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Standardization of a non-invasive methodology to measure cortisol in hair of beef cattle

D. Moya [a], K.S. Schwartzkopf-Genswein [a,*], D.M. Veira [b]

[a] Agriculture and Agri-Food Canada, P.O. Box 3000, Lethbridge, Alberta, Canada T1J 4B1
[b] Agriculture and Agri-Food Canada, Agassiz, British Columbia, Canada V0M 1A0

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ABSTRACT

A study was conducted to (a) determine if beef cattle hair contains cortisol at measurable concentrations, and (b) identify the effect of hair location and collection method on hair cortisol concentrations. Hair samples (0.5 g) from the head, neck, shoulder, hip, and switch were collected from twelve Angus cross bulls (313.1 ± 14.7 kg BW) using two sampling methods: plucking, to ensure collection of the hair follicles; and clipping, using an electric razor to ensure collection of the hair as close as possible to the skin. After two washings with isopropanol, hair samples were ground with a ball mill for 5 min at 22 Hz, sonicated with methanol for 30 min, and incubated on a shaker for 18 h, at 50 °C and 100 rpm. The supernatant was pipetted off and evaporated in a block heater, at 45 °C under a stream of nitrogen. Samples were reconstituted with phosphate buffered saline before quantification of cortisol with a competitive immunoassay. The described method was successful in detecting cortisol in all the hair samples, with concentrations ranging from 0.30 to 5.31 pg/mg. The intra-assay coefficient of variation (CV) ranged from 3.6% to 6.0%, while the inter-assay CV ranged from 5.4% to 11.2%. The cortisol concentration was greater ($P < 0.05$) in the hair from the tail (1.99 ± 0.189 pg/mg) compared with the head and the shoulder (1.14 and 0.82 ± 0.189 pg/mg, respectively), and in the hair from the neck and the hip (1.50 and 1.59 ± 0.189 pg/mg, respectively) compared with the shoulder (0.82 ± 0.189 pg/mg). Cortisol concentration was greater ($P < 0.01$) in hair samples collected by clipping (2.35 ± 0.176 pg/mg) than by plucking (1.75 ± 0.176 pg/mg). There was a day × location interaction ($P = 0.01$), where the hair from the head, neck and shoulder had a lower cortisol concentration at d 28 than at d 1 of the experiment. Data show a significant positive association between cortisol concentration in saliva samples and its level in hair from the hip ($r = 0.52$) and the tail ($r = 0.63$). There was also a trend for a positive association between fecal glucocorticoid metabolites and cortisol concentration in the hair from the neck and the tail ($r = 0.46$ and 0.47, respectively). Results indicate that hair can be used as matrix to measure cortisol levels in beef cattle. Clipping hair from the tail seems to be the most suitable way for measuring cortisol concentration in hair.

1. Introduction

Hormonal variables have been widely used to assess the levels of distress experienced by cattle exposed to a range of stressors, including common management procedures such as branding (Schwartzkopf-Genswein et al., 1997), dehorning (Petrie et al., 1996), castration (González et al., 2009), or

Abbreviations: HPA, hypothalamic-pituitary-adrenocortical; PBS, phosphate-buffered saline; RIA, radioimmunoassay

* Corresponding author. Tel.: +1 403 317 3354; fax: +1 403 382 3156.
E-mail address: karen.genswein@agr.gc.ca (K.S. Schwartzkopf-Genswein).

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transport (Tarrant et al., 1992). The most commonly used indices are those representing changes in the activity of the hypothalamic-pituitary-adrenocortical (HPA) system, and especially cortisol concentration.

Cortisol is usually extracted from blood, saliva, urine or fecal samples. However, unlike with other species, measures obtained from restrained or handled beef cattle are confounded by the effects of animal handling, which itself can induce stress (Moberg and Mench, 2000). The use of hair to assess long term levels of cortisol secretion has been studied in humans (Sauve et al., 2007; D'Anna Hernandez et al., 2011), rhesus macaques (Davenport et al., 2006, 2008), cats and dogs (Accorsi et al., 2008; Bennett and Hayssen, 2010), and dairy cows and heifers (Comin et al., 2011; González de la Vara et al., 2011). As far as we know, there has been no attempt to quantify cortisol levels in hair taken from beef cattle. Assessing cortisol concentrations in the hair would be a simple non-invasive technique to assess cortisol changes over long-term periods, without being subject to the influence of circadian variation or the interference of the momentary stress of capture during the sampling procedure.

Sauve et al. (2007) reported large but insignificant variations in cortisol levels of hair taken from different parts of the human head. There has been no attempt to quantify cortisol levels in hair taken from different parts of the body of other species. Subjective observation in cattle suggests that texture and growth of hair differ between various locations on the body, which may have an effect on the final deposition of cortisol.

