JAST (Journal of Animal Science and Technology) TITLE PAGE

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ARTICLE INFORMATION	Fill in information in each box below
Article Type	Research article
Article Title (within 20 words without abbreviations)	Effect of low frequency oscillations during milking on udder temperature and welfare of dairy cows
Running Title (within 10 words)	Low frequency oscillations on udder temperature and welfare of cows
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Competing interests	No potential conflict of interest relevant to this article was reported.
Funding sources	Not applicable.
State funding sources (grants, funding sources, equipment, and supplies). Include name and number of grant if available.	
Acknowledgements	Not applicable.
Availability of data and material	Upon reasonable request, the datasets of this study can be available from the corresponding author.
Authors' contributions	Conceptualization: Sederevičius A., Bubulis A.,
Please specify the authors' role using this form.	Data curation: Oberauskas V., Musayeva K.
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	A. Software: Oberauskas V., Vėžys J. Validation: Sederevičius A., Oberauskas V., Musayeva K., Jūrėnas V., Bubulis A., Vėžys J.		
	Investigation: Sederevičius A., Žemaitis J. Bubulis A., Vėžys J. Writing - original draft: Musayeva K., Vėžys J.		
	Writing - review & editing: Sederevičius A., Oberauskas V., Želvytė R., Žymantienė J., Musayeva K., Žemaitis J., Jūrėnas V., Bubulis A., Vėžys J.		
Ethics approval and consent to participate	The experimental protocols and animal care/handling used in this study comply with the Directive 2010/63/EU, the European Union legislation on the protection of animals used for scientific purposes and is approved by the State Food and Veterinary Service (2021-05-04 No. B6-(1.9.)-1131).		

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7 ABSTRACT

8 The study aimed to investigate the effect of low-frequency oscillations on the cow udder, milk parameters, and animal 9 welfare during the automated milking process. The study's objective was to investigate the impact of low-frequency 10 oscillations on the udder and teats' blood circulation by creating a mathematical model of mammary glands, using 11 milkers and vibrators to analyze the theoretical dynamics of oscillations.

12 The mechanical vibration device developed and tested in the study was mounted on a DeLaval automatic milking 13 machine, which excited the udder with low-frequency oscillations, allowing the analysis of input parameters 14 (temperature, oscillation amplitude) and using feedback data, changing the device parameters such as vibration 15 frequency and duration. The experimental study was performed using an artificial cow's udder model with and without 16 milk and a DeLaval milking machine, exciting the model with low-frequency harmonic oscillations (frequency range 17 15 - 60 Hz, vibration amplitude 2 - 5 mm). The investigation *in vitro* applying low-frequency of the vibration system's 18 first-order frequencies in lateral (X) direction showed the low-frequency values of 23.5 - 26.5 Hz (effective frequency 19 of the simulation analysis was 25.0 Hz). The tested values of the first-order frequency of the vibration system in the 20 vertical (Y) direction were 37.5 - 41.5 Hz (effective frequency of the simulation analysis was 41.0 Hz), with higher amplitude and lower vibration damping. During in vivo experiments, while milking, the vibrator was inducing 21 22 mechanical milking-similar vibrations in the udder. The vibrations were spreading to the entire udder and caused 23 physiotherapeutic effects such as activated physiological processes and increased udder base temperature by 0.57 °C 24 (P <0.001), thus increasing blood flow in the udder. Used low-frequency vibrations did not significantly affect milk 25 vield, milk composition, milk quality indicators, and animal welfare.

The investigation results showed that applying low-frequency vibration on a cow udder during automatic milking is a non-invasive, efficient method to stimulate blood circulation in the udder and improve teat and udder health without changing milk quality and production.

29 Further studies will be carried out in the following research phase on clinical and subclinical mastitis cows.

30 Keywords: low-frequency vibrations, milk parameters, animal welfare.

32 INTRODUCTION

33 The number and variety of vibration applications in the medical treatment of blood flow have increased over the last 34 decades. However, robust evidence on the significance of vibration effects (especially treatment effectiveness) is still 35 lacking [1-5]. The treatment capabilities of the low power acoustic pulse therapy (APT) have been widely reported and 36 is known to produce various responses in biological tissues, such as angiogenesis and anti-inflammatory effects [1], 37 endorses the healing process in musculoskeletal diseases in humans [2], race horses [3], dogs [4] and partial-thickness 38 wounds in piglets [5]. The low power acoustic pulse therapy pressure $(10\pm15 \text{ megapascal (MPa)})$ was shown to produce 39 new blood vessels and improve tissue function with long term effects [1]. In race horses, low power APT improved 40 lameness disorders such as insertional desmopathies, tendinopathy, osteoarthritis myopathies, arthrosis and 41 podotrochlosis syndrome [2]. In dogs, APT was used for shoulder lameness, degenerative joint disease, Legg Calve 42 Perthes disease (degeneration of the head of the femur bone in the hind leg) osteoarthritis and spondylosis [4]. The 43 influence of shock waves on the reepithelialization of partial-thickness wounds was studied in Yorkshire piglets. The 44 stimulating effect of low-energy shock waves coincides with significantly increased vascularization of the upper dermis 45 and thicker layer of the newly formed epithelial cells covering the wound [5].

