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IDENTIFYING ANIMAL BEHAVIOUR INDICATORS AND ESTABLISHING ZOOHYGIENIC STANDARDS FROM THE ANIMAL WELFARE PERSPECTIVE

- doctoral thesis summary -

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ИДЕНТИФИКУВАЊЕ НА ИНДИКАТОРИ ЗА ОДНЕСУВАЊЕ НА ЖИВОТНИТЕ И ВОСПОСТАВУВАЊЕ ЗООХИГИЕНСКИ СТАНДАРДИ ОД АСПЕКТ НА БЛАГОСОСТОЈБАТА НА ЖИВОТНИТЕ

Клучни зборови: благосостојба на животни, однесување на животни, зоохигиена, прецизно фармерство, акцелерометрија, социјални мрежи, анализа на социјални мрежи

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SUMMARY

The implementation of animal welfare standards, enhancing of animal hygiene (zoohygiene) practices and farm management, including the health management are exclusively dependent of relevant monitoring and assessment. This involves conducting appropriate measurements integrated into indicators that might be also established as protocols for achieving objective assessment of the animal state and farms in general. Animal welfare, including animal hygiene standards are generated in compliance with the existing knowledge about the animal needs and environmental influences accompanied by the societal values of the broader public. There are vast possibilities for measurements and maybe even more not known indicators that could indicate an animal's needs, their current state, as well as, their quality of life. Sensor technology is a promising tool for objective continuous data collection that could be used in detection of certain conditions and behaviours of an animal or could contribute in gaining new knowledge in animal science. Accelerometry, in biomechanics, represents quantitative determination of acceleration and deceleration of the body or part of the body in a living organism while performing certain action. Thus, accelerometry could be potentially used in measuring certain conditions in animals. The animal behavior, as an output generated from combined inputs on the organism, is a perfect indicator for the effects of the existing inputs. These inputs, beside the environmental factors, also include the animal's attributes, such as age, production, body condition, as well as the animal's medical condition. The attributes might have potential for a long-term impact on the animal's behavior, their social interactions and even the spatial distribution in a confined space or in the stalls.

The research subject of this doctoral thesis is consisted of two parts. The first part addresses the use of accelerometry as a tool for measuring certain states in animals. In this context two studies were conducted: the first one is for gait and posture discrimination and the second study is dealing with stride kinematics and acceleration modeling. The second part is focused on determining potential indicators that could be used in assessment of animal welfare and animal hygiene. In regards to this, two different studies were conducted, determining associations between animal's attributes and their behavior and spatial distribution, while the second study is working with social network analysis in a herd of dairy cows. Therefore, the general objective of this doctoral thesis is to develop additional methods of measures and identified potential indicators for assessing the animal's behavior, welfare and animal hygiene by using accelerometry and associations between animal's attributes and behaviours.

The first study is about *gait and posture discrimination in sheep using a tri-axial accelerometer*. The initial step in this study was the hypothesis that the temporo-spatial observation of the leg might provide important information about the general condition of an animal, especially for those such as sheep and other free-ranging farm animals that are difficult to access. Tri-axial accelerometers are capable of collecting vast amounts of data for locomotion and posture observations; however, interpretation and optimization of these data records remain a challenge. The aim of the present study was to introduce an optimized method for gait (walking, trotting and galloping) and posture (standing and lying) discrimination, using

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the acceleration values recorded by a tri-axial accelerometer mounted on the hind leg of sheep. The acceleration values recorded on the vertical and horizontal axes, as well as the total acceleration values were categorized. The relative frequencies of the acceleration categories (RFACs) were calculated in 3-s epochs. Reliable RFACs for gait and posture discrimination were identified with discriminant function and canonical analyses. *Post hoc* predictions for the two axes and total acceleration were conducted using classification functions and classification scores for each epoch. Mahalanobis distances were used to determine the level of accuracy of the method. The highest discriminatory power for gait discrimination yielded four RFACs on the vertical axis, and five RFACs each on the horizontal axis and the total acceleration vector. The classification functions showed the highest accuracy for walking and galloping. The highest total accuracy on the vertical and horizontal axes was 90% and 91%, respectively. Regarding posture discrimination, the vertical axis exhibited the highest discriminatory power, with values of RFAC (0, 1] = 99.95% for standing; and RFAC (−1, 0] = 99.50% for lying. The horizontal axis showed strong discrimination for the lying side of the animal, as values were in the acceleration category of (0, 1] for lying on the left side and (−1, 0] on the right side. The algorithm developed by the method employed in the present study facilitates differentiation of the various types of gait and posture in animals from fewer data records and produces the most reliable acceleration values from only one axis within a short time frame. This study introduces an optimized method by which the tri-axial accelerometer can be used in gait and posture discrimination in sheep as an animal model.

