Livestock Science 155 (2013) 130–136

Contents lists available at SciVerse ScienceDirect

Livestock Science

journal homepage: www.elsevier.com/locate/livsci

On the relationships between glucocorticoids and feed efficiency in beef cattle

Y.R. Montanholi [a,*], R. Palme [c], L.S. Haas [a,1], K.C. Swanson [b], G. Vander Voort [a], S.P. Miller [a,**]

[a] Department of Animal and Poultry Science, University of Guelph, 50 Stone Road East, Guelph, Canada N1G 2W1
[b] Department of Animal Sciences, North Dakota State University, PO box 6050, Fargo 58108-6050, USA
[c] Department of Biomedical Sciences, University of Veterinary Medicine, Veterinärplatz 1, 1210 Vienna, Austria

ARTICLE INFO

Article history:
Received 10 August 2012
Received in revised form
1 April 2013
Accepted 3 April 2013

Keywords:
Cortisol
Fecal cortisol metabolites
Feedlot confinement
Handling distress
Residual feed intake

ABSTRACT

Improvement in the utilization of feed in livestock is an important target of breeding and nutritional programs. Recent evidence indicates a potential association between feed efficiency and fecal cortisol metabolites, which could eventually be used as an indirect assessment of this trait. This evidence is more comprehensively evaluated in here with samples for plasma cortisol (PC; ng/ml) and fecal cortisol metabolites (FCM; ng/ml) collected more often during the entire finishing phase in beef steers. Individual daily feed intake of 112 steers fed a high-moisture corn-based and haylage diet was measured over 168 d. Body weight, blood and fecal samples were collected every 14 d and ultrasound measures of backfat thickness and longissimus muscle area were taken every 28 d. Four productive performance traits were calculated: daily dry matter intake (DMI), average daily gain (ADG), feed to gain ratio (F:G) and residual feed intake (RFI). At the end of the feedlot phase, steers were ranked according to RFI and samples were analyzed for PC and FCM from the 32 steers with greatest and 32 steers with lowest feed efficiency. In addition, a sub-group of 12 steers from each of these two groups with divergent feed efficiency were subjected to hourly blood sampling for 24 h. Less efficient steers had greater DMI, F:G and consumed 1.5 kg/d more DMI ($P<0.05$) than steers with improved feed efficiency. No differences ($P>0.10$) in PC over the 12 biweekly sampling periods between steers with divergent feed efficiency were observed. However, a trend toward significance between 19:00 and 02:00 h over the hourly sampling evaluation was noticed, with the sub-group of more feed efficient steers presenting higher levels of PC in this period of the day ($P=0.08$). On the other hand, FCM levels displayed a distinct pattern between RFI groups over the biweekly sampling period, with more efficient cattle presenting greater levels of these metabolites ($P<0.05$). This study reinforces the positive association between improved feed efficiency and FCM levels over the finishing phase; and the lack of association between feed efficiency and PC when single samples are collected every two weeks through a single jugular venipuncture performed after handling the cattle for sampling. Further studies to develop sampling protocols for assessing FCM as an indicator trait for feed efficiency are warranted, as well as, studies to understand the role of endogenous glucocorticoids in the performance of the bovine.

* Corresponding author. Tel.: +1 519 824 4120x56378; fax: +1 519 836 9873.
** Corresponding author.
E-mail addresses: ymontanh@uoguelph.ca (Y.R. Montanholi), miller@uoguelph.ca (S.P. Miller).
[1] Present address: Faculdade de Medicina Veterinária da UFRGS—Avenida Bento Gonçalves, 9090—Porto Alegre 91540-000, Brazil.

1871-1413/$ - see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.livsci.2013.04.002

1. Introduction

The cost to feed beef cattle is a producer's single greatest investment, representing the main fraction of the total variable costs in intensive beef cattle production systems (Miller et al., 2001). Furthermore, there is a moderate to high heritability in feed efficiency (Herd et al., 2003; Schenkel et al., 2004), suggesting the possibility of genetic improvement. However, the high costs and intensive labor associated with the direct assessment of feed efficiency in beef cattle are limiting factors for more effectively selecting feed efficient cattle (Herd and Arthur, 2009), suggesting the need of alternatives for assessing feed efficiency. Additionally, new phenotypes for assessing feed efficiency could facilitate the spread of genomic selection for this trait (Snelling et al., 2011), through the identification of target metabolic phenomena to be genotyped.

Several studies have been conducted aiming to identify reliable indicators for feed efficiency such as feeding and social behavior (Golden et al., 2008; Montanholi et al., 2010) infrared thermography imaging (Montanholi et al., 2009), hormones and metabolites (Richardson et al., 2004), mitochondria function (Kolath et al., 2006) and genomics (Sherman et al., 2010). Results from these studies suggest a few approaches that could be used for screening beef cattle for feed efficiency. In particular, the associations between glucocorticoids (fecal cortisol metabolites) and feed efficiency discovered by Montanholi et al. (2010) deserves additional study due to the ability of discriminating high- and low-feed efficient groups of cattle and the possibility of relatively easy implementation by the beef industry.

