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EVALUATION OF ANIMAL-BASED PARAMETERS OF WELFARE IN TWO HUNGARIAN DAIRY FARMS USING PRINCIPAL COMPONENT ANALYSIS

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Abstract

The aim of this study was to give information about the characterization of parameters of heart rate variability (HRV) and some traditional parameters on two Holstein Friesian farms (farm 1, farm 2). Both were small-scale farms. The average daily milk yield, body condition of the animals, locomotion and temperament tests and cardiac activity measurements were made in autumn 2013. The physical activity of the animals was also recorded (lying, lying and ruminating, standing, standing and ruminating, feeding). Principal component analysis (PCA) was performed using the SPSS 20 (SPSS Inc., Chicago, IL) statistical software. Rotation of factors was done by the Oblimin method. The latent variables were calculated from the correlation matrix of the parameters. In the interpretation of PCs, only loadings greater than 0.6 were considered practically significant. The daily milk yield of cows was 25.5±4.41 kg and 23.5±8.29 kg on farm 1 and 2, respectively. Means for body condition score, temperament, heart rate, RMSSD, HF, LF/HF were recorded: farm 1: 3.1 score; 1.3 score; 73.42 beat/min; 14.19 ms; 23.52; 9.95 ratio; and farm 2: 2.7 score; 1.7 score; 74.05 beat/min; 18.75 ms; 28.53; 6.47 ratio respectively. Parameters recorded for the two farms were similar. On farm 1, the following four significant PCs (explain together over 80% of the total variance) were involved in the analysis: *traits of HRV (1), body condition-temperament score (2), daily milk production (3), RMSDD (4).* The first principal component (36.0% of total variance) can be interpreted as a linear combination of HF, HR and LF/HF examined parameters. On farm 2, the following four principal components (explain together 81.2% of the total variance) were defined: *traits of HRV (1), body condition and daily milk production (2), temperament (3), RMSDD (4). The first principal component in the case of farm 2* corresponds to a group of three parameters: LF/HF, HF and HR. This principal component explained 29.5% of the all variance.
Keywords: Holstein Friesian cow, HRV parameters, milk production, body condition

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Állatjóléti paraméterek értékelése két magyar tejelőfarmon főkomponens-elemzéssel

Összefoglalás

Ennek a tanulmánynak a célja a pulzusszám (HRV) paramétereinek és néhány hagyományos paraméterének jellemzése volt két holstein-fríz gazdaságban (1. farm, 2. farm). Mindkét gazdaság kisebb méretű volt. 2013 őszén megmérték az átlagos napi tejhozamot, az állatok kondícióját. Az állatok mozgását és temperamentumát megnézték és mérték szívritmusukat. A fizikai aktivitást is meghatározták (fekvő, fekvő és kérődző, álló, álló és kérődző, táplálkozási). A főkomponens elemzést (PCA) az SPSS 20 (SPSS Inc., Chicago, IL) statisztikai szoftver alkalmazásával végezték. Az Oblimin rotálási eljárás lett alkalmazva. A háttérváltozók kiszámítása a korrelációs mátrix alapján történt. A főkomponensek szakmai tartalmának meghatározásakor a 0.6-nél magasabb súlyértékeket tekintettük jelentősnek. A tehenek napi tejhozama az 1. és a 2. gazdaságban 25,5 ± 4,41 kg és 23,5 ± 8,29 kg volt. A kondíció pontszám, a temperamentum, a pulzusszám, az RMSSD, HF, LF/HF értékeket feljegyezték: farm 1: 3,1 pont; 1,3 pont; 73,42 ütem/perc; 14,19 ms; 23:52; 9,95 arány; és 2. farm: 2,7 pont; 1,7 pont; 74,05 ütem/perc; 18,75 ms; 28.53; 6,47 arány. A két gazdaság paraméterei hasonlóak voltak. Az 1. gazdaság esetében az első háromfőkomponens (a teljes variancia több mint 80%-át magyarázza meg együtt) bizonyult jelentősnek: a HRV tulajdonságai (1.), a kondíció és temperamentum pontszámai (2.), a tejtermelés (3.), valamint az RMSSD (4). Az első főkomponens (a teljes variancia 36.0%-a tömörül benne) a HF, HR és LF/HF paraméterek lineáris kombinációjaként értelmezhető. A 2. gazdaság esetében a következő négy főkomponens (magyarázott variancia: 81,2%) bizonyult jelentősnek: a HRV tulajdonságai (1.), a kondíció és a napi tejtermelés (2.), temperamentum (3.) és a RMSSD (4). Az első főkomponens a 2. gazdaság esetében a három paraméterből álló mutatócsoportnak felel meg: LF/HF, HF és HR. Ezen főkomponens 29,5%-ot magyaráz meg a teljes varianciából.
Kulcsszavak: holstein-fríz tehén, HRV paraméterek, tejtermelés, kondíció

Introduction

The importance of animal welfare and behavior in addition to the production level is getting noticed nowadays in dairy farming. It is important to emphasize that we can measure more and more parameters with special instruments, even in farm conditions.

