INTERIM SCIENTIFIC REPORT

on the implementation of the project TE 14/2022 '***Evaluating stress and welfare in cattle and water-buffalo: mapping physiological, behavioural and vocal indicators'*** *code* **PN-III-P1-1.1-TE-2021-0027**

Funding:	**State budget**
Programme name in PNCDI III:	**Programme 1 - Development of the national R&D system**
Subprogramme name:	**Subprogramme 1.1 - Human Resources**
Project type:	**Research projects to stimulate young independent teams**
Project title:	**Evaluating stress and welfare in cattle and water-buffalo: mapping physiological, behavioural and vocal indicators**
Total contract value:	**449.704,00 lei**
Contract duration:	**24 months**
Contracting authority:	**Executive Unit for the Financing of Higher Education, Research, Development and Innovation (UEFISCDI)**
Contractor:	**Institute of Research and Development for Bovine (ICDCB)**
Phase 1:	**Research on the use of vocal parameters and infrared-thermography to assess stress and welfare in bovines**
Implementation period	**05.15.2022 - 12.31.2022**
Acronym:	**BovineTalk**
Project code:	**PN-III-P1-1.1-TE-2021-0027**
Contract number:	**TE 14 / 2022**

AIM of the TE 14/2022 PROJECT: to investigate whether vocal parameters in cattle and water-buffalo, linked with other physiological and behavioural responses, can be indicative of well-being and stress, and whenever this indicators could ultimately be used as tools for assessing objectively animal welfare. To the best of our knowledge, this is the first project to investigate cattle and water-buffalo vocal parameters in order to develop science-based non-invasive welfare indicators.
Our hypothesis is that, individual distinctiveness and emotional state of the animals are encoded in their vocalizations, and that bioacoustics profiles of large domestic ruminants can be used as reliable indicators for behaviour, welfare and stress, in various farming contexts.

OBJECTIVES of the BovineTalk PROJECT are:

i) use of vocal and infrared-thermography (IRT) parameters in evaluating stress and welfare of cattle;
ii) use of stress biomarkers and accelerometry data in monitoring the health status of cattle;
iii) use of vocal and IRT parameters to assess stress and welfare in water buffalo;
iv) use of stress biomarkers and their correlation with vocal and IRT parameters in water-buffaloes.

ACTIVITIES implemented in Phase 1:

Activity 1.1 - Collection of sound emissions in cattle and analysis of vocal parameters;
Activity 1.2 - Use of IRT investigation in the assessment of the health status of cattle and water buffalo;
Activity 1.3 - Validation of bioacoustic and IRT indicators in cattle;
Activity 1.4 - Specialisation of the human resources involved in the project through the implementation of a scientific internship;
Activity 1.5 - Dissemination of partial results through the publication of a peer-reviewed scientific article and participation in conferences with presentations.

RESULTS OBTAINED IN PHASE 1 OF IMPLEMENTATION: RESEARCH ON THE USE OF VOCAL PARAMETERS AND INFRARED-THERMOGRAPHY TO ASSESS STRESS AND WELFARE IN BOVINES

RESULTS PLANNED FOR PHASE 1 (according to the project implementation plan):

- Database of vocal emissions in cattle;
- Database of thermographic images and their correlation with the health status of cattle;
- Scientific internship in bioacustics and the analysis of vocal parameters in farm species;
- Participation in two international conferences, presenting partial data from the project;
- Scientific article submitted for publication.

The implementation of the BovineTalk project activities planned in phase 1 took place mainly in the Experimental Farm and the Production Systems Laboratory of the Research and Development Institute for Bovine Balotesti, and to a lesser extent in the Experimental Farm of the Research and Development Station for the Buffaloes Sercaia (exclusively for the validation of the infrared thermographic method to assess stress in lactating buffalo cows).

In order to comply with the legislation in force and international good practice on research involving animals, the approval of the RDIB Ethics Committee for monitoring the TE14/2022 project was obtained. Furthermore, all project activities complied with the EU Directive 2010/63 on the protection of animals used for scientific purposes.

The animals involved in the study were as follows:

- multiparous dairy cows, Romanian Black and White HF breed, lactations II-IV, 94 heads;
- un-weaned calves 0-3 months, 25 heads;
- multiparous water buffalo cows of the Romanian Buffalo breed, lactations II-IX, 68 heads.

