

## "Ss. CYRIL AND METHODIUS" UNIVERSITY IN SKOPJE FACULTY OF VETERINARY MEDICINE IN SKOPJE DOCTORAL SCHOOL VETERINARY MEDICINE



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15	Miroslav Kjosevski, DVM
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26	ZOOHYGIENIC STANDARDS FROM THE ANIMAL WELFARE PERSPECTIVE
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49 50	ИДЕНТИФИКУВАЊЕ НА ИНДИКАТОРИ ЗА ОДНЕСУВАЊЕ НА ЖИВОТНИТЕ И ВОСПОСТАВУВАЊЕ ЗООХИГИЕНСКИ СТАНДАРДИ ОД АСПЕКТ НА
51	БЛАГОСОСТОЈБАТА НА ЖИВОТНИТЕ
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54 55	Клучни зборови: благосостојба на животни, однесување на животни, зоохигиена, прецизно фармерство, акцелерометрија, социјални мрежи, анализа на социјални мрежи
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99	PhD Mentor: Prof. Vlatko Ilieski, PhD
100	full professor at the Faculty of Veterinary Medicine in Skopje, Ss. Cyril and Methodius
101	University in Skopje
102	
103	
104	
105	Commission members:
106	
107	Prof. Misho Hristovski, PhD, chairmen
108	full professor at the Faculty of Veterinary Medicine in Skopje, Ss. Cyril and Methodius
109	University in Skopje
110	
111	
112	Prof. Vlatko Ilieski, PhD, mentor
113	full professor at the Faculty of Veterinary Medicine in Skopje, Ss. Cyril and Methodius
114	University in Skopje
115	
116	Assoc. Prof Aleksandar Dodovski, PhD
117	associate professor at the Faculty of Veterinary Medicine in Skopje, Ss. Cyril and Methodius
118	University in Skopje
119	
120	
121	Prof. Christoph Winckler, PhD
122	full professor at the University of Natural Resources and Life Sciences, Vienna (BOKU)
123	
124	
125	Prof. Lazo Pendovski, PhD
126	full professor at the Faculty of Veterinary Medicine in Skopje, Ss. Cyril and Methodius
127	University in Skopje
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## 132 SUMMARY

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134 The implementation of animal welfare standards, enhancing of animal hygiene (zoohygiene) practices and farm management, including the health management are exclusively dependent 135 136 of relevant monitoring and assessment. This involves conducting appropriate measurements 137 integrated into indicators that might be also established as protocols for achieving objective 138 assessment of the animal state and farms in general. Animal welfare, including animal hygiene 139 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 140 141 vast possibilities for measurements and maybe even more not known indicators that could 142 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 143 144 certain conditions and behaviours of an animal or could contribute in gaining new knowledge 145 in animal science. Accelerometry, in biomechanics, represents quantitative determination of 146 acceleration and deceleration of the body or part of the body in a living organism while 147 performing certain action. Thus, accelerometry could be potentially used in measuring certain 148 conditions in animals. The animal behavior, as an output generated from combined inputs on 149 the organism, is a perfect indicator for the effects of the existing inputs. These inputs, beside 150 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 151 long-term impact on the animal's behavior, their social interactions and even the spatial 152 153 distribution in a confined space or in the stalls.

154 The research subject of this doctoral thesis is consisted of two parts. The first part addresses 155 the use of accelerometry as a tool for measuring certain states in animals. In this context two 156 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 157 determining potential indicators that could be used in assessment of animal welfare and animal 158 159 hygiene. In regards to this, two different studies were conducted, determining associations 160 between animal's attributes and their behavior and spatial distribution, while the second study 161 is working with social network analysis in a herd of dairy cows. Therefore, the general 162 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 163 164 accelerometry and associations between animal's attributes and behaviours.