The second study for accelerometry addressed the *stride kinematics and acceleration modeling of walking and galloping in sheep*. The current knowledge for locomotion in quadrupeds is becoming more useful in assessing mental and physical state of the domestic and wild animals. However, collecting and analyzing data from different gaits by non-invasive and observer bias-free technique is quite difficult. Therefore, finding suitable tools for precision livestock farming and for locomotion studies in different species is one of the major challenges today. The stride is consisted of complete cycle of leg movements and represents the basic unit of the gait. Additionally, tri-axial accelerometers are devices that record the acceleration values of the moving object in time. By combining these two properties this study develops an acceleration model of a stride for counting steps and measuring kinematic parameters (stride, stance and swing duration and duty factor) of the hind leg in sheep during walking and galloping. The stride acceleration model was constructed on the basis of previously defined eight key acceleration points for walking (four points for each phase, stance and swing) and seven key acceleration points for galloping (three points in stance and four points in galloping). The percent error of the model in terms of counting steps compared with the video observation was 2.08% for walking and 0.97% for galloping. The measured acceleration values during the stride for the hind leg in sheep indicate that the stride duration was 0.81±0.19 seconds and the duty factor was 63.30±6.98% in walking, while in galloping 0.40±0.08 seconds and 32.51±10.42%, respectively. The accuracy of the stride analysis for walking was compared with findings in other experimental studies using different tools. By using the developed method, for the first time the stride kinematic parameters in galloping sheep was measured. Thus, we demonstrated that single accelerometer mounted on the leg of the animal provides detailed stride analysis in quadrupeds. This information could possibly be used for early detection of lameness,

monitoring the animal health and welfare, farm management or in terrestrial locomotion studies.

The study concerning the *associations between behaviors, including spatial distribution, with the attributes of dairy cows* was the first of the two conducted studies related to animal behavior. The individual, as well as social, animal behavior is a direct response of the received inputs from the environment or from the animal's state itself. Therefore, the animal's attributes like age, body condition, milk production, pregnancy etc. have certain impact on the individual behavior. Additionally, in sick animals the behavior is changing into so called sickness behavior that represents adaptive stereotypical behavioural response to infection or injury caused by immunological and central nervous system. Thus, behavioural changes caused by the attributes or sickness can be used as indicators for detection of these states in cattle. One of the current challenges in ethology is to determine the long-term associations between the animal's attributes, including health conditions, with the animal's behaviours. Additionally, animal's movement and usage of the available space is depending of the location properties in terms of food and water resources, vegetation, soil properties etc. On the other hand, sickness and social rank among the animals could have impact on their movements and utilization of the available space. Thus, the spatial distribution of the animals has a potential to be used as an indicator for some of their attributes. The objective of this study was to identify the existing associations between the attributes and medical history with the individual behavior of dairy cows, including their spatial distribution in loose housing system with cubicles.

This study was performed in a commercial farm for dairy cows with loose housing system with cubicles. The data needed for this study were sampled by observation of the animal's behaviour and by collecting information concerning the attributes of the observed animals, including their medical history. The continuous behavior observation on 91 dairy cows was performed from video recordings of four cameras placed in the confined area in a period of 14 hours/day, from 07:00 –21:00 o'clock for two consecutive days. The identification of the animals was based on their special skin and color markings on the body from the photos taken in different projections and summarized into a catalog. Specialized software was used for the behavioural observations and recording of all predetermined behaviours defined in the ethogram. Information about the animals i.e. the cows' attributes were taken from the farm management software. For the purpose of spatial distribution analysis, the location of the observed animal was continuously recorded in the available space during the observation. The whole observational space was divided into eight zones with similar surface area in each zone in the feeding (ZR 1 – ZR4) and lying (ZL1 – ZL4) alleys. Moreover, the row of each cubicle where the animal lied down was recorded, whether it was in the middle towards the feeding alley or at the side towards outside. The processing of the behavioural records yielded 47 dependent variables and after the analysis of the data from the farm management software, in total 20 attributes were identified as appropriate for further use in the study.