Vocal emission recording in cattle was performed using the following equipment (Fig. 1):

- Sennheiser MKH 416-P 48 U3 super-cardioid broadcast microphone (40-20,000 Hz);
- Rode NTG2 phantom power microphone (20-20,000 Hz);
- Marantz PMD661MKIII 4-channel audio recorder with file encryption;
- DIGITAL SLR DR-70 audio recorder with 4 channels and linear audio recording.

Contexts studied in the Experimental Farm of RDIB Balotesti:

- isolation of cows and calves (I - visual isolation & II - visual and auditory isolation);
- stress habituation (monitoring of cows and calves in isolation for 4-6 hours);
- positive contexts in cows (return to group, communication with conspecifics, anticipation of feeding);
- positive contexts in calves (return to gropu, anticipation of feeding, feeding of milk, view of animal-caretaker, housing in group pens);
- positive contexts in heifers 12-18 months (anticipation of feeding, estrus period, social interactions).

After recording the sounds, the files were tagged according to context and animal, and then analysed using Praat® bioacoustic analysis software. After analysis with the specific software, a sound emission database was built, for each sound emission a number of 24 parameters were calculated as follows: call type (closed-mouth or low-frequency and open-mouth or high-frequency); Mean F0; Max F0; Min F0; Range F0; Q25%; Q50%; Q75%; Fpeak; sound duration (s); AM var; AM rate; AM extent; harmonicity; F1 mean; F2 mean; F3 mean; F4 mean; F5 mean; F6 mean; F7 mean; F8 mean; formant dispersal and wiener entropy (as recommended by Torre M. P. et al, Appl. Anim. Behav. Sci. 163: 58-68, https://doi.org/10.1016/j.applanim).

Figure 1. Vocalization recordings in 0-3 months calves at ICDCB Balotești (left photo) and in lactating cows at INRA*E* France (right photo)

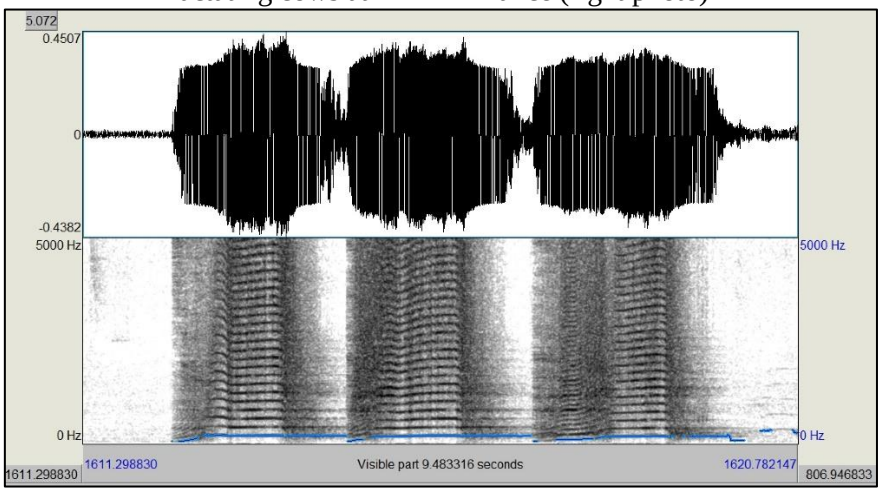

Figure 2. Oscillogram and spectrogram of a sound emission by adult cow, open-mouth vocalization (high frecuency call) in negative context (isolation)

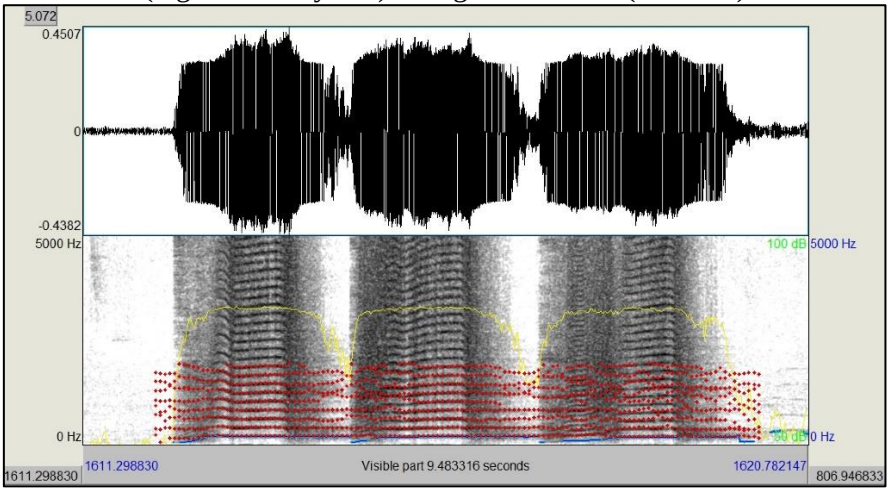

Figure 3. Oscillogram and spectrogram of a sound emission by adult cow (same as Fig. 2), sound intensity shown in yellow and sound pulse in red

