UNIVERSITY "Ss. CYRIL AND METHODIUS" IN SKOPJE
FACULTY OF VETERINARY MEDICINE - SKOPJE

ISAE
International Society for Applied Ethology

# BEHAVIORAL SIGNS FOR STRESS AND PAIN IN ANIMALS

## PROCEEDINGS
## The Joint East and West Central Europe ISAE Regional Meeting 2013

co-organized by

AWARE
Animal Welfare Research in an Enlarged Europe

8-10 October 2013
Skopje, Macedonia

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## INTERNATIONAL ORGANIZING AND SCIENTIFIC COMMITTEE



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# FOREWORD

Dear Colleagues and Friends,

On behalf of Organizing Committee, we are pleased to welcome you on the Joint East and West Central Europe ISAE Regional Meeting 2013 that is held from 8-10 October in Skopje, Macedonia. The organizers are the Faculty of Veterinary Medicine at the University of Ss. Cyril and Methodius in Skopje, the FP7 project AWARE and the International Society for Applied Ethology.

The program offers unique opportunity for plenary lectures, scientific presentations and discussions about various topics in applied ethology and animal welfare science. We believe that this meeting is an excellent opportunity for renewal of old and making new contacts between ethologists and animal welfare scientists from central Europe and the Balkan countries.

The capital town of Skopje is ready to welcome all participants offering unique experience through a blend of beautiful architecture, historical museums, old churches and above all traditional Macedonian food.

We kindly welcome you in Skopje for this unique and stimulating event!

The Organizing Committee!

## *PROGRAM*

| | | |
|---|---|---|
| | ***Day I – 08.10.2013(Tuesday)*** | |
| 17:00–19:00 | ***Registration*** | |
| 20:00 | ***Welcome cocktail*** | |
| | ***Day II – 09.10.2013(Wednesday)*** | |
| 09:00–09:30 | ***Opening of the conference*** | |
| | **Session 1: Horses**<br>*Chairman* Marek Spinka | |
| | | **Plenary Lecture** |
| 9:30-10:30 | Jitka Bartošová | *They still behave like horses: Ethology and behavioural ecology in horse breeding* |
| | | **Oral Presentations** |
| 10:30-10:50 | Mira Suwala | *Different horse for different user? Survey on equine mental traits* |
| 10:50-11:10 | Aleksandra Gorecka | *Test for reactivity in horses: application in safety assessment* |
| 11:10-11:30 | Jana Dubcová | *The effect of inbreeding on horse fitness and viability: Do the inbred mares have lower fitness and weaker offspring?* |
| 11:30-11:50 | Martina Komárková | *Inbreeding may alter maternal care in domestic horses* |
| 11:50-12:10 | Richard Policht | *Acoustic divergence in domestic horses* |
| 12:10-13:30 | Lunch | |
| | **Session 2: Pigs**<br>*Chairman* Lubor Košťál | |
| | | **Plenary Lecture** |
| 13:30-14:30 | Frauke Ohl | *All animals are unequal: Understanding the biological relevance of inter-individual variation for animal welfare management* |
| | | **Oral Presentations** |
| 14:30-14:50 | Gudrun Illmann | *Calling by domestic piglets during simulated crushing: A signal of need?* |
| 14:50-15:10 | Pavel Linhart | *Acoustic features of piglet "scream" and "grunt" calls reflect level of situation urgency* |
| 15:10-15:30 | Mario Ostovic | *The impact of rubber flooring system on gilt postural behavior* |
| 15:30-15:50 | Astrid Vom Brocke | *Oral behavior of pigs as an indicator for tail biting risk on farm* |

| | | |
|---|---|---|
| 15:50-16:30 | ***Poster session + Coffee/Tea*** | |
| 16:30 | ***Excursion + Social evening*** | |

***Day III – 10.10.2013(Thursday)***

**Session 3: Ruminants I**
*Chairman* Christoph Winckler

| | | |
|---|---|---|
| | | **Plenary Lecture** |
| 9:00-10:00 | Laura Green | *Progress in understanding lameness in sheep and farmers' attitudes to its control* |
| | | **Oral Presentations** |
| 10:00-10:20 | Dinu Gavojdian | *Resting behaviour and milk cortisol levels in post-parturient ewe* |
| 10:20-10:40 | Ivan Dimitrov | *Assessment of animal welfare trough behavioural and cognitive parametrs in dairy sheep on farm conditions* |
| 10:40-11:00 | Csilla Budai | *Effects of genotype by environment interactions on pre-weaning survival rates of lambs* |
| 11:00-11:20 | Anke Gutmann | *Variation of latency to resting values and of differences to non-resting heart rate variability in dairy cows* |
| 11:20-11:40 | Marek Špinka | *Pay respect to the elders - age, more than weight, determines dominance in female beef cattle* |
| 11:40-12:00 | Radka Šárová | *Farmers' perception of lameness on Czech dairy farms* |
| 12:00-13:30 | Lunch | |

**Session 4: Ruminants II**
*Chairman* Vlatko Ilieski

| | | |
|---|---|---|
| | | **Oral Presentations** |
| 13:30-13:50 | Christoph Winckler | *Inter-observer agreement after training in qualitative behavior assessment following the Welfare Quality® protocol for dairy cattle in three different countries* |
| 13:50-14:10 | Ludovic Cziszter | *Effects of heat stress on rumination behavior in lactating dairy cows* |
| 14:10-14:30 | Stephanie Lürzel | *The influence of positive interactions on the human-animal relationship and welfare in calves* |
| 14:30-14:50 | Francisco Ceacero | *Pre-orbital gland opening as a measure for handling habituation in red deer calves* |
| 14:50-15:30 | ***Poster session* Coffee/Tea*** | |

| **Session 5: Poultry**<br>*Chairman* Gudrun Illmann | | |
|---|---|---|
| | | **Oral Presentations** |
| 15:30-15:50 | Lubor Košťál | *Anticipation of reward in Japanese quail: The effects of environmental enrichment and dopamine and opioid receptors blockers* |
| 15:50-16:10 | Maria Horváth | *Estimating cognitive bias in Japanese quail using operant conditioning techniques: Methodological issues* |
| 16:10-16:30 | Katarína Píchová | *Cognitive performance of lying hens reflect their welfare* |

| **Session 6: Dogs**<br>*Chairman* Vladimir Petkov | | |
|---|---|---|
| | | **Oral Presentations** |
| 16:30-16:50 | Shanis Barnard | *Animal-based indicators to assess dog welfare in long-term shelters* |
| 16:50-17:10 | Vucinic Marijana | *Common fear types in Mongrel dogs directly adopted from shelters or public places in Belgrade* |
| 17:10 | ***End of conference***<br>***Gala Dinner*** | |

| **Poster Presentations** | | |
|---|---|---|
| P1 | Uzunova Krasimira | *Investigations on behavioral disorders in broiler chickens affected by coccidiosis* |
| P2 | Binev Rumen | *Investigations on some forms of stereotypic movement (psychological) disorders in animals kept in zoos* |
| P3 | Radisavljević Katarina | *Cortisol concentration in free-roaming dogs after transport and housing in new environment* |
| P4 | Sossidou Eva | *Assessment protocol for sheep transported over long distance throughout Europe* |
| P5 | Mikus Tomislav | *Animal welfare in briefing of indigenous Croatian coldblooded horse breeds* |
| P6 | Jahja Ardita | *Efect of physical activity of laying hens on egg quality* |
| P7 | Djimrevska Ana | *The effect of acute heat stress and period of recovery over the glycogen metabolism in rats* |
| P8 | Shushleski Damjan | *The influence of acute heat stress on hepatic oxidative status and serum lipide profile in rats* |
| P9 | Velkovski Marjan | *Heat stress induced changes in key molecular mediators of cellular stress response in rat's heart* |

# CONTENT

**Session IV: Ruminants II**

ORAL PRESENTATIONS



**Session V: Poultry**

ORAL PRESENTATIONS



**Session IV: Dogs**

ORAL PRESENTATIONS

**POSTER PRESENTATIONS**

# Session I
# Horses

# THEY STILL BEHAVE LIKE HORSES: ETHOLOGY AND BEHAVIOURAL ECOLOGY IN HORSE BREEDING

Bartošová Jitka

*Department of Ethology, Institute of Animal Science, Praha Uhříněves, Czech Republic; Department of Animal Science and Ethology, Faculty of Agrobiology, Food and Natural Resources, Czech University of Life Sciences Prague*

**ABSTRACT**

Current scientific knowledge only hardly competes with traditional farm routine in horse breeding. While increasing number of horse farms has incorporated better satisfying of horse behavioural needs to the housing system, daily regime or equitation, this is often not the case of breeding management. Under natural conditions, female's choice of reproductive partner occurs and active behaviour plays a key role in the course of sexual encounter and timing of copulation. Compared to this, an unfamiliar stallion is often introduced to the mare in horse breeding and courtship is mostly restricted to copulation. Inhand mating is commonly managed in order to minimize spontaneous behaviour of the mare, either resistant or proceptive, and it often employs pain-inducing restraint devices such as nose or ear twitch, chain shanks on a halter or hobbles. Mares mated out of home stables experience changes in physical and social environment including potential risk of male infanticide. Pregnant as well as lactating mares are often exposed to regrouping associated with social tense. Abrupt weaning induces acute stress in foals as well as their mothers (being mostly pregnant again) and may have long-lasting detrimental effects on the young stock. These all parts of common horse breeding practice more or less ignore behavioural needs and compromise welfare of the horses. Despite common belief of the breeders they do not lead to optimal breeding results. The plenary talk will review the current knowledge on behavioural processes evolved by natural selection that still play role in common breeding practice and have significant impact on behaviour, welfare and reproductive success in domestic horse mares. Understanding these principles would significantly improve both, horse welfare and reproductive outcome.

**Key words:** domestic horses, breeding management, horse welfare, reproductive success

# DIFFERENT HORSE FOR DIFFERENT USER? SURVEY ON EQUINE MENTAL TRAITS

Suwala Mira, Gorecka - Bruzda Aleksandra

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*Department of Animal Behaviour, Jastrzebiec, Poland*

**ABSTRACT**

**Introduction**

Mental traits are crucial for using horses in many fields, from hippotherapy to top sport competitions. However, detailed preferences of different groups of horse users have not been investigated yet. We propose to classify equine mental traits into temperament and character. We define the temperament as simple, innate properties of the whole nervous system, present early in life and stable during the life time, whereas the character includes more complex properties, acquired through learning. We distinguished eight dimensions: Energy (EN), Fearfulness (FR), Sensitivity (SN), Adaptability (AD) for temperament and Submissiveness (SB), Aggression (AG), human Contact Seeking (CS) and Self-Reliance (SR) for character. Aim of the present study was to assess ideal level of each dimension for different groups of horse users.

**Materials and Methods**

To investigate preferred level of each dimension, online survey was developed. Survey was available online (www.ebadania.pl) for two months (2012). It contained questions on ideal level of each dimension of a horse used in respondent's discipline assessed on 1-7 scale (very low: 1; very high: 7, with exception of FR and AG, which were assessed conversely'-'). Survey was filled out by 768 respondents (668 women, 100 men) representing 32 different groups from horse industry (14 equestrian occupations, 10 disciplines, multiple choice). The most represented groups were leisure (n=482), show jumping (303) and dressage (255). The less represented groups (n<30) were races (23), endurance (20), trainers of natural horsemanship (19) and farriers (11). Wilcoxon signed-rank test (dimensions) and Mann–Whitney U test (particular group vs other repondents) were used.