In 1996, Nakamura et al. [6] were the first researchers to claim that vibration used for research affects blood flow 46 47 differently from vibration excited in the professional environment. The research showed that whole-body vibration 48 influenced the blood flow in arteries. Most studies evaluated and analyzed the influence of high-frequency vibration on the human body, whereas only a few studies investigated low-frequency vibration effects [6-8]. Other researchers 49 50 studied whole-body vibration effects on the blood flow in legs and found that the body was more sensitive to 5 Hz 51 vibration than 50 Hz vibration. The influence of vibration intensity on the heart and blood vessel activity during 52 physical workload was also proven [7-8]. Osawa and Oguma [9] found that whole-body vibration had a positive effect 53 on arterial blood flow and other parameters of the circulatory system.

Many studies are investigating the effects of vibration on the whole body [7-9] however, there is a lack of studies on
vibration effects on certain/specific body parts. The uniqueness of this study is that we have determined the influence
of locally-generated low-frequency (15-60 Hz) mechanical oscillations on cow udder.

57 The known devices developed by foreign scientists for exciting the udder by the acoustic or electromagnetic waves of
58 a particular frequency cause harmonic and directional oscillations of the frequencies directed to a target body area, e.g.,
59 to the udder, to be excited [10-11]. The device produces low power pulses with capability of deep tissue penetration
60 that allows the pressure wave to be distributed over a large treatment area of the cow's udder capable of producing
61 therapeutic effect [10, 12].

62 Carefully selected physiologically resonant frequencies can both suppress disharmonious fluctuations that occur in
 63 affected cells during inflammation, and normalize metabolic processes, accelerating the transport of inflammatory

64 products due to the known effect of increasing vascular permeability [13], while recording weak currents caused by

65 potentials of the udder cells themselves [14, 15].

66 The use of non-invasive methods ensures animal welfare and health, and the effects of acoustic low-frequency 67 vibrations on the mammary glands are likely to stimulate blood circulation in cow udder and reduce the incidence of 68 subclinical mastitis in dairy cows.

This is a novel pilot study, and of some interest to veterinary professionals working in the dairy sector. The objective of this study was to validate the operating principle, optimize the dimensions of the vibrating system, and investigate the dynamics of the 3D model of the cow udder, to investigate the impact of low-frequency oscillations on the udder and teats' blood circulation by creating a mathematical model of mammary glands, using milkers and vibrators to analyze the theoretical dynamics of oscillations, to investigate the impact of low-frequency oscillations on the milk yield and quality parameters, and animal welfare.

75

76 MATERIALS AND METHODS

77 The experimental studies were implemented in three stages:

In the first stage, the analytical investigation and calculations were performed with the 3D dynamic model of the cow udder excited with an unbalanced rotating body during the automatic milking process (Fig.1). The experiment aimed to validate the operating principle, optimize the dimensions of the vibrating system, and investigate the dynamics of the model.

82 Then the experimental model of a cow's artificial udder (natural rubber udder of cow-NRUC MODEL produced by 83 LSMU) (Fig. 2) was developed. The udder was made from 3 mm thick rubber with four teats with holes. The artificial 84 udder holds up to 20 litres of milk. The milking rack is designed to replicate a cow's hind leg and consists of a frame 85 with a cover plate between which the rubber udder is clamped.

For evaluating the frequency response of the vibrating system, the studies were performed on the developed artificial cow's udder with and without milk by applying unbalanced vibration motor oscillations in various directions of the artificial udder - in lateral (*X*-axis) and vertical (*Y*-axis), with four directions on a-X axis, b-Y axis, c-X axis, and b-Y axis (Fig. 2). More about the research methodology in the chapters "Mathematical dynamic model of an unbalanced rotor" and "Experiment tests *in-vitro*".

- 91 In the third stage, the application of the testing rig for experiments with a low-frequency harmonic oscillation device
- 92 on a milking system (DeLaval Harmony) on a live animal was used. More about the research methodology in the
- 93 chapter "Experimental tests *in-vivo*." Invention: Cow Udder Stimulation System. Applicants: Kaunas University of
- 94 Technology and Lithuanian University of Health Sciences. Date of submission of application No. 2021-542:12.8.2021.

96 Dynamic model of an unbalanced rotor

97 The dynamic modeling of the udder excited with an unbalanced rotating body is based on the so-called Jeffcott model 98 of an unbalanced rotor [16]. It presents a model and consists of an unbalanced rotor attached to the teat cup of the 99 milking unit, of which one end is connected to a teat and the other - to a claw by a milk tube (Fig. 1a). The model has 100 three degrees of freedom described by three differential equations of the second order. The rotor has only two degrees of freedom because an unbalanced vibratory motor is attached to the teat cup with the mass m as a mass point. It can 101 102 move only in the plane perpendicular to the rotor's axis. The dynamic model in Fig.1b presents the vibratory motion 103 of the mass m in the X-Y plane, and the horizontal Z-axis coincides with an unbalanced rotor's axis. During rotation, 104 the center of gravity moves along an orbit trajectory.



Fig. 1. Diagram of a milking unit with an unbalanced vibratory motor for excitation of the udder: (a) 1 – udder,
2 – teat, 3 – mouthpiece, 4 – unbalanced motor, 5 – vibrators, 6 – shell, 7 – short milk tube, 8 – stand; (b) its simplified
2D dynamic model with vibratory motion in the X-Y plane; (c) milking unit (claw) and pipeline milking system; 9 –
long pulse tube, 10 – milking unit, 11 – long milk tube (milk hose), 12 – claw, 13 – short milk tube, 14 – short pulse
tube, 15 – teat cup shell [17].