Glucocorticoids are continuously released from the adrenal gland into the blood stream as a response to the activation of hypothalamic–pituitary–adrenocortical axis (Eiler, 2004). These hormones have important roles in the metabolism by regulating protein, fat and carbohydrate metabolism, muscle maintenance, the immune system, etc (Sapolsky, 2002). Cortisol and its metabolites can be assessed in several matrices (Palme, 2012). Blood plasma cortisol (PC) and fecal cortisol metabolites (FCM) represent a relatively stressful sampling and a minimum distress sampling procedure, respectively (Möstl and Palme, 2002). Additionally, FCM reflect the long-term response, representing the cortisol that was released into the blood stream about 12 h before sampling and PC represents the immediate response of the adrenal gland (Palme et al., 1999, 2005). Montanholi et al. (2010) found that high-feed efficient steers had higher FCM than low-feed efficient steers and no differences in PC between the two feed efficiency groups.

Montanholi et al.'s (2010) study was performed with a relatively small number of animals, with limited phenotypic diversity for feed efficiency and small number of samples over time, thus indicating the need of a more comprehensive investigation in order to verify the consistency of such association (different, larger population and longer sampling periods) since levels of cortisol are known to be influenced by age (González-de-la-Vara et al., 2011), body composition (Delgiudice et al., 1990) and season of year (Gwazdauskas, 1985) to name a few. In addition, it is known that cattle vary in stress responsiveness to handling and physical restraining (Curley Jr. et al., 2008). These potential sampling biases on the assessment of baseline of PC levels can be minimized by getting the animals used to sampling procedure (Hopster, 1999), as well as, by using venous catheterization instead of successive venous punctures that cause fluctuations on PC level due to the considerable distress (Hopster et al., 1999). Particularly in the case of PC, frequent blood collections represent a more desirable sampling approach to evaluate the circadian cycle concentrations due to the great fluctuations, under physiological conditions, which can be observed for this hormone over the circadian period (Macadan and Eberhart, 1972). Therefore, the objectives of this study were (1) to verify if the relationship between feed efficiency and FCM holds in a larger population of cattle with samples collected more often and over longer period during the finishing phase in beef steers and; (2) to evaluate the relationship between circadian and biweekly concentrations of PC with feed efficiency.

2. Material and methods

2.1. Animals, management, experimental design and sampling

The experiment followed recommendations as outlined by the Canadian council of animal care guidelines (1993) and was approved by the University of Guelph animal care committee. A total of 112 crossbred beef steers were fed for 168 d at the Elora Beef Research Centre, University of Guelph, Canada. The average age of the steers at the start of the experiment was 275 ± 25 d (mean $\pm$ standard deviation) and the initial and final body weights were 338 ± 44 and 519 ± 51 kg, respectively. The overall breed composition of the steers was 58.3% Angus, 30.6% Simmental and 11.1% other European breeds (i.e. Hereford, Gelbvieh and Piedmontese). Steers were originated from Elora Beef Research Center (University of Guelph), from New Liskeard Research Station (University Guelph) and from three commercial herds.

Steers were housed in groups of 16 animals, sorted at the start of the trial by body weight (pen $1=296 \pm 9$; pen $2=306 \pm 8$; pen $3=313 \pm 12$; pen $4=326 \pm 15$; pen $5=344 \pm 18$; pen $6=362 \pm 16$; and pen $7=423 \pm 19$), in indoor pens bedded with wood shavings. Each pen contained four automated feeding stations (Insentec, B.V., Marknesse, The Netherlands). Prior to the start of the testing period, each steer was fitted on the right ear with a radio frequency transponder button (Allflex U.S.A. Inc., Dallas–Fort Worth, U.S.A.), which identified animals to the automated feeding system allowing for individual feed intake events to be recorded continuously. These measures were computed to calculate the individual daily dry matter intake, which was used for calculating feed efficiency measures.

Steers were allowed to adjust to the facilities, feed and feeding system for a minimum of 15 d prior to the start of the trial. In addition, three steers had reduced daily feed intakes for extended period due to chronic illness and were excluded from the experiment. Steers were fed a high moisture corn-based diet (dry matter basis) with 78%

of high moisture corn, 13.5% of haylage, 5% of soybean meal and 3.5% of a premix containing soybean meal, monensin, salt and trace minerals premix described in details by Montanholi et al. (2010). The average chemical composition of the diet was: 38.5% of moisture; 12.8% of crude protein; 13.2% of neutral detergent fiber; 6.7% of acid detergent fiber; 89.8% of total digestible nutrients and; 42.2% of starch. Diet was offered for ad libitum consumption throughout the test period and feeders were filled daily between 09:00 and 10:00 h.

Body weight, blood and fecal samples were collected every 14 d during the testing period. In total, 12 biweekly samples of blood and feces were collected per animal between 8:00 and 12:00 h, with samples from all the animals collected in the same morning. Additionally, ultrasound scanning (backfat thickness and longissimus muscle area) for assessing body composition was conducted every 28 d over the 168 d of testing, as described in details by Montanholi et al. (2009). Blood samples were collected through jugular venipuncture, upon moving the entire group of steers, housed in the same pen, to the handling facility and with the animal restrained in a squeeze chute (Silencer® Hydraulic Squeeze Chute; Moly Manufacturing Inc., Lorraine, U.S.A.), using a 10 ml blood collection tube (Vacuntainer®; BD Inc., Franklin Lakes, U.S.A.) containing sodium heparin mounted on a 2.5 cm 20 GA needle. Blood samples were stored in ice until centrifugation (3000*g* for 20 min) to separate the blood plasma, which was stored at −80 °C until further analysis for PC. Immediately after collecting the blood sample, the fecal sample was collected through rectal palpation and stored at −20 °C and processed as described by Palme et al. (2000) for further analysis for FCM.

