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BIOCHEMICAL AND PHYSIOLOGICAL RESPONSES IN DAIRY COWS AFTER TRANSRECTAL EXAMINATION

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ABSTRACT

The evaluation of physiological stress indicators provides valuable insights into the stress responses of dairy cows subjected to routine handling procedures such as transrectal examination. This study investigated changes in cortisol levels, heart rate, and rectal temperature in dairy cows with different behavioral profiles following transrectal examination. All measured indicators showed significant increases after the procedure, except the temperature, indicating a clear physiological stress response. The average cortisol level is 2.30±0.26 U/ml, and after the rectal examination it is 2.45±0.34 U/ml. The level of white blood cells increases by 0.7%, the levels of ALAT increase by 7.33* U/ml, the levels of ASAT by 15.67* U/ml, the levels of glucose increase by 0.63 U/ml. From the physical indicators – the systole increases by 12.5*mm/Hg, the diastole – 3.33*mm/Hg, the pulse increases by an average of 10.84 beats/min. The average temperature before the rectal examination is 37.7°C, after the rectal examination it drops to 36.93°C. The results can contribute to refining handling practices to improve animal welfare.

Key words: transrectal examination, cows, stress responses.

Introduction

Stress is generally recognized as a reflex response that inevitably occurs when animals are exposed to adverse environmental conditions, leading to various negative consequences ranging from discomfort to death. The primary mechanisms of the stress response involve increased glucocorticoid secretion and activation of the sympathetic nervous system (Heinrich, *et al.*, 2020). The hypothalamus synchronizes the release of hormones regulating adrenocorticotropic hormone (ACTH) and catecholamines, resulting in complex biochemical and physiological stress reactions (Spencer et Deak, 2017). Veterinary procedures, such as rectal palpation, are known to be potent stressors in cattle, triggering a physiological cascade that activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (Mormède *et al.*, 2007). The mechanical stimulation and handling associated with rectal palpation induce an acute stress response, leading to a rapid increase in cortisol secretion from the adrenal cortex. Elevated cortisol levels serve as a primary biomarker of stress, reflecting the activation of the HPA axis in response to perceived threats or discomfort (Smith et Vale, 2006). Beyond hormonal changes, rectal palpation elicits various physiological and behavioral responses indicative of acute stress. Increased heart rate and blood pressure are commonly observed due to heightened sympathetic nervous system activity (Shaffer and Ginsberg, 2017). Additionally, rectal palpation may cause changes in respiratory rate, pupil dilation, and muscle tension, further demonstrating the activation of the autonomic stress response (Fernandez-Novo *et al.*, 2020). In addition to cortisol, other blood indicators, including ALT, AST, glucose, and white blood cell counts, are essential for

monitoring stress responses in cattle. Increased heart rate and arterial pressure are common signs of acute stress, while alterations in ALT, AST, and glucose levels reflect metabolic and hepatic adaptations. White blood cell analysis provides further insights into immune function, which is often compromised under prolonged stress exposure. The aim of the present study was to evaluate the effects of rectal palpation on cortisol levels, hematological parameters, heart rate, body temperature and blood pressure in dairy cows.

Materials and Methods

Animals and Housing

For this study, 13 clinically healthy cattle of the Bulgarian Black and White breed – the main dairy breed in the country, developed through selection and crossing with Holstein-Friesian cattle were examined. The cows were raised under standard conditions for the breed and production system, with controlled feeding, care and sexual exploitation, in accordance with the regulatory requirements for humane treatment and reproductive health. They were milked twice a day by machine milking.

Experimental Design

The rectal palpation procedure was conducted in a controlled environment using the traditional technique. The animals in the study group were placed in the same stocks. The rectal examination was performed with examination gloves initially lubricated with an appropriate gel. The fingers were shaped into a cone and gently touched the anal sphincter while waiting for the animal's response. After passing the anal sphincter, any feces or debris in the intestinal tract were carefully evacuated, ensuring that no air was introduced. Once the rectum was emptied, and pelvic bones, cervix uteri, uterus and both ovaries were palpated for five minutes each.

Measuring temperature, blood pressure, pulse and collection of blood samples were done from each animal one hour before and immediately after the rectal palpation procedure, via jugular vein puncture into heparinized and regular tubes for measuring white blood cell (WBC) and into tubes with clot activator for measuring alanine aminotransferase (ALT), aspartate aminotransferase (AST), Glucose – (Glu) as well as plasma cortisol concentrations. The circulating WBC levels were measured from whole blood (2 mL), while ALT, ASAT, glucose and cortisol levels were measured from serum.

Hematological Analysis

WBC were measured from whole blood using an automatic blood count device Mindray BC-30 Vet.