111

105

112 A simplified dynamical model of the milking unit with an unbalanced motor is presented in Fig.1a and 1b. The model 113 also contains flexural rigidity and damping of the milk hose and teat, which can be considered spring and damper 114 producing vibration of/in the unbalanced rotor. In this way, the unbalanced vibratory motor is connected with a teat-115 cup attached to the udder and a claw through springs and dampers. The oscillations in the X and Y or Z directions are 116 actuated by time-variable radial components of the rotating vector of harmonic force. It is a consequence of the 117 unbalanced rotating rotor (Fig. 1b). The rotor axis is perpendicular to a rotating vector of the harmonic force $F_c = m_o$ $e \,\omega^2$ where m_o and e are unbalance mass and eccentricity, respectively, and ω is the angular velocity of the rotor. α_x, α_y 118 119 and α_{z} are anglers between the axis of a rotor and the X-axis, Y-axis, and Z-axis, respectively.

120 Some parameters of the dynamical model were determined experimentally, and used in the theoretical investigation

121 and calculations. These parameters were obtained using the DeLaval Harmony model milking unit. They are shown in

122 Table 1.

123

124 Table 1. Parameters used in the theoretical calculations

Parameters					
kx	Stiffness in X axis	250	N/m		
k _y	Stiffness in Y axis	625	N/m		
m	Mass of the construction	0.36	kg		
mo	Mass of the unbalanced rotor	0.05	kg		
c_x	Damping coefficient in X axis	2.3			
cy	Damping coefficient in Y axis	6			
e	Ecentricity of mass m ₀	0.005	m		

¹²⁵

126 Motion equations for the described substitute model are derived from the Newton's Second Law:

127 $m\ddot{x} + b_x\dot{x} + k_xx = F_c \sin\alpha_x \cos(\omega t)$

128 $m\ddot{y} + b_y\dot{y} + k_yy = F_c sin\alpha_y sin(\omega t)$

129
$$m\ddot{z} + b_z\dot{z} + k_zz = F_c \sin\alpha_z \sin(\omega t)$$

130 where x, y, z – displacements in the X-axis, Y-axis and Z-axis, respectively; b_x , b_y , b_z – damping in the X-axis, Y-axis

(1)

(2)

(5)

(3)

(4)

131 and Z-axis, respectively; k_x , k_y , k_z - stiffness in the X-axis, Y-axis and Z-axis, respectively. For the centrifugal force F_c ,

132 caused by unbalance, we can derive:

$$133 \qquad F_c = m_o r_o \omega^2$$

- 134 In this case, we further analyzed a simplified dynamic model with oscillations in the X-axis and Y-axis. It was assumed
- that the Z-axis coincides with the axis of the unbalanced rotor, and motion in this axis is negligible compared to the
- 136 motion in *X*-axis. The equation of motion in the *X* direction can be written as:

137
$$m\ddot{x} + b\dot{x} + kx = m_0 e\omega^2 \sin(\omega t)$$

138 where *m* is the mass of the vibrating structure, *b* is the damping present in the system, and *k* is the system's stiffness.

139 Steady-state amplitude *X* and phase angle due to vibration caused by rotating unbalance is given by

140
$$X = \frac{m_0 \omega^2 e}{\sqrt{(k - m\omega^2)^2 + (b\omega)^2}}$$
(6)

141 The dynamics in the *Y*-axis were investigated analytically using the same methodology with the stiffness and damping142 coefficients in the *Y* direction.

143

144 Experimental tests in-vitro

In the second stage, the experiment was conducted on an artificial cow udder model produced by LSMU to validate the proposed model. An unbalanced vibratory motor was made for the experimental research and applied to the DeLaval Harmony model milking unit. Four vibrators were attached to each teat cup. Only one vibrator of four was powered during one measurement, and only one - was powered with different frequencies and amplitudes. Measurements were done on all four teats independently. Fig. 2 presents the testing rig setup for the excitation of lowfrequency oscillations on the artificial cow udder model.

151



Fig. 2. Setup of the testing rig for the application of low-frequency harmonic oscillation device on the artificial
cow udder model: (a) experimental setup, (b) basic view of the model (stand): 1 – *X*-axis accelerometer KD35 (RFT
GmbH, Germany), 2 – computer, 3 – power supply HY1803D (V&A Instruments Ltd., China), 4 – oscilloscope
PicoScope 3424 (Pico Technology Ltd., GB), 5 – teat of the model, 6 – unbalanced vibratory motor, 7 – *Y*-axis
accelerometer KD35 (RFT GmbH, Germany), 8 – milker device (Harmony model), 9 – artificial cow udder model.

158

The frequency responses were measured with an empty artificial cow udder model, and then, to simulate natural udderconditions, the artificial udder model was filled with milk.

161 Experimental tests in-vivo

162 In the third stage, the same low-frequency oscillations (range 15 - 60 Hz) of the directional harmonic oscillation device

- 163 were applied on the milkers (DeLaval Harmony model) and tested on the cow during the milking process (Fig. 3). Four
- vibrators were used on each teat cup, but only one was driven in the determined frequency range during measurement.
- 165 Measurements were done on all four teats independently.



