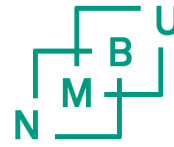




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Book of abstracts



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Nordic ISAE 2024

Session keynote speaker

Tuesday 6th February 0900 – 0945

Chair: Inger Lise Andersen

Key note lecture, abstract

Let me out! Exploring the motivations for prosocial behavior in pigs

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Farm animals are highly social species who can discriminate among individuals, are sensitive to each other's emotions and rely on other group members for safety and support. Farm animals are therefore highly relevant, but largely over-looked model species for studying the processes mediating prosocial behaviors that benefit others. Helping is of special interest for the study of prosociality because it appears to be motivated by an understanding of the needs of others. We developed a novel helping paradigm in which animals are tested in social groups in their home environment, where they can decide to open a door in a helping context, to release a conspecific trapped in a compartment, or in a non-helping context, to access an identical but empty compartment. We used this paradigm to investigate motivations for helping in $n = 75$ pigs (*Sus scrofa domestica*) tested in eight social groups. Pigs spontaneously learned to open doors without training and were more likely and quicker to open a door to free a trapped pig than to open a door to an empty compartment. Pigs who were more attentive to the trapped pig were more likely to help. Trapped pig who gave more distress signals were more likely and quicker to be released. Several results suggest that pigs may understand the needs of others, but other results are consistent with simpler explanations for helping based on selfish motivations. I discuss these different interpretations of the results and present our next steps to determine whether helping persists when we vary the potential benefits or costs of helping. I also discuss the potential applications of research on prosocial behavior for promoting the spread of positive emotions in farm animals.

Nordic ISAE 2024

Session 1 Pigs

Tuesday 7th February 0945 – 1215

Knut E. Bøe

The New Year's resolution of the finisher pig: physical activity, health and behaviour.

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New Year's resolutions often imply adding something new, such as a hobby or habit, or removing something old in order to improve our physical health and mental well-being. Pigs do not have New Year's resolutions of course, but the positive impact of being able to control their environment and pursue activities that increase well-being while avoiding negative experiences is maybe a common denominator for our 2024 resolutions and the motivations of the pigs. I chose the metaphor of New Year's resolutions in order to highlight how pigs in traditional production systems in some sense have little control over their environment. When pigs were moved out of their natural habitat in order to be protected from pathogens we tried to add indoors what we took them away from outside: we added controlled feeder systems, water and a dry resting space, and more recently the importance of rooting material has been acknowledged and attempts made to allow pigs to perform the highly motivated behaviour of rooting. However, there is one feature of the natural environment that we have not been able to add indoors, and that has not received much focus, namely that of physical activity. In the wild, pigs are active with rooting, digging and walking many hours a day, and can cover long distances (Stolba and Wood-Gush 1989; Podogorski et al., 2013). This is not possible in a traditional small pen. In my presentation I will give a brief summary of the link between the immune system, neurophysiology and behaviour, before I use examples from human public health and from controlled sports medicine studies to argue that the possibility to be active is a very important prerequisite for good animal welfare.

Fentanyl increases locomotion, stiff gait, and repetitive behaviours in pigs

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Treatment of pain is central in maintaining good animal welfare in a clinical and a lab animal setting. Pigs are frequently used in surgical research and are also in need of analgesics for procedures carried out in the field. Fentanyl is a potent opioid analgesic, although has the potential to induce side effects that can potentially limit their use and make evaluation of their efficacy difficult. In humans fentanyl is known for its potential to cause muscle rigidity, and in anaesthetized pigs tremors have been coupled to fentanyl. In awake animals, increased locomotion has been described in several species. A direct effect of fentanyl on behaviour could interfere with pain relief assessment. Knowing how conscious pigs respond to fentanyl would aid in understanding what kind of behavioural alterations can be expected from fentanyl alone. Uncovering the mechanism behind locomotor side effects would enable us to decipher their relation to analgesia. We therefore aimed at investigating behavioural effects of fentanyl, and the effect of a serotonin antagonist ketanserin, in pigs.

Fourteen mixed breed pigs, weighing 17-25 kg were included in a randomised blinded prospective, balanced two-group study. Ten pigs received first 5 and then 10 $\mu\text{g kg}^{-1}$ of fentanyl intravenously. Later ketanserin 1 mg kg^{-1} or saline was given intravenously as a third injection. While four control pigs received three injections of saline. Behaviour was video recorded. Distance moved was automatically measured, and behaviours manually scored from video recordings. Distance moved after fentanyl or saline injections was compared in a linear mixed model. Median and range for frequency and duration of distinct behavioural patterns were calculated. Alpha was set at 0.05.

Fentanyl inhibited resting and playing and induced different repetitive behaviours. Pigs showed individual patterns in which behaviours they repeated (manipulating the water, jumping on the walls and rearing are some examples). The mean (SD) distance moved in the control group and fentanyl group was 21.3 (13.0) and 57.8 (20.8) metres respectively ($p < 0.05$ for pairwise comparison). A stiff gait pattern was seen after fentanyl injection for median (range) 254 (165-305) seconds per 10 minutes, which was reduced to 0 (0-4) seconds after ketanserin administration. Fentanyl had psychomotor effects on the pigs, and both increased their distance walked and also seemed to alter their motivation as reflected in reduced playing and resting and an increase in seemingly non-goal-directed behaviours. These direct motor and behavioural effects induced by fentanyl can potentially interfere with post operative pain evaluation in pigs. There is a possible involvement of serotonin in fentanyl-induced psychomotor effects in pigs.

Effects of litter amount and different types of rooting material on welfare of fattening pigs on commercial farms

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Information about how rooting and bedding material types and management routines around their provision (amount, frequency) influence the pigs has not been well documented. The aim of this field study (n=87 pig farms; n=648 pens; n=5769 pigs; about 8 pens per farm) was to investigate associations between these factors. We predicted that all of these factors would be associated with bite marks on pigs (≥ 1 red stripe, including healed wounds). Proportions of pigs with bite marks on the body, tail, and ears were analysed using a generalised linear mixed model with binomial distribution. Rooting material types (1 vs. >1 of: chopped or long straw, silage, hay, newspaper), rooting material distribution frequency (≤ 1 daily vs. >1 daily), and amount of bedding (sparse, low, or moderate amount of wood shavings, or high amount of straw bedding), as well as the interactions between them, were included as fixed effects in the model. Provision of newspaper resulted in a lower proportion of pigs with bite marks on the body ($P=0.050$), and provision of hay reduced the proportion of pigs with bite marks on the tail ($P=0.015$) and ears ($P=0.002$), compared to the other rooting material types. With increasing amount of bedding, the proportion of pigs with body ($P<0.001$), tail ($P=0.048$) and ear ($P=0.031$) bite marks was lower. A lower proportion of pigs had body bite marks when given more than one rooting material at a frequency of more than once daily compared with providing only one rooting material type at a frequency of up to once daily ($P=0.020$). Similarly, the proportion of pigs with body bite marks was lower when providing a combination of more than one rooting material type and a greater amount of bedding ($P<0.001$). Furthermore, the proportion of pigs with body bite marks was lower when pigs were given more bedding along with more frequent provision of rooting materials ($P<0.001$). Our results suggest that the pigs receiving more than one type of rooting material, distributed more than once daily, in addition to ample bedding material over the solid-floored resting area, had better welfare.