After calculating feed efficiency values for the remaining 109 steers (using performance and feed intake data collected over 168 d of trial), blood samples were collected from the 12 steers with the greatest and 12 steers with poorest feed efficiency on an hourly basis over 24 h in groups of four animals grouped by feed efficiency (2 HE and 2 LE) and by age (priority to the older steers). This sampling was carried out over six periods of 24 h. Steers were adjusted to individual stalls for 2 d. Steers were considered ready for the circadian blood sampling when displaying a minimum of 75% of the regular daily DMI, based on the average intake of the last 15 d prior to the start of the adaptation. Feed and water were offered for ad libitum consumption inside of a head chamber (indirect calorimeter), which was being used for other purpose at the same time. Steers were loosely tied within each head chamber and allowed to lie down. A jugular catheter (14GA 2.1 × 133 mm, Angiocath®; BD Inc., Franklin Lakes, U.S.A.) was placed in the right jugular vein of the steers, between 10:00 and 11:40, after mild sedation with xylazine (0.02 mg/kg; Rompun®, Bayer Inc., Bergkamen, Germany) and a local ring block anesthesia with lidocaine 2% (2 ml/steer; Lidocaine Hydrochloride Injection USP, Alveda Pharmaceuticals Inc., Toronto, Canada). The catheter was fixed in place with two sub-dermal stitches and protected with gauze and wrap. The tubing attached to the catheter was attached in between the shoulders of the steers with tape in order to minimize any distress during blood sampling. Catheter and tubing were protected from clogging with heparinized saline (30 USP units/ml, Heparin Sodium Injection, USP, Pharmaceutical Partners of Canada Inc., Richmond Hill, Canada; 0.9% NaCl), with 5 ml injected after each blood collection to flush the entire tubing and catheter. Blood collection started at 12:00 and concluded at 11:00 in the following day using the same tubes for storing and processing the samples as described before. During the 24 h blood collection, luminosity in the room where the steers were housed individually was maintained similar to the group pens.

2.2. Productive performance traits

The feed intakes recorded by the automated feeding system were first summarized by animal to determine the individual average daily feed intake during the testing period. The 2% lowest daily records on an individual basis (6.0 ± 1.8 kg, group average $\pm$ SD, of feed intake as fed basis) and obvious outliers, due to mechanical or software issues were excluded. These represented 2.6% of all daily records. The individual average daily dry matter feed intake (DMI) over the testing period was calculated on the remaining records.

Average daily gain (ADG) was determined by a regression of body weight on days on test, with 12 observations per animals at intervals of 14 d. Feed intake to gain (F:G) was computed using the average DMI and ADG. Residual feed intake (RFI) was calculated using the original model described by Koch et al. (1963) and models combining ultrasound traits, such as described by Montanholi et al. (2009). The model including total gain during the test period for backfat thickness and for ribeye area had the highest values for the regression coefficient ($R^2 = 0.63$) and lower for Bayesian information criteria and was selected for this study (data of the other models tested is not shown). The regression equation for predicted feed efficiency was:

$DMI_{predicted} = 2.082 + 0.012$(average body weight; kg) $+ 1.237$(ADG; kg/d)$+ 0.068$(total gain backfat thickness; mm)$+ 0.010$(total gain ribeye area; cm^2)+RFI

The RFI values were calculated by subtracting observed DMI from predicted DMI.

2.3. Determination of glucocorticoids

Concentrations of glucocorticoids were measured similarly as outlined by Montanholi et al. (2010). Plasma cortisol (ng/ml) was measured directly using a commercially available radioimmunoassay kit (Coat-A-Count, Diagnostic products Corporation, Los Angeles, U.S.A.). The intra- and inter-assay coefficients of variation were 4.7% and 9.4%, respectively. From the total of 109 steers, only the top 32 efficient and the bottom 32 inefficient steers, based on RFI values defined above, had their blood samples analyzed for the testing period (biweekly samples). The 24 steers subjected to the 24 h hourly sampling had their blood plasma analyzed over the circadian period for PC.

The determination of FCM (ng/g) was performed with a group-specific 11-oxoetiocholanolone enzyme immunoassay, measuring 11,17-dioxoandrostanes (Palme and Möstl,

Table 1
Comparisons between productive performance and other characterization data (means±standard error) of high- and low-feed efficient groups (n=32 in both groups) of steers.