Fig. 3. Setup of the testing rig for the application low-frequency harmonic oscillation device on a live animal
(cow): (a) structural scheme, (b) milker with a special created vibrator and two accelerometers, (c) experiment during
milking process. 1 – cow udder, 2 – milker with the vibrators and accelerometers, 3 – holder, 4 – unbalanced vibratory
motor, 5 – oscilloscope PicoScope 3424 (Pico Technology Ltd., GB); 6 – computer, 7 – power supply HY1803D
(V&A Instruments Ltd., China), 8 – X-axis accelerometer computer, 9 – Y-axis accelerometer.

The power supply changed voltage with a step of 0.2V (1.2V - 3.8V), which resulted in the vibrator's operating frequency change from 15 Hz to 60 Hz. The tests were performed using the accelerometers KD35 (RFT GmbH, Germany). Two single-axis accelerometers were used on the testing rig to measure the vibration simultaneously in the lateral (*X*) and vertical (*Y*) directions. All the vibration data was recorded with an oscilloscope and analyzed using PC.

178 Animals and farm

179 The study was carried out on an organic dairy herd of 1330 Holstein-Friesian cows in 2021. The dairy parlors were 180 equipped with an online computerized DeLaval Herd milking system. About 14 thousand kg of milk was milked during 181 one milking with an average milk yield of 22.9±2.1 kg. Thirty multiparous lactating Holstein cows (mean 182 653.15 ± 100.24 kg of body weight, mean 137.93 ± 98.53 days in milk, mean 2.40 ± 1.40 of parities) were selected in the 183 experiment. Cows were randomly assigned to control and experimental groups. The data collection period lasted for 184 about two weeks. We analyzed all collected data on both, cows not treated with low-frequency vibration devices during 185 milking (control group n=15) and cows treated with low-frequency vibration devices during milking (experimental 186 group n=15). Milk yield was measured in real-time at each milking session by the automated milking system installed 187 at the milking parlor. The cows were milked twice a day at 05:00 and 17:00 h in a milking parlor. The animals were 188 kept in a loose housing system and were fed a feed ration throughout the year at the same time balanced according to

their physiological needs. The composition and feeding value of diet for all cows were as follows: Dry Matter (DM)

190 content of silage 55.6%, Metabolic Energy 9.87 MJ/kg DM, Net Energy for Lactation (NEL) 5.76 MJ/kg DM, Crude

191 Protein 13.1% DM, Crude Fat 2.75% DM, Crude Fibre 26.8% DM, Ash 8.6% DM. Cows had unlimited access to fresh

192 water and were fed a total mixed ration (TMR) *ad libitum*.

During the experiment *in vivo*, we analyzed the effect of low-frequency vibrations and evaluated daily milk yield, milk composition and quality, udder surface temperature, and dairy cows' welfare. Milk samples (n=60) were collected from each cow for the milk's composition and quality analysis. For determining somatic cell count, the milk samples (50 mL) were preserved with bronopol (2-bromo-2-nitropropane-1,3-diol and 2-bromo-2-nitropropanol) in micro tabs and analyzed by the flow cytometric analysis method using a Somascope cell counter (Foss, 3400 Hillerød, Denmark), according to the LST EN ISO 13366-1:2008+AC:2009 microscopic method standard. The lactose, fat, urea and milk protein levels were determined using the spectrophotometric method with infrared meter LactoScopeFTIR (FT 1.0.

200 2001; Delta Instruments, Netherlands).

201 Temperature measurement

Thermography provides valuable information for identifying simple thermal patterns resulting from changes in blood flow, which can be used to detect the inflammation or pathology presence in a target area. In this study, we used an infrared thermography camera T450sc (FLIR Systems Inc., USA) to determine the skin surface temperature changes in the mammary gland. The thermal image was captured when the entire test area (hindquarters of the udder) was visible on a camera screen. The average temperature of the mammary gland test surface was determined from the selected points in the thermogram.

The comparative analysis of frequency-amplitude characteristics in the lateral (*X*) and vertical (*Y*) directions showed the higher amplitude of vibrations at the 25 Hz frequency, which coincided with the resonance frequency in the lateral (*X*) direction. It was selected as the operating frequency for the thermography tests. The temperature measurements were taken using the 25 Hz frequency vibrations before and during the milking process.

212 Animal welfare assessment

213 The cow's grimace scale was assessed before and during the milking process. Cows' welfare was calculated by applying

the cow pain scale. For this purpose, cow discomfort was analyzed by facial expression, ear-head-eyes-nose position

- score-scale (0-2) adapted by Gleerup et al. 2015 and 2017 [18,19]. Moreover, back position evaluation was determined
- 216 where pain scale score meanings were as follows:
- 217 Facial expression: 0 attentive, neutral look, focused on a task, e.g., eating, ruminating, sleeping; 1-2 strained,
- tense/worried face, furrows above, eyes and wrinkles above the nostrils.