Studies about heart rate variability

The first application of measuring heart rate variability (HRV) was used in human medicine (*ESC-NASPE Task Force,* 1996). The heart rate variability as a measure of autonomic regulation of cardiac activity for assessing stress and welfare in farm animals was proven by *von Borell et al.,* 2007).

The heart rate (HR) and parasympathetic nervous system (PNS) indices of cardiac function are reliable measures of animal welfare in dairy cattle (*Kovács et al.,* 2014a). The root mean square of successive differences (RMSSD) between IBIs and high frequency (HF) component of HRV are used to detect tendencies in PNS activity. Poincaré measure standard deviation 1 (SD1) also represents vagal tone (*Tarvainen et al*., 2014). The relative power of the

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low frequency (LF) component and HF (LF/HF ratio) gives information about sympathetic nervous system (SNS) and the SNS/PNS balance (*van Ravenswaaij-Arts et al.*, 1993).

Earlier HRV studies in cattle reported the effects of pathological conditions of cardiac function, while current works are available on the physiological and behavioural aspects of pain evoking husbandry procedures (*Kovács et al.*, 2014b). Previous studies on short-term physiological and behavioural responses of animals in relation with breed and milking systems (*Hagen et al.*, 2005), milking technology (*Kézér et al.,* 2015), or as the effects of responsiveness towards humans (*Sutherland et al.*, 2012, *Kovács et al*., 2015) were carried out on small-scale farms.

Importance of temperament

Temperament is defined as a behavioural reaction of animals when handled by humans. Temperament of cattle can be investigated in restrained (e.g. scale test and crush test) and non-restrained tests (e.g. flight speed test, open field test and pound test) (*Burrow*, 1997). It is not always possible to relate behaviours in a restrained situation to behaviours in a non-restrained situation because some of the animals that are difficult to handle in a paddock demonstrate a moveless response when restrained (*Burrow and Corbet*, 2000).

Cattle are not easy to be provoked, however, they can respond by flight and aggression to a novel situation. The reaction of animals can be trampling, pushing, kicking and flicking tail, but this can depend on their previous positive or aversive experience (*Boivin et al.*, 1998).

More factors are known to influence temperament to a certain extent. In Hungary *Czakó* (1978), *Tőzsér et al.* (2003), *Holló et al.* (2004) as well as *Szentléleki et al.* (2005) have already reported the relatedness of cattle's temperament to some of their traits. Temperament between and within of some beef cattle breeds can be significantly different, as confirmed by several studies. *Morris et al.* (1994) proved the difference between Angus and Hereford breeds in temperament at weighing. Angus individuals were more nervous than Hereford. *Tőzsér et al.* (2004) applied scale test and flight speed test to assess temperament of Hungarian Grey and Charolais cattle. Significant difference between the breeds was determined at 0.001 significance level: Hungarian Grey steers were calmer (mean score: 1.37, mean time: 4.81 sec) compared to Charolais steers (mean score: 2.0, mean time: 2.71 sec). However, *Holló et al.* (2004) could not show any difference between temperament of Holstein Friesian and Hungarian Grey fattening bulls kept under same conditions.

Human-animal relationship remarkably affects not only the behaviour of animals but their production and welfare, as well (*Seabrook*, 1994, *Le Neindre et al.*, 1996). Careful handling is necessary in animal housing systems, since safe treatment of animals is crucial during medical and pregnancy examinations, claw treatment, weighing etc. carried out in everyday farm practice (*Györkös and Kovács*, 2004). Accordingly, to handle an animal that has not got accustomed to humans presents a risk to the safe of farmer as well as animal welfare. In different housing technologies it is possible to improve temperament and treatment of calves by decreasing stress factors and increasing handling efficiency (*Boivin et al.*, 1994, *Burrow*, 1997, *Györkös and Kovács*, 2004).

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Utilization of principle component analysis in cattle breeding

According to the literature, the most important fields where PCA was used in cattle breeding are the following ones.

