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Relationship between periparturient diseases, metabolic markers and the dynamics of hair cortisol concentrations in dairy cows

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ABSTRACT

Hair cortisol concentration (HCC) might represent a promising marker for retrospective welfare assessment of dairy cows. The objective of the study was to explore the dynamics of HCC in diseased and healthy cows from eight-week ante partum (AP) to eight-week post partum (PP). Twenty-four pregnant cows were followed from drying off to week eight PP. Tail hair was used to measure cortisol at five different time points. The occurrence of peripartum diseases, lameness and the body condition score (BCS) were monitored on a weekly basis. Blood β-hydroxybutyric acid, non-esterified fatty acids, calcium and insulin-like growth factor-1 (IGF-1) concentrations were measured. The temperature-humidity index (THI) was continuously recorded.

The median values of HCC in all cows were 0.4, 0.3, 0.6, 0.8 and 0.5 pg/mg at weeks eight, four AP, calving, weeks four, eight PP, respectively. There was no association between HCC and the occurrence of peripartum diseases ($P \geq 0.05$). A positive correlation between HCC and BCS loss ($P < 0.01$) and THI ($P < 0.05$) was observed. The occurrence of peripartum diseases was associated with low IGF-1 during the study period but no relationship was found between cortisol and IGF-1 levels ($P \geq 0.05$). Brown Swiss cows showed higher HCC ($P < 0.01$) at weeks eight, four AP, and week four PP and lower average milk yield ($P < 0.05$) than Holstein–Friesian cows. In conclusion, HCC was not a suitable marker for peripartum diseases but it could reflect a stress response, which is linked to BCS loss, heat stress and breed.

1. Introduction

Periparturient period is a critical period for dairy cows. During this time, animals are exposed to various stressors and have the highest risk for the development of diseases (Mordak and Anthony, 2015; Sordillo and Raphael, 2013). Nearly all high milk-producing cows undergo a state of negative energy balance (NEB) around parturition, when the energy requirement for maintenance and lactation exceeds the energy intake (Butler and Smith, 1989). The susceptibility to infectious and metabolic diseases is increased by NEB (Drackley, 1999; Duffield, 2000). For monitoring metabolic health, indicative metabolic markers are used, such as blood β-hydroxybutyric acid (BHB), non-esterified fatty acids (NEFA) and calcium levels. When there is no prospective disease monitoring, there are a high number of unreported cases (Drackley, 1999; Idris et al., 2021). Additionally, environmental conditions and especially heat stress (HS) affect the well-being of dairy cows (Idris et al., 2021). The temperature-humidity index (THI) is widely used to investigate effect of HS on dairy animals (Polsky and von Keyserlingk, 2017). High producing dairy animals are considered heat stressed when THI values are ≥72 (Armstrong, 1994). Many studies have been focused on the optimization of prospective disease monitoring (LeBlanc et al., 2006), while less is known about the retrospective welfare monitoring.

The concentration of cortisol in blood has been widely adopted as a stress indicator (Daley et al., 2000; Huzzey et al., 2012). However, blood sampling needs animal handling, which itself can be a stressor affecting the results (Beagley et al., 2010). In addition, blood cortisol concentration follows a diurnal pattern so that results depend on the time of sampling (Thun et al., 1981). Hair cortisol concentration (HCC) might

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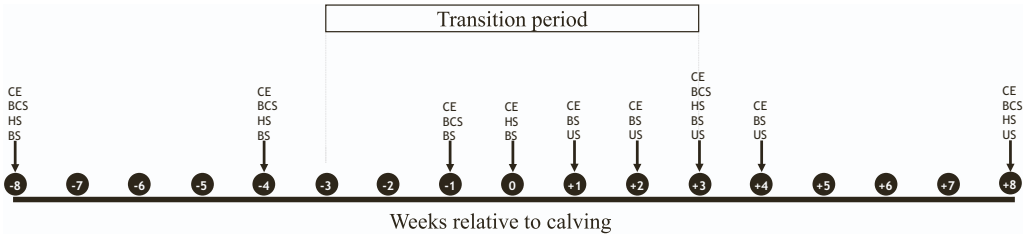

Fig. 1. Schematic representation of sampling schedule from Brown Swiss and Holstein–Friesian cows ($n = 24$) during the study period. The time points of clinical examinations (CE), body condition scoring (BCS), hair (HS) and blood sampling (BS) are indicated. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 1
Distribution of the diseases in the breeds (Holstein–Friesian (HO) and Brown Swiss (BV)) and parity groups.

