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Evaluation of visible eye white and maximum eye temperature as non-invasive indicators of stress in dairy cows

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ABSTRACT

The aim of this study was to investigate if visible eye white and eye temperature measurements are feasible non-invasive physiological indicators of acute stress in cows when they are exposed to cattle crush treatment for claw trimming.

In the experimental setting, 30 cows of two breeds (Red Holstein and Brown Swiss) were exposed to a non-stressful (feeding) and a stressful situation (claw trimming in a cattle crush) for 10 min each. We took pictures of the eyes at 0, 5 and 10 min after starting of exposure to measure the percentage of visible eye white (from photographs) and the maximum eye temperature (from thermographs). Heart rate and heart rate variability parameters were recorded continuously throughout both situations. Twenty minutes after the beginning of each situation, saliva samples were taken to determine the cortisol concentration.

As expected, sympathetic activity and cortisol concentration were higher in the claw trimming than in the feeding situation. However, neither maximum eye temperature nor percentage of visible eye white differed between treatments. Instead, the results of these measurements differed between the breeds. The maximum eye temperature increased during and after both situations in Brown Swiss cows, whereas in Red Holstein cows, it increased after (but not during) both situations. Furthermore, we found that Red Holstein cows had a lower percentage of visible eye white than Brown Swiss in general. This finding might be due to differences in eye coloration patterns, with Red Holstein cows having more contrast between eye white and iris and Brown Swiss having less contrast because of their darker eye white. This breed effect might have masked potential treatment effects.

1. Introduction

Stressful situations lead to an activation of the hypothalamic-pituitary-adrenal axis (HPA; Selye, 1936) and stimulation of the autonomous nervous system (ANS; Cannon, 1935; Moberg, 2000) with the sympathetic and parasympathetic branches (Stewart et al., 2005). This activation can be measured by means of hormonal secretions after stress exposure (Friend, 1980; Owen et al., 2005; Sheriff et al., 2011; Ursin and Olff, 1993;).

One of the most used stress indicators is the concentration of corticosteroids and catecholamines in plasma. Disadvantages of sampling plasma are well known. It is an invasive method and animals have to be restrained, which in turn may affect hormone concentration (Alam and Dobson, 1986; Möstl and Palme, 2002). Nevertheless, non-invasive methods are available to detect corticosteroid metabolites in saliva, milk, feces or hair (Möstl and Palme, 2002; Palme, 2012; Palme et al., 1996). However, limitations of these methods have been discussed thoroughly (Mormède et al., 2007; Negrão et al., 2004; Sheriff et al., 2011). The concentration of corticosteroids alone does not reflect the full picture because it is increased not only during punishing but also during rewarding situations such as mating or hunting (Mormède et al., 2007; Sapolsky et al., 2000). To overcome this problem, additional indicators can be used to assess the response to a potential stressor, including heart rate variability (such as regulation of sympatho-vagal tone), maximum eye temperature and especially behavioral changes.

However, regulation of the sympatho–vagal tone seems to be context specific and interpretations should thus include other stress-indicating variables (Patt et al., 2016).

Eye temperature as stress indicator has been investigated in several species (humans: Pavlidis et al., 2000, 2002; horses: Bartolomé et al.,

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2013; Cook et al., 2001; McGreevy et al., 2012; cows: Schwartzkopf-Genswein et al., 2012; Stewart et al., 2005, 2007, 2008a,b, 2009, 2010). The exact neuroendocrine pattern, however, has not been understood fully.

Artificially injecting exogenous adrenocorticotropic hormone had no immediate effect on maximum eye temperature in calves (Stewart et al., 2007). Therefore, it is thus assumed that in cattle, changes in maximum eye temperature are not due to activation of the HPA axis alone, but that other factors are involved such as a sympathetically mediated response of the ANS (Stewart et al., 2007, 2008b) or emotionally relevant stimuli (Mason, 1971; Stewart et al., 2005, 2007, 2008a,b). The limitations of the non-invasive stress indicators highlight the need for additional indicators to allow a more feasible stress assessment.

Percentage of visible eye white as an indicator of emotional state was shown to cover the entire spectrum of the contentedness axis from frustration to satisfaction (Sandem et al., 2002), with the visible eye white area increasing when cows are exposed to frustration or show startle reactions and decreasing when cows are satisfied and content. Application of the anxiolytic substance diazepam reduced the percentage of visible eye white in frustrated cows (Sandem et al., 2006).

Furthermore, exit speed was positively correlated with cortisol concentration (Curley et al., 2006), and percentage of visible eye white was positively correlated with exit speed (Core et al., 2009).

The proportion of visible eye white is controlled by the sympathetically controlled *Musculus tarsalis* that lifts the eyelid (Patel et al., 2008; Proctor and Carder, 2015; Sandem et al., 2006). Accordingly, Reefmann et al. (2009) found a positive correlation between heart rate and relative eye aperture and a negative correlation between heart rate variability and eye aperture. A reduction of visible eye white thus is assumed to be triggered by a sympathetic deactivation or a parasympathetic activation. Visible eye white might thus allow a reliable assessment of stress responses in cattle.