Biochemical Analyses

Blood was collected in a tube with clot activator and centrifuged at 3500 rpm for 5 minutes. Serum cortisol concentrations were measured using an ELISA kit. Glucose ALT, ASAT levels were analyzed in serum using a semi-automatic Mindray BA-88A analyzer.

Physiological parameters

Blood pressure and pulse were measured at the a. coccygea with an automatic blood pressure monitor. Temperature was measured rectally with a digital thermometer.

Results

The variations of the hematological and biochemical markers before and after rectal palpation are summarized in Table 1. Serum glucose and cortisol concentrations have significantly and markedly increased ($P < 0.05$). The glucose levels increased with 0.63 U/ml and cortisol levels with 0.61 ng/ml. White blood cell levels values increased from 6.95% to 7.64%. Liver function markers also increase – for ALT we report an increase of 7.33* U/ml with a significant difference, and for AST – 15.67 U/ml increasement.

Table 1: Hematological and biochemical parameters in Bulgarian black and white breed after and before rectal palpation procedure. Results are expressedas means ± standard errors.

Parameters	Before rectal palp	After rectal palp	Variation
WBC % mm^3	6.95±1.12	7.64±1.66	0.69
ALAT U/ml	27±5.44*	34.33±2.87*	7.33
ASAT U/ml	75.33±15.73	91±17.66	15.67
Glu U/ml	5.47±0.64*	6.1±1.27*	0.63
Cortisol ng/ml	2.3±0.36*	2.91±0.15*	0.61

**p < 0.05*

The results of the examined physical parameters are summarized in Table 2. We report an increasement in the pulse from 79 to 90 beats per minute. The systolic pressure is increased by 12.44* mmHg, and the diastolic by 3.3mmHg. The only parameter that shows a decreased result after the rectal examination is body temperature, which drops by almost a whole degree.

Table 2: Physiologycal parameters in Bulgarian Black and White breed after and before rectal palpation procedure. Results are expressedas means ± standard errors.

Parameters	Before rectal palp	After rectal palp	Variation
Puls bpm	79.16±21	90±15.83	10.84
Systol mmHg	117.16±3.76*	129.6±8.26*	12.44
Diastol mmHg	87±7.09	90.3±5.53	3.3
Body temp	37.75±0.4	36.93±1.07	0.82

**p < 0.05*

Discussion

Rectal palpation is a widely used method among veterinarians for physical examination, pregnancy diagnosis, and assessing reproductive potential in cattle. Although it is an essential diagnostic tool, the procedure can cause discomfort and trigger physiological and behavioral stress responses (Momont, 1990; Baillie and al., 2008). Stress is an adaptive biological reaction aimed at maintaining homeostasis in response to external stimuli (Romano and al., 2007). While this mechanism

serves a protective function, excessive stress can lead to disruptions in biological processes, potentially compromising animal health and overall well-being.

One of the primary biomarkers of stress is cortisol, the most significant corticosteroid hormone in hoofed animals. Under normal conditions, only 10% of circulating cortisol exists in its free and active form, while the remaining 90% is protein-bound, primarily to albumin and corticosteroid-binding globulins (Nakao and al., 1994). However, during stress, free cortisol levels can rise to 20–30%, significantly impacting physiological functions (Kagan and Levi, 1974). The activation of the hypothalamic-pituitary-adrenal (HPA) axis is a critical component of the stress response in cattle. When an animal perceives a stressor, such as rectal palpation, the hypothalamus releases corticotropin-releasing hormone (CRH), which stimulates the anterior pituitary gland to secrete adrenocorticotropic hormone (ACTH). ACTH then acts on the adrenal cortex, triggering the release of cortisol into the bloodstream (Carroll et al., 2014). Cortisol plays a vital role in regulating metabolism, immune function, and cardiovascular activity during stress. Under normal physiological conditions, cortisol levels fluctuate within a baseline range. However, during acute stress, the adrenal glands can rapidly increase cortisol secretion by tenfold or more (Herskin *et al.*, 2007). In one study using sheep, serum cortisol concentrations peaked (from 2.5μg/dL or 69nmol/L) 20min after the procedure (Damian and Ungerfeld, 2011). We find an increase of 0.61 nmol/ml which corresponds to 61nmol/L, but the sample was taken immediately after the rectal examination. This surge is part of an adaptive response designed to mobilize energy reserves, suppress non-essential functions (such as digestion and reproduction), and enhance an animal's ability to cope with stressors. Several studies have demonstrated that various husbandry practices, including transportation, handling, and veterinary procedures, significantly elevate plasma cortisol levels in cattle (Crookshank *et al.*, 1979; Robertson *et al.*, 1994; Fidan *et al.*, 2010). Specifically, rectal palpation has been shown to be a potent stressor, as evidenced by the sharp rise in cortisol levels immediately following the procedure (Nakao and al., 1994; Waiblinger *et al.*, 2004). This increase can persist for an extended period, depending on the duration and intensity of the stressor, as well as the animal's individual stress tolerance. Our results confirm previous findings that rectal palpation is a significant stressor in cattle, as evidenced by the marked increase in plasma cortisol levels. Prolonged or repeated activation of the HPA axis due to frequent stress exposure can have negative consequences. Chronic elevations in cortisol levels can impair immune function, reduce reproductive efficiency, and disrupt metabolic balance, ultimately affecting the overall health and productivity of cattle. Given these findings, stress mitigation strategies – such as minimizing handling time, ensuring proper restraint techniques, and using desensitization protocols – may help reduce the physiological burden of procedures like rectal palpation.