Incorporation models assume that blood-borne substances enter the hair through passive diffusion from capillaries into growing cells of the hair follicle, and subsequently become deposited in the hair shaft (Cone, 1996). However, other possible mechanisms of entry proposed include the diffusion from sweat or sebum secretions after its formation (Pragst and Balikova, 2006), or the sequestration of glucocorticoids in the hair shaft derived from a peripheral HPA axis homolog in the skin and hair follicle cells (Keckeis et al., 2012). In any case, including the follicle as part of the hair sample could have significant impact on cortisol concentration of the sample (Meyer and Novak, 2012). While using electric clippers is the easiest way to harvest hair, there is always a residual length of hair and hair follicles left behind. On the other hand, plucking hair is a more difficult method of sampling, but it perhaps provides a truer value of cortisol concentrations.

Our overall objective was to establish the validity of hair for measuring cortisol in beef cattle, and identify the effects of the collection method and hair location on hair cortisol concentration.

2. Materials and methods

All procedures described in this study were approved by the Animal Care and Use Committee of the Lethbridge Research Centre, and animals were cared for in accordance to the Canada Council of Animal Care guidelines (2009).

2.1. Animals

Twelve Angus cross bulls (313.1 ± 14.7 kg BW) were housed together in a pen for a minimum of 6 weeks before the beginning of the trial to become accustomed to the environment at the Agriculture and Agri-Food Canada Research Centre in Lethbridge (AB, Canada). Pens measured 21 m × 27 m, with 15 m^2 of concrete pavement in front of the feed bunk, and 12.6 m^2 of pen space per animal. Cattle were fed a total mixed backgrounding ration consisting of 170 g/kg rolled barley and 800 g/kg barley silage (as fed) and supplement containing minerals and vitamins to meet requirements (NRC, 2000). Feed was delivered once daily at 0900, and fresh water and a bedded area away from the feed bunk were available at all times.

2.2. Sampling and procedures

On day 1 of the experiment, hair samples were collected from the locations described in Fig. 1 by using two sampling methods: plucking (PK, $n=6$), by using tweezers to ensure the collection of the hair with its follicle; or clipping (CL, $n=6$), by using an electric razor to cut the hair as close to the skin as possible. Four weeks later, the same sampling procedure was repeated using only the electric razor, as the hair was not long enough to be collected with the tweezers in the amount needed for its later analysis, especially in the neck or hip areas. Once collected, hair samples were stored in plastic bags until analysis. A subset of 250 mg from each sample was washed twice for 3 min in 5 mL of Isopropanol (Ricca Chemical Company, Arlington, TX, USA), dried and covered for 5 d at room temperature. Hair cortisol extraction was then performed according to the method described by Koren et al. (2002) modified by Accorsi et al. (2008). Briefly, hair was put in 10 mL metallic cylinder with a 12-mm mill ball, and ground with a mixer mill (MM 200, Retsch Inc., Newtown, PA, USA) at 22 Hz for 5 min. Twenty milligrams of the minced hair was placed in a 5-mL glass vial, and 1 mL of Methanol (EMD Chemicals Inc., Billerica, MA, USA) was added. The vials were sonicated for 30 min and incubated on a shaker for 18 h, at 50 °C and 100 rpm. A total of 0.8 mL of the supernatant was pipetted off and evaporated in a block heater, at 45 °C under a stream of nitrogen. Samples were reconstituted with 100 μL of phosphate-buffered saline (PBS) before quantification of cortisol in duplicate with an enzyme immunoassay kit (Salimetrics LLC, State College, PA, USA), according to the

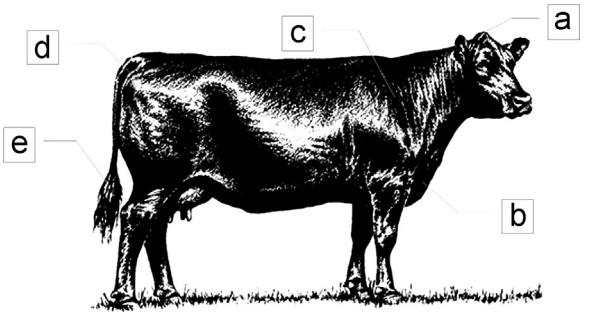

Fig. 1. Hair sampling locations: (a) head, hair from the "nuchal crest"; (b) neck, hair located in the brisket area, over the jugular area; (c) shoulder, hair located over the spine of the scapula; (d) hip, hair located over the Femur–Ischium junction; and (e) tail, hair from the last third of the tail and the switch.