This study introduced the so called favorable (preferred) zones by the animals in the confined area according to the following criteria: average time of the herd spent in each zone; predominant zone chosen by each individual; correlation between the duration spent in each

zone with the time spent in feed bunks in the feeding alley or lying duration in the lying alley; and correlation between the zones and the number of agonistic interactions. For determining the associations between behaviours and animal's attributes some sort of triangular approach was used where the association was considered as existing if the same association is confirmed by three different analyses: 1. *Spearman* rank correlation; 2. the iterative partitioning method of *non-hierarchical K-means clustering* and 3. *Principal Component Analysis (PCA)*. Later, the results from the conducted analyses were summarized into four categories: no associations; not conclusive; partial (positive or negative) associations and association (positive or negative) between the attributes and behaviours.

The herd of dairy cows involved in the study had heterogeneous structure in terms of age (from 2 to over 9 years); origin - 36% animals were bought and the rest were born on farm; body condition (74% were animals with body condition score of 3 – 4); pregnancy - from non-pregnant animals to the third trimester of pregnancy; milk production - from 5 - 13 thousands litters in 305 days lactation. Additionally, 66% from the animals at least once had higher number of somatic cell counts in milk, 41% at least once were diagnosed with mastitis; 19% had lameness in their lives; while 51% of cows had at least one incidence of reproductive disorder. The behavioural observation revealed that the animals spent: approximately 4.5 hours in lying, 5 hours at the feed bunks, standing 1.5 hour and 18 minutes in moving, while the automatic brush was used on average about 5 minutes with great individual variations. The conducted correlation for determining associations between the attributes and animal's behaviours identified 70 significant correlations. The cluster analysis found 79, from which 49 were important associations and 30 partially important associations. Whereas, according the findings of the last analysis - PCA, six principle components defined by the animal's attributes as active variables, were in correlation with nine behaviours as dependent (supplementary) variables. Thus, the summarized results from the three analyses reported that out of 760 tests for associations between attributes and dependent variables, in 569 there was no association indicated from any of the three analyses. In total, 25 (12 positive and 13 negative) associations were confirmed by each of the three analyses, and 164 were partial (67 partially negative and 97 partially positive) associations which were not confirmed by all analyses.

Specific zones/locations in the confined area were identified as preferred by the dairy cows with the spatial distribution analysis. These zones in the lying alley were those that are centrally located. The similar tendency was detected in the feeding area, yet, according to the set criteria, the peripheral zone where supplementary mineral cubes were placed was the second favorable zone. These findings indicate that spatial distribution of dairy cows is highly influenced by the environment in the intensive farm systems. Considering the individual behaviours and attributes, older animals spent more time in standing with lower time spent in feed bunks. The older animals received less head butts and displacements from the other members of the herd, hence positioning themselves at the higher social rank in the herd, also confirmed by the social network analysis. Older animals mostly preferred to lie down in the outer cubicles, i.e. closest areas of the stall to the outside. Regarding the space utilization, the older animals are making the optimal choice between the lowest level of social conflict in the zone and their zone's preferences. Recently bought dairy cows showed tendency to spend more time in the favorable

zones, longer stay at the feed bunks and increased number of agonistic interactions. Though, these parameters are decreasing, some even to the submissive level, as the animal takes its social rank in the herd. When considering the body condition as an attribute, lesser usage of the automatic brush was detected in animals with lower body condition score. In regards to the milk production as an attribute (overall milk production and milk production in current lactation), the animals with higher production lay down in the most favorable zone. The dairy cows with medical histories of mastitis, increased number of somatic cells in milk, lameness, different treatments and other disorders had partial associations very similar with the behaviour of older animals. Accordingly, the prolonged standing and less time spent at the feedbanks were detected for these animals. However, certain behaviours of the animals were specific for some conditions, such as: the animals with mastitis in the current lactation had lesser lying time, while the lying duration is increasing in the animals at reconvalescent stage; cows with history of lameness have higher number of scratching events, and those that hade lameness during the current lactation have more self-grooming (self-licking). Furthermore, the partial associations in pregnant animals were indicating reduced time for lying and moving, and prolonged standing in animals with higher pregnancy state, also these animals were in the zones with lower number of animals i.e. zones with less social conflicts. Higher licking of other cows together with behaviours not defined in the ethogram (other behaviours), as typical signs for estrous behaviour, was found in non-pregnant cows. The findings of this study represent data based developed hypotheses convenient for future research and validation. The identified associations have potential to contribute in the management of animal hygiene, health, welfare and behaviour of the dairy cows in intensive farm systems.