Traits (abbreviation; unit)	High feed efficiency	Low feed efficiency	*P* value
Dry matter intake (DMI; kg DM/d)	9.12 (±0.14)	10.66 (±0.20)	<0.001
Average daily gain (ADG; kg/d)	1.91 (±0.04)	1.92 (±0.06)	0.7265
Feed to gain ratio (F:G)	4.83 (±0.13)	5.63 (±0.17)	<0.001
Residual feed intake (RFI; kg DM/d)	–0.74 (±0.07)	0.76 (±0.09)	<0.001
Body weight start trial (kg)	330.7 (±7.8)	335.1 (±11.0)	0.6906
Body weight end trial (kg)	513.7 (±8.4)	520.2 (±11.9)	0.5827
Backfat thickness start trial (mm)	2.89 (±0.28)	3.01 (±0.40)	0.7757
Backfat thickness end trial (mm)	12.49 (±0.81)	13.07 (±0.57)	0.4806
Ribeye area start trial (cm^2)	59.11 (±1.00)	58.83 (±1.40)	0.8417
Ribeye area end trial (cm^2)	108.00 (±1.39)	108.41 (±1.97)	0.8389
Age start trial (d)	257 (±3.4)	266 (±4.8)	0.6909

1997). The intra- and inter-assay coefficients of variation were 10.1% and 11.9%, respectively. This enzyme immunoassay has previously been validated in cattle (Palme et al., 1999) and successfully applied in this species (Palme et al., 2000; Pesenhofer et al., 2006; Montanholi et al., 2010). Similarly to the PC determination, the top 32 and the bottom 32 feed efficient steers, based on RFI, had their fecal samples analyzed for FCM over the testing period.

2.4. Statistical analysis

Data were analyzed using SAS software (2003). Preliminary regression analysis conducted using the general linear model procedure to evaluated effects of breed composition indicated absence of significant breed effect ($P > 0.10$) on the traits evaluated (data not shown). Therefore, breed composition were excluded from the analyses detailed below. The general linear model procedure was also used to compare means of the feed efficiency groups (32 steers with high- (HE) and 32 steers with low-feed efficiency (LE)) and the feed efficiency sub-groups (12 steers with high- and 12 steers with low-feed efficiency). Correlation analyses, within each feed efficiency group, were conducted between average circadian PC and PC considering only samples collected between 8:00 and 12:00 over the circadian cycle, with average PC from the 12 biweekly sampling events and with the last PC biweekly sample. These analyses were performed using the correlation procedure. In addition, the repeated measures over time for FCM and PC during the testing period and for PC only during the circadian cycle sampling were analyzed through random regression using the mixed procedure, according to the following model:

$$Y_{ijtk} = \mu + \mathrm{RFIc}_i + \sum_{k=0}^{nf} \phi_{jtk}\beta_k + \sum_{k=0}^{nf} \phi_{jtk}\gamma_{jk} + \varepsilon_{ijtk}$$

where Y_{ijtk} is the k-th glucocorticoid level (either PC or FCM) measured at the t-th time (t=0–12 biweekly sampling and t=0–24 hourly sampling for testing period phase and circadian sampling, respectively) at the j-th steer from the i-th feed efficiency group (i=high- and low-feed efficiency); μ is the overall mean for the trait; RFIc_i is the fixed effect of the i-th RFI class; $\sum_{k=0}^{nf}\phi_{jtk}\beta_k$ are fixed regression coefficients; $\sum_{k=0}^{nr}\phi_{jtk}\gamma_{jk}$ are the k-th random regression for animal j; ϕ_{jtk} is the k-th linear polynomial for glucocorticoid level of steer j at time t; nf and nr are the order of the linear polynomial for fixed and animal effects regressions; and ε_{ijtk} is the residual random effect associated with the t-th measure. Degrees of freedom were adjusted using the Satterth method. The unstructured covariance structures used was based upon the comparison of different structures using Bayesian Information criterion. There was no evidence of pen effect ($P > 0.05$); therefore, pen effect was removed from the models. For all analysis data were considered statistically significant when $P < 0.05$.

3. Results

Table 1 presents the comparisons between productive performance and other characterization traits by feed efficiency group (HE and LE). The difference in feed intake between LE and HE groups based on RFI was 1.5 kg/d (or 252 kg less intake per HE steer over the entire finishing period) of DMI, while achieving the same body weight and body composition based on ultrasound assessment, as RFI was calculated with the inclusion of the total gain in ribeye area and in backfat thickness during the 168 d of feeding test. The RFI values of the HE and LE sub-groups, submitted to the circadian evaluation of PC, were −1.10 and 1.06 kg DM/d, respectively ($P < 0.001$).

Fig. 1 shows PC levels over the 12-biweekly sampling events by feed efficiency group. The curves for HE and LE display similar levels over time, and as a result the PC levels between HE and LE did not differ (HE=23.9 and LE 22.8 ng/ml; P=0.54). Conversely, the FCM levels assessed over the same period (Fig. 2) displayed an overall significant difference between the two feed efficiency groups, with more feed efficient steers having higher levels of FCM than less feed efficient steers (HE=32.2 and LE=19.2 ng/g; P=0.02). However, it is important to note that between the biweekly sampling periods 4 and 7 the difference in FCM concentrations of the two RFI groups was merely numeric ($P > 0.10$). Furthermore, these two variables (biweekly PC and FCM) were studied considering only the 24 steers used in the circadian period study and comparable results to the original groups of 32 steers were observed (PC; HE=25.2 and LE 21.2 ng/ml; P=0.42 and; FCM; HE=33.8 and LE=17.8 ng/g; P=0.04).