165 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 166 167 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 168 169 to access. Tri-axial accelerometers are capable of collecting vast amounts of data for 170 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 171 172 for gait (walking, trotting and galloping) and posture (standing and lying) discrimination, using

173 the acceleration values recorded by a tri-axial accelerometer mounted on the hind leg of sheep. 174 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 175 176 (RFACs) were calculated in 3-s epochs. Reliable RFACs for gait and posture discrimination 177 were identified with discriminant function and canonical analyses. Post hoc predictions for the 178 two axes and total acceleration were conducted using classification functions and classification 179 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 180 the vertical axis, and five RFACs each on the horizontal axis and the total acceleration vector. 181 182 The classification functions showed the highest accuracy for walking and galloping. The 183 highest total accuracy on the vertical and horizontal axes was 90% and 91%, respectively. 184 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 185 186 horizontal axis showed strong discrimination for the lying side of the animal, as values were in 187 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 188 189 the various types of gait and posture in animals from fewer data records and produces the most 190 reliable acceleration values from only one axis within a short time frame. This study introduces 191 an optimized method by which the tri-axial accelerometer can be used in gait and posture 192 discrimination in sheep as an animal model.

193 The second study for acceleromtry addressed the stride kinematics and acceleration modeling 194 of walking and galloping in sheep. The current knowledge for locomotion in quadrupeds is 195 becoming more useful in assessing mental and physical state of the domestic and wild animals. 196 However, collecting and analyzing data from different gaits by non-invasive and observer bias-197 free technique is quite difficult. Therefore, finding suitable tools for precision livestock farming 198 and for locomotion studies in different species is one of the major challenges today. The stride 199 is consisted of complete cycle of leg movements and represents the basic unit of the gait. 200 Additionally, tri-axial accelerometers are devices that record the acceleration values of the 201 moving object in time. By combining these two properties this study develops an acceleration 202 model of a stride for counting steps and measuring kinematic parameters (stride, stance and 203 swing duration and duty factor) of the hind leg in sheep during walking and galloping. The 204 stride acceleration model was constructed on the basis of previously defined eight key 205 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 206 207 percent error of the model in terms of counting steps compared with the video observation was 208 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  $0.81\pm0.19$  seconds and the duty 209 210 factor was 63.30±6.98% in walking, while in galloping 0.40±0.08 seconds and 32.51±10.42%, 211 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 212 213 time the stride kinematic parameters in galloping sheep was measured. Thus, we demonstrated 214 that single accelerometer mounted on the leg of the animal provides detailed stride analysis in 215 quadrupeds. This information could possibly be used for early detection of lameness,

216 monitoring the animal health and welfare, farm management or in terrestrial locomotion 217 studies.

218 The study concerning the associations between behaviors, including spatial distribution, with 219 the attributes of dairy cows was the first of the two conducted studies related to animal 220 behavior. The individual, as well as social, animal behavior is a direct response of the received 221 inputs from the environment or from the animal's state itself. Therefore, the animal's attributes 222 like age, body condition, milk production, pregnancy etc. have certain impact on the individual 223 behavior. Additionally, in sick animals the behavior is changing into so called sickness 224 behavior that represents adaptive stereotypical behavioural response to infection or injury 225 caused by immunological and central nervous system. Thus, behavioural changes caused by 226 the attributes or sickness can be used as indicators for detection of these states in cattle. One of 227 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, 228 229 animal's movement and usage of the available space is depending of the location properties in 230 terms of food and water resources, vegetation, soil properties etc. On the other hand, sickness 231 and social rank among the animals could have impact on their movements and utilization of 232 the available space. Thus, the spatial distribution of the animals has a potential to be used as an 233 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 234 235 cows, including their spatial distribution in loose housing system with cubicles.