Table 1. Means and dispersion indices for vocal parameters in adult cows (n=10, 10 sounds analysed per animal) subjected to isolation for 4 hours, sounds emitted with open mouth (high frequency calls), *preliminary results*

Vocalization parameter	**High-frequency calls within the first hour after isolation**			**High-frequency calls within 3-4 hours after isolation**		
	Mean±SEM*	**Min.**	**Max.**	**Mean±SEM***	**Min.**	**Max.**
F0 (Hz)	**183.86±7.24[a]**	165.42	205.31	**196.0±11.0[b]**	170.8	234.3
Max. F0 (Hz)	259.86±7.96[a]	240.88	284.89	250.4±17.1[a]	208.8	293.1
Min. F0 (Hz)	**93.5±11.4[a]**	71.4	136.2	**80.20±7.42[b]**	66.45	108.30
Gama F0	166.39±9.79[a]	144.99	199.78	170.2±21.7[a]	100.5	212.1
Q25% (Hz)	**310.4±49.2[a]**	212.4	431.9	**211.0±10.7[b]**	185.4	247.0
Q50% (Hz)	**439.1±59.9[a]**	275.9	608.1	**360.9±23.5[b]**	297.5	430.1
Q75% (Hz)	**1071.0±230[a]**	561.0	1740.0	**824.0±103.0[b]**	522.0	1116.0
Peak F (Hz)	122.7±11.7[a]	95.2	162.4	119.0±14.0[a]	98.9	173.8
Duration (s)	1.851±0.243[a]	1.370	2.684	1.656±0.177[a]	1.108	2.058
Variability AM	42.15±7.79[a]	21.98	65.82	35.18±8.70[a]	14.16	56.60
Rate AM (s^{-1})	10.856±0.879[a]	8.754	13.410	10.352±0.696[a]	8.577	12.150
Degree AM (dB/s)	3.826±0.506[a]	2.014	4.908	3.461±0.848[a]	1.291	5.101
Harmonicity (dB)	9.59±1.13[a]	5.39	11.69	8.22±1.48[a]	3.57	11.72
Mean F1 (Hz)	**320.4±21.6[a]**	272.5	382.7	**286.8±16.6[b]**	256.0	350.6
Mean F2 (Hz)	603.2±15.4[a]	550.6	638.1	626.8±22.4[a]	551.8	685.6
Mean F3 (Hz)	985.0±26.8[a]	886.5	1042.1	966.8±25.6[a]	882.3	1033.3
Mean F4 (Hz)	1361.7±28.4[a]	1274.3	1438.6	1345.2±20.9[a]	1274.4	1389.0
Mean F5 (Hz)	1724.2±29.2[a]	1625.7	1791.9	1728.0±21.8[a]	1655.4	1770.4
Mean F6 (Hz)	2117.1±28.2[a]	2022.4	2182.4	2119.6±11.5[a]	2081.0	2148.2
Mean F7 (Hz)	2535.6±22.0[a]	2465.4	2583.7	2524.7±10.4[a]	2501.4	2557.9
Mean F8 (Hz)	2853.1±18.6[a]	2807.1	2918.0	2862.1±27.3[a]	2784.9	2935.4
Dispersal (Hz)	361.82±5.05[a]	349.67	376.45	367.90±5.13[a]	355.45	382.77
Wiener entropy	**-1.69±0.22[a]**	-2.22	-1.05	**-1.48±0.07[b]**	-1.72	-1.27

** Note: For means with different superscript the p-value is ≤0.05*

Table 2. Means and dispersion indices for vocal parameters in adult cows (n=10, 10 sounds analysed per animal) subjected to isolation for 4 hours, sounds emitted with closed mouth (low frequency calls), *preliminary results*