**Results**

The average choice of respondents was: EN (5.7±0.9), FR- (5.3±1.3), SN (4.9±0.8), AD (6.4±0.9), SB (5.5±1.0), AG- (6.3±1.0), CS (6.3±0.8), SR (5.3±1.1). Except for AD-AG-CS and FR-SR (P>0.001), all dimensions significantly differed in their ideal level. All 32 groups of respondents preferred high level of all dimension except for FR and AG where low level was chosen.

Many of the differences between groups were significant but they concerned mostly dissimilarities in decimals and resulted probably from high number of participants.

**Conclusion**

The average participant, as well as all investigated groups of users, declared that horse for their discipline should be energetic, not fearful, sensitive, highly adaptive, submissive, not aggressive, social to humans and active selfreliant. Even if equestrian disciplines may seem to need the horses of different psychological potential, the expectations of all studied groups of users regarding temperament and character were actually the same. It can be concluded, that in contrary to physique, psyche is very versatile, especially studied dimensions.

**Key words:** preferences, temperament, character, horse

# TESTS FOR REACTIVITY IN HORSES: APPLICATION IN PERCEIVED SAFETY ASSESSMENT IN EQUITATION

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*Department of Animal Behaviour, Jastrzebiec, Poland*

**ABSTRACT**

**Introduction**

Several tests for reactivity have been developed for experimental assessment of different behavioural characteristics of the horse. Although many of them were intended to be helpful in equitation, practical verification of usefulness of such tests was rarely done. Present study is aimed at field application of novel object test (NO) and novel surface test (NS) to assess perceived safety, but also the satisfaction of the rider.

**Materials and Methods**

Thirty two horses from three riding schools were used. The horses were led through 30 m path including on its 15$^{th}$ m a colour cartoon box (NO test) or colour blanket (NS test) to be crossed. After one month, the horses were tested under the saddle in walk and trot with the same protocol by three different riders. The latencies (in seconds) to pass the path in hand and while ridden (averaged) were correlated. The obedience of the horse, attention to the rider, attention to surroundings, experienced safety, sympathy for a tested horse and the satisfaction of the rider were scored from low to high (0-3) and were correlated with each other and with the results of NO and NS tests.

**Results**

The result of NO test was not related to the rider's feeling of safety during the walk and trot. The obedience of the horse in walk ($r_s$=-0.38) and trot ($r_s$=-0.48) was perceived significantly lower in horses that took longer to cross the path with the box. Such horses were less attentive to the rider ($r_s$=-0.48) and the ride was less satisfactory ($r_s$=-0.57) to riders. High latencies to cross the blanket were predictive of experience of less safety of the riders during mounting the horse ($r_s$=-0.37), in walk ($r_s$=-0.66) and trot ($r_s$=-0.66). Similarly, these horses were less obedient in walk ($r_s$=-0.67), in trot ($r_s$=-0.62), were more attentive to the surroundings ($r_s$=0.47) than to the rider ($r_s$=-0.39), aroused less sympathy ($r_s$=-0.66) and the ride was less satisfactory ($r_s$=-0.64). Generally, the satisfaction was related to perceived safety during walk ($r_s$=0.56) and trot ($r_s$=0.67) and contributed to the feeling of sympathy for the horse ($r_s$=0.69).

**Conclusion**

The NO and especially the NS test are valid in the assessment of safety of riders, which is related to experienced level of satisfaction from the ride and sympathy for a horse. Thus, they are promising tools for testing the horses designated to riding centres.

**Key words:** horse, reactivity tests, equitation, safety, satisfaction

# THE EFFECT OF INBREEDING ON HORSE FITNESS AND VIABILITY: DO THE INBRED MARES HAVE LOWER FITNESS AND WEAKER OFFSPRING?

Dubcová Jana[1], Komárková Martina[2,3], Bartošová Jitka[2]

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*[3]Department of Zoology, Faculty of Science, Charles University in Prague, Czech Republic*

## ABSTRACT

Mating between related individuals, so called inbreeding, is often used in breeding of domestic species for manipulating their behavioural or physical characters. The results of this practice may have both positive and negative effects. Most studies have previously focused on changes in fitness or reproductive traits in wild animals, but research focused on viability of domestic breed is also needed. The aim of our study was to test the reproductive parameters of the limited population of the Czech autochthonous breed - Kladruby horse. This population comprised approximately 750 individuals registered in the studbook, and shows high values of inbreeding coefficient based on pedigree (1.4 to 18.674 in the10th generation). Initially, we tested how the females inbreeding coefficient value affects the following reproduction-related variables: the percentage of live-born foals per mating (natality-M), the percentage of live-born foals per pregnant mare (natality-P), the percentage of pregnant mares per mating (PMM) and early postnatal mortality (EPM). Other tested variables were mare affiliations (paternal and maternal lineage). Secondly we tested how the mare's and offspring's inbreeding coefficient affected birth weight of the foals. This analysis included the age of the mare at parturition, season and affiliations of the foal (maternal and paternal lineage).

We analyzed data from 83 mares which mated 627 times. From 483 pregnancies, 405 foals were born; 25 of them died shortly after birth. The statistic analyses revealed that none of our tested reproduction-related variables was affected by mare's inbreeding coefficient. On the other hand we obtained unexpected results: Natality-M and EPM variables differed significantly among paternal lineages of the mares ($p=0.034$, $p=0.086$ respectively, PROC GENMOD, SAS). PMM variable differed marginally among maternal lineages of the mares ($p=0.054$, PROC GENMOD, SAS). We did not find any influence of inbreeding coefficient, neither of the mare nor the foal, on foal's birth weight.

Our preliminary results are encouraging for the breeders, as we haven't found any deprivation of reproductive behaviour in this highly inbred breed of domestic horses in general. However more detailed analysis directed to each maternal and paternal lineage separately is needed to identify potential reproductive problem and to define future breeding strategy.

**Key words:** Kladruby horse, inbreeding, reproduction

# INBREEDING MAY ALTER MATERNAL CARE IN DOMESTIC HORSES

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**ABSTRACT**

The evidence about the inbreeding depression in small or isolated populations is still increasing. Mating between closely related individuals may negatively affect viability and reproductive parameters of their offspring as well as later parental care (e.g. milk production). However attention should be paid not only to endangered species, but also to domestic animals, which have been selectively bred for specific physical and behavioural traits for long time, and have high inbreeding levels. Moreover many now domesticated species have specific natural mechanisms to avoid inbreeding, as is, for example, female natal dispersal in horses. However the effect of the level of inbreeding on behavioural traits, especially those essential for offspring survival, as maternal or reproductive behaviour, have not been sufficiently studied yet. In our study we focused on the influence of inbreeding on maternal care in early foal ontogeny. We expected differences in nursing behaviour of the mares; highly inbred mares should provide shorter suckling bouts, terminate and reject them more than less inbred ones. Our model animals were domestic horses with various level of inbreeding (inbreeding coefficient 1.4 to 12) belonging to Czech autochthonous breed, Kladruby horse, with 750 living individuals and the pedigree known to the tenth generation.

We observed the following suckling behaviour: suckling attempts rejected by the mother ("unsuccessful suckling"), suckling length and frequency and the percentage of suckling terminated by the mare/foal/herdmate. In two seasons 10 848 suckling solicitations (79 foals, 59 mares) were recorded. Neither suckling length, nor its frequency and suckling termination were affected by the mare's inbreeding coefficient. However number of suckling rejected by the mother was lower for more inbred mares compared with those less inbred ($n=108$; $p<0.03$; logistic regression, PROC GENMOD, SAS). Although the number of unsuccessful suckling is low, it indicates willingness to allow suckling in mares with higher inbreeding level. Our study shows that suckling is relatively robust to the negative effects of inbreeding. On the other hand it suggests that the intensity of maternal care may be a compensating mechanism for lower viability of inbred offspring. This result may be valuable for common breeding practise, but also for the breeding programs of endangered wild ungulate species.

**Key words:** horse, foal, suckling, inbreeding

# ACOUSTIC DIVERGENCE IN DOMESTIC HORSES

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**ABSTRACT**

We tested whether pronounced morphological variability of horses caused by human's artificial selection was followed also by variation in their vocalization. We analyzed 1047 whinnies of 120 individual horses belonging to ten breeds. These breeds represent a horse variety both in morphology and history. The results showed significant differences in whinnies of different horse breeds. Whinnies of Shetland pony were the most distinct from calls of other breeds. Such result was in concordance with the distinction based on morphological parameters. Whinnies of primitive Hucul horse belonged among the most correctly classified ones (number of whinnies classified into the correct breed). Classification results of both Old-Kladruber horse colour forms were very different: whinnies of the grey Old-Kladruber horse revealed the least successfully classification whilst calls of the black Old-Kladruber horse showed one of the best classification output. Surprising was the extreme vocal distinction both of the heaviest breeds, the Czech-Moravian Belgian and Silesian noriker which is contrary to their morphological similarity confirmed also in discrimination analysis. Relationship between morphological and acoustical parameters revealed not so higher correlations ($r<-0.57$), similarly the age had not significant effect. Our results do not confirm the hypothesis of acoustic distinction in horse breeds based simply on their morphology. However whinnies of old breed, the Shetland pony, were the most distinct from all others, the other old breeds which were Thoroughbred and Old Kladruber horse clustered together with modern Czech warmblood. Our results seem to not confirm the second hypothesis of vocal distinction based on history time of breed establishing. Significant difference among horse breeds indicate the process of vocal distinction during the process of artificial selection.

# Session II
# Pigs

# ALL ANIMALS ARE UNEQUAL: UNDERSTANDING THE BIOLOGICAL RELEVANCE OF INTER-INDIVIDUAL VARIATION FOR ANIMAL WELFARE MANAGEMENT

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**ABSTRACT**

Animal welfare issues are a matter of intense and often controversial debate. In assessment of the welfare status of individuals, however, welfare generally is understood as an 'universal' state, that is, a state that would be experienced identically by each individual when being exposed to identical conditions. We suggest that such a construct is oversimplistic.

In this paper, and following others, we argue that welfare can only be defined in relation to an animal's own perception of its welfare status or well-being. We further suggest that welfare should not be considered as a static, or rather passive status but should embrace a more dynamic view of welfare such that a welfare issue arises only when an animal or a group of animals have insufficient opportunity (freedom) to respond appropriately to a potential welfare 'challenge' through adaptation by changes in its own behaviour.

We then explore the mechanisms by which animals may assess their own well-being and show that there exists considerable variation in what individual animals may determine for themselves as satisfactory or unsatisfactory in terms of their welfare status in given conditions. Instead of considering individual welfare as an 'objective' or indeed 'universal' state under given environmental conditions, we conclude that welfare is the result of a subjective self-assessment, that, importantly may vary significantly between individuals.

Acceptance of such variation between individuals in possible welfare status even under identical external conditions clearly has significant implications when striving to deliver acceptable welfare at a group or population level. We suggest in addition that an individual's welfare status can be assessed by outside observers only in terms of observations of behavioural adaptation to changing environmental circumstances.

