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Effect of rectal examination on the behavior of multiparous cows (*Bos taurus taurus*) assessed on the basis of selected stress indicators

ARKADIUSZ GRZECZKA[1], GRACJAN WOZNIAK[1], SZYMON GRACZYK[1], JOANNA WYSZKOWSKA[2], BARTŁOMIEJ M. JAŚKOWSKI[3], MAREK GEHRKE[4], JĘDRZEJ M. JAŚKOWSKI[4]

[1]Institute of Veterinary Medicine, Nicolaus Copernicus University in Torun, Gagarina 11, 87-100 Toruń, Poland
[2]Department of Animal Physiology and Neurobiology, Faculty of Biological and Veterinary Sciences, Nicolaus Copernicus University in Torun, Gagarina 11, 87-100 Toruń, Poland
[3]Department of Reproduction and Clinic of Farm Animals, Faculty of Veterinary Medicine, Wroclaw University of Environmental and Life Sciences, Plac Grunwaldzki 49, 50-366 Wrocław, Poland
[4]Department of Diagnostic and Clinical Sciences, Institute of Veterinary Medicine, Nicolaus Copernicus University in Torun, Gagarina 11, 87-100 Toruń, Poland

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Grzeczka A., Wozniak G., Graczyk S., Wyszkowska J., Jaśkowski B. M., Gehrke M., Jaśkowski J. M.

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Summary

Rectal examination can be a source of stress for cows. However, the literature lacks descriptions of cow reactions to a short or prolonged rectal examination, which undoubtedly occur in veterinary practice. Hence, the purpose of this work was to determine changes in the heart rate, blood pressure, and eye temperature values in multiparous cows resulting from rectal examinations of varying duration.

The data were collected during the work of an experienced veterinarian. Heart rate, eye temperature, and blood pressure were evaluated in multiparous cows before and after rectal examination. In group I of cows (n = 12), the effect of a short per rectum examination (of up to 1 minute) was evaluated. In group II (n = 12), the examination lasted 3 minutes, and in group III (n = 12) it took less than 15 seconds.

Cows responded with an increase in the number of beats per minute and a minimal increase in eye temperature. In the group subjected to the shortest examination (group III), no statistically significant increase in the heart rate was observed (from 75.4 ± 6.7 to 76.7 ± 7.3), while its increase differed minimally between the cows subjected to the normal examination (group I) (from 76.2 ± 6.7 to 82.4 ± 3.7) and those that had undergone the prolonged examination (group II) (from 72.9 ± 3.6 to 81.7 ± 4.5). The lowest increase in eye temperature was also noted in group III (from 36.2 ± 0.3 to 36.2 ± 0.3). Similarly, no change in pressure was observed in group III (from 103.75 ± 11.92/57.25 ± 18.1 to 102.0 ± 5.87/58.75 ± 7.39), but significant changes were observed in group I (from 79.0 ± 6.82/49.25 ± 3.63 to 100.5 ± 5.94/50.75 ± 5.97).

The data obtained expand the knowledge of the behavior of cows during rectal examination. Our results are also a valuable source of information for veterinarians involved in the reproduction of domestic cattle.

Keywords: heart rate, blood pressure, eye temperature, cattle, stress, rectal examination

Stress is a state of mental and physical tension arising as a result of being threatened and having physiological needs made difficult or impossible to meet (19). Acute stress is described as a short-lived negative expression of a response to a stressor that makes it possible to restore physiological balance rapidly and completely. Chronic stress, on the other hand, is characterized by the loss of the body's adaptive capacity to the acting stimulus (46). In wildlife and livestock populations, moderate stress is an indispensable part of life. It increases locomotor effort during escape from a predator, during contact with new individuals in a herd, or is an element of a response to unfavorable environmental conditions, e.g. too high or too low temperature in farm buildings (1, 34). Frequent exposure to stress-like disease processes can lead to low productivity in many livestock species, such as pigs, cattle, horses, sheep, and goats (1, 11, 13, 14, 26, 33, 34, 39, 52). Similarly, in dairy cows, a sudden change in milking time during the day can cause stress manifested by behavioral changes, including kicking or lifting of the legs, and reduced production (35). An effect of chronic stress are increased cortisol levels (35, 38). There are also several sensitive and simple physical parameters to assess animal responses, such as the heart rate, blood pressure, and temperature, for example the temperature of the eye (27, 48, 49). The absence of significant deviations from the physiological values of these indicators is equivalent to the absence of stress (3).