- Ear position: 0 both ears forward/one ear forward or back and the other listening; 1- ears back/asymmetric ear
- 220 movements, both ears back/moving in different directions; 2 both ears to the sides and lower than usual, pinna facing
- slightly down.
- Head position: 0 held high; 1- lower than withers; 2 very low.
- 223 Nose: nostrils dilatated; facial muscles relaxed, not in pain (1-2).
- Eyes: 0 clear eyes, bright, healthy/no pain; 1-2 tense muscles above the eyes/in pain.
- 225 Back position: 0 normal; 1- slightly arched back; 2 arched back.
- 226

227 Statistical analysis

- 228 For the theoretical equations, we used an analytical method. For the result analysis in vivo we used R statistical software
- 229 (v. 4.1.2). In terms of animal welfare, Pearson's χ^2 test was used to compare the observed cows' emotional body
- responses. One-way ANOVA and *t-test* were used to analyze milk yield, quality, and udder temperature. Significance
- 231 was declared at $p \le 0.05$ throughout this study. Data are presented as means and SD.
- 232

233 RESULTS

- 234 To determine amplitude-frequency characteristics of the udder model filled with milk and without milk, theoretical and
- 235 live animals were used. The results were obtained in two X (lateral) and Y (vertical) axes directions (Fig. 4). Milkers
- were applied on all four teats, but only one teat was excited during one vibration session. The resonance frequency was
- about 22.5 26.5 Hz on X-axis and Y-axis about 37.5 41 Hz (Table 2). The experimental udder model filled with
- 238 milk showed higher frequency values than the model without milk 6% at the lateral (X) direction and 8.5% at the
- vertical (Y) direction.



Fig. 4. Frequency-amplitude characteristics: (a) in *X*-axis when the model (stand) was without milk; (b) in *Y*-axis
when the model (stand) was without milk; (c) in *X*-axis when the model (stand) was filled with milk; (d) in *Y*-axis
when the model (stand) was filled with milk. LF is left front, LB – left bottom, RF – right front, RB – right bottom.

We can confirm that the frequencies on a live animal are nearly the same as on the model or in the theoretical calculations. The result was such because the cow udder was not affected by the resonance frequency system; only the structure of the milker and its rubber parts mainly were excited by the oscillations. The results are shown in Figure 5.

247



248

Fig. 5. Frequency amplitude characteristics: (a) in *X*-axis, on a live animal (cow); (b) in *Y*-axis, on a live animal
(cow). Only one teat was affected during one session of vibration; the teats are indicated as follows: LF- left front, LBleft bottom, RF-right front, RB-right bottom.

252

Also, using theoretical calculations, we get nearly the same results as in the experiments, and the character of the curves

is mostly the same. It is shown in figure 6.





Fig. 6. Frequency-amplitude characteristics using theoretical calculations on the *X* and *Y* axes.

258 We get a higher resonance frequency of 39.00 – 39.86 Hz (Table 2) on the *Y*-axis because the stiffness of the milker is

- higher on this axis.
- 260 The theoretical and experimental results show that the amplitudes on the X-axis are much higher (about 50% 70%)
- than on *Y*-axis. The amplitudes on a live cow are higher in both axes than in other results.
- 262

263 Table 2. Average resonance frequencies, Hz

	X axis	Y axis
Stand with the milk	24.97	39.86
Stand without the milk	24.48	39.05
Alive animal (cow)	24.33	39.63
Theoretical calculations	25	39

²⁶⁴

The mechanical vibrations, excited by the vibrator in the milker during the milking process of live animals, propagate 265 266 through the teats to the udder and produce a physiotherapeutic effect by increasing the udder surface temperature. The 267 obtained results showed that the surface temperature of the udder during milking of the cows in the experimental group 268 increased by 1.1°C compared to the surface temperature of the udder before milking. Meanwhile, the surface 269 temperature of the udder at the end of milking of the control group cows increased by 0.53 °C compared to the surface 270 temperature of the udder before milking. The data of temperature studies showed (Fig.7) that mechanical vibrations 271 during milking induced a more intense increase in the surface temperature of the udder (0.57 °C (P < 0.001)) compared 272 to the temperature before milking.



274 Fig. 7. Changes in superficial mammary gland temperature during milking

275

In the present study using the new device and applying the mechanical vibration of low frequency (25 Hz), we noticed
that cows' facial expression, the position of eyes, ears, head, back, and tense of nostrils did not change (Table 3). Lowfrequency vibration did not adversely affect any animal welfare parameters, including the cows' emotional state.

279

280 Table 3. Results of cows' welfare assessment between groups

		Gr	oups	
Parameters	Points	1	2	Pearson's chi-squared test
		Total	points	
Facial expression:				
Nose	0	15	11	$x^2 - 25062 df - 1 n > 0.05$
	1	0	4	$\chi = 2.3902, \text{ ul} = 1, p > 0.03$
Eyes	0	13	14	$x^2 - 0$ df - 1 p - 1
	1	2	1	$\chi = 0, uI = 1, p=1$
Ear position	0	15	15	$r^{2}-0$ df -1 r^{-1}
	1	0	0	$\chi = 0, \mathrm{di} = 1, \mathrm{p} = 1$
Head position	0	15	15	$v^2 - 0$ df -1 $v - 1$
	1	0	0	$\chi = 0, \text{ al } = 1, p = 1$
Back position	0	15	15	$\chi^2 = 0, df = 1, p = 1$

281 G

Group 1 – control group; Group 2 – experimental group

The effect of low-frequency vibrations on the milk yield, milk composition, and quality was analyzed. The study results revealed that the average somatic cell count and urea values of milk had increased by 3.5% and 2.88%, respectively, compared to the milk not affected by low-frequency vibrations, but this difference was not significant. Mechanical vibrations also had no significant effect on the milk yield and quality parameters mean values (Table 4).