In modern dairy farming, cows are regularly exposed to potentially acute stressful situations such as regrouping or restraint for artificial insemination, medical treatments and claw trimming. Cows that were squeezed in a cattle crush had higher plasma cortisol levels 1 h after squeezing compared with their baseline values (Szenci et al., 2011; Thun et al., 1998). Furthermore, claw trimming led to higher fecal cortisol concentration in comparison with baseline concentrations before claw trimming (Pesenhofer et al., 2006). Accordingly, treatment with an analgesic and sedative drug led to lower cortisol concentrations, but also to lower heart and respiratory rates during claw trimming (Rizk et al., 2009, 2012).

The aim of this study was to validate visible eye white and maximum eye temperature as non-invasive stress indicators. For this aim, we assessed visible eye white and maximum eye temperature in addition to heart beat parameters and saliva cortisol concentration in dairy cows during claw trimming and in a control situation (undisturbed feeding).

2. Materials and methods

2.1. Animal husbandry and management

The study was conducted in March 2015 at the Research Station Agroscope Taenikon, Switzerland. The dairy herd consisted of 52 lactating dairy cows (Brown Swiss and Red Holstein) kept in a cubicle barn with permanent access to an outdoor run. The cows were fed a total mixed ration 7 times daily at a self-locking feed rack. They were locked routinely in the feed rack for approximately 1 h after feed delivery.

On three consecutive days, 10 cows each were selected resulting in a sample of 30 cows (18 Brown Swiss and 12 Red Holstein, parity number 3.38 ± 1.89, 150.93 ± 66.48 days in milk).

2.2. Experimental procedure

All experiments conformed to the Swiss Animal Welfare Legislation and were ethically approved by the cantonal veterinary office (TG 05/2014).

In a cross-over design, we exposed the cows to a claw trimming and to a control situation (undisturbed feeding). In both situations, we took pictures of the cows' eyes, collected saliva samples and recorded heart rate (HR) and heart rate variability (HRV). Measurements were done only during morning hours.

2.2.1. Control treatment

Data collection started at around 7:30 to 8:00 a.m. when the focal cows were self-locked in the feed rack. When the focal cow was feeding calmly, three pictures each were taken at t_0, and approximately 5 min (t_5), and 10 min (t_{10}) later. Saliva was collected approximately 20 min (t_{20}) after t_0. The cows were considered one after the other in a random order.

2.2.2. Claw trimming treatment

Claw trimming was done by two experienced claw trimmers. The cows were guided individually to the cattle crush that was located adjacent to the outdoor run, not visible to the herd. When the cow had entered the crush with all four legs, a girth was attached around her chest, and one hind leg and the opposite front leg were lifted and restrained (Fig. 1). After finishing trimming of the first two claws, these legs were released and the other two legs were lifted. At the end of trimming, all legs were released, the chest girth was opened, and the cow left the crush and returned to the home pen.

As soon as a cow had entered the crush, data collection started with t_0 = cow in crush with all four legs; t_5 = approximately 5 min after legs were lifted, t_{10} = 10 to 15 min after entering crush (claw trimming was finished and all four claws touched the ground again). The cows were released immediately thereafter and returned freely to the home pen for feeding. Saliva was collected in the home pen, approximately 20–30 min after the beginning of claw trimming (= 5–10 min after releasing from the crush (t_{20})).

2.2.3. Picture capturing

Pictures were taken from the left side of the cow, in approximately 1 m distance, with an angle of view of 90° (for details see: Figs. X, Y, Suppl. material), using a fixed focal length (as suggested by Schaefer et al., 2012). Each time (t_0, t_5, t_{10}) three pictures were captured and only clear pictures with an angle of view of 90° were used further.

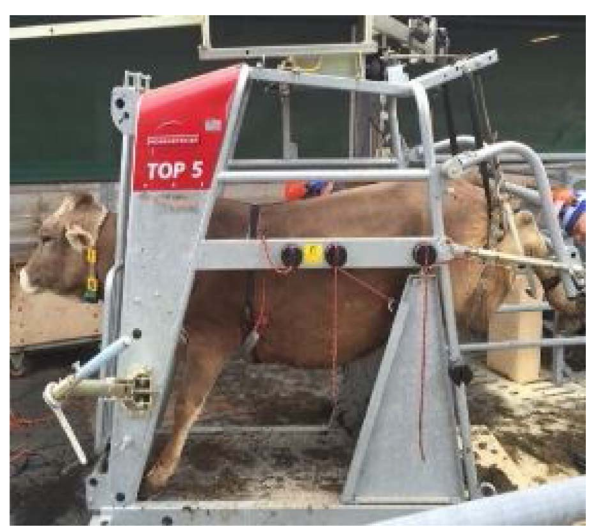

Fig. 1. Walk-in cattle crush used in the study.