	Breed		Parity	
Disease or condition	HO (n = 16)	BV (n = 8)	Primiparous (n = 4)	Pluriparous (n = 20)
Acute mastitis	1 (6%)	2 (25%)	0 (0%)	3 (15%)
Puerperal metritis	3 (19%)	2 (25%)	2 (50%)	3 (15%)
Lameness	6 (38%)	2 (25%)	1 (25%)	7 (35%)
RFM[1]	1 (6%)	1 (13%)	0 (0%)	2 (10%)
Respiratory problems	2 (13%)	1 (13%)	2 (50%)	1 (5%)
Tail amputation	1 (6%)	0 (0%)	0 (0%)	1 (5%)
> one condition	6 (38%)	3 (38%)	2 (50%)	7 (35%)

[1] RFM = retained foetal membranes.

represent a stable non-invasive marker of long-term chronic stress as it accumulates in the hair (Braun et al., 2022; Burnett et al., 2015; Tallo-Parra et al., 2015).

HCC follows dynamic pattern during the cows' production cycle with increased concentrations around the time of calving (Braun et al., 2017b). Moreover, a study concluded that HCC was higher during summer than winter months in dairy animals (Uetake et al., 2018). It is still not entirely clear, however, whether HCC can be used as a retrospective marker of the animal's unspecific disease history. In some studies, HCC was not a useful indicator of disease (Braun et al., 2017b) or lameness (Fischer-Tenhagen et al., 2018), whereas other studies found associations between cortisol concentrations and diseases and lameness (Burnett et al., 2015; Comin et al., 2013). So far, there are only a few studies considering dry period so that knowledge on the health-status related dynamics of HCC before and after calving is unclear.

Therefore, the objective of the study was to explore the dynamics of HCC in diseased and healthy cows over a period of eight-week ante partum (AP) to eight-week post partum (PP).

2. Materials and methods

All animal procedures were approved by the Committee of Animal Experiments of the Canton Zurich, Switzerland (Animal license numbers: eTV30236, ZH131/18).

2.1. Study farm and housing

The study was conducted between January and September 2019 at AgroVet Strickhof, University of Zurich. Cows were housed in a free-stall barn with straw-bedded cubicles. Herd A (63 cows) and herd B (60 cows) were housed on different sides of the barn and milked with a conventional and a robotic milking system, respectively (DeLaval AG, Sursee, Switzerland). Animals were fed a total mixed ration and had ad libitum access to water. The 305-day herd milk yield of herd A and B was 34.1 ± 9.4 kg and 42.3 ± 8.8 kg, respectively (mean ± SD). Additionally, weekly and monthly average milk yield were used for the analysis.

2.2. Experimental design

Clinically healthy pregnant cows (Holstein–Friesian (HO), $n = 16$; Brown Swiss (BV), $n = 8$) with no more than five lactations (primiparous: $n = 4$, pluriparous: $n = 20$) were included in the study. Study animals were followed from week eight AP (dry off) until week eight PP. Throughout the experiment, sample collection, gynaecological and clinical evaluations and the assessment of body condition score (BCS) were performed (Fig. 1). Cows were assigned into different groups as healthy and diseased. Healthy ($n = 13$) cows were not affected by retained placenta, clinical or puerperal metritis, endometritis, mastitis, hypocalcaemia, hyperketonaemia, lameness or any other clinical disease. Diseased cows ($n = 11$) suffered from one or more periparturient diseases. Table 1 shows the distribution of diagnosed diseases. All examinations were done by the same persons (MT, KW).

2.3. Clinical examinations

A general examination was performed at weeks eight, four, one AP and at weeks one, two, three, four and eight PP. The rectal temperature was measured. Additionally, heart and respiration rates, rumination and faecal scoring were documented (Peter, 2002). Respiratory problems were characterized with increased respiration rate associated with fever and the existence of increased lung sounds during auscultation.

The udder was examined by inspection and palpation. The shape and symmetry of the udder were inspected (Peter, 2002). Milk samples were taken for diagnosis of mastitis using the California Mastitis Test (CMT) (Sargeant et al., 2001). Acute mastitis was diagnosed when an animal had a swollen, warm, painful quarter and abnormal milk in the presence of systemic symptoms (Siivonen et al., 2011).