A notable increase in white blood cell (WBC) count following rectal palpation is likely linked to the physiological stress response, particularly the contraction of the spleen. The spleen serves as a reservoir for red and white blood cells, and its contraction is primarily mediated by catecholamines (such as epinephrine and norepinephrine), which are released in response to stress (Bush and al., 1987). When the sympathetic nervous system is activated during acute stress, catecholamines stimulate α-adrenergic receptors in the splenic capsule, leading to a rapid release of stored cells into circulation. This results in a transient elevation in circulating WBCs, particularly neutrophils, which are the first line of defense in the immune system. This result was confirmed by Yeni *et al.*, 2005 reporting an increase of WBC with almost 2 %. We note an increase in white blood cell levels of 0.69%. The increase in WBC count observed during stress is a well-documented phenomenon in cattle and other species. It is often associated with a shift known as stress leukogram**.** This leukocyte

shift is largely mediated by cortisol, which not only enhances neutrophil release but also suppresses lymphocyte and eosinophil production. While an acute rise in WBCs is a normal adaptive response, chronic stress and prolonged cortisol elevation can weaken the immune system, making cattle more susceptible to infections (Waiblinger *et al.*, 2004).

Plasma glucose levels in ruminants originate from multiple sources: approximately 44% from the absorption of organic acids in the rumen, 33% from post-ruminal glucose absorption, and 23% from other carbon sources such as amino acids converted in the liver. Stress-related hormone release, particularly catecholamines and glucocorticoids, stimulates glycogenolysis and gluconeogenesis, leading to hyperglycemia. In this study, glucose concentrations increased following rectal palpation, likely due to elevated cortisol secretion. These findings align with previous studies on stressed animals, including gazelles, sheep, cattle, and deer (Marco et Lavin, 1999; Fidan *et al.*, 2010). Additionally, alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels increased post-examination. This rise is likely due to stress-induced metabolic changes and increased muscle activity during handling and restraint. Elevated ASAT levels, in particular, may also indicate muscle strain or minor tissue damage, as this enzyme is present in both liver and muscle tissues. The observed changes in these biochemical markers further emphasize the physiological impact of rectal palpation and reinforce the need for stress management strategies in cattle handling. Our results manisfes an increase in ALAT by 7.33 U/ml and in ASAT by 15.67 U/ml. von Borell *et al.*, 2007 report report the same trend for enzyme elevations in stressful condition. Chen *et al.*, 2016 reported an increase in ALAT by 12.7 U/ml and in ASAT by 27.7 U/ml.

Our results show that rectal palpation affects key physiological parameters in cattle. Heart rate and blood pressure increased following the procedure, indicating a typical stress response driven by sympathetic nervous system activation. Such increases are also reported by Pilz et al, 2014, who found that blood pressure rose by 29mmHg and pulse by 30 bpm. Kovács *et al.*, 2014 again found an increase in the physical parameters of dairy cattle after medical manipulations. In contrast, body temperature showed a slight decrease, which may be attributed to peripheral vasodilation and heat dissipation as part of the body's adaptive mechanisms to stress. These findings further highlight the physiological impact of rectal palpation and reinforce the need for stress management strategies in cattle handling (Al-Tamimi et al, 2003).

Conclusion

Rectal palpation in cattle induces a physiological stress response, as evidenced by a significant increase in plasma cortisol levels. This stress reaction is accompanied by an elevated white blood cell (WBC) count, likely due to splenic contraction. Additionally, alterations in heart rate, blood pressure, and a slight decrease in body temperature suggest autonomic nervous system involvement. The observed increase in ALT and AST levels may indicate cellular stress and heightened metabolic activity. These findings confirm that rectal palpation acts as a stressor in cattle, leading to physiological changes that should be considered in veterinary practice and livestock management. Implementing strategies to minimize stress, such as improved handling techniques and potential use of stress-reducing interventions, could help mitigate its negative effects on animal welfare and productivity.

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