# CALLING BY DOMESTIC PIGLETS DURING SIMULATED CRUSHING: A SIGNAL OF NEED?

Gudrun Illmann[1], Kurt Hammerschmidt[2],
Marek Špinka[1] and Céline Tallet[3,4]

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**ABSTRACT**

Many deaths of live-born piglets are caused by the sow crushing piglets during the first 3 days post partum when she changes posture. When a piglet gets trapped it starts screaming immediately. However, the responsiveness of the sows toward screams was found to be variable which could be caused by differences in the scream characteristic. The study examined whether piglet distress vocalizations vary with age, body weight and health status, according to the predictions of the honest signaling of need evolutionary model. Piglet vocalizations were recorded during manual squeezing (a simulation of being crushed by mother sow) on Days 1 and 7 after birth in piglets from 15 litters. We predicted that during squeezing, younger, lighter and sick piglets would call more intensely because they are in higher risk of dying during crushing and therefore they benefit more from the sow's reaction to intensive vocalization. Calls were analyzed in their time and frequency domain. The rate of calling, call duration, proportion of high-pitched calls and eight acoustic parameters characterizing frequency distribution and tonality were used as indicators of acoustic signalling intensity. Piglets which experienced 'squeezing' on Day 1 produced more intense acoustic distress signalling than on Day 7. On Day 1, piglets called more often ( $F_{1, 55}$=7.1, P=0.01), and had a higher proportion of high-pitched calls ($F_{1, 55}$=14.8, P= 0.0001) than Day 7. The high-pitched calls were significantly longer (P=0.013) and had a significant higher dominant frequency band (DFB1 start and DFB1 mean, P<0.01) on Day 1 than on Day 7. Lighter piglets called more during squeezing than heavier piglets ($F_{1,55}$=7.1, P=0.011). Health status did not significantly affect any of the indicators of intensity of vocalization during squeezing. In summary, the model of honest signalling of need was confirmed for younger and lighter piglets but not for sick piglets. The results suggest that sow might react more towards the vocalization of younger and lighter piglet during a real crushing.

**Key words:** Sus scrofa, distress vocalization, ontogeny, signalling of need

# ACOUSTIC FEATURES OF PIGLET "SCREAM" AND "GRUNT" CALLS REFLECT LEVEL OF SITUATION URGENCY

Linhart Pavel and Špinka Marek

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**ABSTRACT**

Vocalizations have long been getting attention as possible indicators of animal emotional states. It has been already shown, that piglets (*Sus scrofa domesticus*) increase number of "scream" calls in stressful or painful situations. Question remains, however, whether the specific acoustic features of these "scream" calls and also "grunt" calls that are more widespread across different situations could reflect the level of situation urgency.

We restrained piglets on their backs and recorded their behaviour and vocalizations during one minute. Restrain was done on scales and piglet was kept at the position with the smallest force possible. We recognized three situations based on apparent urgency level: low urgency (LOW; piglet is lying still), medium urgency (MED; piglet is fighting for escape), and maximum urgency (MAX; piglet is fighting with maximum force; force was assessed based on weight read from scales). Three scream calls and three grunt calls per piglet, each from the different urgency level, were analysed; 50 piglets were tested in total. Differences in five basic parameters in the three urgency levels (duration, amplitude, peak and central frequency, and harmonic-to-noise ratio) were analysed separately for each call type with linear mixed effect models (piglet identity and litter as random factors). MAX screams were loudest, longest, they had the highest pitch and the lowest tonality. Grunt calls from the three urgency levels differed in pitch; MAX grunts had the highest pitch.

We show that fine acoustic structure of single scream or grunt call reveals information about piglet emotional state. Grunt calls can be used more generally as they are emitted in many different contexts by piglets unlike scream calls associated mainly with acute threat context. Our results are further consistent with other studies suggesting that urgency is encoded similarly across species.

**Key words:** emotions, vocal communication, domestic pig, stress indicator, welfare

# THE IMPACT OF RUBBER FLOORING SYSTEM ON GILT POSTURAL BEHAVIOUR

Ostović Mario[1], Ekert Kabalin Anamaria[2], Menčik Sven[2], Mikuš Tomislav[3], Pavičić Željko[1]

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**ABSTRACT**

Flooring comfort influences many aspects of animal welfare including lying behaviour and frequency of posture exchange. Breeding females in intensive piggeries are mainly housed on concrete fully or partly slatted floors. These housing systems may result in discomfort, restlessness, more frequent posture exchange and injuring, in particular with the simultaneous effect of severe spatial restriction. The aim of the present study was to investigate if and to what extent the use of rubber mats affects gilt welfare by estimating their postural behaviour.

Control and experimental groups (5 gilts each) were observed during 28-day production cycle in service unit in autumn season. Both groups were accommodated in concrete slatted floor individual stalls, additionally coated with adjusted textured rubber mats in experimental group. Postural behaviour of gilts was recorded from 8.30 a.m. to 12.30 p.m. on cycle days 1, 8, 15 and 28, using digital camcorders and analysis of camera images. Basic data processing was done by use of descriptive statistics methods and normality of data distribution was verified by Kolmogorov-Smirnov test. Statistical significance of between-group differences was assessed by Student's t-test.

Control gilts spent most of the time standing and experimental gilts lying down, with a statistically significant between-group difference on all measurement days ($P<0.05$ all). The predominant form of lying behaviour in experimental group gilts was lateral posture in comparison with control sternal position. The gilts of both groups spent least of the time sitting. The duration of sitting and the frequency of posture exchange yielded no significant between-group differences, except for cycle day 1, when the gilts in concrete stalls were sitting for a significantly longer time, and cycle day 8, when the gilts in matted stalls showed a significantly higher frequency of posture changing ($P<0.05$ both).

In conclusion, rubber mats increased lying comfort of gilts, suggesting this type of flooring system to improve their welfare.

**Key words:** gilt, rubber flooring, behaviour

# ORAL BEHAVIOUR OF PIGS AS AN INDICATOR FOR TAIL BITING RISK ON FARM

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**ABSTRACT**

Tail biting is a widespread, multifactorial welfare and economical problem in pigs. Animal-based indicators may help to define key risks on individual farms. This contribution presents an observation method of oral behaviour and its relationship to enrichment as well as to tail and ear lesions in fattening pigs.

During the course of a tail biting prevention tool study in Germany ("SchwIP"), oral behaviour of pigs was scan sampled while standing in front of pens. Observations were summarised per pen as the ratio of pigs manipulating enrichment vs. pigs manipulating other pigs or anything else in the pen. After behaviour observations, prevalence of tail (blood, swelling, amputation) and ear lesions was assessed. Pens without enrichment were excluded from further calculations and the remainder classified into pens with enrichment objects only (O; e.g. wood; n=345), and pens with enrichment material (e.g. straw) with or without additional objects (M; n=64). This left 409 pens from 141 fattening farms for analysis, which consisted of Wilcoxon rank sum tests and Spearman correlations.

Manipulation ratio was higher in M pens (median=1.00, Q25=0.33, Q75=1.75) compared to O pens (median=0.36, Q25=0.25, Q75=0.67; $p<0.001$). Ear lesion prevalence was lower in M pens (median=0, Q25=0, Q75=0) than in O pens (median=0, Q25=0, Q75=10, $p<0.001$), as was prevalence of swollen tails (M: median=0, Q25=0, Q75=0, O: median=0, Q25=0, Q75=6.25, $p=0.024$). Prevalences of blood on tail or amputated tails did not differ significantly.

Manipulation ratio correlated negatively with all four lesion types, yet rather weakly (blood on tail: $r_S=-0.11$, $p=0.030$; swelling $r_S=-0.06$, $p=0.258$; amputation $r_S=-0.15$, $p=0.003$; ear lesions $r_S=-0.16$, $p=0.002$, n=409)

In summary, it can be concluded that oral behaviour scan sampling can be a useful indicator for enrichment quality in fattening pigs, which should be added to on-farm tail biting risk assessment.

**Key words:** tail biting, behaviour observation, on-farm, enrichment

# Session III
# Ruminants I

# PROGRESS IN UNDERSTANDING LAMENESS IN SHEEP AND FARMERS' ATTITUDES TO ITS CONTROL

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**ABSTRACT**

Lameness is a highly prevalent condition in sheep, it causes pain and if left untreated, ewes lose body condition and are less productive (Wassink et al., 2010). Sheep farmers identify lameness as the disease of most concern in their flock and report that managing lameness is frustrating (Goddard et al., 2006; Wassink et al., 2003). Over the past 14 years we have studied lameness in sheep in GB. Throughout this time we have also worked with farmers to understand their knowledge and attitudes towards managing lameness in sheep.

In 2004, the mean prevalence of lameness in sheep in England was 10% footrot accounted for 90% of lameness (Kaler and Green 2008a). Sheep farmers were using a variety of prevention and control measures and farmers who reported low prevalence of lameness (2%) were using systemic and topical antibiotic to treat sheep with footrot and interdigital dermatitis (Kaler and Green 2008). This was in agreement with earlier work (Wassink et al., 2003, 2004). In 2005 – 6 we ran a clinical trial on one flock of 800 sheep for 18 months, 400 were treated with systemic and topical antibiotic and 400 with foot trimming and topical treatment. Over the 18 months the prevalence of lameness fell from 8% to 2% in the group where sheep, only when lame with footrot, were treated with systemic and topical antibiotic. These ewes were less likely to be barren or die and they also had more lambs that survived and grew faster (Wassink et al., 2010). In a separate clinical trial we identified that the most rapid recovery from footrot occurred when sheep were treated with systemic and topical antibiotic with no trimming of hoof horn (Kaler et al., 2010a). This treatment also reduced repeated cases of lameness and prevented foot conformation from becoming poor (Kaler et al., 2010b).

In our work with farmers we have identified that farmers can recognise lame sheep but make a separate decision on whether to catch and treat lame sheep (Kaler and Green, 2008b; King and Green 2011). The more severely lame sheep are when farmers treat them, and (not surprisingly) the greater the number lame before farmers treat lame sheep, the higher the flock prevalence of lameness (Kaler and Green 2008b). Farmers using individual treatments of lame sheep with systemic and topical antibiotic are highly satisfied with their use of time and money, whilst farmers using footbathing and vaccination are less satisfied and have higher prevalence of lameness (Wassink et al., 2010b).

Most recently we are working with industry and farmers to understand barriers and motivators to adoption of best practices to minimise lameness in sheep.

# RESTING BEHAVIOUR AND MILK CORTISOL LEVELS IN POST-PARTURIENT EWES

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**ABSTRACT**

Lambing period imposes great stress on the ewe's organism due to changes that occur during a short time-frame, such as: pre-parturient period, lambing process itself, debut of lactation, lamb(s) rearing, involution of the genital tract, isolation, regrouping. The objectives of the current research-trial were: i) to evaluate the effects that the lambing process has on the resting behaviour of post-parturient ewes; ii) to test if milk cortisol could be used as an indicator for assessing stress in lactating ewes. Turcana breed secundiparous ewes (n=6) were monitored during the first 21 days following lambing, ewes were housed on deep straw bedding in two collective pens, with a space allowance of 1.7 $m^2$/head. Ewes had *ad libitum* access to water and to high quality pasture hay and to 400 g/head of barley. Video-recordings were made for 24 hours at 7 days intervals, using a surveillance system CC9622BIR equipped with four video-cameras with infrared vision. Milk samples were collected at 14 hours following lambing and at 7, 14 & 21 days, respectively. Samplings were done at 7:00 in order to avoid daily fluctuations of the cortisol levels due to the circadian rhythm. Hormonal analyses were done based on electro-chemo-luminescence Roche method. Data were statistically analyzed by non-parametrical Mann-Whitney test using MiniTab 14® software. All decisions about the acceptance or rejection of statistical hypothesis have been made at the 0.05 level of significance. The study was performed in accordance with the EU Directive 2010 /63 /EU for animal experimentation. Ewes spent lying on average 513.8±25.40, 637.5±21.00, 627.2±16.80 and 602.7±13.10 minutes during first 24 hours after lambing and during 7, 14 & 21 days, respectively. Post-parturient Turcana ewes devoted significantly ($p \leq 0.05$) less time to resting behaviour during first 24 hours after lambing, compared to days 7, 14 & 21 of the experiments. At 14 hours following lambing, cortisol level was of 7.78±0.47 µg/dl, and decreased to 5.08±0.72 µg/dl on the 7th day of the experiment ($p \leq 0.05$), then to 2.75±0.50 µg/dl on the day 14 ($p \leq 0.05$) and up to 1.61±0.43 µg/dl in the last day of experiments ($p \geq 0.05$). It has been concluded that cortisol from milk could prove a suitable indicator of sheep adrenal-cortex activity that may be applied to measure stress in lactating ewes. Both resting behaviour and milk cortisol levels are influenced by the lambing process in multiparous ewes.