Rooting material to finishing pigs – which type improves welfare the most?

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Pigs are highly motivated to explore their environment and will spend much of their active time rooting and foraging. In this study, we aimed to investigate the effects of different rooting materials on behaviour and welfare of finishing pigs; fibrous pellets, peat, straw, hay, a weekly rotation of these materials, or controls with sawdust only. We followed 360 finishing pigs (TN70 x Duroc) for 12 weeks, kept in pens (12 m²) of 10 pigs per group, and provided with rooting material twice a day. Behaviour was registered from video recordings during the first 60 min after material provision, with video analysis for 10 s every 6 min (semi-instantaneous scan sampling and 1/0 sampling). Bite marks on tail, ears and body were registered in weeks 1, 2, 3 and 12.

Pigs provided with rooting material, except for fibrous pellets, showed more exploration ($P<0.001$), play behaviour ($P<0.001$), and tail wagging ($P=0.010$) than controls. The weekly material rotation resulted in the highest level of exploration throughout the experiment period. Rooting material also resulted in less tail biting ($P=0.002$) and manipulation of pen fittings ($P<0.001$) compared to controls. Pigs with straw and rotation of materials showed less ear biting ($P<0.001$) and had fewer observations with the tail tucked between the legs (straw only, $P<0.001$). However, aggression was not reduced ($P=0.036$). Positive social contact between the pigs was lower with rooting materials compared to controls ($P=0.002$). The proportion of pigs with bite marks on tail was lower with peat, straw, and hay, than controls ($P<0.001$). Rooting materials, except for fibrous pellets, resulted in a lower proportion of bite marks on body compared to controls ($P=0.018$), but no significant effect was found on ear bite marks.

Although rotation of materials led to most exploration throughout the finishing period, the higher level of bite marks emphasizes that all materials in use should be suitable and accessible for the pigs. Considering both behaviour and welfare, straw was the single material with best qualities as rooting material in this study.

Genetic parameters for novel behaviour traits based on feeder data.

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¹*Norsvin, Hamar*

Precision phenotyping of behavior in pigs is of key interest for improving animal welfare and production efficiency in pork production. Individual behavior measurements for genetic studies require continuous recording of many individuals, which is time consuming and labor intensive. On the other hand, feeder data from individual feeder recorders with radio frequency identification system (RFID) provides detailed information on feed intake, meal frequency, time per meal and body weight per animal automatically. The aim of this study was to develop novel interaction traits indicating dominant behaviour, based on feeder data alone. An interrupted meal was defined as a short meal (<2min) before another animal is quickly replacing the first (<1min). The number of interruptions were counted for both the provider and the victim on a large dataset, consisting of close to 9 million visits from 2019 to 2023. A continuous model of hunger, based on feeding history was developed and used to classify different types of interruptions in “fight of resources” in the case of both animals were hungry or “dominant behaviour” in the case where the interruptor was not hungry. This led to definition of four interaction behaviour traits: “defeated”, “conqueror”, which are the victim and provider of a fight of resources, and “submissive” and “dominant” in the case of dominant behaviour. The number of such classifications were counted per day over a period of 60 days for both Duroc and Landrace and analysed in various genetic evaluations, using ASReml. These analyses were conducted using a pedigree that contained all animals with data (4,498 Duroc and 4,440 Landrace) and five generations of ancestors. The results show medium to high heritabilities (0.14-0.37) for interaction behaviour traits in pigs.

The Danish emergency response when an animal transport vehicle overturning

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The transportation of live animals within and between EU member states is a significant practice. Denmark alone exported an annual average of 76-89 million animals between 2017 and 2021. Globally, this trade is increasing, and the majority is transported by road in vehicles. These journeys are not without risks, as accidents on the road pose a threat to the welfare of the transported animals. We aim to shed light on the emergency response implemented in Denmark when accidents involving animal transport vehicles occur, emphasizing key elements essential for an efficient resolution. The research draws on two primary sources: 1) data from the Danish police documenting accidents involving pigs since 2017; and 2) interviews conducted with individuals present at the accident scenes or participants in a Danish working group dedicated to enhancing emergency responses within pig transport.

Annually in Denmark there have been 2-9 incidents of pig transport vehicles overturning predominantly concentrated in Central (43%) and Southern Denmark (36%). While it is evident that a vehicle overturning with animals onboard causes acute negative welfare, the subsequent course of action is crucial for potential for the animals to recover or to be euthanised promptly. Recognizing the unique nature of each accident, the development of a strict protocol is impractical. On-site decisions are typically made by task leaders from the police and firefighters, with the best possible outcomes for the animals achieved through collaboration with professionals possessing animal expertise and an understanding of vehicle design.

A significant challenge arises in accessing the animals, a task often consuming the most time and necessitating considerations for the safety of the involved personnel. The presentation underscores key aspects of communication and trained personnel, addressing questions such as: 1) Who receives specific information and when? and 2) What professionals are required on the scene, and what competences should they possess? While accidents involving animal transport vehicles are rare, they present a unique challenge to firefighters, veterinarians, and livestock drivers, who may not encounter such emergencies frequently. As such, societal prioritization is essential in determining the frequency of training exercises for these professionals, ensuring preparedness for rare but critical scenarios. In conclusion, Denmark has a functional emergency response system for pig transport accidents, significantly strengthened by voluntary contributions from the agricultural industry, yet still with room for improvements. Similar efforts could enhance the welfare of other species regularly transported nationally as well as across EU borders.

Immediate behavioural response in pigs at gas stunning with foam

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There are several stunning methods used for the commercial slaughter of pigs. The two most common are electric stunning and stunning with carbon dioxide gas. It has been shown that there are several problems with the stunning methods used today. This master's thesis was conducted as a part of a broader study aiming to evaluate the use of a new technique for gas stunning with high-expansion foam. This study aimed to investigate the differences in pigs' immediate reaction to high-expansion foam when stunned with nitrogen, argon or carbon dioxide.

The study included a total of 36 pigs, divided into three groups (n=12 per group). The three groups were exposed to three different gas stunning treatments; nitrogen, argon, or carbon dioxide. An ethogram consisting of 12 behaviours was conducted for video-based recording of behaviours. The observations started at the initiation of the foam in the stunning box and continued until the foam filled the entire stunning box.

The result show that the pigs expressed higher frequencies of exploration behaviours when exposed to nitrogen and argon while they expressed higher frequencies of escape attempts when exposed to carbon dioxide. These findings suggest potential advantages of using nitrogen or argon for stunning pigs from an animal welfare point of view. Understanding the behavioural response can contribute to refining stunning techniques, emphasizing the importance of considering the wellbeing of animals during stunning in development and implementation of slaughter practices.