Vocalization parameter	**Low-frequency calls within the first hour after isolation**			**Low-frequency calls within 3-4 hours after isolation**		
	Mean±SEM	**Min.**	**Max.**	**Mean±SEM**	**Min.**	**Max.**
F0 (Hz)	83.80±2.05[a]	77.71	89.25	81.10±3.03[a]	72.35	89.62
Max. F0 (Hz)	95.59±4.41[a]	85.75	106.61	91.71±5.56[a]	75.33	104.34
Min. F0 (Hz)	69.91±1.98[a]	65.63	75.37	66.33±1.39[a]	61.65	69.01
Gama F0	25.67±5.99[a]	11.83	40.00	25.38±5.24[a]	12.82	39.66
Q25% (Hz)	139.1±34.0[a]	84.0	254.4	136.3±14.1[a]	110.8	183.5
Q50% (Hz)	307.0±95.0[a]	102.7	640.9	334.9±69.2[a]	187.1	567.9
Q75% (Hz)	**1201.0±481.0[a]**	172.0	2462.0	**883.0±212.0[b]**	358.0	1544.0
Peak F (Hz)	99.2±13.7[a]	78.8	153.4	110.5±23.9[a]	75.2	203.8
Duration (s)	1.082±0.129[a]	0.784	1.478	1.025±0.060[a]	0.874	1.202
Variability AM	35.94±8.39[a]	15.66	66.68	38.50±18.58[a]	8.86	65.76
Rate AM (s^{-1})	7.58±1.02[a]	5.10	10.70	7.74±1.18[a]	5.15	10.70
Degree AM (dB/s)	5.08±1.38[a]	2.52	9.91	4.86±0.69[a]	3.10	6.57
Harmonicity (dB)	10.82±2.35[a]	4.17	16.84	8.01±2.21[a]	1.20	14.43
Mean F1 (Hz)	304.9±25.1[a]	234.9	356.7	319.1±32.5[a]	254.9	440.6
Mean F2 (Hz)	749.5±30.0[a]	667.4	840.6	730.6±48.6[a]	578.9	884.4
Mean F3 (Hz)	1211.7±13.6[a]	1179.2	1241.8	1157.7±26.5[a]	1073.4	1226.8
Mean F4 (Hz)	1611.1±9.9[a]	1586.4	1635.4	1575.7±22.3[a]	1505.3	1636.2
Mean F5 (Hz)	2076.6±39.9[a]	1986.7	2225.3	2011.5±10.7[a]	1974.0	2039.7
Mean F6 (Hz)	**2580.5±18.6[a]**	2512.4	2618.7	**2483.1±9.88[b]**	2445.2	2503.3
Mean F7 (Hz)	**3058.8±28.9[a]**	2978.2	3141.1	**2930.0±35.3[b]**	2856.1	3050.1
Mean F8 (Hz)	3276.4±40.4[a]	3156.9	3363.9	3252.3±99.7[a]	2890.2	3476.1
Dispersal (Hz)	424.50±8.3[a]	400.03	447.00	419.0±18.6[a]	349.9	454.6
Wiener entropy	-1.59±0.35[a]	-2.44	-0.74	-1.11±0.13[a]	-1.44	-0.66

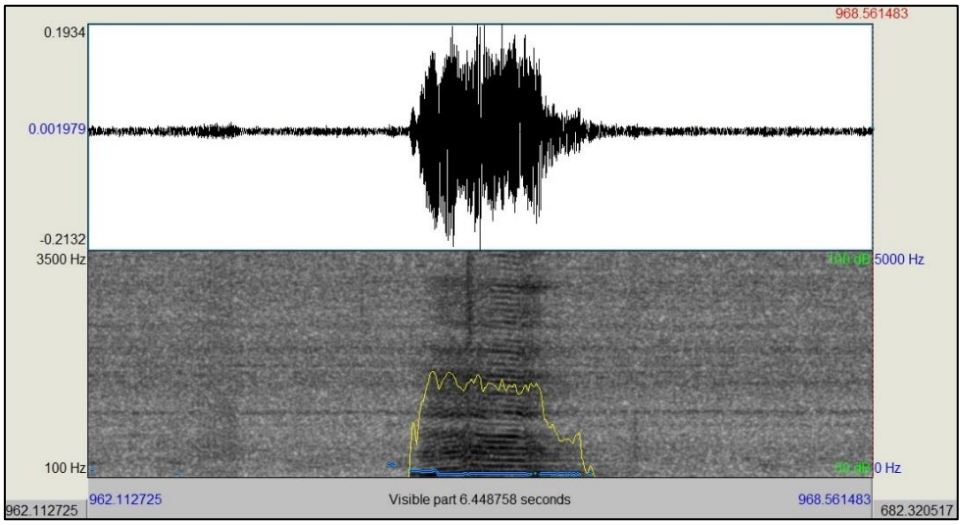

Figure 4. Oscillogram and spectrogram of an adult cow sound emission, low-frequency vocalization, sound emitted with mouth closed

