

Stress Response in Dairy Cows Related to Rectal Examination

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ABSTRACT

Background: Currently, rectal palpation is the cheapest and most convenient method which is using for detecting most of internal diseases, gynecologic conditions and artificial inseminations in dairy cows. However, rectal palpation procedure is probably painful, resulting in physiological and behavioral stress reactions. The life and metabolic activity can be affected by the oxidative stress which is one of the stress-related negative outcomes and characterized by the accumulation of radical oxygen species. Many factors such as stress, diet, antibiotics and husbandry practices effect animals' health and growth performance. The aim of the study was emphasized to the effects of rectal palpation related to stress response and oxidant /antioxidant status in dairy cows.

Materials, Methods & Results: The study was conducted on ten 3-4 years old, non-pregnant Holstein dairy cows. The rectal palpation procedure was performed in a closed area using the traditional method. Blood samples were taken from each animal, an hour before and just after the rectal palpation procedure for detecting white blood cell, red blood cell, hemoglobin levels, hematocrit value, total antioxidant status, total oxidant status and nitric oxide, glucose and total cholesterol levels, as well as the plasma cortisol concentrations. WBC, RBC, Hct and Hb values were measured from whole blood by using an automatic blood count device. Plasma TAS and TOS levels were determined using a commercially available kit. Nitric oxide decomposes rapidly in aerated solutions to form stable nitrite/nitrate products. Plasma nitrite/ nitrate concentration was measured by a modified method of Griess assay. Plasma cortisol concentrations were determined using an ELISA kit. Glucose and total cholesterol levels were analyzed in serum by using an auto-analyzer. White blood cell levels and hematocrit values were significantly increased just after the rectal palpation procedure compared to the baseline values ($P < 0.05$) whereas no significant difference was found in red blood cell levels and hemoglobin concentrations. Serum cholesterol concentrations have decreased after rectal palpation, serum glucose and cortisol concentrations have significantly and markedly increased ($P < 0.05$), the relevant variation factors were 13.41% and 23.26% respectively. Moreover, a remarkable and significant increase in the circulating TOS compared to the baseline values ($P < 0.05$ variation factor 31.78%) was observed after rectal palpation. In parallel, the nitric oxide concentrations were also significantly increased after rectal palpation procedure ($P < 0.05$ variation factor: 13.51%) On the other hand, the total plasma antioxidant status was depressed and the variation intensity remained weak (variation factor: -7.24%).

Discussion: In the present study the results indicate that the rectal palpation procedure will be considered as a stressful condition, as evidenced by the strong increases in circulating cortisol, TOS and NO_x concentrations associated with decreases in TAS concentrations. In addition, increased plasma cortisol concentrations in response to rectal palpation could be an additional factor responsible for the oxidative stress amplification. A more comprehensive identification of the physiological changes during rectal palpation could be beneficial for further researches in terms of accurate management practices in cattle practices and industry. Moreover, the determination of the oxidative stress parameters could provide novel approaches for the evaluation the stress in cattle.

Keywords: rectal palpation, dairy cow, total oxidant status, total antioxidant status, cortisol.

INTRODUCTION

Suffering, pain and stress are being uncomfortable conditions for animals and animal welfare targets a life away from these. Currently, absence of stress response is accepted as an indicator for welfare in animals [3]. Stress has generally been revealed as a reflex response that occurs unavoidable when animals are exposed to adverse environmental situations and which is the cause of many inadequate outcomes, ranging from discomfort to death [11]. Changing of feeding behavior, development of gastric and intestinal ulcers, hypertension, reproductive dysfunction, malnutrition, electrolyte imbalance and immune deficiency may occur related to stress responses [1].

Enhanced secretion of glucocorticoids and increased sympathetic nervous system activity are related to the major stress alterations involvement. Synchronized control of hypothalamic releasing hormones of ACTH and of catecholamines, results in biochemical and physiologic aspects of stress [30]. Most of veterinary procedures, such as rectal palpation is indicated that increase secretion of cortisol from the adrenal cortex in cattle. Animal's physiolog and behavior in a manner typical of an acute stress response is affected by rectal palpation [29,40].