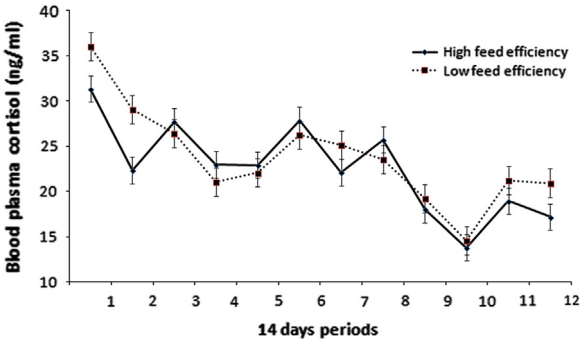

Fig. 1. Plasma cortisol levels over the 12-biweekly sampling events by feed efficiency group, vertical bars indicate standard error. No difference between feed efficiency groups for plasma cortisol ($P > 0.05$).

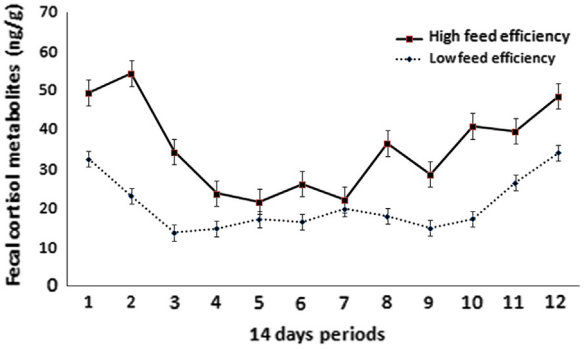

Fig. 2. Fecal cortisol metabolites levels over the 12-biweekly sampling events by feed efficiency group, vertical bars indicate standard error. Difference between feed efficiency groups for fecal cortisol metabolites ($P < 0.05$).

The circadian evaluation of PC concentrations is represented in Fig. 3. Both feed efficiency groups had similar patterns over the 24 h sampling and the overall PC concentration did not differ between the two groups (HE = 12.7 and LE = 11.9 ng/ml; $P = 0.71$). However, between 19:00 and 2:00 h a trend toward significance was observed with the more feed efficient steers displaying higher PC levels than less feed efficient steers (HE = 22.1 ng/ml and LE = 14.1 ng/ml; $P = 0.08$). This trend occurred about 12 h prior to the usual time of fecal sampling conducted during the biweekly sampling period.

In addition, for the 12-biweekly sampling events, the average PC was in general obviousously higher than the PC average over the circadian period for the 24 steers submitted to both sampling approaches. The average PC levels were 110% (range: 12–385%) higher for the biweekly sampling in comparison to the circadian average. A comparisons between average PC considering only samples collected between 8:00 and 12:00 and the last PC biweekly sample indicated that PC levels from the biweekly sampling were also of greater magnitude 121% (range: 41–454%). No significant correlations, within each feed efficiency group, ($P > 0.10$) were observed between PC assessed biweekly and circadian levels of PC as described above. Additionally, a comparison between the averages of the first three and last three biweekly samples indicated

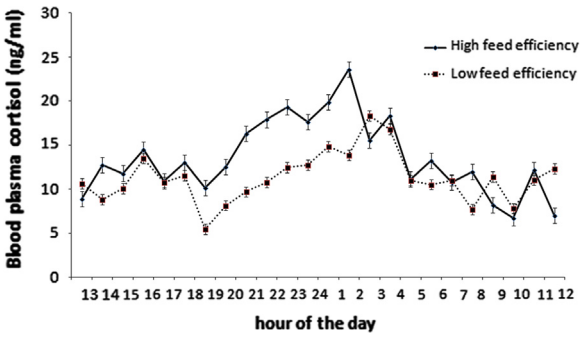

Fig. 3. Plasma cortisol levels over the circadian period by feed efficiency group, vertical bars indicate standard error. No difference between feed efficiency groups for plasma cortisol over the circadian period ($P > 0.05$) and trend toward significance between 19:00 and 02:00 ($P = 0.08$).

greater concentrations of PC for the former (29.95 vs. 17.17 ng/ml; $P < 0.001$).

4. Discussion

The similar patterns of PC over the 12-biweekly sampling events by feed efficiency group (Fig. 1) suggest that steers with distinct feed efficiency have indistinguishable cortisol responses to handling, restraining and blood collection distress. Previous results from our group, in feedlot steers (Montanholi et al., 2010) also observed a similar lack of association between PC and performance traits. In contrast, Richardson et al. (2004) reported correlations ($P < 0.05$) of −0.58 and −0.40 between PC and DMI and RFI, respectively, based on a single PC determination during the feedlot phase. Plasma cortisol levels are subject to sudden and great changes within minutes (von Holst, 1998). According to Hopster et al. (1999) cortisol baseline levels may be assessed in cattle only if blood collection occurs within 1 min of first approaching the cow, which is unpractical in commercial operations. These authors (Hopster et al., 1999) also observed that jugular puncture may induce an increase in cortisol concentration, which seems to depend on the handling experience of the animals and on individual differences. It seems that this observation also applies to the current study, it is notorious on Fig. 1 that levels of PC were lower for both group of steers (HE and LE) at the end of the biweekly sampling, after being handled several times, in comparison to the start of the trial.