Nordic ISAE 2024

Session 2 chicken and dogs

Tuesday 7th February 0945 – 1215

Chair: Cecilie M. Mejdell

Sex differences in play ontogeny in young chickens

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Play often indicates positive affective states and therefore has the potential of acting as an indicator of positive welfare. It is known that young chickens display different play behaviours, however, to the best of our knowledge, potential sex differences have not been investigated. Therefore, the aim of the present study was to investigate potential effects of sex on play ontogeny in young chickens, by comparing its occurrence in males and females of a commercial hybrid of White Leghorn (WL). Eighteen chicks from each sex were hatched in the same incubator and then housed in sex-separated groups of 6 each. Six groups of three chicks were randomly created for each sex, and the same three chicks were then moved to enriched play arenas twice per week, from day 6 until day 53 post hatch. The frequency of 14 different play behaviours, categorized as locomotor play, social play and object play were recorded during 30 min. From our previous studies on play ontogeny in young chickens, using the same method, we know that play peaks between 25-40 days of age. Furthermore, we know that play is affected by early stress, domestication and tameness (unpublished data). In these studies, every group of four birds constituted the independent statistical replicates. However, sex-mixed groups were studied, whereby we have no knowledge of potential sex differences. By the time of the conference, these results will be available and presented.

Enhancing laying hen welfare: shaping coping ability through choice in the rearing environment

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During laying hen rearing and production, exposure to some degree of environmental challenge is almost inevitable. Thus, it is desirable to rear hens in a way that enhances their coping abilities. Given the connection between the concepts of choice and control, we hypothesized that hens given more choices of litter and perch types ("Multi-choice") during rearing would exhibit improved coping abilities in response to environmental challenges, compared to those in standard conditions with one perch and litter type ("Single-choice"). We randomly assigned 16 pens (each containing 22-23 chicks) to one of these two environmental conditions. After 4 weeks, half of the pens were switched to the opposite condition, resulting in four treatments. In week 16, all groups were relocated to 16 standard laying pens. Coping abilities were assessed through tonic immobility (TI) tests during rearing (weeks 3, 6 and 15) and a repeated startle test (repeated umbrella openings in the pen doorway over 10 minutes) at the end of the experiment (week 27), as well as physiological assessments of heterophil:lymphocyte (H/L) ratios, lytic capacity and natural antibodies (nAb) against sheep red blood cells (SRBC) in weeks 6, 14, 17 and 27. The results suggested that the hens experiencing a reduction in resource choice during rearing (Multi to Single) responded differently than hens in the other treatment combinations. Hens from the Multi to Single treatment remained closer to the umbrella and foraged more during the repeated startle test in week 27 ($P \leq 0.001$) and showed a higher nAb titre against SRBC ($P \leq 0.05$) during week 14, compared to hens from the other treatments, suggesting improved behavioural and physiological coping abilities. The same hens also displayed a higher H/L-ratio during week 14 compared to hens from the non-switching rearing treatments ($P \leq 0.05$), and in week 26 compared to the birds reared continuously with "Single-choice". While the H/L findings may indicate sustained stress following loss of choice, the H/L ratios were generally low suggesting that a detrimental chronic stress state was not reached. No treatment effects were observed on lytic capacity, tonic immobility measures or initial flight response in the repeated startle test. In conclusion, providing choice in the early rearing environment may enhance functional development, improving hens' ability to cope with subsequent environmental challenges.

Naturalistic environmental enrichment as a potential buffering mechanism for early stress in commercial hatchery hatched chicks

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Laying hen chicks hatched in commercial hatcheries are exposed to a highly industrialized process that elicits a stress response that has both short- and long-term effects on behavior, physiology, and affective states. This exposure to early stress could potentially lead to future welfare issues. Providing a more complex environment has the potential to improve coping abilities and positive affective states. Rearing environments in the industry are in stark contrast to the natural environment of fowl as they are often static, homogenous, and therefore offer little to no opportunity for choice or a wide array of behaviors.

This study aims to assess the effects of providing naturalistic environmental enrichment to commercial hatchery hatched chicks and its potential to buffer early stress.

A total of 58 chicks were hatched in a commercial hatchery. Twenty-nine chicks were allocated to control pens (CC) with wood shavings, perches, and *ad libitum* feed and water. The other 29 chicks were assigned to enriched pens (EC) which were additionally supplemented with UVA/UVB light, peat, fake grass, fake plants, and daily scatterings of muesli and mealworms. Chicks underwent their respective treatments for 4.5 weeks after which we conducted a cognitive judgment bias test (CJB). Weight data was analyzed using a non-parametric Mann-Whitney U-test. CJB results were analyzed with a generalized linear model with treatment (EC/CC) as a factor, using the normal distribution and the link function 'identity'.

Enriched chicks (EC) were significantly heavier than control chicks (CC) at 8 ($U = 648.5$, $p = 0.003$) and 15 ($U = 606.0$, $p = 0.01$) days of age. However, no differences in weight between the treatment groups were found on any of the other weighing instances during the experiment. In the CJB there was a significant effect of stimulus in latency to approach ($W\chi^2 = 202.602$, $df = 3$, $p < 0.001$), where the chicks were fastest to approach the mirror and slowest to approach the picture of the owl, while the ambiguous stimuli were in-between. There was also a significant effect of treatment on latency to approach the stimuli where EC were faster compared to CC ($W\chi^2 = 4.557$, $df = 1$, $p = 0.033$), indicating a more negative bias in CC who did not receive naturalistic environmental enrichment.

In conclusion, providing more naturalistic elements to the rearing environment of commercially hatched chicks has the potential to improve positive affective states and buffer stressful experiences incurred at commercial settings.

The effects of early play stimulation on cognitive capacities of White Leghorn chicks

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Play is a widespread phenomenon in the animal kingdom with evidence for play from all vertebrate classes. The adaptive value of this behavior, however, remains unclear. In the past century, numerous theories have been put forward, ranging from releasing surplus energy to training species-specific behaviors. However, none of these theories can fully explain the functions of play. A more recent theory suggests that play allows the brain to encounter many different and surprising situations that provide the brain with opportunities to learn about the environment and form predictions about it.

This theory has, however, to our knowledge, not been tested previously. To start exploring the connection between play and cognition, we compared the cognitive capacities of White Leghorn chicks that were stimulated to play in the first five weeks of their lives with chicks that did not receive a play treatment. More specifically, we wanted to test the connection between different types of play and cognitive domains. To achieve this, we designed two play treatments: object players that were provided with a variety of toys during their treatment, and social players that were released into an arena with plenty of space, as this has previously been shown to trigger social play. Subsequently, all three treatments (control, object players, social players) were tested in two experiments of the social domain—a dominance and a social information transfer test—and two of the physical domain—a spatial memory and a motor self-inhibition test.

We found no improvement of cognitive capacities in either play treatment compared to the control chicks in the physical domain. However, object players appeared to be more attentive to social information and social players were dominant over the other two treatments in a competitive situation. Additionally, social players appeared to be more explorative and less neophobic during these tests.

Taken together, these findings indicate that chicks that were allowed to play during their juvenile period, and specifically playing socially, were more explorative, less neophobic, and appear to be dominant over conspecifics. This might allow them to exploit their environment more efficiently, which could in turn affect their welfare as they might be more resilient to stress and have more access to resources. More studies will be needed to assess the long-term effects of play on cognitive capacities in chickens.

