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Evaluation of selected biophysical parameters of the teat skin of dairy cows

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Summary

The aim of the research was to assess transepidermal water loss (TEWL), epidermal hydration (EH) and teat skin pH in Holstein-Friesian (HF) dairy cows of various ages and with varying degrees of teat skin damage. The research was carried out on one farm in 3 groups of 18 animals each: B1 – cows in the 2nd or 3rd lactation with a teat damage score of 2; B2 – cows in the 5th or 6th lactation with a teat damage score of 3; and B3 – cows in the 5th or 6th lactations with a point assessment of teat damage of 4. The Burmeister classification was used (1). In each cow, the parameters were assessed four times throughout the summer. Measurements were performed using a 5-head Courage Khazaka Multi Probe digital camera with the Windows Adapter MPA 5 software (D-50829 Köln, Germany). The TEWL values were lowest in group B1 (average = 28.5 g/m^2/h) and significantly higher in groups B2 (33.28 g/m^2/h) and B3 (39.48 g/m^2/h). The highest EH values were obtained in group B1 (24.85%), and they were significantly higher than those in groups B2 (15.63%) and B3 (15.39%). Skin pH values in all groups of cows ranged from 6.96 to 7.2. The research allowed us to conclude that TEWL, EH and pH are sensitive indicators of the condition of the epidermal barrier of the teat skin and its stability. The parameters tested in this study may be useful in clinical practice for monitoring the condition of teat skin and assessing the progress of skin lichenisation.

Keywords: teat skin, dairy cows, TEWL, EH, pH

The skin of dairy cows' teats is exposed to a number of factors due to their intensive exploitation (18, 20, 22, 32). External factors directly related to the milking process, unfavourable environmental and zoohygienic conditions in the barn predispose the teat skin to lesions (20). They disturb the keratinisation of the epidermis and reduce skin elasticity. Hyperkeratosis and hyperplasia of the skin make it thickened, hard and cracked, and therefore susceptible to changes in the composition of the microbiome and infections. It is a risk factor for the development of mastitis (14, 17, 23).

Clinical changes indicating damage to the teat skin may be short- or long-term (1, 20, 24, 31). Short-term changes, such as erythema and swelling, are most often related to milking and should disappear after it is completed. Long-term changes, such as abrasions, petechiae and wounds, persist for many weeks (1, 11, 19, 24). Literature data indicate that long-term and repeated exposure of the teat skin to adverse effects of irritating environmental factors is a common problem in highly productive herds of dairy cows (12, 14, 20, 24, 25). Their peak occurs in the 3rd-4th month after calving. Lesions deepen with each subsequent lactation. As shown by the findings of other authors, chronic skin lesions of teats are correlated with the milk yield of cows (14, 20, 24, 25).

Literature data indicate that the assessment of teat skin by inspection and palpation is subjective and is largely based on the experience of the researcher. It should be supplemented or replaced by other non-invasive methods that offer an objective assessment of biophysical skin parameters without aggravating skin lesions. The assessment of transepidermal water loss (TEWL), the degree of epidermal hydration (EH) and teat skin reaction (pH) are recognised biophysical skin parameters useful in clinical practice (4, 33, 39). Therefore, the aim of the study was to assess TEWL, EH and teat skin pH in Holstein-Friesian (HF) dairy cows of different ages and with various degrees of teat skin damage. The results can be used to evaluate

the usefulness of these parameters for the prevention and detection of early forms of teat skin damage in dairy cows.

Material and methods

Animals. The research was carried out on Holstein-Friesian (HF) cows from a single farm with a herd of 106 animals in the Lublin Voivodeship. The farm uses a bedding tether housing system in short stands (120 × 180 cm). The bedding consists of cereal straw, which is changed twice a day. Gravity ventilation is used in the barn. The feeding system is based on TMR (total mixed ration) administered once a day after morning milking. The farm uses a conventional mechanical tube milking system. Milking takes place twice a day, at 5:00 a.m. and 5:00 p.m. During each milking, pre-dipping is used with Kenopure washing and disinfecting preparation (Cid Lines, Belgium) with pH = 2.25 and 10% lactic acid content, as well as disposable paper towels for wiping the teats. For post-dipping, the Iodium TX (Hypred Polska) preparation is used with pH = 5.5, an active iodine concentration of 5000 ppm and the addition of glycerine as an emollient and lanolin as a lubricating agent.