Veterinary medicine procedures, as well as organizational and husbandry activities, can cause changes in animal behavior. Some behaviors can be considered a consequence of stress (4, 27). Transrectal palpation is one of the basic activities in veterinary gynecology (4). This examination is used, among others, for the assessment of the reproductive organ, functional structures of the ovaries, and diagnosis of pregnancy.

Views vary on the stress caused by examination of the reproductive tract. Dairy cows, for the most part, are in regular contact with humans and are examined by rectal palpation relatively frequently. Defensive reflexes observed in cattle (back arching, neck straightening, prolonged lying down) are rare even in transvaginal examination (29, 30). Hence the belief that this examination is minimally invasive, and the accompanying stress is low. However, according to some, rectal palpation examination can be a potential stressor for cows. Others report that there may be behavioral changes and a decline in performance (41). Wainblinger et al. (51) found that stress reactions in cows during rectal palpation of insemination can be reduced by prior positive treatment and mild interactions during the procedure. In the available studies, less attention is paid to the influence of the testing technique and the time of the test on the cow's reaction. Prolonged testing, however, can be, at least theoretically, a source of unnecessary stress. Such situations may occur, for example, during student education (17, 22).

In view of the above, the authors assumed that multiparous cows, which had previously undergone repeated rectal examinations, experienced little or no stress, irrespective of the duration of the examination.

The study aimed to determine the effect of rectal examination of various duration on the values of the heart rate, blood pressure, and eye temperature in cows.

Material and methods

Animals. Data were collected from 36 multiparous Polish Holstein-Friesian cows (aged 3-4 years). This was a control study of cows to be subjected to assisted reproduction technology (ART) depending on the diagnosis of functional ovarian structures. The females were selected in advance from among dozens of cows by assessing their temperament on a 3-point scale according to Danchuk (9) and eliminating those rated „1".

Ethical approval. The research conformed with the local and EU animal welfare regulations and did not require additional approval, which was confirmed by the Animal Welfare Advisory Team in Wrocław, Poland.

Description of the study and animal welfare. The data collected allowed the cows to be divided into three groups. In group I of cows (n = 12), the effect of a short per rectum examination (of up to 1 minute) was evaluated. In group II (n = 12), the examination lasted 3 minutes, and in group III (n = 12) it took less than 15 seconds. Before testing, the animals were accustomed to the presence of a four-person team for 10 minutes. Before each test, the glove was generously coated with a slip gel (ExplorationGel-JFarm). The tests were carried out very carefully to avoid unnecessary stress. For the same reason, all methods of taming the cows were dispensed with and limited to a gentle grip on the weak point. All examinations in groups I, II, and III were performed by an experienced veterinarian.

Data collection

Time measurement. During each examination by an experienced veterinarian, a designated person measured the time of palpation with a stopwatch.

Heart rate. In each group, the resting heart rate was measured three times before the per rectum examination, at 1-minute intervals. Subsequent tests were performed immediately after the examination and after 2 and 4 minutes. The heart rate was measured manually on the caudal artery.

Blood pressure. Blood pressure was measured with an OMRON M2 BASIC - CHEM-CR24 upper-arm blood pressure monitor (Alameda Healthcare, Poland). The sleeve of the device was placed on the tail approximately 10-15 cm below its root at the level of the ischial processes. Blood pressure was measured three times: before the examination, immediately afterwards, and 4 minutes later.

Thermal imaging. Thermal images of the ocular surface were taken with a FLIR TG165 camera (FLIR Systems, Inc. 27700 SW Parkway Ave. Wilsonville, OR 97070, USA) to monitor temperature changes before and after the examination (immediately and in the 4th minute after the examination). The value of emissivity was set to 0.95. A lateral thermographic image was recorded at a distance of 1.0 m from the animal's head (Fig. 1). The thermographic images were analyzed by Flir Tools software (ver.

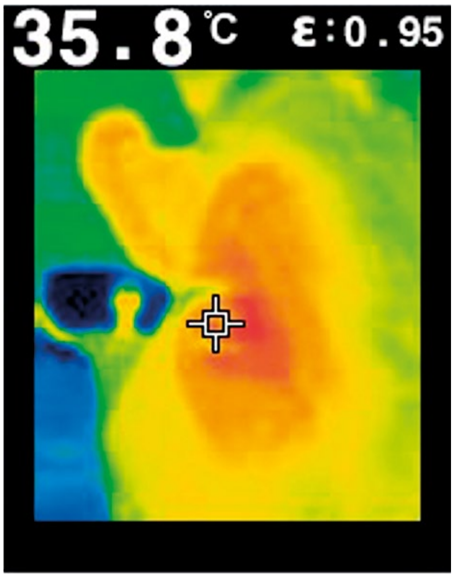

Fig. 1. Example of a thermal image (infrared thermogram) of the head region

6.4.18039.1003). To exclude the effect of different environmental conditions, only cows from one enclosure with comparable humidity and temperature conditions were tested.