- Tracing Cattle Breeds with PCA Ancestry Informative SNPs (*Lewis et al.,* 2011).
- Assessing footprints of natural selection through PCA analysis in cattle (*Moravčíková et al.,* 2017).
- PCA is capable of determination of the early detection of mastitis and lameness in dairy cows (*Meikley et al.,* 2013) and of the incidence of diseases in Norwegian Red Cattle (*Zarnecki et al.,* 1985).
- PCA can be utilized for productive and reproductive traits of Holstein cattle (*Castano et al.,* 2013) and of Red Sindhi dairy cattle breed (*Mello et al.,* 2019).
- PCA was performed on the standardized breeding values for growth and reproductive traits in beef cattle (*Boligon et al.,* 2016).
- Rear shape in 3 dimensions summarized by PCA is a good predictor of body condition score in Holstein dairy cows (*Fischer et al.,* 2015).
- *Tőzsér et al.* (2000) used PCA for investigation of conformation traits of weaned Charolais calves in Hungary.
- PCA results of two analyses on Holstein-Friesian and Hungarian Fleckvieh cows (*Tőzsér et al.,* 2001) clearly confirmed, that the variables for the deposition of fat and adipose tissue cellularity have to be included into the prediction model.
- PCA is important for describing the body measurements and body indices in the Pasundan cows (*Putra et al.,* 2020).
- Evaluation of the results of self-performance test in Limousin breeding candidates by PCA (*Tőzsér et al*., 1997).

There's no information about using PCA for parameters of HRV in cattle breeding in the literature.

The aim of our study was the characterization of the HRV and some traditional parameters using by principal component analyses (PCA) in two commercial dairy farms.

Materials and methods

Farms, animals and housing

Measurements were carried out in two commercial dairy farms (farm 1, farm 2) in Hungary on Holstein- Friesian cows produced more than one calf. Both farms were of smaller scale. Cow population, housing conditions, feeding regime and milking system of the farms are summarized in *Table 1*. According to the rule of ICAR, the average daily milk yield was measured. Focal animals were selected from clinically healthy cows. Cows that were in estrus were not involved in the study. Averages for focal animals were similar in age parity, days in milking and body condition score for both farms. Body condition of the animals was scored using the 5-point scoring system (from very lean to fat) (*Hady et al.,* 1994). Conventional temperament assessment was performed in this study.

In order to avoid any effects of high ambient temperature on cow's cardiac activity, measurements were made in autumn 2013. The study was ethically approved by the Department of Epidemiology and Animal Protection of the Directorate of Food Chain Safety and Animal Health at Central Agricultural Office.

Preparation of IBI data collection

IBIs were recorded using a mobile recording system, which included a Polar Equine T56H transmitter with electrodes a Polar H2 heart rate sensor and a Polar RS800 CX heart rate monitor (POLAR, Kempele, Finlande). After soaking the body surface under the electrodes with tap water, transmitters and the electrodes were positioned on the thoracic region, one electrode on the cardiac area, and one over the right scapula, as advised by *von Borell et al.*, (2007) in their review. Electrode site were covered with ultrasound transmission gel without shaving the skin. On each farm, devices were fitted to cows after the morning milking (between 8.00 and 8.45 a.m. according to the milking schedule of the different farms). IBI recording started after a 2-h acclimatization period and lasted until returning from the evening milking (between 19.30 and 21.00, depending on farm).

Behavioural observations

In parallel with IBI recordings, behaviour was continuously recorded by visual observation and classified with regard to posture. The following levels of physical activity were determined: lying, lying and ruminating, standing, standing and ruminating, feeding. We used the same protocol on each farm. After fixing the HR receivers, focal cows were released back to production groups. Animals were identified by the number on their hind legs and backs drawn on at the time of fixing the HR monitors. The cows were observed by a maximum of four persons at a time. A maximum of eight cows were observed at a time. Observers stood at least 6 m from the cows. They used watches, which were synchronized with the HR receivers to register the exact starting and points of the animal's actual behaviours or activity.

Processing of IBI data

The segments of IBI recordings matching the periods of uninterrupted display of the studied posture/activity were used for HRV analysis. A 2-min interval after any kind of disturbance or social interaction and a 5-min interval after changing posture were excluded from analysis. We examined periods of 5 min as recommended for analysis of HRV in earlier reviews. IBI samples for each level of physical activity were chosen balanced for morning and afternoon periods for individuals on both farms. Number of IBI samples was also balanced for levels of physical activity and for farm. Longer periods of recording were subdivided into several 5-min segments. The Kubios HRV software (2.2.) was used for HRV analysis. Besides time domain measures (HR, RMSSD) for computing frequency-domain HRV, IBI data were subjected to Fast Fourier Transformation of power spectrum analysis. The HRV spectrum was calculated with the Welch's periodogram. Spectral parameters included the normalized power of HF and LF/HF ratio.