2.4. Assessment of uterine health, body condition score and lameness

During the first three-week PP, vaginal examinations were performed for the assessment of vaginal discharge by hand and thereafter, vaginoscopy was carried out as described by Prunner et al. (2014). Retained foetal membranes were diagnosed as a failure of the expulsion of the foetal membranes and rectal temperature above 39.5 °C within 24 h after delivery of the calf (Beagley et al., 2010). A gynaecological examination was performed during the first four weeks PP on a weekly basis and at week eight PP. The examination consisted of a vaginal inspection, rectal palpation and ultrasonographic examination of the genital tract (Iscan-2, Draminski S.A., Olsztyn, Poland) as described previously (Heppelmann et al., 2013; Prunner et al., 2014). The diagnosis of puerperal and clinical metritis was made on the presence or absence of systemic symptoms of diseases and the quality of uterine discharge at the vaginal examination as defined by Sheldon et al. (2006). The diagnosis of clinical endometritis was performed based on rectal palpation and vaginoscopy at week four PP. The discharge was subjectively evaluated and classified into vaginal discharge score (VDS) 0 to 3

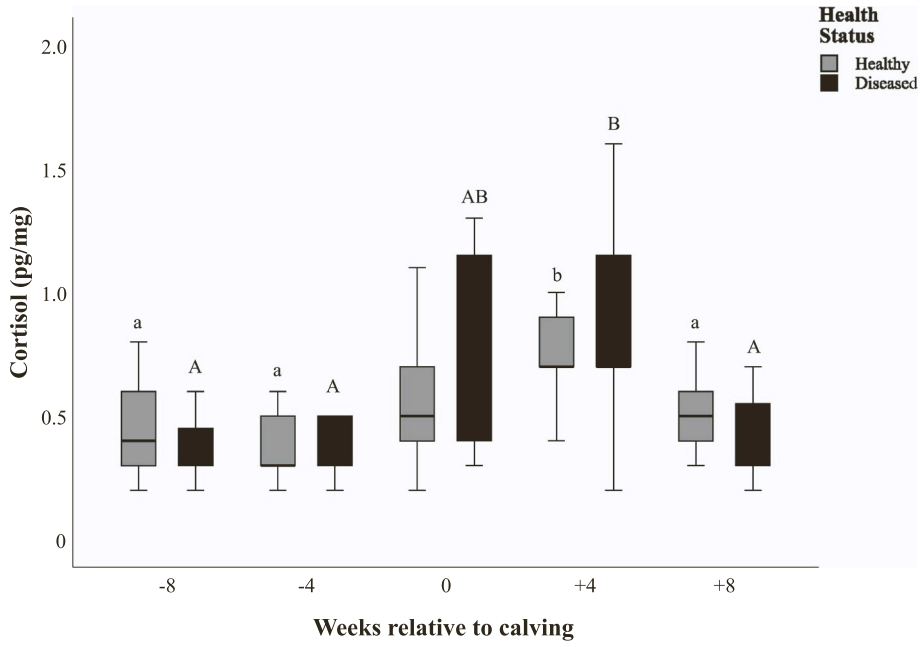

Fig. 2. Hair cortisol concentration in healthy ($n = 13$) and diseased ($n = 11$) dairy cows between eight-week before parturition and eight-week after parturition. Different letters indicate differences between times related to calving within the same group of cows ($P < 0.05$). Lowercase letters were used for the group of healthy cows and uppercase letters for diseased cows. There was no difference between healthy and diseased cows ($P \geq 0.05$).

as previously described (Williams et al., 2005). Cows with VDS ≥ 2 at week four PP were regarded as having clinical endometritis (CE) (Sheldon et al., 2006).

BCS assessment was performed at week eight, four, one AP and at week one, four and eight PP as described previously (Edmonson et al., 1989). Lame cows were diagnosed visually in accordance with changes in gait, posture or behaviour according to a locomotion scoring system (Sprecher et al., 1997).

2.5. Blood hormone and metabolite analysis

Blood samples were collected at drying off, weeks four and one prior to expected calving date, 12 to 24 h PP and then weekly until week five PP. Blood samples were obtained from the coccygeal blood vessels into evacuated tubes (Greiner Bio-One GmbH, Kremsmunster, Austria). After centrifugation at 2000*g* for 15 min/4 °C, plasma and serum were stored at −20 °C until analysis.