Dairy cows of different ages were selected for the study, and after scoring the degree of teat damage according to the classification of Burmeister et al. (1), they were divided into 3 groups of 18 animals each: B1 (cows in the 2nd or 3rd lactation with a teat damage score of 2), B2 (cows in their 5th or 6th lactation with a teat damage score of 3) and B3 (cows in their 5th or 6th lactation with a teat damage score of 4). In each cow, the biophysical skin parameters were assessed four times (measurements 1-4) at intervals of several days in the summer (in June), at an average air humidity in the barn of 55% and a temperature of 18°C. Pre-dipping and post-dipping were not performed in experimental animals for 24 hours before the test, and the parameters were measured at equal intervals between milkings, that is, 6 hours after the morning milking and at the same time 6 hours before the evening milking, that is, between 11 a.m. and 12 p.m.

The biophysical parameters of the skin were measured using a 5-head Courage Khazaka Multi Probe digital camera with the Windows Adapter MPA 5 software (D-50829 Köln, Germany). The device is standardised for humidity of 50-70% and temperature of 10-22°C, and has sensors for indoor measurement. The camera is equipped with the following heads:

A. CK Skin Tewameter TM 300 measures transepidermal water loss (TEWL) by the „open chamber" method using Fick's diffusion law. It assesses the temperature and humidity gradient on the skin surface. The measurement is made after 15 seconds of head adaptation after contact with the skin. The average result from 10 measurements performed automatically at 1 second intervals is given by the camera's computer program in g/m^2/h.

B. CK Corneometer CM 825 assesses skin hydration (EH) by the indirect capacitive method based on testing the water content in the stratum corneum on a surface of 49 mm^2 of skin and a depth of 10 to 40 µm. The result is given in the range from 0 to 130%. The average result from 10 measurements performed automatically at 1 second intervals is provided by the computer program.

C. CK Skin-pH-Meter pH 905 measures skin pH by reading the potential difference measured by an active electrode that is in contact with the skin and a reference electrode filled with a buffer solution. The measuring range of the probe is pH from 0 to 12, and the optimal conditions for measurements are a temperature of 10-40°C and a relative air humidity of 30-70%. The measurement is performed after a 15 second adaptation of the head, which has to be cleaned with distilled water after each measurement. After the head vertically touches the skin, 5 measurements are taken automatically at 1 second intervals. The average reading value is provided by the camera's computer program.

Statistical methods. The results obtained were statistically analysed using Microsoft Excel for Windows 2010 and Statistica 10.0 Pl. Mean values ($\bar{x}$) and standard deviation (± SD) were determined. The level of significance of differences is presented at $p < 0.05$. To analyse the differences between the variables, parametric methods were used for data with a normal distribution and non-parametric methods were used for the others. In order to demonstrate statistically significant differences between the parameters, Student's t-test was performed for parametric observations and the Mann-Whitney U test for non-parametric observations.

The research was approved by the 2nd Local Ethical Committee for Animal Experiments in Lublin (Resolution No. 2/2014 of January 21, 2014).

Results and discussion

The mean values ($\bar{x}$) and standard deviation (± SD) obtained in the tests, as well as the min-max values of control measurements (C) of biophysical parameters in groups B1, B2 and B3 are presented in Table 1. Statistical analysis of the results obtained in groups B1, B2 and B3 showed statistically significant differences between animal groups in TEWL and EH values.

Results of TEWL measurement in groups B1, B2 and B3. The results are presented in Table 1. The lowest mean TEWL value from four measurements was obtained in cows in their 2nd or 3rd lactation with a teat damage score of 2 (group B1), and the highest in cows in their 5th or 6th lactation with a teat damage score of 4 (group B3), which is graphically presented in Figure 1.

In group B1, the minimum TEWL value was 13.2 g/m^2/h and the maximum was 50.4 g/m^2/h. The average value of all measurements was 28.50 g/m^2/h. In this group of cows, the lowest average TEWL value was obtained in measurement 4, and the highest in measurement 2 (Tab. 1). In this group of cows, statistically significant differences in TEWL values were found between measurements 2 and 3 ($p = 0.041$) and 2 and 4 ($p = 0.004$).

In the B2 group of cows, the lowest TEWL value recorded was 13.4 g/m^2/h, and the highest was 65.5 g/m^2/h. The average value of all measurements in cows from group B2 was higher than in cows from group B1 and amounted to 33.28 g/m^2/h (Fig. 1). In cow group B2, the lowest mean value was obtained in measurement 3, and the highest in measurement 2. In cow group B2, a statistically significant difference in TEWL values was observed between measurements 2 and 3 ($p = 0.002$).