**Key words:** resting behaviour, milk cortisol, post-parturient ewes, sheep welfare

# ASSESSMENT OF ANIMAL WELFARE TROUGH BEHAVIOURAL AND COGNITIVE PARAMETRS IN DAIRY SHEEP ON FARM CONDITIONS

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**ABSTRACT**

Animal emotions and their behavioural components are crucial to the concept of animal welfare.

The fourth principle of the Welfare Quality® Project "Appropriate behavior" was assessed on six farms with a total of 1040 dairy sheep.

Signs of pleasure (feeding, affiliative behaviours) and fear (human, machine milking) have been used to assess the level of welfare during lactation period. Welfare was measured through a Complex Score (CS), reflecting the main behavioural traits of each animal during machine milking in milking parlour. Traits were: 1) Feeding reaction towards foraging by hand (F); 2) Reaction "positioning teatcups" (PT); 3) Spontaneity and Persistency of taking place (SP). Each trait was assessed by four-degrees criteria. Observations were carried out on four consecutive days during morning milking. On the basis of CS, three levels of welfare ($p<0.001$), were established: High (H), Intermediate (I) and Low (L). Human – animal relations and social interactions were additionally assessed by the Beliaev's test (fear of human) and cognitive negative (new ambiance and close confinement) and positive (supplementary feeding) tests.

Statistical analyses were performed using one-way analysis of the variance (ANOVA). Significant effects were further analysed using post hoc comparison of means. Significance was set at $P<0.05$.

Behavioural data were examined using Principal Components Factor Analysis followed by orthogonal (VMAX) rotation of data.

Trait F was significantly different between animals of H/L and H/I levels of welfare, (minimum $P<0.01$) in all farms. The values of the trait T were

significantly contrasted between H/L and H/I levels (min. $P<0.05$). Trait SP was significantly different between animals of H/L, L/I and H/I levels of welfare, (minimum $P<0.01$) in all farms. Cognitive negative (new ambiance and close confinement) and positive (supplementary feeding) stimulus before milking affected the spontaneity and persistency of taking place in the parlour.

It is suggested that the higher occurrences of emotional and social negative behaviours recorded in L are clearly an indicator of poor welfare.

Over 25% of the animals (mainly L) in the dairy flock had higher emotional sensitivity, revealing on this base different problems concerning the milk let down, level of milk production and technology of milking.

**Key words:** welfare assessment, emotions, behavior, cognition, dairy sheep

# EFFECTS OF GENOTYPE BY ENVIRONMENT INTERACTIONS ON PRE-WEANING SURVIVAL RATES OF LAMBS

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**ABSTRACT**

Lamb welfare and survival are critical factors in determining the success of the sheep industry. Mortality rates in unweaned lambs is one of the major concerns for breeders. Limits reported of this trait ranging typically between 5 to 15 % in most European countries. Low selection response of the trait is due to the low heritability (0.03 – 0.07). Nowadays the ,,easy care philosophy" is slowly starting to be accepted by the sheep breeders. Easy care sheep breeds (e.g. Dorper) were developed for lower costs and selectively bred for high welfare traits such as easy lambing and high lamb survival rates. Different breeds show different inter-animal characteristics which may favour survival, therefore throughout cross-breeding, survival rate of the lambs could be improved. The aim of the current research was to investigate the effects of genotype-by-environment interactions on pre-weaning survival rate in three lamb genotypes, meat-specialised, easy care Dorper (DO) and the prolific Bluefaced Leicester (BL) and the Romanian indigenous Turcana (TA), under extensive rearing system. The research was performed in a commercial farm from western Romania, where the TA (n=140), $F_1$DOxTA (n=124) and $F_1$BLxTA (n=119) lambs were kept under identical extensive rearing conditions and were weaned at the age of 90 days. Turcana purebreds registered a survival rate of 94.29±0.019%, followed by $F_1$DOxTA cross-breed lambs with 91.94±0.024% and the $F_1$BLxTA dual-breeds with 85.71±0.032%, respectively. TA lambs survival rate until the age of 90 days was the highest, but not significant ($p \geq 0.05$) when compared to DO sired lambs, while differences registered between TA and $F_1$BLxTA genotypes were statistically significant ($p \leq 0.05$). The main reason to produce the $F_1$BLxTA cross-breeds is to use the females in developing the stratified sheep production system, the prolificacy being the most important trait of the genotype. However, higher survival rates of the cross-breeds could not be ignored since half of the $F_1$ progeny are male lambs that will be marketed. Dorper performed well under the Romanian management conditions and proven to be adaptable to new rearing conditions. It was concluded that Turcana and Dorper genotypes perform better in terms of survival rates of un-weaned lambs, which positively impacts welfare and profitability.

**Key words**: bluefaced leicester, dorper, easy-care, lamb mortality, turcana

# DO AGE, TIME OF THE DAY AND PRE-LYING BEHAVIOUR AFFECT THE TIME NEEDED TO OBTAIN STABLE HRV RESTING VALUES AND DIFFERENCES TO NON-RESTING VALUES IN DAIRY COWS?

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**ABSTRACT**

Heart rate variability (HRV) is a promising measure to get insight into animals' inner state and HRV during specific situations is usually compared to resting values. In Holstein cows these baseline values are subject to significant variation depending on e.g. circadian rhythm and age. The aim of the present study was therefore to investigate whether age, time of the day, and pre-lying behaviour also affect the time span to reach stable HRV resting values and differences in HRV as compared to non-resting.

In total 128 lying bouts (7±2.5 mean±SD) of 17 cubicle-housed Holstein Friesian dairy cows (four each in 1st, 2nd, 4th lactation, five in 3rd lactation (lac1-4)) were sampled from video recordings throughout the daylight period on three to four consecutive days. HRV was measured using Polar RS800CX® and further processed in Kubios HRV® to determine heart rate (HR), root mean square of successive differences (RMSSD), and standard deviation of interbeat-intervals (SDNN) during the first 15 minutes of lying (phase 1-6, minutes 0-5, 1-6, 2-7, 3-8, 5-10, 10-15), and during the first 5 minutes of standing post-lying.

As measure of latency, the phase that does not differ significantly from the subsequent phases was determined separately for each lactation and pre-lying-behaviour (active vs. standing calm) using mixed models for repeated measures with phase, period (pre- and post-afternoon milking) and their interaction as fixed factors). Similarly, differences between phase 6 and post-lying were analysed separately for each lactation.

Independent of lactation number, pre-lying-behaviour and phase, HR was higher post-milking (all $p<0.05$). RMSSD and SDNN were lower post-milking than pre-milking for lac3 and lower post-milking in lac1, 2 and 4 only after calm pre-lying-behaviour (all $p<0.05$). HR-latency differed depending on pre-lying-behaviour such that cows standing calmly before lying achieved stable values earlier than those being active (lac1: phase 1 vs. 2, lac2: 2 vs. 3, lac3:

2 vs. 4, lac4: 2 vs. 3). For RMSSD and SDNN, no significant differences between phases were found. Difference in HR between phase 6 and post-lying was greater in lac3 than in lac1 und lac2 (pairwise comparisons: p=0.05 and p=0.08).

The effects of activity level during pre-lying phase and of lactation number on HR-latency most probably reflect physical fitness and should be taken into account. However, excluding the first 10 minutes of lying bouts appears to be sufficient to achieve reliable baseline levels. Lactation number affected HR-differences, i.e. the autonomic nervous system of older animals may react more pronounced although differences of other HRV measures did not differ significantly.

**Key words:** dairy cows, difference, heart rate variability, latency, resting value.

# SOCIAL DOMINANCE IN FEMALE BEEF CATTLE - DETERMINED BY BODY WEIGHT OR BY RESPECT FOR AGE?

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**ABSTRACT**

Dominance hierarchies in groups of social animals can be based either on asymmetries that are important for agonism (such as body weight) or on more "conventional" cues (such as age), which is respected despite it having little relationship to the animal's fighting abilities. We investigated how social dominance is determined by age and weight in a herd of 29-39 beef cows over a period of ten years, focusing on all levels of the dominance hierarchy (individual, dyadic and group). At the dyadic level, age superiority had a stronger influence on the direction of social dominance in pairs than did weight superiority. Older cows were dominant in 73.6% of those dyads studied, even when the younger cow was heavier. At the group level, the strong influence of age on dominance produced a hierarchy that was very stable and strongly transitive. At the individual level, path analysis confirmed that the dominance index of a cow was more strongly associated with her age than with her weight. Our findings show that beef cows, for the most part, do not use their physical strength to attain dominance over older, but lighter, herdmates. This results in a stable age-based hierarchy, which might serve a universally shared function that promotes the smooth functioning of the herd and/or the expression of experience by older cows.

**Key words:** beef cows, social dominance, age, body weight, conflict resolution

# FARMERS' PERCEPTION OF COW LAMENESS ON CZECH DAIRY FARMS

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**ABSTRACT**

**Introduction**

Lameness is a serious economic and welfare problem in dairy cows. Published studies show underestimation of lameness prevalence and high variability in approach to lameness problem in farmers. The understanding of these attitudes might be crucial for reducing the lameness prevalence on dairy farms. The aim of our study was to assess the farmers' perception of lameness problem on Czech dairy farms.

**Methods**

We scored randomly selected lactating cows for lameness (40-80 cows per farm) on fourteen Czech dairy farms while cows were encouraged to walk in a barn. We used 3-level scale: 0 (not lame), 1 (moderately lame) and 2 (severely lame) according to the Welfare Quality® protocols. In the analyses, we used "Non lame" (0) and "Lame" (1+2). Farmers were questioned about 1. number of lame cows in their herd, 2. the percentage of lame cows in the herd that they would see as a problem (5%, ≥10%) 3. how quickly do they react when an animal starts to get ill (1-5; waiting a while – react immediately), 4. how effective would they assess their own health strategy? (1-5; bad-good).