Behavior and animal welfare in dogs involved in animal-assisted interventions in Swedish schools

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The research aims to address the importance of dogs' well-being and their interactions in educational environments, which is a relatively unexplored area. The work investigates how school dogs function and interact with pupils, especially those experiencing problematic school attendance and explores signs of both positive and negative welfare in these dogs. The method includes observations of video recordings of the dogs' behavior during interactions with students, as well as data from protocols on their well-being. The recordings were made in the beginning, in the middle, and at the end of each period of sessions. The video recordings of each session were also divided into three parts and those are analyzed separately. The study involves a behavioral analysis, providing an understanding of the dogs' experiences and reactions in the school environment. Descriptive statistics have been made and some analyses with chi-square tests.

The results indicate that school dogs predominantly showed signs of positive welfare, with common behaviors including seeking contact with students and wagging their tails. However, there were variations in behaviors among different dogs, emphasizing the importance of considering individual differences. The study also observed less desirable behaviors, such as barking or walking away, but these occurred to a lesser extent. For some of the behaviors, there was a difference between the different sessions or parts of the sessions. For example, the behavior “perform a command from student” was significantly more common during the last third of the session ($p < 0.005$). A behavior that was different between the third session was “wagging tail”, which occurred less during the last sessions ($p < 0.05$). Furthermore, the behavior “physical contact with the pupil” was most common in the first part of the session ($p < 0.005$).

This research contributes to the understanding of dogs' welfare in educational settings and emphasizes the need for continued attention to the well-being of dogs. It also highlights the importance of individualized care and training for school dogs to ensure a positive experience for both dogs and students.

Dogs' effect on healthy test-persons' immediate physiological responses during pain trials.

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Studies of how dogs influence physiological responses in humans in challenging situations have become increasingly popular, but results are often contradictory. The studies vary regarding methodology and often the contact with the dog is not defined or considered. Thus, more basic research on how interaction with dogs may influence different human physiological responses during challenging situations is needed. The present study investigated how the presence of a dog affected immediate physiological responses in healthy humans during pain infliction.

Sixty healthy participants (14 men, 46 women, age: 18-66 years) were enrolled in the study, but two participants were excluded after enrolment. They were divided into two groups; “with dog” (WD) and “no dog” (ND) and exposed to two thermal heat pain trials each with either support (S) or no support (C), randomised and counterbalanced. The WD-S included a dog within reach and a person initiating conversation, and ND-S a person initiating conversation. Both WD-C and ND-C included neither dog nor person. During all pain trials, a researcher responsible for inflicting pain was present. A standard poodle and three golden retrievers unfamiliar to the participants were included in the study. After individual pain calibration (60-70 mm on a 0-100 mm visual analogue scale, no pain - worst imaginable pain), the participants were exposed to two 5-minute pain trials with a 20-minute break between. Heart rate and heart rate variability were recorded continuously. Blood pressure (BP) and salivary cortisol were sampled before and after each test-situation, and baseline measures were sampled. The physiological data were analysed using linear mixed effects models including behavioural observations.

The analysis is ongoing and will be presented in its entirety at the Nordic ISAE conference. Preliminary results reveal from highest to lowest heart rate to be; WD-S (83±14 bpm), WD-C (75±13 bpm), ND-S (69±8 bpm) and ND-C (67±10). For heart rate variability; ND-C (58±20 ms), ND-S (55±24 ms), WD-C (48±24 ms) and WD-S (44±14 ms). For post-test systolic BP; WD-S (121±16 mmHg), ND-S (119±14 mmHg), WD-C (118±15 mmHg) and ND-C (117±11 mmHg). For post-test diastolic BP; ND-S (78±7 mmHg), WD-S (77±13 mmHg), ND-C (77±7 mmHg) and WD-C (77±9 mmHg). For saliva cortisol; WD-C (906±829 pg/mL), WD-S (893±623 pg/mL), ND-S (874±513 pg/mL) and ND-C (843±579 pg/mL). The preliminary findings suggests that when a person is in pain, the presence of a dog and support person may result in higher arousal and more stressed nervous system compared to sitting alone.

Social Preference and Coping in Hens: Domestication Effects in Red Junglefowl and Chickens

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Studies of domestication have unveiled transformative traits in animals, driven by selection pressures influenced by human needs. The concept of 'domestication syndrome,' includes changes in appearance, physiology, and behavior, emphasizing the impact of human-driven evolution. The key to successful domestication is tameness, which is defined as inherited low fear of humans. This manifests as decreased aggression, reduced fearfulness, heightened sociability, and increased playfulness. In the context of chicken domestication, social structures undergo significant transformation compared to their wild counterparts. Red Junglefowl exhibit a hierarchical social structure with more rigid social groups, centered on temporary harems and territorial behavior. Domestic chickens, however, often lack a natural stability, and is characterized by flexible dynamics from human needs. Animals displaying reduced emotional sensitivity to environmental changes exhibit higher tameness, reinforcing the adaptability required for human interaction. The reduced responsiveness to fear-inducing stimuli serves as an adaptation to living in environments with frequent invasions of personal space. The hypotheses of this experiment was that domestic birds would show better social coping skills in a novel social setting, with less agonistic behaviour towards unknown birds, and that we will see less social preference in the less domestic lines of birds, due to the need to flock and feeling secure in a group. Our experimental setup involves a straightforward arena where test animals can eat, perch, and explore in proximity to both unknown and familiar pairs of birds. A 30-minute video recording captures data, including agonistic behaviors and social exploration. Analysis involves recording variables such as behavior frequency and duration, with a focus on understanding preferences. A linear mixed model, incorporating group (White Leghorn, Red junglefowl, High-fear of humans selected Red junglefowl, Low-fear of humans selected Red junglefowl) and sex as potential explanatory factors, is employed. This study contributes to the broader understanding of social preference and coping with social novelty in domesticated hens, shedding light on the intricate interplay between domestication syndrome traits, behavioral adaptations, and the evolving dynamics of social structures in response to human-driven selection pressures. It's important to note that the results are still under analysis, and a comprehensive conclusion is pending.

Nordic ISAE 2024

Session keynote speaker

Wednesday 7th February 0900 – 1000

Chair: Judit Vas

Key note lecture

Farm animal vocalizations as a window to their emotions and welfare

E.F. Briefer

Behavioural Ecology Group, Section for Ecology and Evolution, Department of Biology, University of Copenhagen, Denmark

Developing accurate indicators of animal emotions is crucial to provide an accurate assessment their welfare. Vocalisations constitute promising indicators of affective states. Indeed, they can be recorded non-invasively and have been shown to reflect both the valence (positive versus negative) and the arousal (bodily activation) of the emotion experienced by vocalising animals across species. I will describe the results of a large study, where we investigated similarities in the vocal expression of emotions in several species of farm animals (horses, pigs, goats, cattle and sheep), as well as the perception of emotions within and between species, including human perception of ungulate emotions. More recently, we have also incorporated "machine learning algorithms" to investigate the potential of these techniques for automatic classification of emotions within and across species. These results reveal the potential of vocalisations to be used as non-invasive indicators of welfare. In addition, they present interesting insights into the evolution of vocal expression of emotions and the factors that may influence cross-species perception of emotions.