Since a measure for welfare of the animals has not yet been determined, using the oxidative stress parameters could be a new approach in determining the stress. However there was no published article investigating the effects of rectal palpation procedures on animal welfare using biomarkers of oxidative stress in dairy cows.

The aim of the present investigation is to evaluate the effects of rectal palpation procedures on oxidant-antioxidant status and stress in dairy cows.

MATERIALS AND METHODS

Animals and Protocol Design

The study was conducted on ten 3-4 years old, non-pregnant Holstein dairy cows. All animals were carefully monitored and the study protocol was carried out in accordance with the Helsinki Declaration as revised in 1989.

The rectal palpation procedure was performed in a closed area using the traditional method. The animals in the study group were only placed in same stocks. Rectal examination was performed with ex-

amination gloves initially lubricated with appropriate gel. Than the fingers became cone shaped and touched the sphincter ani while waiting the animal's response. After passing the anal sphincter at once, any feces or debris in the intestinal tract was evacuated carefully with care being taken not to let air in. When the rectum was emptied, the peristaltic contractions of rectum, intestinal mucosa, left kidney's mesenterial ligaments, cervix uteri, uterus, and pelvis bones were palpated for five minutes respectively.

Blood samples were taken from each animal, an hour before and just after the rectal palpation procedure, by puncture of the jugular vein into heparinized and regular tubes for measuring white blood cell (WBC), red blood cell (RBC), hemoglobin (Hb) levels, hematocrit (Hct) value, total antioxidant status (TAS), total oxidant status (TOS) and nitric oxide (NOx), glucose and total cholesterol levels, as well as the plasma cortisol concentrations.

The circulating WBC, RBC, Hct and Hb levels were measured from whole blood (2 mL) whereas glucose and total cholesterol levels were measured from the serum and other biochemical parameters from the plasma obtained after centrifugation of blood (1000 g, 10 min at room temperature) and stored at -30°C.

Hematological Analysis

WBC, RBC, Hct and Hb values were measured from whole blood by using an automatic blood count device¹.

Biochemical Analyses

Plasma TAS and TOS levels were determined using a commercially available kit² developed by Erel [12]. Nitric oxide decomposes rapidly in aerated solutions to form stable nitrite/nitrate products. Plasma nitrite/ nitrate concentration was measured by a modified method of Griess assay, described by Miranda *et al.* [26]. Plasma cortisol concentrations were determined using an ELISA kit³. Glucose and total cholesterol levels were analyzed in serum by using an auto-analyzer⁴.

RESULTS

As reported in Table 1, white blood cell levels and hematocrit values were significantly increased just after the rectal palpation procedure compared to the baseline values ($P < 0.05$) whereas no significant difference was found in red blood cell levels and hemoglobin concentrations.

Table 1. Hematological parameters in Holstein dairy cows (n = 10) after and before rectal palpation procedure. Results are expressed as means ± standard errors.

Parameters	Before Rectal Palpation	After Rectal Palpation	Variation Factor (%)
WBC (mm ³)	7457.14 ± 551.55*	10614.29 ± 1358.29*	42.33
RBC (10 ⁶ /mm ³)	6.57 ± 0.35	6.70 ± 0.46	1.97
Hct (%)	34 ± 2.28*	37.44 ± 2.07*	10.11
Hb (g/dL)	10.83 ± 0.48	10.85 ± 0.52	0.18

**P* < 0.05. The variation factors were given by the following formula: variation factor (%) = 100 [Ca – Cb] / Cb where Ca and Cb were the concentrations observed after rectal palpation procedure and before rectal palpation procedure, respectively.

The variations of the biochemical markers before and after rectal palpation procedure are summarized in Table 2. While the serum cholesterol concentrations have decreased after rectal palpation, serum glucose and cortisol concentrations have significantly and markedly increased (*P* < 0.05), the relevant variation factors were 13.41% and 23.26% respectively. Moreover, a remarkable and significant increase in the

circulating TOS compared to the baseline values (*P* < 0.05 variation factor 31.78%) was observed after rectal palpation. In parallel, the nitric oxide concentrations were also significantly increased after rectal palpation procedure (*P* < 0.05 variation factor: 13.51%) On the other hand, the total plasma antioxidant status was depressed and the variation intensity remained weak (variation factor: -7.24%).