Tab. 1. Values of the biophysical parameters of dairy cows' teat skin. Group B1: cows in the 2nd or 3rd lactation with teat damage score = 2, B2: cows in the 5th or 6th lactation with teat damage score = 3, and B3: cows in the 5th or 6th lactation with teat damage score = 4

Pomiar		B1 (n = 18)			B2 (n = 18)			B3 (n = 18)		
		TEWL (g/m²/h)	EH (%)	pH	TEWL (g/m²/h)	EH (%)	pH	TEWL (g/m²/h)	EH (%)	pH
1	$\bar{x}$	32.78	26.32	6.85	33.97	16.08	6.98	46.30	14.55	6.43
	SD	6.58	2.08	0.33	7.98	1.70	0.59	7.23	9.18	0.77
	Min-Max	24.7-40.3	24.4-29.4	6.5-7.3	26.5-45.5	13.9-41.2	6.2-7.6	21.7-56.7	5.7-46.3	6.3-8.5
2	$\bar{x}$	37.38*	24.70	6.98	40.33*	14.47	7.61	37.58	22.70	7.34
	SD	10.77	4.38	0.56	30.71	1.81	0.55	9.47	8.85	0.21
	Min-Max	21.4-50.4	20.6-30.2	6.3-7.7	20.1-65.5	11.3-26.3	6.6-8.3	23.7-96.3	14.3-32.5	6.4-8.5
3	$\bar{x}$	23.02*	23.25	6.91	20.47*	16.87	7.19	38.53	14.57	7.62
	SD	6.78	6.22	0.32	4.95	2.09	0.36	17.34	3.63	0.85
	Min-Max	13.2-31.8	16.2-30.5	6.4-7.1	13.4-42.9	14.2-28.3	6.5-7.5	24.9-79.2	9.3-36.3	6.9-8.9
4	$\bar{x}$	20.80*	25.13	7.09	31.80	15.09	7.04	35.52	9.73	7.20
	SD	2.70	5.67	0.26	4.72	2.15	0.41	7.80	3.12	0.43
	Min-Max	17.4-25.3	18.1-29.9	6.6-7.4	18.6-48.8	12.4-23.7	6.2-7.5	27.9-74.8	6.1-49.8	6.3-7.8

Explanation: * – indicates statistically significant differences (p) between subsequent measurements

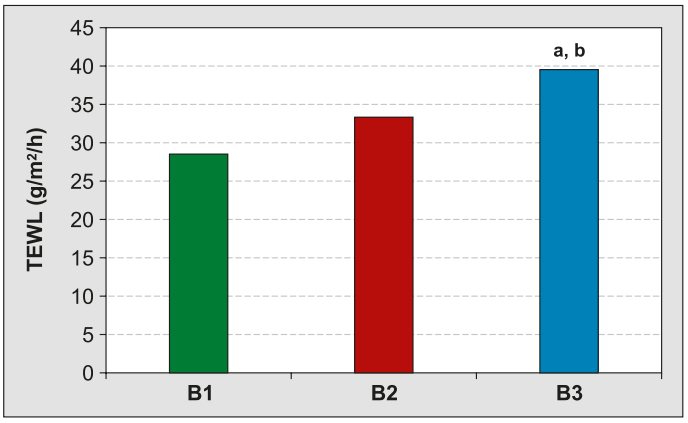

Fig. 1. Average TEWL values measured in cow groups B1, B2, and B3, taking into account the significance of differences: a – between groups B1 and B3 (p = 0.0008); b – between groups B2 and B3 (p = 0.003)

However, in group B3, the lowest TEWL value obtained was 21.7 g/m²/h, and the highest was 96.3 g/m²/h. The average value of all measurements was 39.48 g/m²/h (Fig. 1). In that group, the lowest mean TEWL value was obtained in measurement 4, and the highest in measurement 1 (Tab. 1). In group B3, no statistically significant differences in TEWL values were found between consecutive measurements.

Statistical analysis of TEWL results obtained in the three study groups showed statistically significant differences between groups B1 and B3 (p = 0.0008) and groups B2 and B3 (p = 0.003), which is graphically presented in Figure 1.

Results of measuring epidermal hydration (EH) in groups B1, B2 and B3. The EH results are presented in Table 1, and the average values are shown in Figure 2. Epidermal hydration was best (average = 24.85%) in cows in their 2nd or 3rd lactation with a teat damage score of 2 (group B1) and much worse in groups B2 (average = 15.63%) and B3 (average = 15.39%), which is presented graphically in Figure 2.