The actual and potential use of animal welfare indicators in Norwegian farming practice

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There is a growing need for good welfare indicators to assess farm animal welfare. Assessment methods have shifted from an initial focus on measuring resource inputs to measuring outcomes associated with the welfare state of the animal. MatPrat, Norwegian Egg and Meat Council, has supported the creation of a database that aggregates existing indicators used to assess welfare of the main, land-based food producing animals. The aim was to develop a tool that aggregates existing indicators to assess welfare, to document these in a systematic way and to provide insights that can improve animal welfare in Norway and globally. The structure applied to the collated data was based on the four Principles for assessing welfare (Good Feeding, Good Housing, Good Health and Appropriate Behaviour) and the derived twelve Criteria (EU Welfare Quality project). Main sources of information were reports from the European Food Safety Authority (EFSA), describing welfare aspects for farmed animals and housing systems (published from 2007 to 2012, and the 2022 and 2023 updated reports), supplemented with information from other scientific sources. Each indexed welfare indicator was scored on several dimensions, e.g., type of indicator (either a welfare outcome or a resource/management input measure, and for outcomes, whether they were behavioural or health/physical/production-related measures). Indicators were also assessed on their use in Norwegian farming practices. National experts used local legislation, current industry-led animal welfare assessment programmes -especially for cattle, pigs and poultry-, scientific literature, and consultation with Norwegian researchers and practitioners. The database currently contains a total of 381 unique welfare indicators across a variety of farm animals (dairy cows: 54; dairy calves: 49; pigs (all types): 95; beef cattle: 47; broiler chickens: 55; laying hens: 41; sheep: 40). Of the total list of indicators, 88.9% were classed as welfare outcome measures and 53.5% as behavioural indicators. The assessment on use in Norway found that 68.7% of indicators were in use (averaged across species), meaning that for some species around 50% of potential indicators are not currently used in farming practice to measure animal welfare (but may be used in research). The database contains a wealth of additional information related to assessing animal welfare, that can be a valuable tool for use by various stakeholders to assess welfare. The use of animal welfare indicators in Norway appears to be substantial, but there is scope to apply more indicators to better quantify and thereby improve farm animal welfare.

Nordic ISAE 2024

Session 3 Cattle

Wednesday 7th February 1030 – 1200

Chair: Margit Bak Jensen

Impact of dairy cow personality traits and concentrate allowance on the adaptability, behaviour, and production of dairy cows trained to use a free-traffic automated milking system

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The objectives of this study were to determine: 1) if dairy cow personality traits and concentrate allowance affect how well dairy cows adapt to an automated milking system (AMS) following training; and 2) if these factors were associated with the behaviour and performance of cows after AMS training.

Twenty-nine Holstein cows (218 ± 49 DIM) of mixed parity who were naïve to an AMS were enrolled in this study and assigned to 1 of 2 dietary treatments; a basal partial mixed ration (PMR) common to both treatment groups, with a concentrate allowance in the AMS of: 1) 2.0 kg/d (L-Tx); or 2) 6.0 kg/d (H-Tx). To ensure a distribution of cow personality types within each treatment, cows were initially classified as dominant or subordinate (based on their success in feed bin displacement tests prior to the study), and then were allocated to each treatment within each classification. Cows were trained to use the free-traffic AMS over 72 h and were milked in this system for 63 d after training was complete. The cows were trained by being individually brought to the AMS entrance 3x daily for 72 h and encouraged to enter by physical pressure on the hindquarters. Between 42 and 63 d after AMS introduction, each cow was assessed for personality traits through observing responses to a novel environment, novel object, and novel human.

Principal components analysis of the personality assessment revealed two factors (interpreted as boldness and activeness) that together explained 85% of the variance. More active cows produced less milk ($P=0.02$) and active cows within the H-Tx had a 3.9 times greater risk of kicking off teat cups during AMS training ($P=0.05$). However, more active cows also had a 1.4 times lesser risk of teat cup kickoffs in the 8 wk after training ($P=0.04$). During the 8 wk after training, the H-Tx cows produced 5.1 kg more milk per day compared to the L-Tx ($P=0.02$) but had 2.0 kg/d lesser PMR intake ($P=0.05$) and were 2.9 times more likely to have premature AMS visits ($P=0.03$). The results indicate that more active cows may take longer to adapt to an AMS compared to less active cows. Greater amounts of concentrate in a free-traffic AMS may promote greater milk yield, regardless of personality, in mid- to late-lactation cows. Dairy cow personality traits are a factor that should be considered to optimize feeding strategies and transition to an AMS.

Effect of light environments on melatonin and IGF-1 in lactating Norwegian red cows

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Light programs are commonly used in dairy barns, but the effects of low light intensities during daytime, high light intensity during nighttime, and lack of shifts in lighting during the day-night cycle are not known. A lack of light cues to indicate the diurnal shift may have an unfavorable effect on the circadian rhythm of cows and thereby a negative impact on their welfare. Melatonin, also known as the dark hormone, is associated with circadian rhythms, and expressed at night under low level light intensities, while IGF-1 mediates the effect of light programs on milk yield. In this study, we aimed to investigate the effects of three different light programs on IGF-1 and melatonin in dairy cows during night and day.

We used 12 mid- to late lactating and Norwegian red cows, who were subjected to the following day/night light treatments for four consecutive days: 200/0, 50/50 and 50/0 lux respectively. Serum IGF-1 and melatonin were sampled from the tail vein once during daytime (12:00) and once during nighttime (00:00) of the last 24h of each treatment period. Cows were milked twice daily, and milk yield was registered at each milking. Data was analyzed in a linear mixed model with treatment and time of day as fixed effects, and the repeated effect of cow.

Preliminary results show that serum melatonin was higher in 50/0 lux compared to both 200/0 ($p < 0.0017$) and 50/50 lux ($p < 0.0079$). Independent of treatment, melatonin in nighttime samples was higher compared to daytime samples ($p < 0.0035$). Surprisingly, IGF-1 remained unaffected by the light programs. It could be speculated that our treatment periods were too short, or treatments too mild, to elicit any changes in serum IGF-1. As expected due to the short treatment period, there were no effects on milk yield.

Our results indicate that high light intensities during daytime reduce serum melatonin levels at night, even though a low light intensity was applied during the dark hours. The 50/50 lux treatment also showed reduced serum melatonin levels, which probably was due to the relatively high light intensity at nighttime. Given the negative effects on melatonin, it is likely that both the 200/0 and 50/50 light programs have an adverse effect on the circadian rhythm and welfare of cows. These findings are important to consider when planning light environments for dairy cows.