On the other hand, the FCM results indicated higher concentrations of these metabolites over the 12-biweekly sampling events in HE in comparison to LE steers (Fig. 2). These results are in line with the findings of Montanholi et al. (2010), where only two fecal samples harvested 56 d apart per steer were analyzed for FCM and found to be associated with feed efficiency. It is also important to note that all measured FCM values were within the range for the bovine and thus did not indicate the occurrence of acute stress prior to sampling (Palme et al., 1999; 2000; Pesenhofer et al., 2006; Rouha-Mülleder et al., 2010), while a negative relationship could be expected between improved performance and levels of glucocorticoids, due to the well known associations between cortisol reactivity and the associated deleterious effects on productive

performance (Purchas et al., 1980). It is important the highlight that FCM levels are indicating the baseline levels of glucocorticoids (Palme, 2012) and not the cortisol reactivity in the way that the current experiment was conducted.

The superior feed efficiency associated with increased levels of glucocorticoids may be explained by the anabolic effects of these substances and by distinct behavioral responses. Glucocorticoids in low doses are reported to be used as growth promoters in cattle (Courtheyn et al., 2002). Thus, the natural higher baseline of FCM in HE steers may explain the better productive efficiency of this group of steers. In the same way, behavioral studies (as reviewed by Koolhaas et al. (1999)) have revealed higher cortisol baseline in animals more likely to have higher energetic efficiency due to differences in coping styles. According to these authors (Koolhaas et al., 1999), animals considered bold have low cortisol baselines and when exposed to stress situations display a sympathetic response (fight and flight response); conversely, shy animals have high cortisol baselines and under stress present a parasympathetic response (conservation-withdrawal response). Therefore, one can suggest that HE steers are considered shy and LE steers could be considered bold.

The reasons of why the FCM concentrations of HE and LE steers were close between periods 4 and 7 (Fig. 2) is not completely clear; however there are two potential explanations. First, this could be the seasonal effect on cortisol secretion, given that during those periods (March and April) the day length increases in a great fashion in the Northern Hemisphere. There are evidences of seasonal effects on cortisol secretion in squirrels (Boswell et al., 1994) and in deer (Ingram et al., 1999) but no evidences has been found in the bovine. Second, this period also coincides with the calving season of a herd with 150 cows at the research station in a facility near (50 m) to the feedlot where the steers were housed. The increased vocalization in the cow herd during the calving season could cause a chronic distress in the steers (as reviewed by Manteuffel et al. (2004)) affecting the baseline levels of cortisol during such period.

Evaluation of the cortisol profile over the circadian cycle revealed that PC, in both groups of steers, is secreted episodically characterized by irregular, short term fluctuations with high individual variability, which is comparable to the study conducted by Thun and Eggenberger (1996) also in steers. A circadian rhythm with higher levels in the night and early morning and lower concentrations over the day was observed. This pattern differs from the results of Thun and Eggenberger (1996) but was similar to the pattern found by Fulkerson et al. (1980) in dairy cows. In addition, one could argue that cortisol levels may have been affected by the mild sedation with xylazine but apparently this is not the case (Fayed et al., 1994). It is also interesting to notice that during the period of raising PC concentration for both groups of steers a trend for higher PC levels for HE steers was observed. During this period (19:00 and 2:00) it was observed that steers have less physical activity in their stall and spend more time lying and ruminating (observation not quantified by the researcher). This period also coincides with the time period 12 h before the time that fecal samples were collected during the 14 biweekly periods. Therefore, this might be providing further evidences that trend of higher PC is associated with greater levels of FCM due to lag of about 12 h between PC and FCM (Palme, 2012).

Despite of the limitations in comparing PC collected over distinct periods, the comparison of the 2 groups of 12 steers that were both sampled over 12-biweekly events and over the circadian period revealed that handling and restraining cattle for blood sampling greatly affects PC. Curley Jr. et al. (2008) provided evidence that fearfulness traits (which is experienced by cattle while being handled, restrained and blood sampled) are directly associated with PC. The tremendous difference in PC by handling and restraining cattle in comparison to jugular catheterization suggests that the first procedure is not recommended for assessing basal values for PC, which was also elegantly demonstrated by Carragher et al. (1997).

Thus, these results underline that FCM are better suited than PC to evaluate baseline adrenocortical activity (Palme et al., 1999) and provide further evidences to support the appropriateness of FCM as a biomarker for feed efficiency in the bovine. Further studies are warranted to evaluate seasonal patterns, differences among animal types (heifers, cows and bulls), influence of different husbandry systems and effects of physiological states on FCM.

5. Conclusions

This study reinforces the positive association between improved feed efficiency and FCM levels over the entire finishing phase; and the lack of association between feed efficiency and PC when single samples are collected every two weeks through a single jugular venipuncture after handling and restraining the cattle for sampling. Further studies for developing sampling protocols for assessing the use of FCM as an indicator trait for feed efficiency and studies to gain better understanding of the role that endogenous glucocorticoids may play in regulating productive performance of the bovine are warranted.