Statistical analysis

Statistical analysis was performed using the SPSS 20 (SPSS Inc., Chicago, IL) statistical software. Principal component analysis (PCA) is a method of data reduction. Principal components are the linear combination of the original variables and in these kinds of analyses original group of variables is transformed into another group. The data set will be shortened by factor analysis and PCA, thus, it could be described in more accurate way. A large sample size was observed in our study: from 457 to 566. A value of Kaiser-Meyer-Olkin was more than 0.6 in all cases and the null hypothesis of Bartlett's test (an observed correlation

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matrix is an identity matrix, in which all of the diagonal elements are 1 and all off diagonal elements are 0) was rejected in both farms. Rotation of factors was done as outlined by the Oblimin method(delta=0) with Kaiser normalization (*Sváb,* 1979). Background variables were calculated from the correlation matrix of the parameters.

Table 1: **Characteristics of Farm No. 1 and 2 where the experiment was carried out**

Farm No. (1)		1 (Nóráp)	2 (József Major)
Herd size (2)		75	80
Housing conditions (3)		Pasture (11)	Old freestall barn, for 100 cows (12)
Bedding (4)		Fold bedded with straw (13)	Rubber mattress (14)
Group size (5)		No grouping (15)	35–40
Average space allowance (m^2/cow) (6)	For the whole barn area, including feeding place (16)	55.0	14.6
	For bedded lying area (17)	27.6	6.2
Feeding regime (7)		TMR, twice a day, grazing (18)	TMR, twice a day (19)
Milking system (8)		2 × 4-stall herringbone milking parlor (20)	2 × 5-stall herringbone milking parlor (21)
Milking frequency (9)		Twice a day (22)	Twice a day (23)
Average daily milk yield (kg) (10)		24.3	23.2

1. Táblázat: A vizsgált két farm jellemzői
telep (1), állomány méret (2), tartási körülmény (3), almozás módja (4), csoportméret (5), átlagos pihenőtér (m2/tehén) (6), takarmányozás rendszere (8), fejések száma (9), átlagos napi

tejtermelés, kg (10), legelő (11), régi kötetlen istálló 100 tehénre (12), pihenő box döngölt agyaggal (13), pihenő matrac (14), nincsen csoport (15), az egész istálló az etetőtérrel együtt (16), az almozott pihenőtérre vonatkoztatva (17), TMR, napi kétszer és legelés (18), TMR napi kétszer (19), 2x4- es halszálkás fejőház (20), 2x5 -ös halszálkás fejőház (21), kétszer egy nap (22, 23)

Results and discussion

Data of experimental animals is shown in *Table 2*. We could conclude that parameters of those traits are similar in the two farms.

Table 2: **Characteristics of experimental animals (means ± SD) in the two farms**

Farm No (1)	Farm 1 (N=566)	Farm 2 (N=457)
Characteristics (2)	Mean ± Std. Deviation	Mean ± Std. Deviation
Daily milk yield (3)	25.5±4.41	23.5±8.29
Body condition score [b] (4)	3.1±0.63	2.7±0.46
Temperament (5)	1.3±0.91	1.7±0.72
Heart rate, beat/min (6)	73.42±9.27	74.05± 1.05
RMSSD, ms (7)	14.19±12.10	18.75±25.34
HF (8)	23.52±20.16	28.53±20.01
LF/HF ratio (9)	9.95±11.70	6.47±8.33

[b]ranking from 1 = very lean to 5 = fat (10)

2. Táblázat: A farmokon vizsgált állatok jellemző adatai (átlag±szórás)

telep (1), jellemzők (2), napi tejtermelés, kg (3), kondíció (4), temperamentum (5), szívritmus (6), RMSSD (7), HF (8), LF/HF arány (9), rangsorolás: 1= nagyon sovány, 5= kövér (10)

The coefficients of correlation were also calculated between the examined traits. Only the most important results of this study are presented here.

A medium strong correlations were found in farm 1 between body condition and temperament ($r= 0.66$, $p<0.001$). Negativ tendency was observed for relationships between HR and HF ($r= -0.59$, $p<0.001$) as well as between HF and LF/HF ($r= -0.66$, $p<0.001$). In farm 2, milk production shows a moderate negative correlation with the body condition score ($r= -0.44$, $p<0.001$). The other traits didn't correlate strongly enough with milk production; their coefficient was less than 0.4.