Characterization of calf position during suckling and allosuckling in a cow-calf contact system

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In this study, we investigated the positions used by calves during suckling and allosuckling in a cow-driven cow-calf contact system. We hypothesized that the positions used by calves would differ between suckling and allosuckling bouts as well as between start and end of the bout. 30 calves were observed during suckling and allosuckling bouts during seven 24-hour periods at 2-8 weeks of age, and position was registered at the start and end of 1071 suckling and 470 allosuckling bouts. Position was characterized according to a clock model, where 12 represented the head of the cow, and the direction of the calf body in relation to the cow body was registered as position 1-11. Position 1 and 11 represented the ‘inverse parallel’ position on either side of the cow body, 2-5 and 7-10 represented intermediary positions, while 6 represented the ‘between hind legs’ position and 3/9 a 90-degree angle between the cow and the calf’s body. For analysis, positions with the same angle relative to the cow body (1/11, 2/10, 3/9, 4/8 and 5/7) were grouped together in order to describe positions irrespective of from which side the suckling or allosuckling occurred.

As hypothesized suckling positions were different for suckling vs. allosuckling events. Suckling calves most often used position 1/11 or 2/10, together representing 90% of suckling bouts (59 and 31%, respectively). Allosuckling calves however, only used these positions in 5 and 23% of bouts, respectively, and used positions 3/9, 4/8 and 5/7 in a larger proportion of bouts (32, 18 and 15% of bouts respectively). Position 6 was used in 7% of allosuckling bouts, which was lower than expected based on previous findings, yet higher than for suckling (0% of bouts). Contrary to our predictions we found that the start and end positions were similar during both suckling and allosuckling.

Results presented clearly show that calf position differs between suckling and allosuckling bouts and presents a new model for scoring position which may be used to inform about bonding quality among dams and foster cows and their calves in cow-calf contact systems. This model allows for swift registration not only of position, but also of preferences regarding left/right sides of the cow.

Suckling behaviour of dairy calves experiencing gradually reduced cow contact

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De-coupling the loss of milk and the loss of maternal contact can mitigate the stress of cow-reared calves at weaning and separation. Fence-line separation is an approach that allows for some dam-calf contact, but abruptly prevents suckling and thus milk intake. However, under natural conditions, frequency and time spent suckling is gradually reduced by the cow and calves are not abruptly weaned off milk. This study investigated whether gradually reducing the daily time that calves have access to their dam reduced the time that calves spent suckling. Fifty-five dairy cows and their calves were enrolled and housed in straw-bedded pens in groups of 4 cow-calf pairs. For seven weeks, cow-calf pairs experienced either full-time contact (23 h/d, 28 pairs) or part-time contact (10 h/d, 27 pairs). From week 8 to 10, half of the cow-calf pairs experienced reduced contact (RC), first to 50% of original contact in week 8 and then to 25% of original contact in week 9 (12 full-time RC pairs, 13 part-time RC pairs). This was obtained by temporarily fence-line separating cow and calf within the straw-bedded pen. The remaining cow-calf pairs continued with unchanged daily contact (UC; 12 full-time UC pairs, 10 part-time UC pairs). Calves could only access milk through suckling. Time spent suckling the dam and other (alien) cows was recorded for 24 h in each of week 8 and 9 for each of the four treatments. The total time spent suckling was reduced for part-time RC calves compared to part-time UC calves (22.9 ± 3.6 vs. 36.5 ± 3.6 min, t -ratio=-2.9, $p=0.027$). For time spent suckling only the dam, there was an interaction between treatment and observation week; part-time UC calves spent more time suckling their dam in week 9 (37.3 ± 3.8 min) than part-time RC calves in week 8 (20.8 ± 3.8 min, t -ratio=-3.3, $p=0.029$) and full-time RC calves in week 9 (19.7 ± 3.5 min, t -ratio=-3.8, $p=0.0073$). In partial support of our hypothesis, gradually reducing daily cow contact time over two weeks reduced suckling time in 9 to 10-week-old calves, but only if the daily contact was already limited (i.e., part-time contact); no difference in suckling time was found between full-time RC and UC calves. Although not measured in this study, milk intake was likely also reduced with reduced suckling time, suggesting that daily time in contact with dam has to be markedly reduced to reduce milk intake and thus prepare calves for complete weaning and separation

Dairy cow personality: What do personality tests really tell us?

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Personality research has become integral to improving the welfare and management of dairy cows. While recent research has developed increased understanding of personality traits and their development, much still remains unclear regarding the methodology and interpretation of behavioural data. In the current study, 49 Holstein and Swedish Red dairy cows were tested twice in a Combined Arena (CA) test, which included a Novel Arena test, Novel Object test, Novel Human test, Human Approach test, and ended with a variation of a Social Runway test. One behavioural variable were selected from each test and analysed to determine the behavioural consistency of individual cows over test repeats, and how data between tests were related to one another. Of the variables analysed, the outcomes for cow behaviour in the two repetitions did not differ apart from number of lines crossed in the Novel Arena test, which decreased in the second repetition: (Repetition 1: 61.82 ± 3.66 , Repetition 2: 34.73 ± 3.93 , $t=6.57$, $df=48$, $p<0.001$). Some of the variables in the different test outcomes were seen to be highly related to each other. A higher number of lines crossed in the Novel Arena test increased the probability of a cow touching a novel object ($F_{1,95}=13.75$, $p<0.001$) and if a cow touched a novel object, there was a higher probability of a cow touching a novel human ($\chi^2=18.31$, $p<0.001$). The proximity in the Human Approach test and the latency to reunite in the Social Runway test did not show a significant relationship to any of the other outcome variables. This suggests that some behaviours may be more relevant when investigating and interpreting cow personality in a multi-test format such as the CA test, than others, as well as that different cow behaviour may reflect the same underlying trait. The consistency of individual cow behaviour suggest that such tests are relevant for the study and investigation of dairy cow personality. In conclusion, dairy cow personality tests allow us to understand individual cow behaviour and how it relates to their personality. This notwithstanding, these tests should be utilized strategically and examined critically in order to ensure that their interpretation is accurately reflecting the personality of the cow.

Identifying behaviour indicators as predictive cues for hazardous situations in cattle handling

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Farm work is one of the most hazardous occupations, and working with cattle leads to the most serious accidents. The injured persons are often crushed, run over, trampled, kicked and gored. Accidents most often occur when moving animals, or during handling in connection with milking, aversive procedures, and when treating sick animals. Many factors contribute to the accident occurring, including the animals' and humans' behaviour, previous experience, as well as the humans' knowledge about and attitude towards cattle. This study aimed to investigate the frequency of hazardous situations when moving cows for milking and hoof trimming.

Four Danish dairy farms with 120-250 cows were included. Thirty randomly selected cows on each farm were tested for fearfulness (Avoidance test). On each farm video, recordings were made of one hoof trimming event and of moving cows to milking morning and evening (for 14 days) From the recordings, the duration of humans being within one cow's length of one or more cows was measured, and the frequency of risky behaviour by both cows (e.g. kicking, pushing, goring, sudden change of direction) and humans (e.g. driving, hitting, pushing, kicking, tail twist, using body to stop the cow) during these instances was calculated.