Conflict of interest statement

All authors of this study do not have any actual or potential conflict of interest.

Acknowledgments

We would like to thank the assistance of the staff at the Elora Beef Research Centre, University of Guelph, for taking care of the steers and assisting with the sampling. Authors acknowledge the financial support received from BCRC (Beef Cattle Research Council of Canada) Agri-Food Canada, OMAFRA (Ontario Ministry of Agriculture, Food and Rural Affairs) and OCA (Ontario Cattlemen's Association). The assistance with the FCM analysis of Edith Klobetz-Rassam and the fruitful discussions with Erich Möstl are also acknowledged.

References

Boswell, T., Woods, S.C., Kenagy, G.J., 1994. Seasonal changes in body mass, insulin, and glucocorticoids of free-living golden-mantled ground squirrels. Gen. Comp. Endocrinol. 96, 339–346.

Canadian Council on Animal Care, 1993. In: Olfert, E.D., Cross, B.M., McWillians, A.A. (Eds.), Guide to the care and use of experimental animals, vol. 1. , Canadian Council on Animal Care, Ottawa, Ontario, Canada.

Carragher, J.F., Ingram, J.R., Matthews, L.R., 1997. Effects of yarding and handling procedures on stress responses of red deer stags (*Cervus elaphus*). Appl. Anim. Behav. Sci. 51, 143–158.

Courtheyn, D., Le Bizec, B., Brambilla, G., De Brabander, H.F., Cobbaert, E., Van de Wiele, M., Vercammena, J., De Wasch, K., 2002. Recent developments in the use and abuse of growth promoters. Anal. Chim. Acta 473, 71–82.

Curley Jr., K.O., Neuendorff, D.O., Lewis, A.W., Cleere, J.J., Welsh Jr., T.H., Randel, R.R., 2008. Functional characteristics of the bovine hypothalamic–pituitary axis vary with temperament. Horm. Behav. 53, 20–27.

Delgiudice, G.D., Mech, L.D., Seal, U.S., 1990. Effects of winter undernutrition on body composition and physiological profiles of white-tailed deer. J. Wildl. Manage. 54, 539–550.

Eiler, H., 2004. Endocrine glands. In: Duke, H.H. (Ed.), Dukes Physiology of Domestic Animals12th Ed. , Cornell University Press, Ithaca, USA, pp. 621–669.

Fayed, A.H., Zakaris, A.D., Hedaya, S.A., El-Ashmawy, I.M., 1994. Effects of xylazine (alpha 2-adrenergic agonist) on the stress response to immobilization and heat in rats. Asian Aust. J. Anim. 7, 397–400.

Fulkerson, W.J., Sawyer, G.J., Gow, C.B., 1980. Investigations of ultradian and circadian rhythms in the concentration of cortisol and prolactin in the plasma of dairy cattle. Aust. J. Biol. Sci. 33, 557–561.

Golden, J.W., Kerley, M.S., Kolath, W.H., 2008. The relationship of feeding behaviour to residual feed intake in crossbred Angus steers fed traditional and no-roughage diets. 86, 180–186.

González-de-la-Vara, M.R., Valdez, R.A., Lemus-Ramirez, V., Vázquez-Chagoyán, J.C., Villa-Godoy, A., Romano, M.C., 2011. Effects of adrenocorticotropic hormone challenge and age on hair cortisol concentrations in dairy cattle. Can. J. Vet. Res. 75, 216–221.

Gwazdauskas, F.C., 1985. Effects of climate on reproduction in cattle. J. Dairy Sci. 68, 1568–1578.

Herd, R.M., Archer, J.A., Arthur, P.F., 2003. Reducing the cost of beef production through genetic improvement in residual feed intake: opportunity and challenges to application. J. Anim. Sci., 81; E9–E17.

Herd, R.M., Arthur, P.F., 2009. Physiological basis for residual feed intake. J. Anim. Sci. 87, E64–E71.

Hopster, H., Van der Werf, J.T., Erkens, J.H., Blokhuis, H.J., 1999. Effects of repeated jugular puncture on plasma cortisol concentrations in loose-housed dairy cows. J. Anim. Sci. 77, 708–714.

Ingram, J.R., Crockford, J.N., Matthews, L.R., 1999. Ultradian, circadian and seasonal rhythms in cortisol secretion and adrenal responsiveness to ACTH and yarding in unrestrained red deer (*Cervux elaphus*) stags. J. Endocrinol. 162, 289–300.

Koch, R.M., Swiger, L.A., Chambers, D., Gregory, K.E., 1963. Efficiency of feed use in beef cattle. J. Anim. Sci. 22, 486–494.

Kolath, W.H., Kerley, M.S., Golden, J.W., Keisler, D.H., 2006. The relationship between mitochondrial function and residual feed intake in Angus steers. J. Anim. Sci. 84, 861–865.

Koolhaas, J.M., Korte, S.M., De Boer, S.F., Van Der Vegt, B.J., Van Reenen, S.G., Hopster, H., De Jong, I.C., Ruis, M.A.W., Blokhuis, H.J., 1999. Coping styles in animals: current status in behaviour and stress physiology. Neurosci. Biobehav. Rev. 23, 925–935.