Infrared thermography (IRT) data recording in cattle and water-buffalo was performed using two FLIR® Pro Thermal mobile cameras with a resolution of 19200 pixels, temperature measurement range from -20°C to +400°C. Thermal imaging data were stored and processed using specific VividIRTM software.

The collection of thermal imaging data in the dairy cattle and the lactating buffalo cows herds included in the study were aimed at the following contexts: pre- and post-milking; isolation of animals; metabolic diseases; mammary gland diseases and disorders (clinical, sub-clinical mastitis, mechanical lesions); lameness with various etiologies; calves 0-3 months at neutral temperatures and heat stress (>35°C); estrus period in cows and heifers; cows in the last 48 hours before calving; before and after weaning of calves; at separation of dam-cow from calf.

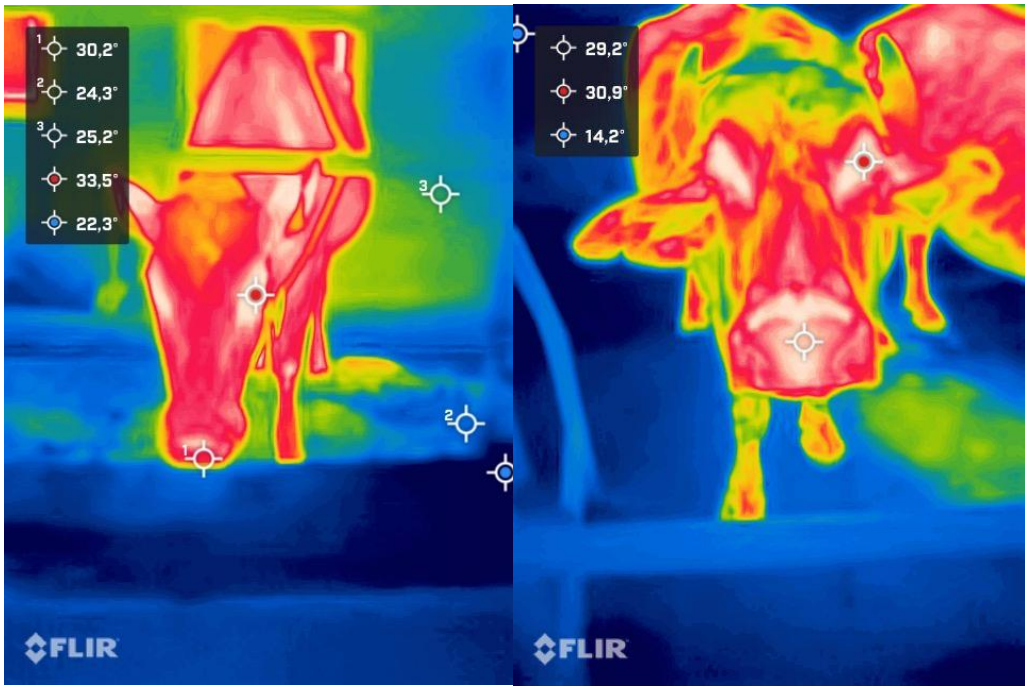

Figure 5. Aspects of the use of infrared thermometry (IRT) in dairy cattle (left photo) and water buffaloes (right photo)

Table 3. Means and dispersion indices for infrared thermal imaging data (IRT) in dairy cows subjected to isolation (n=20), for 4 hours post-milking, with assessment of ocular and nasal regions temperature