287

288Table 4. Milk yield and milk quality results of cows treated with a low - frequency vibration (experimental289group) or without a low - frequency vibration (control group) during milking process. Data are presented as290mean ± standard deviation (SD)

	Milk yield Fat	Fat	Protein	Lactosa	Uroo	Somatic cell
Group		Totem Lactose	Lactose	Ulea	count	
-	kg/day	%	%	%	mg/100 mL	x 10 ³ cells/mL

Control	20.33±4.61	4.53±0.96	3.39±0.57	4.49±0.25	14.47 ± 5.02	50.53±32.33
Experimental	20.36±4.42	4.54±0.85	3.37±0.47	4.44±0.31	15.33±4.81	54.20±37.69
p-value	p=0.984	p=0.989	p=0.925	p=0.594	p=0.633	p=0.777

292 DISCUSSION

293 The researchers interested in physiological animal body responses to vibration have had one primary concern - the 294 possibility to improve peripheral blood flow without pharmacological intervention [1, 10-12].

295 Cows are usually milked for 4-8 minutes, but the duration of milking and the rate at which milk is delivered are highly 296 dependent on the preparation of the udder, the milk yield, the individual characteristics of the cow, the construction 297 and the technical condition of the milkers [20-21]. Many studies have shown that the amount of milk and the duration 298 of milking depend on the position of the udder quarters [22-23]. After analyzing all previous studies, we chose to apply 299 the 8 min long vibration as this time was the average milking time of the whole cow herd.

The results showed that using vibrations of 25 Hz frequency for up to 8 minutes (as long as the milking process takes) increased the udder surface temperature by 0.57°C, thus confirming that low-frequency vibrations stimulate and activate blood flow to the cow's udder [24]. The conducted research showed that whole-body vibration influenced the blood flow in arteries [6, 25-26]. However, such studies were meant to evaluate the influence of high frequency vibration on the human body, and only a few studies described low frequency vibration effects. Other authors, who have investigated whole-body vibration effects on the blood flow in legs, claim that the body is more sensitive to 5 Hz vibration than to 50 Hz vibration [7].

In this study a vibration system to excite the cow's udder has been analyzed theoretically and developed for 307 308 experimental research. Four unbalanced motor vibrators were attached to each teat cup of the milker (DeLaval 309 Harmony model), and the low-frequency range vibrations were induced in the cow udder. The 2D dynamic model of 310 the vibration system has been established, and the frequency response of the vibrator-milker assembly has been solved 311 analytically and verified experimentally. A frequency sweep test was performed on the developed vibration system in 312 the frequency range of 15 - 60 Hz. The calculated and experimentally obtained frequency responses of the vibrations 313 in lateral and vertical directions are close. The test values of the first-order effective frequency of the vibration system 314 in the lateral (X) direction are 23.5 - 26.5 Hz, the effective frequency of the simulation analysis is 25.0 Hz, and the 315 relative error is 6%. The test values of the first-order effective frequency of the vibration system in the vertical (Y) 316 direction are 37.5 – 41.5 Hz, the effective frequency of the simulation analysis - 41.0 Hz, and the relative error - 8.5%. 317 The operational frequency of 25 Hz was defined for a developed vibration system, at which higher amplitude and lower 318 damping of vibrations were gained.

An indicator of the healthy udder of a cow is the components of the milk secretion of the udder: the number of somatic

320 cells, organoleptic components (milk uniformity, and other indicators) [11].

321 This study analyzed the effect of low-frequency vibrations excited on the cow udder during the milking process. Also,

322 we have evaluated some parameters such as milk yield, composition and quality, temperature, and welfare of dairy

323 cows.

One more Acoustic Pulse Therapy device was designed to produce deep penetrating acoustic pulses that are distributed over a large treated area at a therapeutic level [12]. At the experiment daily milk yields of the treated cows increased significantly (p < 0.05) and the percentage of cows with log somatic cell count under 5.6 cells/mL was significantly higher (p < 0.001). Milk of the infected quarters appeared normal with lactose greater than 4.8%, but this difference was not significant [10]. Low-frequency vibrations had no statistically significant effect on the mean values of milk yield, milk composition, and quality in our study.

Welfare has been evaluated on cows under low-frequency vibration during milking. As a cow's face reflects an emotional state, the cow's facial expressions can demonstrate pain and sickness even in the early stages. The facial expression should always be observed when animals are disturbed as it can impact animal welfare, milk production, and reproductive efficiency. Moreover, dairy cows' ear postures are reliable indicators of low arousal and/or positive emotional state [27].

Facial expression is a pain recognition tool in animals, and new technology application has been associated with animal welfare on farms. On-farm use of the facial expression to recognize pain in cattle may override some of the difficulties in animal pain diagnosis and, consequently, allow for rapid and consistent intervention to alleviate suffering [28-29]. Many studies have focused on eye white and ear posture as potential promising indicators for interpreting emotions in dairy cows [30]. Researchers now globally recognize that emotions are part of the complex life of dairy cows [31]. Our study results demonstrate that applying low-frequency oscillations on the mammary gland does not change a cow's facial expression, eye, and ear postures and does not adversely affect cow's welfare.

342

343 CONCLUSIONS

344 The mechanical vibration device developed and tested in the study was mounted on a DeLaval milking machine, which 345 excited the udder with low-frequency oscillations. It allowed the analysis of input parameters (temperature, oscillation 346 amplitude) and, using feedback data, changing the device parameters such as vibration frequency and duration.