Blood plasma insulin-like growth factor-1 (IGF-1) concentration was measured using radioimmunoassay (Vicari et al., 2008). Intra- and inter-assay coefficient of variation was 10 and 15%, respectively. Plasma NEFA, BHB and calcium concentrations were measured using the COBAS MIRA 2 centrifugal analyser (Hoffman La Roche, Basel, Switzerland) according to the manufacturer's instructions using commercial kits from Randox Laboratories Ltd. (Ibach, Switzerland), and calcium was measured using a commercial kit (DiaSys Diagnostic Systems GmbH, Holzheim, Germany).

2.6. Hair collection and analysis of cortisol

Hair was obtained from the tail switch, because in this location it grows faster than at the other sites of the body (Burnett et al., 2014; Heimbürge et al., 2020). Collection of hair samples started at week eight AP. Before sampling, hair was cut with scissors to a length of 2 cm, and remaining hair was collected. Then, every month, regrown hair was sampled 0.5 mm close to the skin by means of Electric clippers (Aesculap Suhl GmbH, Suhl, Germany). Obtained hair samples were dried at room temperature overnight, wrapped into aluminium foil and kept at room temperature until cortisol analysis. HCC was measured using liquid chromatography-tandem mass spectrometry (LC-MS/MS) as described previously by Binz et al. (2016).

2.7. Temperature-humidity index calculations

Numerous environmental measures such as temperature, wet bulb temperature (Twb) and dry bulb temperature (Tdb) were recorded in the farm. THI of the farm was calculated according to following formula (Rowsell, 1972):

$$\mathrm{THI} = (0.15 \times \mathrm{Tdb} + 0.85 \times \mathrm{Twb}) \times 1.8 + 32$$

THI values ≥72 were considered HS for the dairy cows (Armstrong, 1994) and monthly average THI values calculated for the evaluations.

2.8. Statistical analysis

Statistical analyses were conducted using IBM SPSS version 26 (Chicago, IL, USA). The normality of the data was checked visually by histogram and by means of the Shapiro-Wilk test. Differences in quantitative variables were tested using the Student's *t*-test for normally distributed data, and the Mann-Whitney *U* test non-normal data. The effect of time, group and interaction between group and time variables (BCS, milk yield and concentrations of hair cortisol, NEFA, BHB and calcium) were analysed using a general linear model with repeated measurement. Data are presented as mean or median ± standard error of the mean or median and quartiles, depending on the data distribution. Correlations between HCC, BCS loss, milk yield and THI were calculated using Spearman's correlation. Differences were considered statistically significant when $P < 0.05$.

3. Results

3.1. Associations between cortisol concentration and postpartum diseases

The median values of the hair cortisol concentration in all cows were 0.4, 0.3, 0.6, 0.8 and 0.5 pg/mg at week eight AP, week four AP, calving, week four PP and week eight PP, respectively. There was no difference in HCCs between healthy and diseased cows ($P \geq 0.05$). However, there

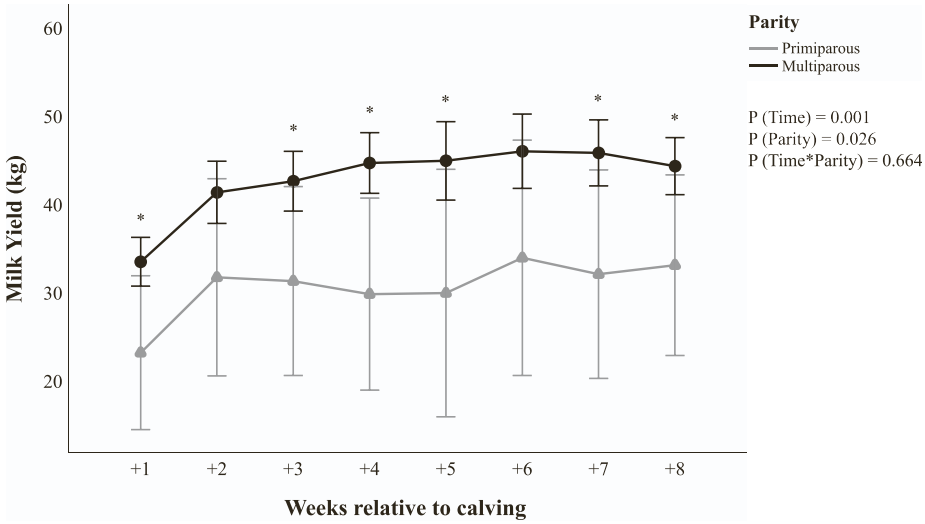

Fig. 3. Average milk yield during the first eight-week postpartum in dairy cows. There were differences between pluriparous ($n = 20$) and primiparous ($n = 4$) cows within indicated weeks (*$P < 0.05$).