236 This study was performed in a commercial farm for dairy cows with loose housing system with 237 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 238 239 medical history. The continuous behavior observation on 91 dairy cows was performed from 240 video recordings of four cameras placed in the confined area in a period of 14 hours/day, from 241 07:00 –21:00 o'clock for two consecutive days. The identification of the animals was based on 242 their special skin and color markings on the body from the photos taken in different projections 243 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 244 245 animals i.e. the cows' attributes were taken from the farm management software. For the 246 purpose of spatial distribution analysis, the location of the observed animal was continuously 247 recorded in the available space during the observation. The whole observational space was 248 divided into eight zones with similar surface area in each zone in the feeding (ZR 1 - ZR4) and 249 lying (ZL1 – ZL4) alleys. Moreover, the row of each cubicle where the animal lied down was 250 recorded, whether it was in the middle towards the feeding alley or at the side towards outside. 251 The processing of the behavioural records yielded 47 dependent variables and after the analysis 252 of the data from the farm management software, in total 20 attributes were identified as 253 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 257 zone with the time spent in feed bunks in the feeding alley or lying duration in the lying alley; 258 and correlation between the zones and the number of agonistic interactions. For determining 259 the associations between behaviours and animal's attributes some sort of triangular approach 260 was used where the association was considered as existing if the same association is confirmed 261 by three different analyses: 1. Spearman rank correlation; 2. the iterative partitioning method 262 of non-hierarchical K-means clustering and 3. Principal Component Analysis (PCA). Later, 263 the results from the conducted analyses were summarized into four categories: no associations; 264 not conclusive; partial (positive or negative) associations and association (positive or negative) 265 between the attributes and behaviours.

266 The herd of dairy cows involved in the study had heterogeneous structure in terms of age (from 267 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-268 pregnant animals to the third trimester of pregnancy; milk production - from 5 - 13 thousands 269 270 litters in 305 days lactation. Additionally, 66% from the animals at least once had higher 271 number of somatic cell counts in milk, 41% at least once were diagnosed with mastitis; 19% 272 had lameness in their lives; while 51% of cows had at least one incidence of reproductive 273 disorder. The behavioural observation revealed that the animals spent: approximately 4.5 hours 274 in lying, 5 hours at the feed bunks, standing 1.5 hour and 18 minutes in moving, while the 275 automatic brush was used on average about 5 minutes with great individual variations. The 276 conducted correlation for determining associations between the attributes and animal's 277 behaviours identified 70 significant correlations. The cluster analysis found 79, from which 49 278 were important associations and 30 partially important associations. Whereas, according the 279 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) 280 281 variables. Thus, the summarized results from the three analyses reported that out of 760 tests 282 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 283 284 were confirmed by each of the three analyses, and 164 were partial (67 partially negative and 285 97 partially positive) associations which were not confirmed by all analyses.

286 Specific zones/locations in the confined area were identified as preferred by the dairy cows 287 with the spatial distribution analysis. These zones in the lying alley were those that are centrally 288 located. The similar tendency was detected in the feeding area, yet, according to the set criteria, 289 the peripheral zone where supplementary mineral cubes were placed was the second favorable 290 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 291 292 attributes, older animals spent more time in standing with lower time spent in feed bunks. The 293 older animals received less head butts and displacements from the other members of the herd, 294 hence positioning themselves at the higher social rank in the herd, also confirmed by the social 295 network analysis. Older animals mostly preferred to lie down in the outer cubicles, i.e. closest 296 areas of the stall to the outside. Regarding the space utilization, the older animals are making 297 the optimal choice between the lowest level of social conflict in the zone and their zone's 298 preferences. Recently bought dairy cows showed tendency to spend more time in the favorable

299 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 300 301 social rank in the herd. When considering the body condition as an attribute, lesser usage of 302 the automatic brush was detected in animals with lower body condition score. In regards to the 303 milk production as an attribute (overall milk production and milk production in current 304 lactation), the animals with higher production lay down in the most favorable zone. The dairy 305 cows with medical histories of mastitis, increased number of somatic cells in milk, lameness, 306 different treatments and other disorders had partial associations very similar with the behaviour 307 of older animals. Accordingly, the prolonged standing and less time spent at the feedbanks 308 were detected for these animals. However, certain behaviours of the animals were specific for 309 some conditions, such as: the animals with mastitis in the current lactation had lesser lying 310 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 311 312 during the current lactation have more self-grooming (self-licking). Furthermore, the partial 313 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 314 315 with lower number of animals i.e. zones with less social conflicts. Higher licking of other cows 316 together with behaviours not defined in the ethogram (other behaviours), as typical signs for 317 estrous behaviour, was found in non-pregnant cows. The findings of this study represent data 318 based developed hypotheses convenient for future research and validation. The identified 319 associations have potential to contribute in the management of animal hygiene, health, welfare 320 and behaviour of the dairy cows in intensive farm systems.