Table 2. Biochemical parameters and oxidant / antioxidant status in Holstein dairy cows (n = 10) after and before rectal palpation procedure. Results are expressed as means ± standard errors.

Parameters	Before Rectal Palpation	After Rectal Palpation	Variation Factor (%)
Glucose (mg/dL)	42.57 ± 5.05*	48.28 ± 4.66*	13.41
Cholesterol (mg/dL)	113.57 ± 15.83	109.57 ± 16.23	-3.52
Cortisol (ng/mL)	2.02 ± 0.04*	2.49 ± 0.05*	23.26
TOS (µmol H ₂ O ₂ /L)	6.04 ± 0.56*	7.96 ± 0.60*	31.78
TAS (mmol Trolox equiv/L)	0.69 ± 0.20	0.64 ± 0.15	-7.24
NOx (µmol/L)	10.06 ± 0.39*	11.42 ± 0.40*	13.51

**P* < 0.05. The variation factors were given by the following formula: variation factor (%) = 100 [Ca – Cb] / Cb where Ca and Cb were the concentrations observed after rectal palpation procedure and before rectal palpation procedure, respectively.

DISCUSSION

One of the most preferred methods among veterinarians for physical examination, pregnancy diagnosis, and evaluation of reproductive ability in clinical practice is bovine rectal palpation [4,28,37]. The rectal palpation procedure can be painful and cause physiological and behavioral stress reactions. [29,40]. Stress has been defined as the cumulative

response of an animal against the interaction with milieu via receptors [17]. Stress has some biological outcomes that have an adaptive purpose, aiming to achieve hemostasis [10,32]. However, they may cause a pre-pathological condition which eventuates with a pathological condition. The pre-pathological condition has been suggested to be clinical changes in biological functions without evident negative outcomes for life-

time [27]. The deterioration of biological functions caused by the stress can compromise animals' health state and life [22,27]. Several hormones play a role in the adjustment of biological functions. Thus, stressful conditions have been reported to stimulate the hypothalamo-hypophyseal-adrenal axis [3], which leads to at least ten-fold increase in the ACTH and cortisol secretions. Corticosteroids are steroid hormones that are produced within the adrenal cortex. In addition, cortisol is the most important corticosteroid hormone of hoofed animals, which has been conventionally accepted as a good stress indicator [32]. Under normal circumstances, only ten percent of circulating cortisol is free and active form. At body temperature, 90% of plasma cortisol is bound to proteins, of which 70% are corticosteroid binding globulins and albumin [31]. However, the free cortisol concentrations have been reported to reach 20 to 30% during the stress response [35]. In several studies, plasma cortisol concentrations have been reported to be increased in cattle husbandry practices [9,16,36] including rectal palpation [29,40]. As significant increases in plasma cortisol concentrations following the rectal palpation procedure were recorded in the present study, it has been suggested that rectal palpation itself would be a stressful condition.

Stress control has been found to alter numerous blood cell parameters. The first stage of the stress response is the activation of the sympathetic nervous system, in which the stimulation of adrenal medulla and the release of catecholamines occur. The spleen contraction that is caused by the action of catecholamines on α -adrenergic receptors located in the capsule frequently leads to an increase in hemoglobin concentration and packed cell volume [3,16]. In this study, despite there was no significant change in RBC and hemoglobin levels, rectal palpation procedure has been suggested to induce strong elevations in WBC depending on the contraction of the spleen, which was reflected by a relative increase in Hct value.

In ruminants, plasma glucose levels come approximately up to 44% because of the organic acid absorption from the rumen and subsequent conversion to glucose in liver, to 33% because of the postprandial glucose absorption, and to 23% because of other carbon sources such as amino acids and subsequent conversion to glucose in liver. Catecholamines and glucocorticoids increase glycogenolysis and gluconeogenesis [15,23] which may secondarily induce hyperglycemia. In this

study, glucose concentration has been increased due to the rectal palpation procedure probably through the increase of cortisol secretion. Our results are consistent with the results of previous studies conducted on stressed animals (gazelle, sheep, cows, and deer) [6,16,18,25].