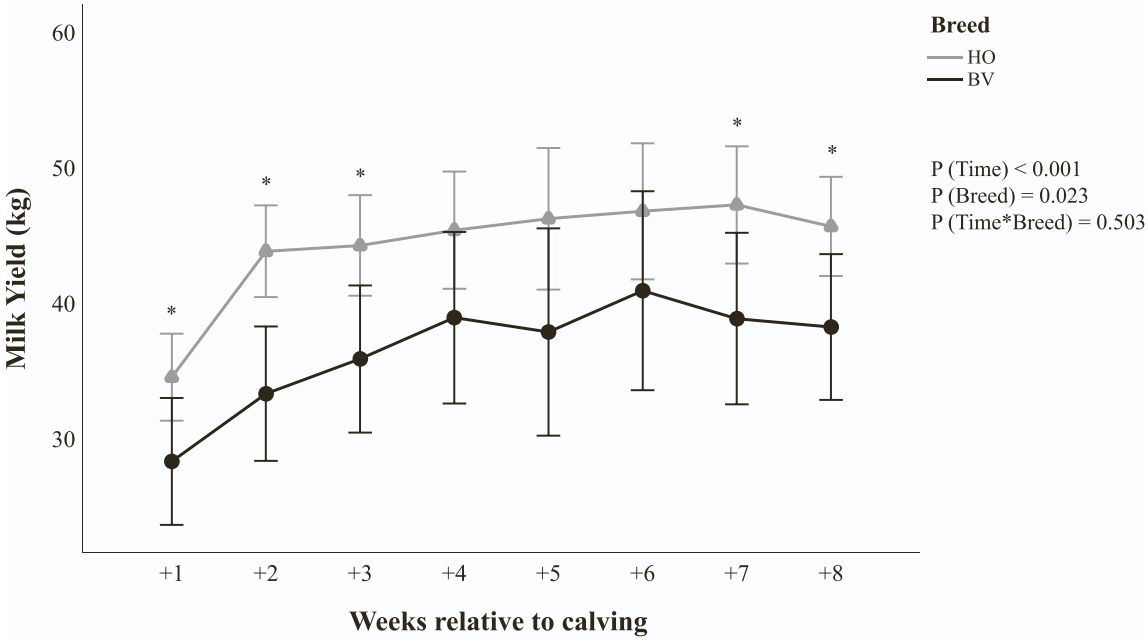

Fig. 4. Average milk yield during the first eight-week postpartum in dairy cows. There were differences between groups (Holstein–Friesian (HO), $n = 16$ and Brown Swiss (BV), $n = 8$) within indicated weeks (*$P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

was an influence of the time relative to calving on HCCs ($P < 0.05$, Fig. 2). Maximum median HCC in healthy and diseased animals were observed at week four PP (1.1 and 2.3 pg/mg).

3.2. Relationship between cortisol concentrations, milk production and breed

The average daily milk yield was 41.7 ± 1.6 kg. There was no association between PP HCC and monthly average milk yield ($P \geq 0.05$). However, weekly average milk yield was influenced by time ($P < 0.01$) and parity ($P < 0.05$), as weekly milk production in pluriparous cows was higher than in primiparous cows, except for weeks two and six PP (Fig. 3). Furthermore, BV cows had a lower average milk yield during the study period ($P < 0.05$, Fig. 4) than HO cows. BV cows had higher HCC than HO cows during the experimental period ($P < 0.01$). HCC was higher in BV cows than HO cows at week eight and four AP as well as at week four PP (Fig. 5).