321 The social network analysis and associations between social interactions and the dairy cows' 322 attributes was the second behavioural and the last study contained in this doctoral thesis. Cows 323 are social herd animals with complex social relations, present allelomimicry for most of the 324 behaviours and clearly defined, stable hierarchical structure. The social environment is 325 comprised of nonrandom and heterogeneous social interactions. These interactions are 326 represented by affiliative and agonistic behaviour in cattle. The behaviour of an individual 327 might be affected by, and is affecting, the presence and behaviour of the other members within 328 their social networks. The social network analysis (SNA) is a method that gives an opportunity 329 for detailed description, analysis and understanding of the social relations in the frames of the 330 social networks. This method enables analysis of the social structure on individual, 331 intermediate and herd level in the social network. The social behaviour of one animal is actual 332 multifactorial output. More precisely, some animal's attributes could be considered as potential 333 factors that have an impact towards the social networks. Those attributes are age, productivity, 334 body condition, health state and medical history, as well as the animal hygiene i.e. housing 335 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 336 337 between the attributes and SNA parameters in dairy cows. The data used in this study were the 338 same as those from the previous behavioural study. More precisely, continuous video 339 observation of the social interactions among 91 dairy cows in the herd coupled with the 340 collected attribute's data, including the medical history. The social network analysis was 341 conducted on the data collected from the continuous observations for 28 hours. On the basis of 342 the recorded social interactions among the observed individuals, three associative matrices 343 were developed: 1) "All social interactions"; 2) "Agonistic interactions" and 3) "Affiliative interactions". Additionally, for each individual the dominant rank within the herd was 344 345 calculated. The SNA was conducted on the three matrices and also the associations were tested 346 between the social interactions and animal's attributes. From all studied attributes, the age of 347 the animal has manifested as an important attribute in all three networks. The animals with the 348 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 349 350 cows toward the younger animals (E-I<sub>index</sub>=0.47). Cows with same or similar gestational period 351 had increased mutual affiliative interactions (autocorrelation r=0.50, p<0.05). Regarding the 352 milk production, the animals with higher milk production received less interactions from the 353 rest of the members in the herd (r<sub>InFarness</sub>=0.23-0.25, p<0.05). Additionally, the members of the 354 herd that had higher number of somatic cell count in the milk during the current lactation had 355 higher number of received affiliative interactions (InDegree =  $4.74\pm8.02$ ) in comparison to the 356 animals without increased somatic cell counts (InDegree =  $2.23\pm2.13$ ), p<0.05. The agonistic 357 social network exhibits highly defined hierarchical structure in the herd of dairy cows. This 358 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 359 360 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 361 not pregnant and potentially are in some of the estrous cycle stages had increased number of 362 social interactions and temporary are centrally located in the networks. There was no 363 significance regarding the associations between some of the disorders like mastitis, lameness, 364 reproductive disorders, other disorders and treatments with the SNA metrics, except increased 365 homophily in affiliative networks for animals with lameness in the current lactations and the 366 367 history of other diseases. There was a positive correlation between agonistic and affiliative 368 social networks (r=0.46, p<0.001) and one interaction in the agonistic network implies 369 probability of 86% for an interaction in the affiliative network. This study confirms the 370 applicability of SNA as a tool for developing indicators for health and animal welfare, as well 371 as for establishing good animal hygiene practices in the management of dairy cows.

372 The four conducted studies within this doctoral thesis confirmed that accelerometry and animal 373 behaviour have the potential to be used for assessment of animal welfare, zoohygiene, health 374 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 375 376 the assessment indicators. Additionally, the identified associations between behaviours and 377 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 378 379 and the applicability of the precision livestock farming in scientific and practical sense. In this 380 context, the development of new algorithms, analytical data processing together with proper 381 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 382 383 lies in establishing a system that will enable monitoring and real-time assessment of the state

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

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