Macadam, W.R., Eberhart, R.J., 1972. Diurnal variation in plasma corticosteroid concentration in dairy cattle. J. Dairy Sci. 55, 1792–1795.

Manteuffel, G., Puppe, B., Schön, P.C., 2004. Vocalization of farm animals as a measure of welfare. Appl. Anim. Behav. Sci. 88, 163–182.

Miller, A.J., Faulkner, D.B., Knipe, R.K., Strohbehn, D.R., Parrett, D.F., Berger, L.L., 2001. Critical control points for profitability in the cow-calf enterprise. Prof. Anim. Sci. 17, 295–302.

Montanholi, Y.R., Swanson, K.C., Schenkel, F.S., McBride, B.W., Caldwell, T. R., Miller, S.P., 2009. On the determination of residual feed intake and associations of infrared thermography with efficiency and ultrasound traits in beef bulls. Livest. Sci. 125, 22–30.

Montanholi, Y.R., Swanson, K.C., Palme, R., Schenkel, F.S., McBride, B.W., Lu, D., Miller, S.P., 2010. Assessing feed efficiency in beef steers through feeding behaviour, infrared thermography and glucocorticoids. Animal 4, 692–701.

Möstl, E., Palme, R., 2002. Hormones as indicators of stress. Domest. Anim. Endocrinol. 23, 67–74.

Palme, R., Möstl, E., 1997. Measurement of cortisol metabolites in feaces of sheep as a parameter of cortisol concentration in blood. Z. Säugetierkd—Inter. J. Mamm. Biol. 62 (Suppl. 2), 192–197.

Palme, R., Robia, C., Messmann, S., Hofer, J., Möstl, E., 1999. Measurement of faecal cortisol metabolites in ruminants: a noninvasive parameter of adrenocortical function. Wien. Tierärztl. Monatsschr.. Vet. Med. Austria 86, 237–241.

Palme, R., Robia, C., Baumgartner, W., Möstl, E., 2000. Transport stress in cattle as reflected by an increase in faecal cortisol metabolite concentrations. Vet. Rec. 146, 108–109.

Palme, R., Rettenbacher, S., Touma, C., El-Bahr, S.M., Möstl, E., 2005. Stress hormones in mammals and birds: comparative aspects regarding metabolism, excretion, and noninvasive measurement in fecal samples. Ann. NY Acad. Sci 1040, 162–171.

Palme, R., 2012. Monitoring stress hormone metabolites as a useful, non-invasive tool for welfare assessment in farm animals. Anim. Welfare 21, 331–337.

Pesenhofer, G., Palme, R., Pesenhofer, R.M., Kofler, J., 2006. Comparison of two methods of fixation during functional claw trimming – walk-in crush versus tilt table – in dairy cows using faecal cortisol metabolite concentrations and daily milk yield as parameters. Wien. Tierärztl. Monatsschr. Vet. Med 93, 288–294.

Purchas, R.W., Barton, R.A., Kirton, A.H., 1980. Relationships of circulating cortisol levels with growth rate and meat tenderness of cattle and sheep. Austr. J. Agric. Res. 31, 221–232.

Richardson, E.C., Herd, R.M., Archer, J.A., Arthur, P.F., 2004. Metabolic differences in Angus steers divergently selected for residual feed intake. Aust. J. Exp. Agric. 44, 441–452.

Rouha-Mülleder, C., Palme, R., Waiblinger, S., 2010. Assessment of animal welfare in 80 dairy cow herds in cubicle housing—animal health and other animal-related parameters. Wien. Tierärztl. Monatsschr. Vet. Med. 97, 231–241.

Sapolsky, R.M., 2002. Endocrinology of the stress response. In: Becker, J.B., Breedlove, M., Crews, D., McCarthy, M.M. (Eds.), Behavioral Endocrinology2nd ed. , The MIT Press, Cambridge, MA, pp. 409–450.

SAS, Statistical, Analysis Systems Institute, 2003. SAS/STAT User's Guide, Version 9.1. SAS Institute Inc, Cary.

Schenkel, F.S., Miller, S.P., Wilton, J.W., 2004. Genetic parameters and breed differences for feed efficiency, growth, and body composition traits of young beef bulls. Can. J. Anim. Sci. 84, 177–185.

Sherman, E.L., Nkrumah, J.D., Moore, S.S., 2010. Whole genome single nucleotide polymorphism associations with feed intake and feed efficiency in beef cattle. J. Anim. Sci. 88, 16–22.

Snelling, W.M., Allan, M.F., Keele, J.W., Kuehn, L.A., Thallman, R.M., Bennett, G.L., Ferrell, C.L., Jenkins, T.G., Freetly, H.C., Nielsen, M.K., Rolfe, K.M., 2011. Partial-genome evaluation of postweaning feed intake and efficiency of crossbred beef cattle. J. Anim. Sci. 89, 1731–1741.

Thun, R., Eggenberger, E., 1996. Abhängigkeit zwischen Cortisol und Testosterone unter Ruhebedingungen, nach akutem Streβ und Hormonstimulierung beim Stier. Schweizer Arch. Tierheilkd. SAT 138, 225–233.

von Holst, D., 1998. The concept of stress and its relevance for animal behaviour. Adv. Study Behav. 27, 1–131.