Statistical analysis. Statistical analysis of the numerical data was performed by generally applicable methods. The Microsoft Office Excel 2016 software was used, and means and standard deviations were calculated. The significance of differences between means was assessed using analysis of variance (ANOVA), and the division of means into homogeneous groups was performed post hoc using the NIR test (Statistica software statistical tools package version 7.1 PL). Differences were considered significant at $P < 0.05$.

Results and discussion

Heart rate. Table 1 shows changes in the heart rate at different stages of the study (Tab. 1) (Fig. 2). The differences between the study groups were minimal. The most similar results were obtained in groups I and II. In group I, the heart rate increased on average from 76.2 beats/min to 82.4 beats/min ($P < 0.05$), and in group II from 72.9 beats/min to 81.7 beats/min ($P < 0.05$). No increase in heart rate values was observed in group III ($P > 0.05$). After 4 minutes, the heart rate returned to baseline values in 41% of the cows, was higher than

Tab. 1. Average heart rate (HR) at rest, immediately after the examination, and 4 minutes after the examination

	Group I	Group II	Group III	ANOVA
HR output	76.2 ± 6.7[a]	72.9 ± 3.6[a]	75.4 ± 6.7	SS effect = 211.468 df = 2 MS effect = 105.730 F = 3.002 p = 0.0541
HR directly after the examination	82.4 ± 3.7[bB]	81.7 ± 4.5[bAB]	76.7 ± 7.3[A]	SS effect = 231.539 df = 2 MS effect = 115.770 F = 3.583 p = 0.0394
HR 4 min after the examination	75.8 ± 4.5[a]	75.3 ± 5.0[a]	76.3 ± 6.9	SS effect = 6.002 df = 2 MS effect = 3.001 F = 0.087 p = 0.9167
ANOVA	SS effect = 352.776 df = 2 MS effect = 176.388 F = 4.878 p = 0.0114	SS effect = 694.040 df = 2 MS effect = 347.020 F = 19.684 p = 0.0000	SS effect = 19.344 df = 2 MS effect = 9.672 F = 0.194 p = 0.8242	–

Explanations: a, b – $P < 0.05$ (within-group comparison); A, B – $P < 0.05$ (between-group comparison)

Tab. 2. Mean temperature value (T) at rest, immediately after the examination, and 2 minutes after the examination

	Group I	Group II	Group III	ANOVA
T output	35.7 ± 0.4[A]	36.2 ± 0.3[B]	36.2 ± 0.3[B]	SS effect = 1.677 df = 2 MS effect = 0.839 F = 6.894 p = 0.0041
T directly after the examination	35.8 ± 0.3[A]	36.4 ± 0.3[B]	36.3 ± 0.3[B]	SS effect = 1.898 df = 2 MS effect = 0.949 F = 9.610 p = 0.0008
T 2 min after the examination	35.8 ± 0.4	36.3 ± 0.4	36.3 ± 0.4	SS effect = 1.132 df = 2 MS effect = 0.566 F = 3.285 p = 0.0541
ANOVA	SS effect = 0.045 df = 2 MS effect = 0.022 F = 0.148 p = 0.8626	SS effect = 0.213 df = 2 MS effect = 0.107 F = 0.823 p = 0.4525	SS effect = 0.003 df = 2 MS effect = 0.002 F = 0.017 p = 0.9834	–

Explanations: a, b – $P < 0.05$ (within-group comparison); A, B – $P < 0.05$ (between-group comparison)