347 Applying low-frequency (X-axes – 25.0; Y-axes – 41.0 Hz) vibration on cow's udder during the automatic milking

statistically significantly increased udder temperature by 0.57° C (p < 0.001) and did not adversely affect the studied

animal welfare indicators, milk yield and quality parameters.

350 The perspective is to carry out experiments on a larger number of cows and to confirm our hypotheses regarding animal

351 welfare, prevention and treatment of mastitis, and increasing the amount of milk produced.

352 Further studies will be carried out in the following research phase on clinical and subclinical mastitis cows.

354 Competing interests

- 355 The authors report no conflicts or competing interests.
- 356 Funding
- 357 This research was funded by the association Santakos slėnis implementing a joint Kaunas University of Technology
- 358 (KTU) Lithuanian University of Health Sciences (LSMU) scientific project PP34/2106 Application of acoustic
- 359 oscillations for the prevention and treatment of bovine mastitis (VIPGAMA).
- 360 Acknowledgements
- 361 Not applicable.
- 362 Availability of data and material
- 363 Upon reasonable request, the datasets of this study can be available from the corresponding author.

364 Authors' contributions

- 365 Conceptualization: Sederevičius A., Bubulis A.,
- 366 Data curation: Oberauskas V., Musayeva K.
- 367 Formal analysis: Sederevičius A., Oberauskas V., Jūrėnas V., Vėžys J.
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- 374 Jūrėnas V., Bubulis A., Vėžys J.
- 375 Ethics Declarations
- 376 The experimental protocols and animal care/handling used in this study comply with the Directive 2010/63/EU, the
- 377 European Union legislation on the protection of animals used for scientific purposes and is approved by the State Food
- **378** and Veterinary Service (2021-05-04 No. B6-(1.9.)-1131).
- 379 Consent for publication
- 380 Not applicable.
- 381 Abbreviations
- 382 APT: Acoustic Pulse Therapy; KTU: Kaunas University of Technology; LSMU: Lithuanian University of Health
- 383 Sciences; JSC: Joint-Stock Company; NRUC: Natural Rubber Udder of a Cow; FLIR: Infrared Thermography Camera.

384 References

- Mariotto S, de Prati AC, Cavalieri E, Amelio E, Marlinghaus E, Suzuki H. Extracorporeal shock wave therapy in inflammatory diseases: Molecular mechanism that triggers anti-inflammatory action. Curr. Med. Chem. 2009; 16:2366–72. https://doi.org/10.2174/092986709788682119
- Crowe OM, Dyson SJ, Wright IM, Schramme MC, Smith RK. Treatment of chronic or recurrent proximal suspensory desmitis using radial pressure wave therapy in the horse. Equine Vet.J. 2010;36(4):313-6. https://doi.org/10.2746/0425164044890562
- McClure SR., Merritt DK. Extracorporeal shockwave therapy for equine musculoskeletal disorders. Compendium.
 2003.68-70.
- http://assets.prod.vetlearn.com.s3.amazonaws.com/mmah/a2/47cf6e30a24ac1a80949760c2dc41b/filePV_25_01_
 68.pdf
- Souza AN, Ferreira MP, Hagen SC, Patricio GC, Matera JM. Radial shock wave therapy in dogs with hip osteoarthritis. Vet. Comp. Orthop. Traumatol. 2016; 29:108–14. https://doi.org/3415/VCOT-15-01-0017
- 397 5. Haupt G, Chapvil M. Effect of shock waves on the healing of partial-thickness wounds in piglets. J. Surg. Res. 1990;49(1):45-8. https://doi.org/10.1016/0022-4804(90)90109-F
- 399 6. Nakamura H, Okazawa T, Nagase H, Yoshida M, Ariizumi M, Okada A. Change in digital blood flow with simultaneous reduction in plasma endothelin induced by hand arm vibration. Int Arch Occup Environ Health.1996;68(2):115-9. https://doi.org/10.1007/BF00381243
- 402 7. Lohman EB, Sackiriyas SB, Bains GS, Calandra G, Lobo C, Nakhro D, et al. A comparison of whole-body vibration and moist heat on lower extremity skin temperature and skin blood flow in healthy older individuals.
 404 Med Sci Monit. 2012;18(7):415-24. https://doi:10.12659/MSM.883209
- 405 8. Lee K, Song C. Determining the posture and vibration frequency that maximize pelvic floor muscle activity during
 406 whole body vibration. Med Sci Monit. 2016;27(22):4030-6. https://doi.org/10.12659/msm.898011
- 9. Osawa Y, Oguma Y. Effects of whole-body vibration on resistance training for untrained adults. J Sports Sci Med. 2001;10(2):328-37. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3761851/
- 409 10. Leitner G, Zilberman D, Papirov E, Shefy S. Assessment of acoustic pulse therapy (APT), a non-antibiotic
 410 treatment for dairy cows with clinical and subclinical mastitis. PLoS ONE. 2018;
 411 https://doi.org/10.1371/journal.pone.0199195
- 412 11. Lyubimov VE, Romanov DV, Tsoi YA, Bulat G, Ziganshin BG, Sitdikov FF. Results of application of frequency 413 resonance therapy for treatment of cow mastitis. BIO Web of Conferences. 2020; 414 https://doi.org/10.1051/bioconf/20201700254
- Leitner G, Papirov E, Gilad D, Haran D, Arkin O, Zuckerman A, Lavon Y. New Treatment Option for Clinical and Subclinical Mastitis in Dairy Cows Using Acoustic Pulse Technology (APT). Dairy. 2021;2(2):256–69. https://doi.org/10.3390/dairy2020022
- 418 13. Lyubimov VE, Alekseevich TY, Vladirimovich RD, Ivanovich OV. The method and therapeutic device for
 419 exposure to a pulsed frequency resonance electromagnetic field for the prevention and treatment of diseases of the
 420 udder of cows, stimulation of lactation. R.F. patent for invention no. 2644826 (publ. 02.14.2018. Bull. No. 5).
 421 https://patents.google.com/patent/RU2644826C1/en
- 422 14. Lyubimov VE. The influence of electromagnetic fields of ultrahigh-frequency on the mammary gland of cows
 423 during machine milking, PhD dissertation thesis (Moscow veter. academy named after K.I. Skryabin, Moscow,
 424 2004)