The lowest epidermal hydration value obtained in group B1 was 16.2%, and the highest was 30.5%. In this group of cows, the lowest average EH value was obtained in measurement 3, and the highest in measurement 1 (Tab. 1). In group B1, there were no statistically significant differences in EH values between measurements.

In cow group B2, the lowest value of epidermal hydration was 11.3%, and the highest was 41.2%. The lowest mean EH value was obtained in measurement 2 and the highest in measurement 3, but, as in group of B1, no statistically significant differences were found between the measurements (Tab. 1). Comparative analysis of EH results in groups B1 and B2 showed that in the B2 cows, that is, those in their 5th or 6th lactation with a teat damage score of 3, EH values were significantly lower than in group B1 (Fig. 2).

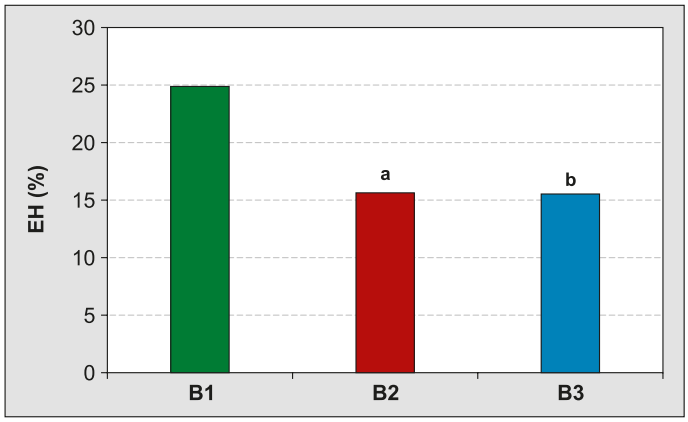

Fig. 2. Average values of EH measurement obtained in cow groups B1, B2 and B3, taking into account the significance of differences: a – significance of differences between group B1 and B2 (p < 0.000003); b – significance of differences between group B1 and B3 (p = 0.00005)

In group B3, the minimum and maximum EH values were 5.7% and 49.8%, respectively, while the average EH values ranged from 9.73 (measurement 4) to 22.70% (measurement 2).

A comparative analysis of EH results obtained in the three groups of cows showed that the results for group B1 of cows with a teat skin score of 2 were significantly higher than those for group B2, in which the score was 3 ($p = 0.00005$), and B3, with a score of 4 ($p < 0.000003$). This is shown in Figure 2.

Results of measuring skin reaction (pH) in groups B1, B2 and B3. The pH values for teat skin in cow groups B1, B2 and B3 ranged from 6.2 to 8.5, with an average of 7.1 (Tab. 1). The lowest mean value (6.96) was found in group B1 and the highest (7.21) in group B2, but the statistical analysis of the results did not show statistically significant differences between the groups (Fig. 3).

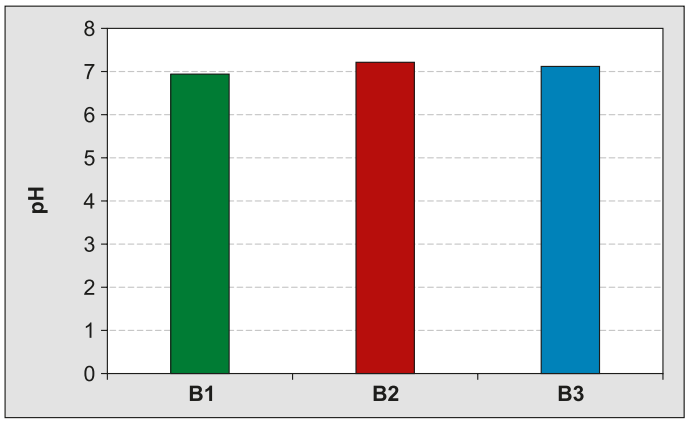

Fig. 3. Mean values of pH measured in cow groups B1, B2, and B3. No statistically significant differences were found

Long-term and repeated exposure of the skin of dairy cows' teats to irritating factors hinders or prevents its full regeneration and causes irreversible pathological processes affecting deeper layers of skin. This leads to the lichenisation of the skin (5, 13, 14, 18, 30). Initially, when exposed to the adverse effects of irritating factors, the teat skin is deprived of its natural, protective hydrolipid layer, which results in its dehydration (EH), an increase in transepidermal water loss (TEWL) and a change in pH. This mechanism is known and frequently described in humans (5, 6, 26, 29, 30). Already in the first hour after a single experimental mechanical damage to the epidermis, an inflammatory reaction develops in the skin, which leads to the immediate activation of repair mechanisms (8-10, 27, 30, 35). Repeated exposures disrupt the proper process of keratinisation and epidermal renewal and may cause the skin to lose its elasticity and suppleness. This condition changes the skin microflora and promotes colonisation by bacteria that can cause various forms of inflammation and, in the case of dairy cows, mastitis (1, 11, 12, 14, 18, 19, 25).