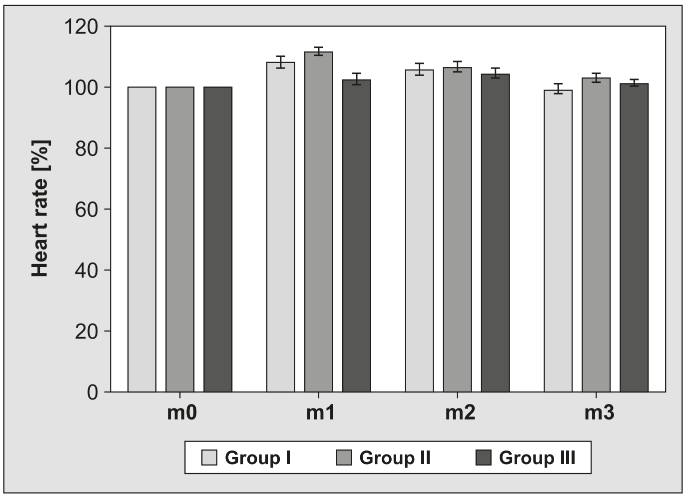

Fig. 2. Changes in the heart rate due to rectal examination in groups I-III
Explanations: Heart rate measured before the examination (m 0), immediately after the examination (m 1), 2 minutes after the examination (m 2), and 4 minutes after the examination (m 2). Values mean ± SEM

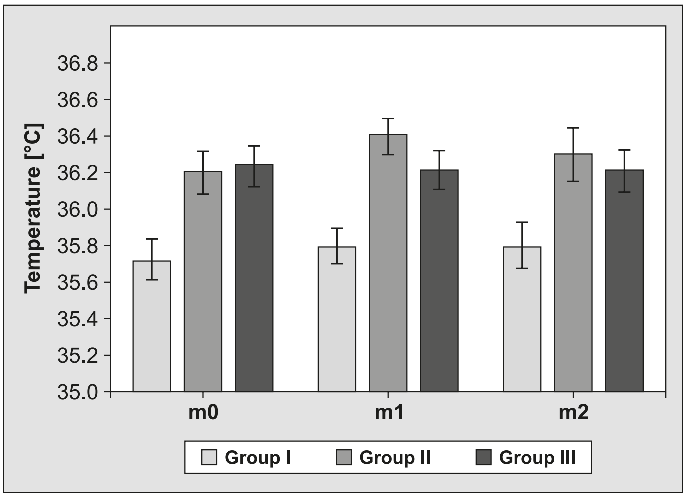

Fig. 3. Changes in eye temperature due to rectal examination in groups I-III
Explanations: Temperature measured before the examination (m 0), immediately after the examination (m 1), and 4 minutes after the examination (m 2). Values mean ± SEM

baseline values by 1 to 3 heartbeats/min in 45% of the cows, and by an average of 4 heartbeats/min in 14% of the cows.

Eye temperature. There was no effect of rectal examination on ocular temperature (Tab. 2) (Fig. 3). In Group I, the baseline values of temperature and in subsequent measurements were significantly lower than those in Groups II and III. In Groups I and III, temperature rose by 0.1°C above the baseline value after palpation, but the change was not statistically significant ($P > 0.05$). In group II, this change was 0.2°C ($P > 0.05$).

Blood pressure. The mean values of systolic/diastolic blood pressure measured immediately before and after rectal examination are shown in Table 3. A significant increase in systolic blood pressure values was observed in group I ($P < 0.05$). The value of systolic pressure after the rectal examination was 21.5 mmHg higher than the baseline value. No changes in systolic pressure values were observed in groups II and III

Tab. 3. Mean values of systolic/diastolic pressure measured immediately before and after rectal examination

Group	Blood pressure before the examination (mmHg)	Blood pressure after the examination (mmHg)	ANOVA (systolic pressure)	ANOVA (diastolic pressure)
I	79.0 ± 6.82^{aA}/49.25 ± 3.63	100.5 ± 5.94^{b}/50.75 ± 5.97	SS effect = 924.500 df = 1 MS effect = 924.500 F = 16.963 p = 0.0062	SS effect = 4.500 df = 1 MS effect = 4.500 F = 0.138 p = 0.7229
II	113.67 ± 15.14/78.66 ± 19.14	104.2 ± 3.97/51.20 ± 6.85	SS effect = 168.030 df = 1 MS effect = 168.030 F = 1.875 p = 0.2198	SS effect = 1414.530 df = 1 MS effect = 1414.530 F = 8.773 p = 0.0250
III	103.75 ± 11.92^{B}/57.25 ± 18.1	102.0 ± 5.87/58.75 ± 7.39	SS effect = 38.272 df = 1 MS effect = 38.272 F = 0.326 p = 0.5859	SS effect = 0.138 df = 1 MS effect = 0138 F = 0.001 p = 0.9810
ANOVA (systolic pressure)	SS effect = 2309.311 df = 2 MS effect = 1154.650 F = 7.612 p = 0.0141	SS effect = 58.500 df = 2 MS effect = 29.250 F = 0.680 p = 0.5266		
ANOVA (diastolic pressure)	SS effect = 1537.400 df = 2 MS effect = 768.735 F = 2.934 p = 0.1107	SS effect = 116.164 df = 2 MS effect = 58.082 F = 0.9716 p = 0.4087		

Explanations: a, b – $P < 0.05$ (within-group comparison); A, B – $P < 0.05$ (between-group comparison)

(P > 0.05). Post-test systolic pressure was 1.75 mmHg higher in group II and decreased by 19.2 mmHg in group II. Diastolic pressure did not change from its baseline values.