The main results of principal component analysis (PCA) are summarized in *Tables 3-4*.

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Table 3: **Eigenvalues, explaind variance, principal components structure (rotated loadings) in Farm 1**

Traits (1)	Components (2)			
	1	2	3	4
Daily milk yield, kg (3)	0.005	-0.030	**-0.999**	0.011
Body condition score (4)	0.017	**0.946**	0.121	0.041
Temperament score (5)	-0.019	**0.818**	-0.186	0.104
Heart rate, beat/min (6)	**-0.842**	-0.282	0.124	0.178
RMSSD, ms (7)	0.045	0.131	-0.008	**0.949**
HF (8)	**0.869**	-0.042	0.068	0.125
LF/HF ratio (9)	**-0.741**	0.297	0.002	-0.181
Eigenvalue (10)	*2.523*	*1.839*	*1.090*	*0.533*
Variance of eigenvalue, % (11)	36.0	26.3	15.6	7.6

3. Táblázat: A sajátértékek, a sajátérték variancia, a faktorok és faktorsúlyok az első farmon jellemzők (1), komponensek (2), napi tejtermelés, kg (3), kondíció (4), temperamentum (5), szívritmus (6), RMSSD (7), HF (8), LF/HF arány (9), sajátérték (10), sajátérték variancia (11), teljes variancia (12)

On farm 1, four factors were determined as follows: *HRV parameters (1), body condition-temperament and temperament scores (2), milk production (3) and RMSSD (4).* In case of factor 1, the individual factor loadings (>0.6) involved in HF, HR and LF/HF played predominant roles (variance of eigenvalue: 36.0%). In the factor 2, the total variance was increased by the body condition (0.946) and the temperament (0.818) as well. In this case, we calculated a relatively high (26.3%) variance of eigenvalue. The factor 3 (variance of eigenvalue: 15.6%) was determined by only one parameter (daily milk yield: -0.999). In the last factor RMSSD involved with very good individual factor loading (0.949). In farm 1, we proved over 85.5% of the total variance.

In farm 2, we also observed four factors as follows: *traits of HRV (1), body condition and daily milk yield (2), temperament (3)* and *RMSSD (4).*In case of factor 1, three individual factor loadings (LF/HF, HF, heart rate) played important roles to the variance of eigenvalue: 29.5 %. In factor 2, the best individual factor loading was calculated for body condition (-0.869). The effects of the daily milk yield (0.824) and body condition in absolute value were similar to the variance. In this case, the variance of eigenvalue was 27.1 %. Factor 3 was determined by one parameter (temperament: -0.977). The variance of eigenvalue (13.6 %) of factor 3 was similar to the one measured for factor 3 in farm 1 (15.6%). In the factor 4 the individual factor loading (0.959) of RMSSD was very high. In this farm, the 81.2% of the total variance was proved.

Table 4: **Eigenvalues, explaind variance, principal components structure (rotated loadings) in Farm 2**

Traits (1)	Components (2)			
	1	2	3	4
Daily milk yield, Kg (3)	0.022	**0.824**	0.168	0.079
Body condition, score (4)	0.068	**-0.869**	0.207	0.145
Temperament, score (5)	0.034	0.009	**-0.977**	0.057
Heart rate, bit/min (6)	**0.681**	0.312	-0.009	0.338
RMSSD, ms (7)	-0.110	-0.071	-0.050	**0.959**
HF (8)	**-0.814**	0.132	0.011	0.279
LF/HF ratio (9)	**-0.860**	-0.084	-0.034	-0.070
Eigenvalue (10)	*2.068*	*1.897*	*0.950*	*0.776*
Variance of eigenvalue, % (11)	29.5	27.1	13.6	11.0

4. Táblázat: A sajátértékek, a sajátérték variancia, a faktorok és faktorsúlyok a második farmon
jellemzők (1), komponensek (2), napi tejtermelés, kg (3), kondíció (4), temperamentum (6), szívritmus (6), RMSSD (7), HF (8), LF/HF arány (9), sajátérték (10), sajátérték variancia (11), teljes variancia (12)

Conclusion

The following conclusions can be drawn from this study.

- In both farms with similar herds foure factors explained a significant percentage of the total variance: 85% and 82%, respectively in Farm 1 and Farm 2.
- In both analyzes, different HRV characteristics were involved with the determinant factor weights in the first factor (0.7–0.9). The importance of the first factors is 36%, and 29% respectively.
- Analyzing the factor weights of the third and fourth background variables, it can be seen that the effect of daily milk yield and temperament is definitely separated and the determining role of RMSDD appears in another factor.

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