Variable	Mean±SEM	DS	CV	Min.	Max.	Q1
IRT nasal region at 0 hours [°C]	27.86±0.546	2.44	8.76	21.60	31.10	26.17
IRT nasal region at 2 hours [°C]	29.87±0.329	1.47	4.93	26.70	32.30	29.07
IRT nasal region at 4 hours [°C]	29.13±0.533	2.38	8.19	22.40	31.90	27.45
Differences 0 vs. 2 hours		*p=0.0055, ** *				
Differences 0 vs. 4 hours		*p=0.0698, NS*				
Differences 2 vs. 4 hours		*p=0.5884, NS*				
IRT ocular region at 0 hours [°C]	31.51±0.459	2.05	6.51	26.10	34.90	30.80
IRT ocular region at 2 hours [°C]	32.54±0.295	1.31	4.05	29.20	34.50	32.10
IRT ocular region at 4 hours [°C]	31.74±0.449	2.00	6.33	27.20	34.30	30.40
Differences 0 vs. 2 hours		*p=0.0482, * *				
Differences 0 vs. 4 hours		*p=0.4902, NS*				
Differences 2 vs. 4 hours		*p=0.2180, NS*				

*Note: Statistical differences were tested with Mann-Whitney U Test, NS p>0.05; * p≤0.05; ** p≤0.01*

Table 4. Means and dispersion indices for infrared thermal imaging data (IRT) in lactating buffalo cows (n=68), pre- and post-milking, with assessment of ocular and nasal region temperature

Milking temperament	IRT nasal region [°C]		IRT ocular region [°C]	
	pre-milking	post-milking	pre-milking	post-milking
Cohort	29.33 ± 0.296	29.47 ± 0.392	31.75 ± 0.192	31.74 ± 0.422
Calm	29.46 ± 0.305	29.31 ± 0.532	31.76 ±0.263	31.61 ± 0.591
Nervous	29.02 ± 0.734	29.86 ± 0.420	31.74 ±0.209	32.06 ± 0.289
Differences calm vs. nervous	*p=0.916, NS*	*p=0.712, NS*	*p=0.958, NS*	*p=0.958, NS*

DVM Madalina Mincu, Young Researcher and PhD student at the Host Institute, member of project no. TE 14/2022, carried out a scientific internship at the National Research Institute for Agriculture, Food and the Environment (INRA*E*) UMR PEGASE Rennes - Saint Gilles, France, in order to specialize in the field of bioacoustics, the internship coordinator on behalf of INRAE was Mrs. Dr. Céline Tallet.

The programme of the scientific internship was as follows:

- 19 - 26 June: introduction to the study of mammalian vocalizations, study of literature and recordings in the experimental dairy cattle farm La Rheu - INRA*E*;

- 27 - 30 June: analysis of vocal parameters recorded during the first week of the study, using specific software (Praat®);

- 27 June: participation in an online meeting (Zoom platform) with Prof. Elodie Briefer from the University of Copenhagen - Denmark, specialist in vocal communication in cattle, in order to learn the elements of statistics applied to bioacoustics;
- 28 - 29 June: participation (online) in the international conference UFAW2022: Advancing Animal Welfare Science, organised by the International Society of Applied Ethology (ISAE).

Main activities implemented:

- Learning the principles of vocal communication in mammals: what is vocal communication? What is the mechanism of sound production in animals? Source-filter theory of sound production;
- Creation of a database with the vocal repertoire of cattle from the La Rheu experimental farm. Sounds were recorded in 4 different contexts: leaving the pasture, returning from the pasture, in the waiting area of the milking parlour and in anticipation of feeding;
- Analysis of recorded sounds with Praat® software.

DISSEMINATION OF RESULTS:

Scientific article:

Mincu M., Gavojdian D., Nicolae I., Olteanu A.C., Bota A., Vlagioiu C., 2022, Water Buffalo Responsiveness during Milking: Implications for Production Outputs, Reproduction Fitness and Animal Welfare, *Animals*, 12(22), 3115; https://doi.org/10.3390/ani12223115 (ISSN 2076-2615, impact factor: 3,231, Q1 '*Agriculture, Dairy & Animal Science*' and '*Veterinary Sciences*' in WoS categories

Attending conferences:

Gavojdian D., Mincu M., Evaluating Cattle Welfare Throughout the use of Behavioural and Vocal Indicators: A Review, *Animal Resources Bioengineering - Multidisciplinary Conference on Sustainable Development* (pp. 46), 26-27.05.2022, Timisoara (oral and poster presentation)

Mincu M., Gavojdian D., Cattle Vocal Parameters as Non-Invasive Animal Welfare Indicators: Potential Uses and Current Developments, *Book of Abstracts Anthrozoology Symposium 5th Edition – Non-human Animals in Open Societies* (pp. 22), 4-5.11.2022, Iasi (oral presentation).

Project team:

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