- 425 15. Subbotina TI, Yashin AA. Resonance effects in the interaction of electromagnetic fields with biosystems. Part II,
 426 Bull. of new med. technol.2018;12(4):152–71.
- 427 16. Schmitz TL, Smith KS. Two Degree of Freedom Forced Vibration. In: Mechanical Vibrations. Springer, Boston,
 428 MA; 2012:1-24. https://doi.org/10.1007/978-1-4614-0460-6_5
- Hazell TJ, Thomas GW, Deguire JR, Lemon PW. Vertical whole body vibration does not increase cardiovascular stress to static semi-squat exercise. Eur J Appl Physiol. 2008;104(5):903-8. https://doi.org/10.1007/s00421-008-0847-y
- 432 18. Gleerup KB, Andersen PH, Munksgaard L, Forkman B. Pain evaluation in dairy cattle. Appl Anim Behav Sci. 2015;171(15):25-32. https://doi.org/10.1016/j.applanim.2015.08.023
- 434 19. Gleerup K. Identifying Pain Behaviors in Dairy Cattle. WCDS Adv. Dairy Technol. 2017;55:231-9.
 435 https://wcds.ualberta.ca/wcds/wp-content/uploads/sites/57/2018/05/p-231-242-Gleerup.pdf
- 436 20. Dzidic A, Weiss D, Bruckmaier RM. Oxytocin release, milk ejection and milking characteristics in single stall
 437 automactic milking system. Livest. Scin. 2004; 86:61-8. https://doi.org/10.1016/S0301-6226(03)00150-7
- 438 21. Gade S, Stamer W, Junge E, Klam E. Estimates of genetic parameters for milkability from automatic milking.
 439 Baltic animal breeding and genetic conference. Palanga. 2005;61-4.
 440 https://www.infona.pl//resource/bwmeta1.element.elsevier-aeeb4586-dac6-3d1c-803d-270d787848ea
- 441 22. Weiss D, Weinfurtner M., Bruckmaier R. M. Teat anatomy and its relationship with quarter and udder milk
 442 low characteristics in dairy cows J. Dairy Sci. 2004;87:3280–9. https://doi.org/10.3168/jds.S0022443 0302(04)73464-5
- Tancin V, Ipema B, Hogewerf P, Macuhova J. Sources of variation in milk flow characteristics at udder and quarter
 Ievels. J. Dairy Sci. 2006;89:978–88. https://doi.org/10.3168/jds.S0022-0302(06)72163-4
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- 25. Chudacek Z. Changes in the thermographic picture of hands of healthy persons after vibrations of 125 Hz. Cech
 Radio. 1989;43(2):98-101. https://pubmed.ncbi.nlm.nih.gov/2731303/
- 451 26. Lythgo N, Eser P, Groot P, Galea M. Whole-body vibration dosage alters leg blood flow. Clin Physiol Funct
 452 Imaging. 2009;29(1):53-9. https://doi.org/10.1111/j.1475-097X.2008.00834.x
- 453 27. Proctor HS, Carder G. Can ear postures reliably measure the positive emotional state of cows? Appl. Anim. Behav.
 454 Sci. 2014;161:20-7. http://dx.doi.org/10.1016/j.applanim.2014.09.015
- 455 28. McLennan KM, Rebelo CJB, Corke MJ, Holmes MA, Leach MC. Constantino-Casas F. Development of a facial
 456 expression scale using footrot and mastitis as models of pain in sheep. Appl. Anim. Behav. Sci. 2016;176:19–26.
 457 https://doi.org/10.1016/j.applanim.2016.01.007
- 458 29. Müller BR, Bellio JCB, Molento CFM. Facial expression of pain in Nellore and crossbred beef cattle. J Vet Behav.
 459 2019;34:60-5. https://doi.org/10.1016/j.jveb.2019.07.007
- 30. Battini M, Agostini A, Mattiello S. Understanding cows' Emotions on farm: are eye white and ear posture reliable indicators? Animals (Basel). 2019;9(8):477. https://doi.org/10.3390/ani9080477

- Proctor HS, Carder G. Measuring positive emotions in cows: Do visible eye whites tell us anything? Physiol. Behav. 2015;147:1–6. https://doi.org/10.1016/j.physbeh.2015.04.011