**Results**

The estimation of lameness given by farmers (mean; 7%) were lower compared to observed prevalence (mean; 31%) (Wilcoxon paired test, $S=39$, $p<0.05$, $n=12$) and the estimated lameness did not correlate with observed prevalence ($r_s=-0.21$ $p>0.05$). The farmers who stated that 5% of lame cows in the herd is a problem estimated significantly lower lameness prevalence in their herds (3.1±1.9%, mean±STD; n=5) than farmers who saw ≥10% as a problem (8.1±7.1, n=6) (Mann-Whitney U test, $Z=-2.27$, $p<0.05$), however, the observed lameness prevalence on their farms did not differ (Mann-Whitney U test, $Z=0.56$, $p>0.05$, $n=12$). The farmers' estimation or the observed prevalence differed significantly neither according to his/her reaction on the situation when animal started to get ill (Mann-Whitney U test, $Z=0.56$, $p>0.05$, $n=12$; Mann-Whitney U test, $Z=-1.6$ $p>0.05$) nor according to his/her estimation of the effectiveness of the health strategy on the farm (Mann-Whitney U test, $Z=1.17$, $p>0.05$, $n=12$; Mann-Whitney U test, $Z=0.24$, $p>0.05$) respectively.

**Conclusion**

The underestimation of lameness prevalence and the lack of link between observed prevalence and lameness estimated by farmers together with farmers' subjective self-assessment of their handling of health problems, which does not seem to have real effect on lameness prevalence suggest a high need of training in lameness detection and external assessment of lameness on dairy farms. Additionally, a comparison of the seriousness of lameness prevalence on different farms (benchmarking studies) could help farmers to deal better with the lameness problem.

**Key words:** lameness, dairy cattle, farm, perception

# Session IV
# Ruminants II

# INTER-OBSERVER AGREEMENT AFTER TRAINING IN QUALITATIVE BEHAVIOUR ASSESSMENT FOLLOWING THE WELFARE QUALITY® PROTOCOL FOR DAIRY CATTLE IN THREE DIFFERENT COUNTRIES

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**ABSTRACT**

**Introduction**

The Welfare Quality®(WQ) protocol aims at assessing farm animal welfare using mainly animal-based measures. One of the measures is Qualitative Behaviour Assessment (QBA), which relies on the ability of human observers to integrate the animals' style of behaving using descriptors, which provide information additional to quantitative measures that is directly relevant for animal welfare. For on-farm use, afixed rating scale of cattle expression with 20 pre-defined terms was developed. It was the aim of the present study to investigate which degree of inter-observer agreement can be achieved after training in the application of the WQ protocol.

**Material and Methods**

During three training courses in France, Finland and Sweden, assessors (n=9, 13 and 10, respectively) were given detailed instructions on the procedure and the use of the rating scale. Additionally, all terms were translated into the local languges and discussed between the attendants. This was followed by training sessions using both video recordings and live observations. Inter-observer agreement was tested using 17 1-min video clips of different herds of dairy cattle. Per country, data were submitted to PCA and scores of the first two dimensions analysed using Kendall Correlation Coefficient W.

**Results**

In all countries, the first principal component (explained variance 31-36%) consistently distinguished between negative and positive mood (‚agitated'/ ‚frustrated'/‚uneasy' vs. ‚content'/‚calm'/‚relaxed'). For the second component (explained variance 17-23%), consistently only terms of ‚positive engagement' achieved high loadings (‚sociable'/‚playful'). Kendalls's W ranged between 0.56 and 0.72 for PC1 and 0.52 and 0.56 for PC2 (all $p<0.001$, $df=16$).

**Conclusions**

The results indicate that with few hours of training reasonable to satisfactory agreement can be achieved for QBA in dairy cattle using a fixed rating list, which is independent from the background of the assessors.

**Key words:** qualitative behaviour assessment, inter-observer reliability, training, welfare quality®

# EFFECTS OF HEAT STRESS ON RUMINATION BEHAVIOUR IN LACTATING DAIRY COWS

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**ABSTRACT**

Rumination is considered a key factor to monitor cow's comfort and health, especially when rumen functions are involved. Lactating dairy cows devote on average 6 to 9 h/day to rumination, most of the rumination process taking place while resting/lying. The behavioural pattern of rumination in lactating cows is being modulated by a series of factors such as: DMI, lactation phase and milk yield, health, housing system, thermal stress etc. In Holstein breed cows the heat stress begins at temperatures of over 25°C, when both the DMI and milk yield are negatively affected. Aim of the current research was to evaluate the effects that heat stress has on the rumination behaviour in lactating Holstein dairy cows when housed in tied stanchion barns. Behaviour of ten lactating multiparous cows (2.9 lactations) in their first 100 days of lactation was monitored during summer 2012, using a four video cameras surveillance system (CC9622BIR). Cows were fed a diet consisting of 25 kg of alfalfa, 4 kg of concentrates and 5 kg of brewer's yeast, and had an average milk yield of 26.4 kg/day. Experiments were replicated twice, first trial (LowT) when the average temperature inside barn was of 24.1°C and second trial (HighT), when the temperature reached an average of 31.7°C per 24 h. Ethograms consisted in measuring the total time devoted to rumination/24 h, the number of rumination periods and the length of the rumination sessions per day. Cows spend ruminating on average 453.0±24.61 minutes per day during LowT trial, and ruminated 350.6±13.82 minutes in HighT trial. Time devoted to cud-chewing was significantly reduced (p 0.01) at high environmental temperature. During the LowT trial cows ruminated in 17.1±0.96 sessions, while during HighT they ruminated in 14.6±0.87 periods, resulting on average in 2.5 less rumination periods when the environmental temperature was high (p 0.05). Average time length of one rumination session was 26.6±0.97 minutes in LowT and 24.8±1.97 minutes in HighT, showing a tendency (p=0.0937) to be lower under the heat stress. It was concluded that the rumination process of the lactating dairy cows was significantly influenced by heat stress.

**Key words:** behaviour, heat stress, holstein, multiparous cows, rumination

# THE INFLUENCE OF POSITIVE INTERACTIONS ON THE HUMAN-ANIMAL RELATIONSHIP AND WELFARE IN CALVES

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**ABSTRACT**

**Introduction**

The human-animal relationship (HAR) in farmed animals has strong implications for welfare and productivity. The aim of the present study was to find out whether HAR and welfare can be improved by providing positive interactions (PI) to calves. Eight groups of calves were kept with reduced human contact. Four of the groups were in addition provided with PI during their first four weeks of life. All groups were tested for their HAR as well as for welfare-related parameters at different ages.

**Material and Methods**

The experiment was carried out with 25 Austrian Simmental calves. Following 14 d of single housing, they lived in groups of four until weaning in week 12. If not enough Simmental calves were born, groups were filled with calves of other breeds, which were not tested. According to usual practice, animals had contact to humans during bucket-feeding, cleaning of pens, medical treatment, as well as disbudding. Each animal of four groups (PI groups) received PI in form of gentle talking and stroking for 2 min on 5 d per week during the first 4 weeks of life, whereas the other animals (control groups) did not.

The HAR was measured by avoidance distance tests (ADT) at 4 and 11 weeks of age and by an arena test with and without presence of a human at 4 weeks of age. Welfare-related parameters included salivary cortisol as well as heart rate variability.

**Results**

Avoidance distances were very low, without any significant differences between the groups. In the arena test, there was a pronounced pattern of less stress-indicative behavior (i. e. vocalizations) during presence of the experimenter, with animals mostly keeping close to her. ANOVA revealed a

highly significant effect of phase, but no significant effect of treatment. There was a main effect of treatment for salivary cortisol concentrations, with control calves having higher levels than PI calves before as well as after the arena test. Heart rate variability data are currently being analyzed.

**Conclusions**

There were no differences detectable between the groups as to their HAR. This is not due to a failure of the PI treatment, but to a ceiling effect, as the control groups had very low avoidance distances, and they showed less stress-indicating behavior in the arena test in the presence of a human than in her absence. The good quality of the HAR in controls may be due to a breed or farm effect, or to an influence of the experimental procedures. Salivary cortisol concentrations were higher in control than in PI calves. This may either be due to a difference in their HAR that is too small to show in behavior or to a physiological effect of PI during early life.

**Key words**: calves, human-animal relationship, HRV, salivary cortisol, avoidance distance

# PRE-ORBITAL GLAND OPENING AS A MEASURE OF HANDLING HABITUATION IN RED DEER CALVES

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**ABSTRACT**

Pre-orbital gland seems to play not only an olfactory function in cervids, but also a visual one. Previous studies have shown that opening this gland is an easy way for the calf to communicate with the mother, indicating hunger, satiety, stress, pain, fear and/or excitement. This visual information can be also useful for farm operators to assess how fast are calves habituating to handling routines, and to detect those calves which are not habituating correctly and may suffer of chronic stress in the future.

We performed two consecutive experiments to clarify if this pre-orbital gland opening is an useful method to understand habituation to handling in farmed deer. In the first one, we measured the opening behaviour displayed by newborns during a initial handling (including weighing, ear-tagging and sex determination). Thirty-one calves born in three different paddocks were subjected to the same routine handling but with different periodicity: every one, two or three weeks. Therefore, the second part of the study consisted in measuring pre-orbital gland opening during the same routine handling (weighing and assessment of body condition) which also included a more stressful procedure as blood sampling. These test were performed when calves were one, three and five months.

93% of the calves opened the gland during the first procedure, and this behaviour was not related to sex, time since birth or birth weight. When handled with one and three months, those calves which opened the gland had experienced a lower number of previous handlings than those which did not ($P = 0.031$ and $P = 0.001$, respectively), but these differences disappeared when

calves were handled with five months. Linear regression models confirmed that the percentage of time opening the gland in a given handling was usually affected by the number of previous handlings, but also by the percentage of time opening in the previous experimental phases. Finally, we also found a strong individual repeatability of this behaviour, since those calves which opened the pre-orbital gland for longer in a given phase also did it in the following.

Our results suggest that a regular assessment of the state of the pre-orbital gland during routine handlings can be used as an indicator of habituation to handling in farmed cervids, and as a tool to detect those calves which are not habituating and may suffer of chronic stress. Therefore, this information can improve the welfare of farmed cervids. This result is especially relevant because few welfare indicators has been proposed by now for a growing industry as deer farming.

**Key words:** Cervus elaphus, habituation, handling routines, pre-orbital gland, stress

# Session V
# Poultry

# ANTICIPATION OF REWARD IN JAPANESE QUAIL: THE EFFECTS OF ENVIRONMENTAL ENRICHMENT AND DOPAMINE AND OPIOID RECEPTORS BLOCKERS

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**ABSTRACT**

Sensitivity of animals to announced reward, reflecting previous experience and measured by anticipatory behaviour, has been suggested as a possible indicator of welfare status. Induction of anticipation of a positive rewarding stimulus has been shown to have stress-reducing effects. The aim of this study was to define the anticipatory behaviour induced by classical trace conditioning in Japanese quail. Adult quail hens (n=14) were trained to anticipate in the conditioning chamber by gradually increasing the conditioned stimulus (CS) – unconditioned stimulus (US) interval from the 1 to 30 s. Anticipatory behaviour consisted mainly of rapid head movements and standing alert. Frequency of the anticipatory behaviours increased during the interval between CS (flashing green spot, 5 s) and US (food, 10 s), as compared with the same interval before CS and after US. Anticipatory behaviours were affected by housing conditions (two-way ANOVA, post-hoc LSD test). Quails housed in wire cages were more sensitive to reward than those housed in the deep litter pen, as reflected by lower anticipatory behaviour in an enriched housing (e.g. standing alert during the CS-US interval did not increase significantly in deep litter pen as compared to wire cages, $p = 0.01$). Treatment with dopamine antagonist haloperidol (0.5 mg/kg i.m.) as well as opioid antagonist naltrexone (5 mg/kg i.m.) suppressed certain part of anticipatory behaviours during the CS-US interval (e.g. frequency of head movements during CS-US interval did not increase significantly in both haloperidol and naltrexone treated birds, as compared to control; haloperidol - $p = 0.05$; naltrexone - $p = 0.052$) suggesting the role of dopaminergic and opioid mechanisms in their control.