During driving to milking and hoof trimming, humans were on average close to one or more cows 6-7% and 17% of the time, respectively. The frequency of cows' and humans' risky behaviour varied between farms, both morning and evening (all $p < 0.001$), as did the types of risky behaviour of both cows and persons. On the farm where the cows were least fearful, more cow-directed behaviour was observed both during morning and evening milking (both $p < 0.001$). The frequency of human behaviour directed at cows when driving cows to hoof trimming was similar to when driving cows to milking, but a higher proportion of rough handling were observed. During hoof trimming the cows' frequency of risky behaviour was 1.2 per min as opposed to 0.15 per min when being driven to milking.

The results suggest that it is possible to identify behavioural indicators of potentially dangerous situations. Future studies should aim to optimize handling of cows, especially when subjecting them to aversive procedures. This could be through education of humans and training of the animals.

App to assess and evaluate animal welfare on-farm using the Welfare Quality® protocols

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In 2021, the WelCow project set out to attain a status of animal welfare in Norwegian dairy herds using the Welfare Quality®(WQ®) protocol. From the initial stages of the planning process, it was clear that a system to electronically register data during the farm visits and then automatically calculate the WQ® scores would be invaluable. Each WQ® assessment takes between four and eight hours, depending on the size of the herd, with most of the time typically used for behavioural measures. Assessing welfare in this manner is time-consuming enough without spending additional time manually processing the data collected. At the time no such systems were available to us, so we aimed to develop our own, and thus the WelCowApp1.0 was created.

The Eik Lab (NMBU Centre for Student Driven Innovation) paired the WelCow project with talented, ambitious computer science students and together we developed an online application to our specifications. The app includes the full WQ® protocol for dairy cattle and has been used on-farm to register data in over 60 dairy herds and to perform the WQ® score calculations for more than 160 dairy herds. WQ® criteria and principle scores are automatically calculated, and a WQ® category assigned, immediately following data registration during the farm visit.

The collaboration was beneficial both for us, as animal welfare scientists, and the students, as budding developers. Our cooperation continues, and an improved WelCow App 2.0 is under development. The second version will include user-specific access to the database (a must for data security), improved visualisation of the assessment results (both WQ® scoring and intermediate data such as prevalences), and the addition of the WQ® protocol for welfare assessment in fattening cattle.

We will present the original WelCow App1.0 and the improved functionality expected with the WelCow App 2.0, with a particular focus on the registration of behavioural measures. We hope to make our app available to fellow animal welfare researchers certified by WQ® around the world, so that they and the animals under their care can avail of the benefits of easy registration and instantaneous feedback within the robust WQ® framework.

Is it all connected? Exploring relationships between happy cows, satisfied workers and good economy on dairy farms.

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The aim of this study was to test the hypothesis that there are positive associations between the welfare of dairy cattle on a farm, caretakers' views and farm economy. Data were gathered during visits to 31 dairy farms in Sweden. Farms were selected from an industry database, according to pre-determined welfare criteria, to potentially span the range from excellent to poor farms. We provide initial results exploring the relationship between two different assessments of dairy cow welfare (from the Welfare Quality (WQ) protocol and a newly developed positive welfare (POS) protocol), results from questions to the staff (on attitudes to animals and job satisfaction) and an analysis of the economic potential of the farm (based on estimates associated with farm costs and incomes). The POS protocol consisted of animal-based indicators selected from the literature as having most potential to be associated with positive welfare.

Composite indicators were built with the package COINr, specifically designed to develop aggregated sets of indicators to assess complex and multifaceted data. We explored relationships at different levels of aggregation. At the highest level of aggregation, we found no correlations between cow welfare assessed according to the WQ protocol, and the POS protocol, the stockperson's attitudes and job satisfaction, and farm economic potential. At the middle level of aggregation, we explored relationships between the four sub-areas (principles) in the WQ protocol, five sub-areas in the POS protocol, three sub-areas for the stockperson and 3 sub-areas related to economic potential. Using hierarchical clustering, five clusters of farms were identified, of which the two most clearly separated clusters (1 and 5) and their key attributes are presented below.

Farms in cluster 1 were characterised by an overall poor farm rank and low levels of the tail positions, ear positions and openness of the eyes indicators in the POS protocol, low scores for the allocation of resources and a poor score for the WQ housing principle. Farms in cluster 5 were characterised by an overall good farm rank and high levels of behavioural synchronization and frequent performance of positive behaviours from the POS protocol, high scores for allocation of resources on the farm, and a positive attitude towards animal welfare by the caretakers. Although complex, this analysis does support links between cattle welfare, stockperson attitude and economic potential on farms. Furthermore, it helps identify which animal-based indicators of positive welfare might be important to include in future assessment protocols.

Nordic ISAE 2024

Session 4 horses and salmonid fish

Wednesday 7th February 1300 – 1500

Chair: Øyvind Øverli

Short term alleviation of girthing in riding horses – effects of hay during saddling

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When saddling, horses can show a range of unwanted behaviors. These behaviors seem to be connected to some kind of discomfort or aversion to the saddle or the situation. They might also show aggressive behaviors directed towards the human performing the saddling. These behaviors are called girthing. There is no known definition of girthing, and no one really knows the underlying causes of the behaviors. There is no known solution to the possible problems and there is little knowledge about the prevalence and magnitude of the behaviors. There is a variety of theories for the reason for girthing. The horse owners are seemingly developing strategies to saddle up their horses in a safer way for themselves and/or more pleasant for the horse. The aim of this study was to find out if there might be a way to make the saddling less aversive for the horses. This is a short-term study, and if successful, this method might help with reducing the behavior in a long term. In other types of horse training food is shown to be successful. This is why it was chosen for this study. The behavior of ten horses were observed. They were saddled during two different conditions, with and without hay. They were also tested for mechanical nociceptive threshold on three different body parts. This was done using a pressure algometer.

The results show that the horses showed less aversive behaviors, less mouth related behaviors and less hoof movement when they were given hay. Therefore, we can assume that the hay is a potentially successful way of alleviating girthing in the short-term. The long-term effects do however need to be studied further.

Equine headshaking – a survey among Norwegian horse owners

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Headshaking in horses is a well-known syndrome, but with an unclear etiology. The aim of this survey was to collect more information from Norwegian horse owners who reported to have horses affected by idiopathic headshaking.

A German group of researchers had distributed an inventory of equine headshaking syndrome and asked researchers in other European countries to participate, using the same questionnaire. We translated the 22 questions and adjusted them to Norwegian conditions. Respondents with headshakers were recruited by informative articles in six Norwegian horse magazines and webpages containing a link to the survey.

A total of 333 horse owners responded. Among the reported affected horses, 49.5% were geldings, 48.6 % mares and 1.8 % stallions. The mean age of the affected horses were 10.6 years varying from one to 24 years. Most of the horse owners (46.2 %) stated that the horse was kept in single boxes inside but were kept outside in groups during daytime. 35.4 % stated that the horse was kept single both inside and outside and 17.4 % stated that the horse was kept in some kind of loose housing system together with other horses. 24.3 % stated that the affected horse was a warmblood riding horse, 22.2 % stated that the horse was a warmblood trotter, 20.4 % a cold blood trotter and the rest of the horses were sport ponies, Icelandic horses or different other breeds.