To assess the cows' response to rectal examination, we chose to measure the heart rate, blood pressure, and eye temperature because these methods are simple, non-invasive, and feasible under barn conditions. Furthermore, these parameters are very sensitive, and changes in their values under stimulus can be immediate. Conducting these tests in an environment familiar to the animals makes it possible to obtain a reliable response to the stimulus. In contrast, testing under laboratory conditions may cause overexcitement due to the change in the environment.

Some authors believe that rectal examination can cause significant stress in cows (7, 51). This is not confirmed by the results of the present study. We assessed the effect of rectal examination on the values of selected physical indices, heart rate, and eye temperature. The results did not show significant differences between short and prolonged examinations. The heart rate increased immediately after the examination, but returned to the baseline value after only four minutes. Similar results were previously presented by Giese et al. (12) and Ille et al. (15). Following rectum palpation by students, the heart rate increased from 80 to 83 beats per minute (12). A slight change in the heart rate was also found after a rectal examination of mares (15). On the other hand, other authors report a significant increase in the heart rate after rectal examination in both lactating and non-lactating cows (by 16.4 and 16.1 beats/min) (21). In a study by Mohr et al. (27) the heart rate after rectal examination increased from its baseline value by 10.2 beats/min in lactating cows and by 14.2 beats/min in non-lactating cows. In our study, the heart rate, after a short increase, decreased again to the baseline value. In 41% of the cows, the heart rate reached the baseline value after 4 min, and in 86% of the animals the heart rate assessed after the rectal examination was comparable to the baseline value or remained slightly above (1-3 heartbeats/min). An increase in the heart rate also accompanies physiological processes, such as calving. It was found that the average heart rate in cows was 82.9 beats/min 12-24 hours before parturition, increased to 107.5 beats/min during parturition, and decreased to 98.3 beats/min 0.5 hours after parturition (20). An increase in the heart rate was also found in cows exposed to 10 mA and 12 mA electric current for 10 seconds. The average baseline heart rate was 72.9 beats/min, whereas after the application of 10 mA current for 10 sec the heart rate increased to 89.6 beats/min. In horses, an increase in the heart rate caused by repeated transport as well as prolonged transport was studied (36, 37). A marked change occurred in both cases. It was shown that the heart rate was higher at the start of transport (36). If the transport of horses was repeated, the increase in the heart rate was highest during the first transport and significantly lower in subsequent transports (37).

A change in blood pressure can be an important effect of stress. There are no papers describing changes in blood pressure due to rectal examination in cattle. Studies in humans show that stress can lead to functional changes, such as increased blood pressure, higher heart rate, and even myocardial infarction (50). Our results revealed a cardiovascular response manifested by a significant change in blood pressure. The changes following rectal examination were statistically significant. However, the pressure decreased (long examination), increased (short examination), or remained unchanged (momentary examination) (Tab. 2). This shows that pressure cannot be a reliable indicator of a cow's reaction to rectal examination. According to researchers, a reduction in vagus nerve tone has a significant effect on blood pressure (8, 50). Chronic stress leads to such situations. Since rectal examination is a short-term stimulus, it appears to cause no significant changes in the blood pressure of domestic cattle.

Body temperature in animals is routinely measured in the rectum with an electronic thermometer (5). There are also other, simpler methods of measurement correlated with internal body temperature, such as the measurement of eye temperature with a thermal imaging camera (10, 13). Thermography is a non-invasive examination. That is why was used, among others, to detect elevated body temperature in cattle during lung diseases, such as BRD. Eye temperature measurement has also been used to measure responses to anesthetics administered to calves and to assess the welfare of domestic cattle (9, 42-44). The studies cited above showed a high correlation between eye temperature and changes in the heart rate (42-44). This method has some limitations. They are due to the influence of environmental factors: wind speed, insolation, and the distance of the thermal camera from the eye of the animal (6). There have also been attempts to use thermography of the nose, eye, and coronal edge of the thoracic limbs to assess the emotional state of the animal (31, 47). Limb warmth was analyzed for heat production. The most conclusive results about heat production were for the foot area (28).