**Key words:** anticipatory behavior, Japanese quail, environmental enrichment, dopamine, opioids

# ESTIMATING COGNITIVE BIAS IN JAPANESE QUAIL USING OPERANT CONDITIONING TECHNIQUES: METHODOLOGICAL ISSUES

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**ABSTRACT**

Emotional states influence cognitive processes. Judgement bias reflects the tendency of a subject to show behaviour indicating anticipation of either relatively positive or relatively negative outcomes in response to affectively ambiguous stimuli.

Here we report our attempt to adopt the concept of cognitive (judgement) bias for the assessment of Japanese quail welfare in relation to housing conditions. Adult hens were first trained in a Go/NoGo task to respond by pecking to a positive stimulus (white circle in half of animals; 80% grey in the other half) associated with a positive event (food) and to refrain from pecking at the negative stimulus (80% grey circle in half of animals; white in the other half) to avoid a negative event (white noise) in the touch-screen operant chamber. Once the birds discriminated successfully both stimuli, they were divided into the two groups according to housing conditions (wire cage vs. deep litter) and after the 2 weeks they were subjected to ambiguous stimuli test in 3 daily sessions. In these tests in addition to the reinforced and punished stimuli a non-reinforced intermediate cues (20%, 40% and 60% grey circles) have been presented.

We have realized four series of experiments (n= 9; 12; 11; 19). Their results are not consistent. In three out of four experiments in agreement with our hypothesis the proportion of birds responding to stimuli in the ambiguous stimuli test was higher in deep litter housed birds than in cage housed ones. The shape of the responses to positive, negative and ambiguous stimuli differed in particular series of experiments. In two series of experiments responses of one group of animals to positive stimulus was low (in the third series the cage group, in the fourth series the deep litter group).

We are working on identification of sources of differences. One possibility is that the time spent in different environments (2 weeks) is not long enough to induce stable changes in quail emotional states detectable by the method. We have used as a reward the same food by which the birds were regularly fed and the birds were food deprived to increase their feeding motivation. Maybe the use of the reward with higher hedonic value (mealwroms) without the need of food deprivation would be better. In the fourth series of experiments the lower response rate of quail kept on deep litter was observed. We assume that quality of litter (we have changed the supplier of wood shavings), content of small particles in litter, may affect the feeding motivation.

Our studies indicate that the use of operant techniques for the assessment of cognitive bias in poultry is very sensitive to various influences and further studies are needed to improve the method.

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# COGNITIVE PERFORMANCE OF LAYING HENS REFLECT THEIR WELFARE

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**ABSTRACT**

The assumption that animals can experience affective states is critical for our concern about animal welfare. Even though experience of emotion cannot be assessed directly (verbal communication), their neural, behavioural and physiological indicators can be measured. Recently, the measurement of cognitive bias has been suggested as an indicator of emotions in animals. The aim of this study was to investigate the influence of two different housing conditions on laying hens by using cognitive bias test based on discriminant operant conditioning. We have designed the touch-screen operant chamber and automatic mealworm dispenser for testing cognitive bias in hens. Sixteen adult laying hens were trained in a Go/NoGo task to discriminate two visual stimuli; to peck a positive stimulus (white circle in 7 hens and 80% grey circle in 9 hens) rewarded by mealworm and not to peck a negative stimulus (80% grey circle in 7 hens and white circle in 9 hens) punished by unpleasant sound. After four sessions all hens significantly discriminated stimuli ($\rho$=0.730±0.006). Proportion of responses to positive and negative stimuli was 97.91±0.95% and 6.25±2.31%, respectively. Then they were divided into two groups with different housing conditions. One group remained in a deep litter box, while another group was moved to enriched cages. After two weeks groups were replaced. The judgement bias was estimated by presenting 3 new, ambiguous stimuli (20%, 40%, 60% grey). Ambiguous stimuli test was performed once in each of three consecutive days 2 weeks after the division and 2, 4 and 6 weeks after the replacement of birds housing conditions. In each test the mean proportion of responses to ambiguous stimuli was higher in hens on deep litter. The biggest difference in the proportion of responses to the presented stimuli was observed four weeks after the groups replacement in near positive stimulus (deep litter hens 64,26±3,30%, caged hens 38,89±14,44%) and middle stimulus (deep litter hens 23,61±3,37%, caged hens 11,11±4,75%). However, the two-way mixed model ANOVA with day of testing as a within-subject factor and housing conditions as a between subject factor did not prove the significant effect of housing on the proportion of

responses to different stimuli. This suggests that although there was clear trend towards higher proportion of responses to ambiguous stimuli („optimism“) in birds housed on deep litter, variation in responses was too high, as indicated also by the significant effect of testing day. Other authors using another method of the judgement bias estimation (spatial judgement task) in laying hens also did not find significant effect of the two environments with different level of enrichment. These stresses need further clarification of the sensibility of this method.

**Key words:** laying hens, emotions, cognition

**This work was supported by APVV-0047-10 and VEGA-2/0192/11.

# Session VI
# Dogs

# ANIMAL-BASED INDICATORS TO ASSESS DOG WELFARE IN LONG-TERM SHELTERS

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**ABSTRACT**

**Introduction**

There is evidence that the quality of life of dogs spending part, or most, of their life housed in shelters, can be impaired whenever the environment is not adequate. Currently, no standard tool is available to evaluate the welfare state of dogs housed in shelter facilities. Being welfare a condition of the animal, measures that are based on the direct evaluation of the animal (e.g. body condition score, presence of abnormal behaviours) can be reliable indicators of its actual welfare state. The 'Shelter Quality' protocol, developed within a research project financed by the Italian Ministry of Health, is a new tool to assess shelter dogs' welfare. The main aim of the project was to identify animal-based measures in an attempt to estimate the actual welfare state of the animals in terms of their behaviour, health or physical condition. In this paper, preliminary descriptive data collected applying the protocol on a sample of shelter facilities in Italy, Romania, Serbia and Montenegro will be presented.

**Materials and Methods**

The 'Shelter Quality' protocol contains a check list of 28 measures. Check-lists were compiled assessing a random sample of dogs from each shelter. Sixteen shelters (11 Italy, 3 Romania, 1 per each Serbia and Montenegro) were evaluated for a total of 784 animals assessed. A descriptive analysis helped assessing the suitability of this new tool in identifying differences among shelter facilities in different Countries.

**Results**

Age class differed among shelter dog populations: 57% of the animals were, on average, geriatrics in Italy, adults (54%) in Romania and young (51%) in Serbia and Montenegro. Resource-based measures were collected to evaluate the appropriateness of the shelter facilities. Most parameters (including bedding

material, presence of harmful edges, cleanliness of drinking water) were, on average, adequate for each Country. Space allowance instead was in some occasions not satisfying minimum requirements (inadequate for 25% of cases in Italy and Romania and around 40% in Serbia-Montenegro). Among scored health parameters, those recorded with a higher prevalence were: inadequate nutritional state (7% of animals were too thin), presence of injuries or hair loss areas on the body, and lameness (around 4%). Other parameters (i.e. evidence of tumours, swelling, diarrhoea or respiratory disorders) were recorded on less than 2% of the cases. Finally, dog-human social interaction was measured. Although most dogs were friendly and sociable, a notable component of fear towards unfamiliar people was recorded across shelters (on average 21% of the total population).

**Conclusion**

These preliminary results show the feasibility of the 'Shelter Quality' protocol to asses some key issues that might affect negatively the welfare of dogs in shelters. Individual analysis of single shelters may allow to spot the origin of the problem, and target the actions for improvement.

**Key words**: shelter, welfare, dog, animal-based indicators

# COMMON FEAR TYPES IN MONGREL DOGS IN BELGRADE

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**ABSTRACT**

**Introduction**

Fear is a reaction to the perception of actual danger that threatens the integrity of an individual. Basic causes of fear in dogs are a genetically-determined, disease-induced or ageing-induced lower tolerance to stressors and anxiety provoking situations, lack of early life experiences due to improper habituation and socialization and negative experiences. Fear can be induced by novel and unknown or by known non-social (inanimate occurrences, places, situations and objects) or social stimuli (people and other animals) that an animal previously remembered as dangerous. Fear-related behaviours can impair the human-pet bond resulting in abandonment, relinquishment, or euthanasia of a dog. The aim of the study was to estimate common types of fears in mongrel dogs.

**Material and Methods**

The same methodology as that use by Blackwell *et al*. (2005) for RSPCA survey of fears and phobias in the domestic dog in UK was applied in this study. Data were collected through a questionnaire survey of mongrel dog owners in different parts of Belgrade. Owners of mongrel dogs (N=213) were chosen from two main sources (owners out walking their dogs and owners visiting small animal practice). The survey was conducted from January 2007 to December 2010. Results are expressed as absolute (n) and relative numbers (%). A one-sample *t*-test between proportions was performed to determine whether there was a significant difference between the percent of mongrel dogs with fears and the percentage of dogs without fears and between the percent of owners who asked veterinarians for advices and treatment and the percentage of owners who did not ask veterinarians for advices or treatments of their fearful pets with behavioural problems.

**Results**

More than two-thirds of dogs (n=148; 69.5%) were directly adopted by their owners form municipal or private shelters. The rest of dogs was previously found by their owners or other family members or owners' acquaintances in public areas (streets, roads, parks, hiding places in settlements *etc*.) and then was adopted directly in homes of their owners (n=65; 30.5%). There was a statistical significant difference ($t$=6.181; df=212; $p$<0.01) between the percent of mongrel

dogs adopted from shelters (69.5%) and the percentage of dogs adopted from public places (30.5%).In the sample of 213 owners of mongrel dogs, 122 of them reported fears in their pets. There was a significant difference ($t$=2.154; df=212; $p$<0.05) between the percent of mongrel dogs with fears (57.3%) and the percentage of dogs without fears (42.7%). Owners reported that their dog was frightened of fireworks, thunderstorms or both of them (n=21; 10%), entrance in veterinary clinic (n=19; 9%), entrance in a car (n=16; 7.5%), sound of vacuum cleaners (n=15; 7%), other unknown dogs (n=12; 5.6%), unknown people (n=10; 5%), sound of washing machines (n=10; 5%), loud noises on TV (n=8; 3.8%), umbrella opening (n=6; 2.8%) and traffic noise (n=5; 2.3%). The most commonly reported behaviour shown by fearful dogs was barking (n=31; 14.5%), trembling/shaking (n=26; 12.2%), seeking out people (n=22; 10.3%), hiding (n=14; 6.6%), escaping behaviour (n=11; 5.2%), howling (n=9; 4.2%), salivation (n=7; 3.3%) and urination/defecation (n=2; 1.0%). There was no statistical significant difference between the percent of owners who asked veterinarians for advices or treatment of their pets (n=68; 32%) and those who did not ask veterinarians for advices or treatment of their fearful pets with behavioural problems (n=54; 25%).