3.3. Associations between cortisol concentration, body condition and metabolic markers

Positive correlations were found between HCCs at calving and BCS loss from week eight AP to week eight PP ($r = 0.58$, $P < 0.05$) and from week eight AP to week one PP ($r = 0.68$, $P < 0.05$). Furthermore, HCC at week four PP was positively correlated with BCS loss from week four AP to week eight PP ($r = 0.56$, $P < 0.05$, Table 2). No significant associations were observed between HCC and NEFA, BHB or calcium concentrations ($P \geq 0.05$). Except for calving period, diseased cows had lower plasma IGF-1 than healthy cows ($P < 0.05$, Fig. 6) but IGF-1 was not related to HCC ($P \geq 0.05$). Following the last week of pregnancy, a

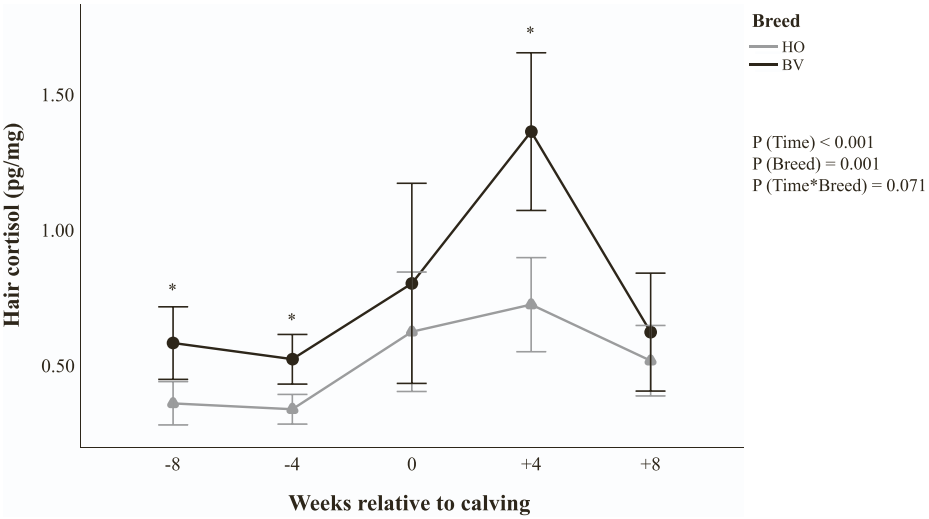

Fig. 5. Hair cortisol changes during the study period in dairy cows. There were differences between groups (Holstein–Friesian (HO), $n = 16$ and Brown Swiss (BV), $n = 8$) within indicated weeks (*$P < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table 2
Spearman correlation between hair cortisol and body condition score (BCS) loss at different time points in dairy cows (postpartum (PP), antepartum (AP) and correlation coefficient (CC)).

		BCS loss			
Cortisol (pg/mg)		week eight ap to week eight PP	week eight AP to week one PP	week four AP to week eight PP	week one PP to week eight PP
Week eight AP	CC	0.43	0.27	0.45	0.43
	P-value	0.06	0.33	0.06	0.09
	N	19	15	19	16
Week four AP	CC	0.34	0.35	0.08	0.10
	P-value	0.16	0.20	0.76	0.71
	N	19	15	19	16
Calving	CC	0.58**	0.68**	0.25	0.27
	P-value	0.01	0.01	0.31	0.24
	N	19	15	19	21
Week four PP	CC	0.35	0.56*	0.13	0.18
	Sig. (2-tailed)	0.14	0.03	0.60	0.43
	N	19	15	19	21
Week eight PP	CC	0.25	0.26	0.00	−0.01
	P-value	0.30	0.36	1.00	0.96
	N	19	15	19	21

significant reduction in the plasma IGF-1 was observed in both healthy and diseased cows, resulting in a time effect on plasma IGF-1 ($P < 0.01$, Fig. 6).

3.4. Association between the temperature-humidity index and cortisol concentration

The association between HCC and THI was evaluated. A positive correlation was found between HCC at week four PP and average THI values from week four AP to week four PP ($r = 0.57$, $r = 0.47$, respectively, $P < 0.05$, Table 3). There was also a positive correlation between HCC at week four AP and average THI values from week one PP to week eight PP ($r = 0.71$ and $r = 0.56$, respectively, $P < 0.05$, Table 3).

4. Discussion

Steroid measurement in hair is a non-invasive technique that gives the possibility to observe long-term stress responses. The objective of the study was to investigate health status-related dynamics of HCCs during the transition period. Therefore, HCC was evaluated, and its relationships with periparturient diseases, metabolic markers, milk production, parity and breed were determined.