The result from the Norwegian survey supports the results from previous international studies, that headshaking is a symptom and probably doesn't reflect one common etiology, and thus there is no single therapeutic treatment. Since headshaking can be associated with pain, such as trigeminal nerve affection, the syndrome should be taken seriously. The stress of social isolation, competitions and frustration during difficult exercises might be contributing factors which are possible for the owner to change and address.

Ethological observation of aggressive social interactions reveals multiple targets for non-invasive monitoring of stress and welfare in farmed Atlantic Salmon (*Salmo salar*)

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Fish exhibit sophisticated cognitive abilities, have anticipation about the future, have perception of time, create mental charts to recognize their surroundings, and possess advanced social skills such as individual recognition, observational learning and collaboration. Except for the ability to feel pain, we know little about fish emotions. Until we learn more about emotional states in fish, we must assume that welfare considerations are just as important for farmed fish as any other production species. Key aspects of fish welfare, including stress response mechanisms, cognitive abilities, and the impact of environmental factors, are currently intensely scrutinized in applied aquaculture settings. In Norway and within the EU there is lack of consensus on how to define and assess fish welfare, and lack of standardization and applicable on-farm documentation systems. The challenge is to find operational welfare indicators based on behavior and appearance and verify these with neurobiological and physiological testing. Non-invasive operational indicators are urgently needed for proactive continuous assessment of fish welfare status. In the present study, we aim to study behavioral and morphological stress responses during agonistic interactions in juvenile Atlantic Salmon. A group of 32 juvenile Atlantic salmon was transferred from circular rearing tanks to eight aquariums (50x50x100cm). Four fish were placed in each of the aquariums and kept in isolation by removable walls. The fish was hand fed daily with one pellet at a time until 1% body weight or rejection of three consecutive pellets to register daily feed intake and latency to first feeding after transfer to a novel environment. After three weeks in isolation the walls between two and two fish were removed for 1.5 hours daily for five days to create pairwise agonistic interactions. On the last day the fish were kept in pairs overnight. Immediately after the last dyadic interaction, the fish were humanely euthanized, and blood, brain and skin samples were collected for physiological analysis of stress. The fish were photographed on the first and the last day of the experiment to analyze changes in skin pigmentation and the fish were videorecorded both during feeding and the agonistic interactions to study behavior. An ethogram was constructed to systematically analyze the behavior and included behaviors such as latency to display, latency to first physical interaction, duration of fight, frequencies of chase, bite, escapes and release of gas bubbles. The vertical positioning in the water column and bottom contact was also registered at 5 min intervals. Chronically stressed social subordinate individuals in the pairwise encounter showed a higher frequency of released gas bubbles compared to dominant fish, and this effect progressed over time in the experimental period. There is also a significant positive correlation between aggressive interactions and the release of gas bubbles. In the experimental aquariums juvenile fish rapidly gained black eumelanin spots on the otherwise silvery skin surfaces and preliminary data show that the process of gaining dark melanin spots was affected by stress and linked to plasma cortisol levels and feed intake. In summary, this study points to several novel indicators of stress and welfare status that are suitable for non-invasive visual monitoring.

Melanin-based skin pigmentation: A visual signal of stress in salmonid fishes?

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Feeding behavior and feed intake are considered well-established indicators of stress responses in animals. This study investigates the intricate relationship between feeding behavior and alterations in melanin-based skin spots in juvenile Atlantic salmon (*Salmo salar*) within a controlled experimental setting. Thirty-two juvenile salmon were relocated from their rearing environment to individualized experimental aquariums, where they underwent a 26-day period of daily hand feeding, with video recording employed to monitor feeding behavior and record daily feed intake. The primary aim was to evaluate the acclimatization of fish to a novel environment. Following a three-week acclimation period, the removable barriers segregating pairs of fish were daily lifted for 1.5 hours over a 5-day span, inducing socially stressful interactions. Photographic documentation of the fish occurred at both the start and end of the experiment, to analyze changes in melanin-based skin spots. Significant variability was observed in the latency to the initiation of feeding (ranging from 3 to 26 days) and overall feeding behavior, encompassing the eagerness to capture sinking pellets. Average daily feed intake increased consistently from the initiation of feeding until the onset of agonistic interactions. Notably, subordinate fish demonstrated a distinct reduction in feed intake consequent to the initiation of stress testing compared to dominant and control fish. Moreover, the total feed intake as a percentage of body mass and percentage growth exhibited a positive correlation with changes in the number of melanin skin spots on the body, further elucidating melanin-based skin spots as potential visual indicators of recent stressful experiences in salmonid fishes. In practical aquaculture settings with tremendous populations sizes, continuous monitoring of behavior of individual fish is a formidable task. However, individual recognition based on melanin spots is under development to aid the monitoring of single individuals in large scale industrial settings. This study underscores the need for a comprehensive ethological approach to ascertain to what degree stress-induced alterations in skin pigmentation is an element of intra-specific social signaling in fish.

Interaction of the physical and social environment shape the neural substrate for individual welfare states

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The serotonergic system in the brain plays a major role in modulating behavioural and neuroendocrinal stress responses in vertebrates. Accordingly, differences in serotonergic signalling are associated with individual differences in stress reactions. Such individual differences are often described as stress coping styles. In fish, there are studies showing that the rearing environment (i.e. environmental enrichment) affects social behaviour and stress coping. In the present study we investigated the effect of rearing temperature, another highly relevant environmental factor, on the serotonergic system and how this affects behaviour in farmed salmon. This was done by keeping 63 post-smolt Atlantic salmon in nine groups (7 fish in each) at control low (12 °C), medium (15 °C), and high (18 °C) water temperatures. Agonistic social interactions (chases and bites, actor and recipient) were observed for four weeks. At the end of the treatment period, brain samples were collected for monoamine analysis without (Control) or after an acute stress exposure (30 minutes of confinement, Stressed). 5-HT and its catabolite 5-hydroxyindole acetic acid (5-HIAA) were analysed in the brain stem of all fish, and the ratio between 5-HIAA and 5-HT was used as indicator of serotonergic activity. In addition, agonistic interactions in the tank (sum of chases and bites, per hour), and proactivity index of the individual (ratio of initialized and received agonistic interactions) were calculated.

Generalized linear models were applied with the 5-HT, 5-HIAA, and their ratio (5-HIAA/5-HT) as outcome variables with temperature treatment, acute stress treatment, agonistic interactions in the tank and individual proactivity index as explanatory variables. We found that higher concentrations of 5-HT were associated with higher temperature treatments ($p < 0.0001$), higher proactivity index ($p < 0.0001$) and lower levels of agonistic interactions in the tank ($p < 0.0001$). Higher 5-HIAA was associated with higher temperatures ($p < 0.0001$), acute stress ($p = 0.009$) and higher proactivity index ($p = 0.019$). We found that the 5-HIAA/5-HT ratio was increased in acutely stressed individuals ($p = 0.0145$) while not affected by the other factors. In conclusion, while the 5-HIAA/5-HT ratio was affected by the acute stress similarly to earlier results, we found that this was not the case for chronic stress factors (social stress and temperature), but these rather influenced the general level of the serotonergic concentrations in the brain. Thus, our results indicate that not just the directly experienced negative social interactions, but the observed interactions influence the serotonergic system.