**Discussion and Conclusion**

These results suggest that mongrel dogs were reported to show signs of a entrance in veterinary clinics or cars (17%), fear of sounds from household devices (15%), social stimuli (10.2%), fireworks, thunderstorms or both of them (10%), umbrella opening (2.8%) and traffic noise (2.3%). Only 68 owners (32%) asked veterinarians for advices or treatments of their fearful pets with behavioural problems. It is well known that untreated fears can develop into phobias, and generalize to other inanimate or animate similar stimuli.

**Key words:** behaviour, dog, fear, mongrel

# POSTER PRESENTATIONS

## P1 INVESTIGATIONS ON BEHAVIOURAL DISORDERS IN BROILER CHICKENS AFFECTED BY COCCIDIOSIS

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**ABSTRACT**

**Introduction**

Coccidiosis is a parasitic disease with acute or extremely acute development, affecting broiler chickens and other avian species. It is caused by protozoa from the family Coccidia, genus *Eimeria*, which affect different part of the intestines of infected birds. They could be encountered in the caecum, duodenum, proximal jejunum, small intestine without duodenum, the distal part of small and the proximal part of large intestine.

The parasitic infection is manifested when coccidiostatic in poultry feed is absent or deficient. Possibly, the infection could occur after application of a nonefficient coccidiostatic drug. The most significant predisposing environmental factor is the high humidity of the inadequately thick permanent litter on which broiler chickens are reared.

Considering the fact that coccidiosis is a disease of the so-called „diseases of environment-technopathy“ / that exhibits mostly at non-specific animal hygiene requirements / the purpose of our research is proving accurate pathognomonic complex that integrating a specific clinical presentation and typical behavior and facilitating the diagnosis of common diseases, without the obligatory till now autopsy of dead birds.

**Materials and Methods**

Targeted behavioral changes are studied in 30-day-old broiler chickens from the hen type shown suffering from coccidiosis mortem and parasitological / isolation of the causative /. Study animals were 8000 in number, grown on deep irremovable bed of straw, 4000 in two monolithic rooms without windows and lighting / light with use of program-3 h illumination with a break of 1 h /, in two different farms. Feeding the birds is carried out by the chain-grooved feeding

and watering of vacuum drinkers. The temperature, relative humidity and velocity of the air in the space defined by the standard animal hygiene methods.

Behavioral activities were examined and described using methods:

- Visual observation, continually for 96 hours / 4 days /;
- Time measurement-measurement during the following behavioral activities, movement, sleep, feeding, watering, defecating;
- Preparation of etogrami - characteristics of the above-behavioral activities in duration;
- Quantitative assessment of behavioral / set formula /.

Results processed the variance-a statistical comparison program and expressed as a percentage /% /.

All research methods are tailored to ensure the welfare of animals.

Results. It was found that the affected individuals / 35%, with known coccidiosis occurring acutely / are kept in an environment with high relative humidity / 80% / in 22-23 pcs. birds M2 / overcrowding / and typical behavior:

1. Hypokinesia, 20% of patients, birds, etc., respectively;
2. Pronounced fatigue-27%;
3. Anorexia, 35%;
4. Increased thirst, 33%;
5. Prostration-25%;
6. Bloody diarrhea, 35%;
7. Collection of chicks in groups / typical cluster / - 35%.

Described behavioral symptoms are accompanied by specific changes in the appearance of the birds with coccidiosis birds and the environment in which they are developed:

1. Pale skin, 35%;
2. Pale comb-35%;
3. Pale legs-35%;
4. Fluffed and ruffled feathers, especially around the anus, blood-stained 35%;
5. Drooping wings, 30%;
6. Head down, 25%;
7. Weight Loss-35%;
8. Bedding, stained with blood in some places;
9. Increased relative humidity;
10. Overpopulation: 100%;
11. Mortality, 60%.

**Conclusions**

It is inferred a characteristic pathognomonic symptom complex of acute occuring coccidiosis in broiler chickens of hens species. But it is pathognomonic if ethological characteristic finding is accompanied by specific changes on the appearance of affected birds / reduced body weight, ruffled feathers, drooping wings, pale comb, feet and skin, faecal blood, high mortality / or the environment / high relative humidity, congestion, local blood-stained bedding /**.**

Only then, the veterinarian could rely upon the established by our team the pathognomonic symptom complex (specific etiology and appearance of diseased birds and their environment) to pose the diagnosis "coccidiosis" without confirmation by necropsy findings and isolation of the causative agent of coccidiosis.

**Key words**: broiler chickens, abnormal behaviour, coccidiosis

**P2**

# INVESTIGATIONS ON SOME FORMS OF STEREOTYPIC MOVEMENT (PSYCHOLOGICAL) DISORDERS IN ANIMALS KEPT IN ZOOS

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**ABSTRACT**

**Introduction**

The purpose of zoos is to acquaint people with animals that could not be seen in their natural environment. Often, these are the only places where some animal species could be really encountered. The deprivation of wild animals from the natural stimuli of the habitat and their life in conditions of limited or no freedom are the main causes for the occurrence of stereotypic movement (abnormal, behavioural, psychic) disorders. On the other side, the artificial surroundings of a zoo did not permit the animals to satisfy their natural behavioural needs. It is acknowledged that a behaviour that is non-provoked, permanently or frequently exhibited, with similar or equal signs, should be classified as a psychic disorder. Recently there is increasing body of evidence that such a disturbed behaviour is exhibited by animals in order to compensate for the deficiency of various stimuli. This fact is particularly specific for zoo environment. It makes animals permanently "frustrated" and for overcoming the boredom, they perform a number of activities. Often, such a behaviour is unsolicited by people but it remains a necessity for animals kept in zoos.

**Materials and Methods**

The investigations were performed within a 5-year period (2008 to 2012) on the territory of the city zoo in Stara Zagora. It is situated on an area of 70 decares and is the home of 72 different animal species. The regimen of feeding and watering is in compliance with their biological requirements.

The behaviour animals with behavioural, disorders was analysed by means of visual and optical observation, 8 h a day, during the light part of the day. The following behavioural activities have been monitored: locomotion, irritability, scratching and vocalisation.

**Results**

During the monitoring period, stereotypic movement behaviour was observed in brown bear (*Ursus arctos*) (n=1) (14%), white-nosed coati (*Nasua narica*) (n=1) (50%), Barbary sheep (goat-antelope) (*Ammotragus lervia*) (n=1) (50%), marten (*Martes foina*) (n=2) (50%), tiger (*Panthera tigris*) (n=1) (50%) and budgerigars (*Melopsittacus undulatus*) (n=3) (25%). The bear, martens and the tiger performed frequent repetitive movements near the fences or between two objects. Simultaneously, having reaching the end of the route, they turned and this was preceded by single or repeated rotations of the head at $180^0$. Another abnormal activity that was often seen was the frequent and stubborn persistent grooming in bears, It was demonstrated as continuous scratching and licking the upper part of the body, especially the head. A similar stereotypic movement activity was observed in budgerigars, which cleaned their feathers so obstinately that in one of the three birds, skin lesions have appeared. The male Barbary sheep, performed occasionally a focused attack to fences and made attempts to jump over them. This behaviour was exhibited in the hours before feeding. During our observations, the coati and the two martens were moved to other habitats, where stereotypical disorders were not observed.

**Conclusions**

1. The commonest stereotypic movement disorders in the animals kept in the city zoo, Stara Zagora, was the locomotor hyperactivity (moving along the fences or on a straight line from one object to another), which was continuous, very frequently repeated and non-provoked.

2. The main causes for the observed disorders are the insufficient area and the lack of stimuli, resulting in uniformity and monotony, and thus, to boredom.

3. The main procedures for prevention and correction of observed stereotypic movement behaviours are: enrichment of the environment, increasing the living area, moving to appropriate premises, considerate and kind attitude of both zoo personnel and visitors to wild animals.

The integrated approach for provision of all necessary living conditions, the compliance with individual and species requirements and specifics is the way to provide psychic comfort to animals and prevent the occurrence of stereotypic movement behaviour in zoos.

**Key words**: stereotypic movement (psychological) disorders, zoo animals

## P3 CORTISOL CONCENTRATION IN FREE-ROAMING DOGS AFTER TRANSPORT AND HOUSING IN NEW ENVIRONMENT

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**ABSTRACT**

**Introduction**

Transport and new uncontrollable or unpredictable social environmental are stressful for dogs and can reduce dog welfare. Particular unpleasant situations for free-roaming dogs are their capture in public areas and caging during transport, vehicle vibrations, traffic noise, unknown environment, unloading etc. All of these events elicit stress responses including endocrine changes in dogs. Therefore, one of the reliable indicators of the stress reaction is cortisol.

The aim of this study was to compare concentration of cortisol in dogs immediately after transport and 24 hours after housing in cages in a new environment of a veterinary practice.

**Material and Methods**

The experiment was performed on 40 female dogs. They were 2 to 4 years old and their body mass varied from 20 to 25 kg. All bitches were involved in the program of birth control of free-roaming dogs in Belgrade, Serbia. Free-roaming dogs were captured in public areas by the communal animal hygiene service and were transported between 30 and 45 minutes to the clinic of a veterinary practice. Blood was sampled two times from each bitch from *vena cephalica antebrachii* for the analysis of biochemical indicator of stress response (cortisol). The first blood was collected from each dog immediately after transport. Sample collected at that time was considered as basal value. The dogs were then transferred in cages (1x1x1 m) in a new environment of a veterinary practice. The second blood was collected from each dog 24 hours after housing in new environment. Normal plasma cortisol levels in dogs vary from 13.79 to 165.54 nmol/l (Feldman, 2004). According to cortisol concentration variations, dogs were divided into six classes: I - (0-<10 nmol/l); II – (10-<30 nmol/l); III – (30-<50 nmol/l); IV – (50-<100 nmol/l); V – (100-150 nmol/l); VI – (150-<200 nmol/l). Concentration of cortisol was determined with chemiluminescence immunoassay (CLIA). Statistical analysis was performed using *t*-test.

**Results**

Average cortisol concentration immediately after transport in dogs of the fifth and sixth class (V-124.33±13.21 nmol/l; VI-170.20±12.02 nmol/l) were significantly higher ($p<0.01$) than after 24 hours in the new environment (V-

94.44±10.16 nmol/l; VI-142.57±10.66 nmol/l). In the second and third class, average cortisol concentration was significantly lower ($p<0.01$) after transport (II-17.10±5.93 nmol/l; III-37.98±4.71 nmol/l) than after 24 hours in the new environment (II-31.86±7.30 nmol/l; III-44.18±1.99 nmol/l). First and fourth class showed no differences between average cortisol concentration immediately after transport (I-4.53±2.19 nmol/l; IV-74.14±16.76 nmol/l) and 24 hours in the new environment of a veterinary practice (I-3.72±1.68 nmol/l; IV-61.11±10.55 nmol/l).

**Conclusion**

Firstly, the obtained results in the study clearly point out great variations in cortisol concentrations in mongrel dogs immediately after transport. Moreover, average blood cortisol concentrations after 24 hours of transport and in the new environment in dogs of all classes decreased except in dogs of the second and the third class. These findings can indicate that differences in cortisol concentrations and stress responses should be consequences of temperament differences in free-roaming dogs. The new housing environment was more stressful for free-roaming dogs in the second and the third class than for dogs in other classes (I, IV-VI). Individual differences like the effects of an unknown breed or earlier life experiences may further attribute to variability in stress responses. It is necessary to improve all treatments with free-roaming dogs that could be potential sources of stressors.