Measured HCCs, ranging between 0.2 and 2.3 pg/mg, were lower than in most of the previous studies in dairy cows, reporting concentrations ranging between 1.9 and 41.7 pg/mg (Burnett et al., 2014; Burnett et al., 2015; González-de-la-Vara et al., 2011; Peric et al., 2013; Tallo-Parra et al., 2018). Previous studies have reported similar concentrations to our findings (Braun et al., 2017a; Braun et al., 2022). Differences in HCC between studies might be a consequence of the applied analytical methods (immunoassay vs. LC-MS/MS). While LC-MS/MS was used in the study by Braun et al. (2017a) and the present study, the other studies determined hair cortisol concentration using a radioimmunoassay (Comin et al., 2013; González-de-la-Vara et al., 2011; Peric et al., 2013) or enzymatic assays (Burnett et al., 2014; Burnett et al., 2015; Tallo-Parra et al., 2018). Furthermore, hair growth rate differs depending on the location of the body. Hair growth is faster at the tail switch than at hips, shoulders and other body parts (Burnett et al., 2014; Fisher et al., 1985; Heimbürge et al., 2020; Schwertl et al., 2003), which could have an effect on HCC (Burnett et al., 2014).

Regardless of the health status, HCC at week four PP was significantly higher than at weeks eight and four AP. Since our data reflect the cortisol accumulation before sampling in a four-week interval, samples from week four PP provide information on the first four weeks after calving, which is a very critical stage for the cow, when important physiologic and metabolic changes take place. Our findings were similar to those studies observing higher HCC around parturition (Braun et al., 2017b; Burnett et al., 2015).

Measurement of HCC was defined as a biomarker of long-term stress in dairy cows (Comin et al., 2013). In our study, no difference on HCC was found between healthy and diseased cows, which is not supported by previous reports, where researchers found that diseased cows had higher HCCs than healthy (Burnett et al., 2015; Comin et al., 2013). However, Burnett et al. (2015) did not find any association between HCC and subclinical endometritis before and after the presence of the condition. It appears logical that disease severity has an influence on the cortisol level and clinical diseases are rather associated with high cortisol levels than subclinical. It is noteworthy that our result should be interpreted with caution because of the small sample size. Nevertheless, similar to our study, Braun et al. (2017b) stated that HCCs were not different before and after the diagnosis of clinical diseases.

Climatic environment is expressed by THI that is calculated using environmental temperature and relative humidity. Environmental condition are defined as HS at THI $\geq$ 72 (Armstrong, 1994). A limited

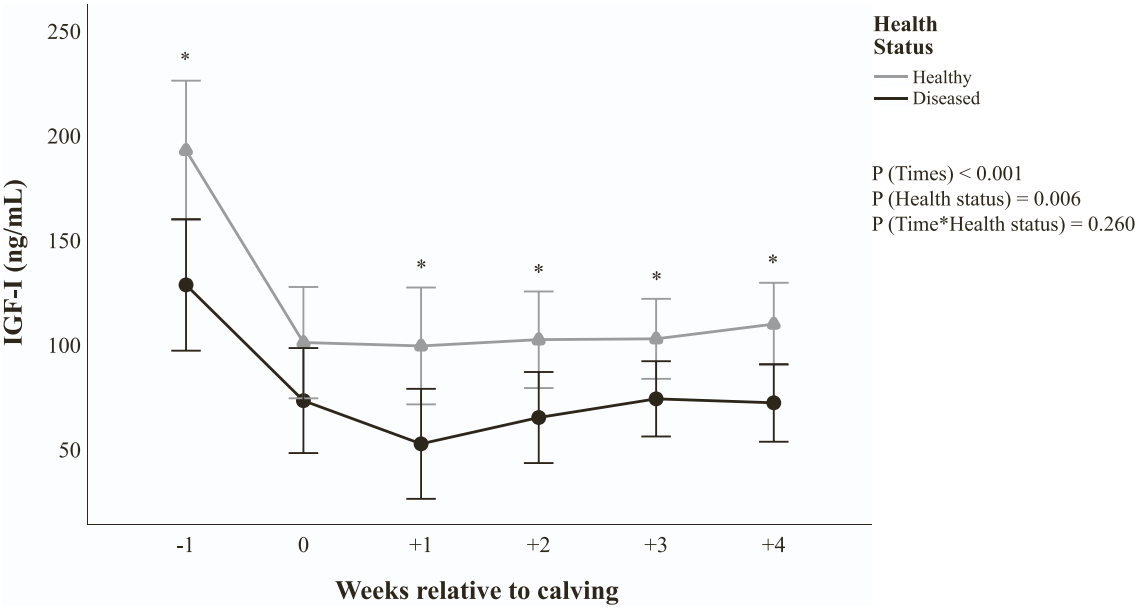

Fig. 6. Insulin-like growth factor 1 (IGF-1) in blood plasma from one-week ante partum to four-week postpartum in dairy cows. There were differences between groups (healthy cows, $n = 13$ and diseased cows, $n = 11$) within weeks indicated by asterisks ($^*P < 0.05$).

