.4

Td: N/C; N/I; C/I - P<0.001

Results: Mean values of the Complex Score in different lactations (ages)

Lactations	N	Mean	Std. Dev.
2	160	35	7.9
3	110	36	8.8
All groups	654	34	9.0

Td: 2/3 - NS;

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Plasma cortisol, lyzozim and complement levels in sheep of different temperaments (*accumulated data of the three tests*)

Indicators	Cortisol ng/ml	Lyzozim mkg/ml	Complement mkg/ml
Temperaments	Calm n- 12	Nervos n- 13	Calm n- 12
\bar{x}	12.30	34.592	0.317
min	0	15.2	0.167
max	27.3	79.6	0.716
td	C/H**		

Conclusions

- The results from the behavioural, cortisol and natural immunity tests confirm the value of using multiple methods to characterize temperament and to study some dairy welfare problems.
- It was found that the plasma cortisol levels measured immediately after challenge were valuable indicators of chronic stress in N (machine milking) and acute stress in N and C (shearing) in terms of improving dairy sheep welfare.

Conclusions

- Over 25% of the ewes in the flock are fearful during machine milking and revealed different problems the most important of which concerns milk let down and reproduction (Dimitrov and Djorbineva, 2003).