The research was aimed at assessing selected parameters of the protective barrier of the teat skin epidermis, as well as their variability during many years of repeated exploitation in HF dairy cows of different ages. This was done based on the analysis of selected biophysical parameters of the epidermis that are used in clinical sciences to detect skin damage and early (subclinical) skin changes (15, 26, 37). In veterinary medicine, the practical use of these parameters poses many problems because the results of measurements depend to a large extent on environmental factors (ambient temperature and environmental humidity) and the measurement method (closed or open measurement systems), which, as in medicine, makes it difficult to obtain reliable results (15, 26, 37). Published data indicate that, in healthy people, TEWL values may range from 8.8 g/m^2/h to 25.0 g/m^2/h, and the EH value from 15% to 78.7%, which depends on the area of the body examined and the measurement technique (2, 15, 26). The results of studies conducted in humans confirm that insufficient hydration of the epidermis impairs the epidermal barrier, and this indicator correlates with high TEWL values (2, 26, 28). Studies conducted in healthy dogs indicate that average TEWL values also depend on the dog breed and the body region examined, ranging from 18.6 g/m^2/h on the sides of the body, 22.3 g/m^2/h on the elbow, 28.2 g/m^2/h on the neck to 35.5 g/m^2/h on the scalp (34, 37, 38). Similarly, the hydration values of dogs' epidermis also vary depending on body area and breed, ranging from 5.7% to 24% (34, 37, 38).

The data on the TEWL and EH of the skin of dairy cows' teats have not been published so far. The available literature includes a number of studies on the assessment of the pH of the teat skin, or rather the external opening of the teat canal, a parameter that undeniably plays a role in the pathogenesis of mastitis (7, 11, 14, 21, 23). Since there are no data in the available literature regarding the research conducted here, only results obtained in other mammalian species can be referred to in discussing the results of the present study. The high TEWL values and low EH values observed in cows with a skin damage score of 3 or 4 compared to cows with a slight degree of damage, i.e. score 2, indicate a progressive dysfunction of the epidermal barrier as a result of continuous exposure to unfavourable environmental factors during use. Likewise, in people suffering from atopic dermatitis (AD), the skin is exposed to the adverse effects of environmental factors and has limited regenerative capacity (16, 26). The average TEWL values in people suffering from atopic dermatitis are high, amounting to 33.5 g/m^2/h, and drop to 21.3 g/m^2/h in periods of remission, while EH does not exceed 40% even in periods of remission, averaging 26.7%. Similar TEWL values were obtained in our studies for cows in their 5th or 6th lactation with a teat score of 3 or 4, while their EH values were much lower than those reported for humans and dogs. In humans, an EH value close to 10-12% is typical of dry skin (xerosis), manifested by visible peeling and cracking of the epidermis (26). Studies conducted on mice (13) have shown that a single mechanical damage to the epidermis, causing qualitative and quantitative changes in lipids in the epidermis and increased transepidermal water loss, stimulates and

accelerates epidermal renewal processes. However, frequent and repeated damage to the same skin area, even if merely through exposure to unfavourable environmental conditions (low air humidity, mechanical or chemical irritation, high skin pH), may prevent full renewal of the epidermis and lead to permanent changes, such as hypertrophy and keratinisation disorders, and, as a consequence, to the irreversible process of skin lichenisation (3, 5, 27, 36). In cows whose teats receive a clinical score of 4 according to the Burmeister scale (1), the symptoms of lichenisation (thickening of the epidermis, cracks, fissures, clefts and warts) may be irreversible, the integrity of the skin's protective barrier may be permanently disturbed, and regeneration mechanisms may be impaired.

The present research allowed us to conclude that transepidermal water loss (TEWL), epidermal hydration (EH) and skin reaction (pH) are sensitive indicators of the condition of the epidermal barrier of the teat skin and its stability. The average values of these parameters depend on the degree of damage to the teat skin and environmental conditions. These parameters of the epidermal barrier may be useful in clinical practice for monitoring the condition of the teat skin and for tests aimed at limiting the progressive lichenisation process, which is economically important.

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