Table 3
Spearman correlation between hair cortisol concentration and exceeded the temperature-humidity index (THI) per hour in dairy cows (postpartum (PP), antepartum (AP) and correlation coefficient (CC)).

THI hours						
Cortisol (pg/mg)		week twelve AP to week eight AP	week eight AP to week four AP	week four AP to week one PP	week one PP to week four PP	week four PP to week eight PP
Week eight AP	CC	−0.592	−0.258	0.254	0.450	0.389
	P-value	0.122	0.472	452	106	0.100
	N	8	10	11	14	19
Week four AP	CC	−0.507	0.078	0.406	0.708**	0.564*
	P-value	0.200	0.830	0.216	0.005	0.012
	N	8	10	11	14	19
Calving	CC	−0.418	−0.499	0.289	0.027	0.200
	P-value	0.263	0.118	0.316	0.914	0.350
	N	9	11	14	19	24
Week four PP	CC	−0.490	−0.020	0.568*	0.472*	0.316
	Sig. (2-tailed)	0.181	0.953	0.034	0.041	0.133
	N	9	11	14	19	24
Week eight PP	CC	0.209	0.267	0.373	0.266	0.101
	P-value	0.590	0.427	0.189	0.271	0.638
	N	9	11	14	19	24

number of studies evaluated an association between hair cortisol concentration and climatic environment (Comin et al., 2011; Ghassemi Nejad et al., 2017; Uetake et al., 2018). In the present study, heat stressed cows had higher HCC than non-heat stressed cows during the first month PP. Our finding is consistent with a previous study (Uetake et al., 2018). Consistent with our result, other researchers showed that cortisol concentration in various biological materials increased during HS (Chen et al., 2018; Idris et al., 2021; Verkerk et al., 1998). This suggests that the effect of long-term exposure to HS could be evaluated using HCC.

There were no associations between metabolic indicators of NEB, such as NEFA, BHB, and HCC. A high BCS during the dry-off period and at calving predisposes cows for higher postpartum BCS loss (Gearhart et al., 1990), resulting in metabolic stress for cows (Bernabucci et al., 2005). It is noteworthy that in the present study, BCS loss between 4 weeks AP and 8 weeks PP was indeed positively correlated with HCC at calving. This indicates that the stress related to BCS loss can also be quantified via HCC measurement. Burnett et al. (2015) found a positive association between BCS and HCC in pluriparous cows. In the present study, the lacking relationships between HCC and BHB or NEFA values could be attributed to the fact that the study farm has a good nutritional management reducing the metabolic impact observed in cases of NEB. In our study, it was observed that diseased cows had lower IGF-1 levels than healthy ones during the first four-week PP but no association was found between IGF-1 and HCC. In a former study, supporting our findings, clinically diseased cows had lower IGF-1 concentration than healthy ones (Piechotta et al., 2014). Therefore, our study confirms that circulating IGF-1 concentration could be a useful indicator of periparturient disorders.

It was also observed in the current study that BV cows had higher HCCs than HO at week eight and four AP and four PP, with a subsequent decrease until week eight PP in BV cows down to the level of HO cows. Our results differ from Braun et al. (2022)'s study that reported a greater HCC in BV cows. Differences between studies could be explained by farm related factors, such as housing, feeding and handling. The fact that BV cows in the present study had lower milk yield than HS, however, supports a breed specific stress response (Del Corvo et al., 2021), which is independent of milk yield. Further studies including a larger sample size are needed to confirm our results.

5. Conclusions

HCC was not associated with the occurrence of metabolic or infectious diseases but a breed-specific stress response has been observed. HCC could be a marker of metabolic stress and HS since the BCS loss and THI were positively correlated with the HCC. Diseased cows present lower IGF-1 PP, but neither IGF-1 nor other metabolic marker were related to HCC. The result of our study provided profound basis for future work to examine associations between HCC and metabolic status, heat stress and breed.

Declaration of Competing Interest

None of the authors has any financial or personal relationships that could inappropriately influence or bias the content of the paper.

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