**Key words:** cortisol, environment, dogs, stress, transport

## P4 ASSESSMENT PROTOCOL FOR SHEEP TRANSPORTED IN LONG DISTANCE ROUTES THROUGHOUT EUROPE

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**ABSTRACT**

This paper presents the Assessment Protocol for sheep transported in long distance routes throughout Europe that was developed within the framework of the project "Development of an EU wide animal transport certification system and renovation of control posts in the European Union- SANCO/2011/GR/CRPA/SI2.610274". The protocol is based on the welfare criteria developed in the Welfare Quality® project, the welfare assessment protocols developed during the EU project "Renovation and promoting high quality control posts in the European Union", the EFSA Scientific Opinion concerning the Welfare of Animals during transport and the available literature on this topic. Moreover, the main current European regulations concerning animal welfare (EC Regulation 1/2005, EU Council Directive 98/58 and Council Regulation 1255/97) are taken into account.

The Protocol covers most of the adverse effects identified for the transport hazards and addresses 12 welfare criteria associated with one of the following four areas: feeding, housing, health and behaviour. The measures were categorized into 3 categories: 1) animal based measures and management based measures recorded during unloading, such as slipping, falling, panting, etc 2) resource and transport measures concerning the journey itself, such as bedding, sharp edges, lighting for animals, etc, and 3) a checklist of measures for drivers on the assessment of fitness to travel at departure, such as lameness, injuries, coughing, etc. Up today, the protocol was tested in Greece and in Italy in 50 cases of sheep transported for 8 or more hours through the main routes of Europe. One assessor was in charge for each case to make direct observations for all parameters included in the Protocol. Preliminary results, derived from descriptive statistics, indicated that deck height was not adequate in 12 % of the trucks while in 8% of the trucks there were sharp edges. There was not sufficient space in 58% of the transports with unshorn sheep and in 38% of the transports with shorn sheep. Moreover, in approximately 50% of the trucks, drinking points were not working and in 42% of the trucks there was no water available. During unloading, the percentage of falling animals and flocks were animals were observed slipping and reluctant to move was considerable (up to 100%). This might be related to a too steep ramp slope (in 20% of the trucks) and to handlers' behaviour. By conclusion, the main problems observed are related to overcrowding, animal handling and deficiencies of truck equipment, mainly ramp slope, deck height and drinker facilities.

**Key words:** sheep transport, assessment protocol, transport behaviour

## P5 ANIMAL WELFARE IN BREEDING OF INDIGENOUS CROATIAN COLD-BLOODED HORSE BREEDS

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**ABSTRACT**

Croatian cold-blooded indigenous horse breeds „Croatian cold-blooded horse“ and „Croatian Posavina horse“ constitute 63% of the total number of horses in Croatia. Great value of indigenous breeds lies in the centuries of their origin and development in the specific growing conditions. They represent the national genetic treasure, whose disappearance would undermine biodiversity. Disappearance or loss of indigenous breeds is present in particular in economically developed countries, including Croatia. Indigenous breeds by the definition of animal welfare - *the ideal level of welfare state is the total physical and mental health of the animal in harmony with its environment*; should have the best ability to adapt to the environment in which they have evolved. In an attempt to prevent the loss of these breeds, Croatia launched a planned breeding programme of indigenous breeds of horses for meat. Because the primary purpose of these horses has been work, the “new” way of farming brings with it new problems in attitudes and care towards animals. Therefore, it becomes a necessity to ensure the breeding requirements with regards to animal welfare as well to their standardization. Such an approach will primarily protect the animals, but will also give consumers confidence that horse meat was produced in accordance with the animal welfare principles.

**Key words:** indigenous breeds, horses, animal welfare, standards, consumers

## P6 EFFECT OF PHYSICAL ACTIVITY OF LAYING HENS ON EGG QUALITY

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**ABSTRACT**

**Introduction**

There is a growing interest in optimizing egg quality characteristics. Especially, enriching eggs with ingredients like omega fatty acids has become a broad field of research. Besides nutrition further factors may enhance deposition of n-3 fatty acids in egg yolks. STEINHILBER (2003) found differences in deposition of n-3 fatty acids to egg yolk for layer breeds and also for husbandry system. Higher n-3 fatty acids content in eggs from organic and free-range farms may be due to feed intake from pasture, higher activity level and/or contact to natural wavelength spectrum. In this aspect, moderate aerobic exercise appears to be of first importance (TURCOTTE, 199). The metabolic consequences of a long term training protocol include the changes in substrate metabolism (GEOR at al., 2002). The aim of the present experiment was, therefore, to test the impact of locomotion activity of hens of deposition of n-3 fatty acids to egg yolks. The effect of exercise on deposition of n-3 fatty acids and other egg quality criteria was investigated.

**Materials and Methods**

*Birds and housing*

In total, 36 young brown non-beak-trimmed laying hens (Bowans brown) were used in the experiment.

*Treatments*

Half of the hens (18 birds) were exposed to exercise by walking on a running treadmill (EG) through the four wks lasting experiment, whereas, the remaining 18 hens in the control group (CG) were permanently kept in the individual cages with very limited walking space. For details of the procedure and results see JAHJA et al. (2010).

**Results**

No significant effect of dietary fat source and running treatment was observed on laying hens performance. Exercise of the birds did not result in differences for either egg weight, yolk proportion or fatty acids profiles indicating a dominating effect of dietary fat source. This was also visible for the interaction.

**Conclusions**

Exercise on the treadmill caused reduced feed intake and egg production, but did not affect either egg weight or yolk proportion. Further research is needed to investigate in more detail the potential of physical activity on the deposition of long chain PUFA to egg yolk.

**Key words:** laying hens, physical activity, fatty acids, yolk

**P7**

# THE EFFECT OF ACUTE HEAT STRESS AND PERIOD OF RECOVERY OVER THE GLYCOGEN METABOLISM IN RATS

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**ABSTRACT**

**Introduction**

In a condition of global climate changes, acute heat stress is part of everyday's living of the animals. We aimed to estimate the effect of acute heat stress (AHS) over carbohydrate related enzymes and substrates in white laboratory rats.

**Material and Methods**

We investigated changes in concentration of some substrates (glycogen and glucose), as well as activity of key hepatic gluconeogenic (glucose-6-phospahtase and fructose-1,6-bisphosphatase) and glycolitic enzymes (glycogen phosphorylase, hexokinase, phosphofructokinase) in intact animals exposed to AHS (41±0,5°C / 1h), with 1h and 24h recovery at ambient temperature (20±2°C). AHS was carried out in a special heat chamber, with regulated air temperature.

**Results**

AHS rose rectal temperature for about 3°C, without any death of the animals. A significant decrease in blood glucose level and liver glucose concentration could be seen after exposure to AHS in both 1h- and 24h-recovery groups. Liver glycogen content and G6P concentration were significantly and dramatically reduced. Nevertheless, the 24h-recovery caused rebound of the glycogen content and additionally spending of G6P concentration compared to 1h-recovery group. AHS has reduced the activity of G6P-ase and F1,6BP-ase, regardless of the period of recovery, but the changes were more evident in the groups with 24h-recovery than in the 1h-recovery groups. There was non-significant increase of the activity of GP*a*, but significant reduction of the HK and PFK activity, regardless of the period of recovery. It is important to stress that 24h-recovery resulted with increment of the enzyme activities compared to 1h-recovery.

**Conclusion**

Acute heat stress with 1h-recovery resulted with intensive glycogenolysis, directed to endogenous glucose production and its further utilization by peripheral tissues. On the other side, 24h recovery imply for slight tendency of normalization of metabolic disturbances caused by heat stress, but still, this is very short time for complete recovery of the metabolic disturbances.

**Key words:** acute heat stress, carbohydrate metabolism, liver, rats

**P8**

# THE INFLUENCE OF ACUTE HEAT STRESS ON HEPATIC OXIDATIVE STATUS AND SERUM LIPIDE PROFILE IN RATS

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**ABSTRACT**

**Introduction**

Exposure to acute heat stress (AHS) of the homeothermic organisms induces significant changes in the oxidative status, as well as lipid profile of the organism. The influence of AHS, depending of different period of recovery et ambient temperature on the activity of key antioxidative enzymes and substrates as well as serum lipid profile in Wistar rats has been studies.

**Material and Methods**

We estimated concentration of hepatic NAD, glutathione, nitric oxide (NO), activity of catalase (CAT) and glutathione peroxide (GPx), as well as serum cholesterol (Chol), triglycerides (TG), HDL and LDL concentration. The animals were exposed to heat in special hot chamber with a regulated temperature of 41±1ºC. All of the parameters were analyzed in function of recovery period of 1h and 24h at room temperature after the AHS, compared to control animals, kept on ambient temperature (20±2ºC).

**Results**

Results showed that in the period of 1hour recovery after the AHS, there was significant increase of the concentration of hepatic glutathione, NO, CAT activity and serum HDL, and on the other hand, reduction of the GPx activity, NAD concentration and serum Chol, LDL and TG. While prolonged recovery (24h after AHS), there was an attempt to normalization the concentration of the estimated parameters, but still significant different from the control animals.

**Conclusion**

Acute heat stress presents one kind of oxidative stress, manifested with significant changes in the key hepatic antioxidative enzymes and substrates Lipide profile disturbances presents another indicator for oxidative changes in the serum. The changes showed significant variation depending on the recovery period after the heat stress.

**Key words:** acute heat stress, oxidative status, lipid profile, liver, serum, rats

**P9**

# HEAT STRESS INDUCED CHANGES IN KEY MOLECULAR MEDIATORS OF CELLULAR STRESS RESPONSE IN RAT'S HEART

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**ABSTRACT**

**Introduction**

Acute heat stress (AHS) as intensive physiological stress induces significant metabolic changes in homeothermic organisms. We aimed to estimate the dynamic molecular and enzyme changes in the rat's heart due to AHS exposure.

**Material and Methods**

The main goals of this work are focused on examining the effects of heat exposure through monitoring the key molecular mediators of cellular stress response (NO, $NAD^+$ and heat shock proteins -HSP72 mRNA) in the heart, as well as the metabolic alterations examined by the specific serum enzyme profile (AST, ALT, ALP and CK) in laboratory Wistar rats. Heat stress treatment (41±1°C/1h) was conducted in special heat chambers, followed by 1h and 24h recovery period at ambient temperature (20±2°C).

**Results**

AHS causes general catabolic effect, followed by an increase of NO and reduction of $NAD^+$, as well as significantly induced expression of HSP72 mRNA in the myocardium 1h after heat stress. Additionally, changes in the activity of serum enzymes markers (increased AST and CK activity) indicate tissue damage caused by the heat stress exposition. The 24h recovery period leads up to $NAD^+$ content increase and reduction of NO content along with higher levels of HSP72 mRNA in myocardium and balanced AST, ALT, ALP and CK activity in the serum.

**Conclusion**

AHS causes significant oxidative damage and impaired redox potential in the heart along with tissue specific metabolic changes detected at 1h-recovery period after applied heat treatment. Reduced oxidative damage intensity and improved myocardial redox potential, combined with normalized serum enzyme profile, indicate that the 24h recovery period leads up to stabilization of the negative oxidative stress effects on the myocardium caused by AHS. We assume that this normalization level is due to significant HSP72 protein induction and their essential stabilizing functions, especially in cellular stress conditions.

**Key words:** acute heat stress, heat shock proteins, serum enzyme profile, heart, rats

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