The social network analysis and associations between social interactions and the dairy cows' attributes was the second behavioural and the last study contained in this doctoral thesis. Cows are social herd animals with complex social relations, present allelomimicry for most of the behaviours and clearly defined, stable hierarchical structure. The social environment is comprised of nonrandom and heterogeneous social interactions. These interactions are represented by affiliative and agonistic behaviour in cattle. The behaviour of an individual might be affected by, and is affecting, the presence and behaviour of the other members within their social networks. The social network analysis (SNA) is a method that gives an opportunity for detailed description, analysis and understanding of the social relations in the frames of the social networks. This method enables analysis of the social structure on individual, intermediate and herd level in the social network. The social behaviour of one animal is actual multifactorial output. More precisely, some animal's attributes could be considered as potential factors that have an impact towards the social networks. Those attributes are age, productivity, body condition, health state and medical history, as well as the animal hygiene i.e. housing conditions and management process. The objective of this study was to measure and present the properties of the social network by applying SNA and identifying the potential associations between the attributes and SNA parameters in dairy cows. The data used in this study were the same as those from the previous behavioural study. More precisely, continuous video observation of the social interactions among 91 dairy cows in the herd coupled with the collected attribute's data, including the medical history. The social network analysis was conducted on the data collected from the continuous observations for 28 hours. On the basis of

the recorded social interactions among the observed individuals, three associative matrices were developed: 1) "All social interactions"; 2) "Agonistic interactions" and 3) "Affiliative interactions". Additionally, for each individual the dominant rank within the herd was calculated. The SNA was conducted on the three matrices and also the associations were tested between the social interactions and animal's attributes. From all studied attributes, the age of the animal has manifested as an important attribute in all three networks. The animals with the same or similar age are located more closely to each other in the affiliative network (autocorrelation r=0.74, p<0.05), while the agonistic behaviour was directed from the older cows toward the younger animals (E-I$_{index}$=0.47). Cows with same or similar gestational period had increased mutual affiliative interactions (autocorrelation r=0.50, p<0.05). Regarding the milk production, the animals with higher milk production received less interactions from the rest of the members in the herd (r$_{InFarness}$=0.23-0.25, p<0.05). Additionally, the members of the herd that had higher number of somatic cell count in the milk during the current lactation had higher number of received affiliative interactions (InDegree = 4.74±8.02) in comparison to the animals without increased somatic cell counts (InDegree = 2.23±2.13), p<0.05. The agonistic social network exhibits highly defined hierarchical structure in the herd of dairy cows. This study found that there was significant positive correlation between the dominance rank with the age (r=0.42, p<0.05) and the milk production in the current lactation (r=0.24, p<0.05). In addition it was noted that more advanced pregnancy leads to stabilization of the social relations which was confirmed by the clustering coefficient (r=0.26, p<0.05). In opposite, cows that are not pregnant and potentially are in some of the estrous cycle stages had increased number of social interactions and temporary are centrally located in the networks. There was no significance regarding the associations between some of the disorders like mastitis, lameness, reproductive disorders, other disorders and treatments with the SNA metrics, except increased homophily in affiliative networks for animals with lameness in the current lactations and the history of other diseases. There was a positive correlation between agonistic and affiliative social networks (r=0.46, p<0.001) and one interaction in the agonistic network implies probability of 86% for an interaction in the affiliative network. This study confirms the applicability of SNA as a tool for developing indicators for health and animal welfare, as well as for establishing good animal hygiene practices in the management of dairy cows.

The four conducted studies within this doctoral thesis confirmed that accelerometry and animal behaviour have the potential to be used for assessment of animal welfare, zoohygiene, health and management in intensive farm systems. The accelerometry highlighted the methods for analysis of locomotion and animal behaviour as measurement that could be integrated within the assessment indicators. Additionally, the identified associations between behaviours and animal's attributes, including the social network analysis, developed hypotheses with potential for creating new assessment indicators. Actually, these four studies have confirmed the need and the applicability of the precision livestock farming in scientific and practical sense. In this context, the development of new algorithms, analytical data processing together with proper interpretation represent the basis for introducing sensor technology and behavioural, including social, analysis in the modern farm systems. The future perspective of the presented studies lies in establishing a system that will enable monitoring and real-time assessment of the state

of the animals and farms which will greatly facilitate the decision making process for proper management.