Under stress, there is a strong activation of the endocrine glands. According to some, it may cause an approximately tenfold increase in ACTH secretion and a significant elevation of corticosteroid levels. Increased activity of the sympathetic nervous system is also observed, resulting in elevated secretion of endogenous catecholamines. Increased activity of these systems affects the values of some hematological and biochemical blood indices (25, 44). During stress, significant changes in the levels of copeptin, a vasopressin-associated protein, have also been shown in humans (16). Subjecting patients to heat stress caused a significant increase in copeptin levels, which was not accompanied by an increase in cortisol concentration (40). It has been suggested that copeptin may be a more sensitive indicator of heat stress than cortisol in a heat-stress situation. The usefulness of determining copeptin levels as a stress marker has not yet been described in animals. However, the measurement of cortisol levels is the most commonly used method to assess stress, including the stress associated with vaginal and rectal examinations (7, 11, 29, 30). Cortisol is the most important corticosteroid hormone in the ungulates. It is widely regarded as a good indicator of stress (24). Increased blood concentrations of this hormone have been observed, among others, in cows that have changed their milking place or have been left unattended by a milker (35). Free cortisol concentration during a stress reaction may increase from 20% to 30% (18). Increased cortisol concentrations in cows are also found during other husbandry practices, such as transport and surgery (7, 11). Cortisol concentration increased at different times after the test or did not increase significantly (41). In some cows, it increased 5-10 minutes after rectal examination, in others after 25 minutes (12). Given that the increase in blood cortisol concentration occurred with some delay after the stressful stimulus, an additional, unrelated stimulation of the hypothalamic-pituitary-adrenal axis cannot be completely excluded. For this reason, a more sensitive stress marker appears to be the heart rate, which better reflects the cow's reaction in real-time. In addition, testing cortisol levels is subject to error due to diurnal variations in its concentration (23). The level of the hormone changes dynamically throughout the day: there are several peaks, occurring at 2-hour intervals. The diurnal rhythm does not exclude an individual cycle of cortisol concentration in different individuals (23). The ultra-daily cycle of fluctuations in cortisol concentration may also depend on the season and the system of animal housing (2). Several different risk factors may indicate that cortisol is not an appropriate response marker to a short-acting stimulus. Some studies suggest that cortisol measurement may be more useful in cases of prolonged stress, e.g. during transport. At the same time, it has been shown that animals can adapt to a stress factor (36). For example, the first transport of animals was accompanied by a significant increase in fecal cortisol levels. During subsequent transports, however, the increase in hormone concentration was not pronounced (36). Similar relationships, taking into account animal adaptation, were obtained by examining fecal cortisol levels after long transports (36). The high initial values of the hormone argued for the onset of significant stress. However, such values were not recorded at later stages of transport, which suggested a rapid adaptation of the animals (37).

In addition to the previously mentioned pulse examination, animal observation is a good way to measure the cow's response to rectal examination (29, 30). Observation is based on a subjective assessment of the cow's behavior and assigning its behavior to a point scale. The results of such observations indicate that rectal examination has no significant effect on the cow's behavior. However, it cannot be excluded that the animal may feel some discomfort during the examination, which is shown by back bending and neck straightening. Temperament, which varies according to age, may play a role in the assessment of the cow's behavior during rectal examination (30). Changes in the behavior of cows were noted on the day following rectal examination. The animals chewed more frequently and lay down longer during the day (41). However, this does not directly suggest stress. Prolonged lying time may be associated with movement problems, e.g. lameness or visceral pain (32). In addition, lying time is prolonged in the summer as a result of heat stress (45).

A cow's reaction to rectal examination is a complex reaction: the sum of the activities of the nervous and endocrine systems. In our study we found that: 1. Depending on the duration of rectal examination, a cow shows different values of the heart rate, eye temperature, and blood pressure. 2. The most reliable indicator of a cow's response to rectal examination is the heart rate. 3. The standard rectal examination (performed routinely by veterinarians) is too short to cause changes in eye temperature. The data obtained may be useful for veterinarians and dairy cattle breeders. Despite minimal differences in parameters, different ways of responding to stress should not be discredited, especially in view of the imperfect knowledge of the psychology and emotional development of dairy cows.

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Corresponding author: Arkadiusz Grzeczka, DVM, Institute of Veterinary Medicine, Nicolaus Copernicus University in Torun, Gagrina 11, 87-100 Toruń, Poland; e-mail: grzeczka@umk.pl