The vast majority of cholesterol in the body is synthesized in the liver; however cholesterol concentrations depend on the dietary supplementation. The lipolytic glucocorticoids stimulate the fat mobilization from adipose tissue, and thereby cause and increase in the circulating concentrations of free fatty acids [16,19]. Thus, plasma cholesterol concentrations are likely to increase following a stress episode; some authors have also confirmed such an increase [25,32]. However, as cholesterol also play a role in corticoid synthesis, plasma cholesterol concentrations are also likely to decrease during a stress period [34]. Both these increased and decreased serum cholesterol and triglyceride concentrations have been found to be related to stress [2,25]. In this study, there was no difference in cholesterol concentrations following the rectal palpation procedure.

The life and metabolic activity can be affected by the oxidative stress which is one of the stress-related negative outcomes and characterized by the accumulation of radical oxygen species (ROS) [8,33,42]. In fact, ROS are the normal products of cellular metabolism. Nevertheless, endogenous biological or exogenous environmental factors may lead to excessive production of free radicals [14,15]. An imbalance between the ROS production and various antioxidant systems can cause cellular injury and tissue damage, which leads to the alterations in macromolecules (membrane lipids, proteins and DNA), changes in intracellular calcium contents and intracellular pH, all of which eventuate with cell death [13,15,24]. Serum (or plasma) concentrations of each of the different oxidant and antioxidant species can be measured in laboratory conditions, although they are time-consuming, labor-intensive and costly and require complicated techniques. As the measurement of each of the different oxidant and antioxidant molecules is not practical and they have additive oxidant and antioxidant effects, the total oxidant status (TOS) and the total antioxidant capacity of a sample can be measured [12,20,21,39,41,42]. TAS and TOS kits, which have been developed by Erel [12], were used in this study. While there is no report available

on oxidative stress during rectal palpation procedure, some other management procedures have shown to cause oxidative stress. The shearing and dehorning procedure has reported to induce significant increase in blood MDA concentrations, which indicates the occurrence of an oxidative stress in sheep and cattle, respectively [15,16]. Calamari *et al.* [7] reported an increase in plasma levels of TBARS and a decrease in lipid soluble antioxidants in moderately heat stressed, mild-lactating cows during summer. Avci *et al.* [3] proposed that transport might induce oxidative stress via a decrease in GSH and an increase in MDA concentrations of a sheep. Similarly, in the present study, the rectal palpation procedure has induced strong elevations in TOS value, which indicates the occurrence of an oxidative stress. On the other hand, TAS value in this study was not affected significantly. In this study, the increase in plasma cortisol levels in response to the stress induced by the rectal palpation could be an additional factor responsible for the amplification of the oxidative stress, which led to an increase in TOS value that was reflected by a relative decrease in plasma TAS value.

The role of NOx seems to be controversial, because of the likelihood of a tissue dysfunction or injury following the inhibition of NOx. However, high levels of NOx production have been proposed to precipitate the tissue injury [5]. Stimulation of tissue NOx production can also induce adverse events including hypotension, inhibition of intermediary metabolism and the production of the potent oxidant peroxynitrite following radical-radical reaction with superoxide

[38]. An increased superoxide radical content, which yields the transformation of NOx to peroxynitrite, reduces the bioavailability of NOx [43]. Therefore, a further significant increase occurred in plasma NOx concentrations following the rectal palpation procedure in our study.

Notwithstanding this is an enclosed study, our results indicate that the rectal palpation procedure will be considered as a stressful condition, as evidenced by the strong increases in circulating cortisol, TOS and NOx concentrations associated with decreases in TAS concentrations. In addition, increased plasma cortisol concentrations in response to rectal palpation could be an additional factor responsible for the oxidative stress amplification. A more comprehensive identification of the physiological changes during rectal palpation could be beneficial for further researches in terms of accurate management practices in cattle practices and industry. Moreover, the determination of the oxidative stress parameters could provide novel approaches for the evaluation the stress in cattle.

SOURCES AND MANUFACTURERS

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²REL assay diagnostics, Mega Tip, Gaziantep, Turkey.

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Declaration of interest. The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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