

Adrenal cortex reactivity in dairy cows differs between lactational stages and between different feeding levels

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Summary

Changes in ACTH challenge test characteristics in dairy cows changing their physiological status at different lactational stages and different feeding levels were not investigated in terms of repeatability yet. In 23 multiparous Holstein cows (10 cows fed a sole fresh herbage diet without concentrate, 13 cows fed with concentrate), three ACTH challenge tests were performed: once during pregnancy shortly prior to drying off (T1), and in week 3 (T2) and 8 (T3) after parturition. Test characteristics were correlated to performance and metabolic parameters: DMI, BW, energy balance (EB), plasma concentrations of free fatty acids (NEFA) and beta-hydroxybutyrate (BHB). Basal plasma cortisol concentrations were higher at T1 compared with T2 and T3 ($p < .05$). The adrenal cortex sensitivity (expressed as total AUC (AUC_t) of cortisol response after ACTH application) was lowest at T2 compared with T1 and T3 ($p < .05$). Ranking of the individual animals' responses was not repeatable between time points of the ACTH tests. Enhancing the energy deficiency during early lactation by omission of concentrate did not affect baseline cortisol concentrations in plasma, but decreased peak height at T2 ($p < .05$). Baseline plasma cortisol concentrations were positively correlated with cortisol peak values after ACTH application, previous lactation performance, milk yield and BW ($p < .05$). The AUC_t was positively correlated with baseline cortisol concentrations, EB and DMI. Cortisol release after ACTH injection was lower in animals with high plasma concentrations of NEFA, BHB and with higher contents of fat and free fatty acids in milk ($p < .05$). Cortisol peak height after ACTH administration was higher in cows with a more positive EB, higher DMI and lower plasma concentrations of NEFA and BHB. In summary, cortisol responses to ACTH challenges in this study were not repeatable in dairy cows changing their physiological status.

KEYWORDS

ACTH challenge, adrenocortical responsiveness, cortisol, dairy cow, HPA-axis, repeatability

1 | INTRODUCTION

The ACTH stimulation test was established more than 60 years ago in human medicine to diagnose adrenocortical insufficiency and hypothalamo–pituitary–adrenal (HPA) axis function (Jenkins, Forsham, Laidlaw, Reddy, & Thorn, 1955; Thorn, Goetz, Streeten, Dingman, & Arons, 1953). ACTH challenge tests are experimentally used in

farm animals to assess stress response (Otvic & Hutchinson, 2015; Trevisi, Bertoni, Lombardelli, & Minuti, 2013; Verkerk, Macmillan, & McLeay, 1994; Weiss, Helmreich, Möstl, Dzidic, & Bruckmaier, 2004). Munksgaard, Herskin, Løvendahl, and Andersen (2006) summarized findings in non-ruminant species with evidence that diet composition and energy supply impact HPA-axis regulation and stress response. Whereas HPA-axis reactivity changed in growing heifers and steers,

adrenal reactivity of lactating cows seemed not to be affected by energy intake (Munksgaard et al., 2006).

However, the importance of investigating changes in ACTH challenge test characteristics (i.e., total capacity of cortisol release, peak height, time of peak, etc.) in terms of repeatability in dairy cows changing their endocrine and metabolic status was not considered so far. Instead, ACTH tests are mostly performed only once and animals grouped retrospectively as high and low responders (Van Reenen et al., 2005; Weiss et al., 2004). Fisher, Verkerk, Morrow, and Matthews (2002) emphasized the role of cortisol for the short-term stimulation to mobilize body reserves. Hence, the adrenal cortex sensitivity could be indicative for the flexibility in metabolic adaptations to nutrient and energy deficiencies. Furthermore, the explanatory power of ACTH test outcomes at different lactational stages needs more analysis to determine appropriate dates for conducting those.

The objective of this study was to investigate relationships between ACTH test response characteristics and metabolic parameters at different lactational stages in dairy cows. Based on these experimental approaches, we tested the hypothesis that responses to ACTH challenges in dairy cows are repeatable among various lactational stages. We further hypothesized that individual performance and enhanced metabolic load affect adrenal cortex reactivity.

2 | MATERIAL AND METHODS

All experimental procedures followed the Swiss Law on animal protection and were approved by the Committee of Animal Protection of the Canton Fribourg, Switzerland.

2.1 | Animals, feeding and housing

Twenty-three pregnant multiparous Holstein dairy cows (parity 2–7) were involved in the experiments. Animals were housed in a free stall barn and transferred to tie stalls for adaptation 2 days prior to the experiments. Milking was carried out twice daily at 05:30 and 16:30 hr. All dairy cows were fed with fresh herbage and additional concentrate to meet their energy and nutrient requirements until dry off 8 weeks prior to expected calving. From week 8 ante partum onwards, animals were divided into two treatment groups based on parity and milk yield in the previously finished lactation. One group received a sole fresh herbage diet without supplementary concentrate (**ZERO**, $n = 10$), whereas the second group was fed with concentrate to meet nutrient and energy requirements until week 8 after parturition (**CONC**, $n = 13$). More details on the nutrient values and diets fed were reported recently by Zbinden et al. (2016). Dry matter intake (DMI) and milk yield were recorded daily, body weight (BW), energy balance, milk composition, metabolic and endocrine parameters in plasma (glucose, NEFA, BHB, IGF-1) at a weekly basis as described and shown by Zbinden et al. (2016).

In all animals, three ACTH challenge tests were performed: once during pregnancy shortly prior to drying off (**T1**), and in week 3 (**T2**) and 8 (**T3**) after parturition. On the day prior to the ACTH tests, cows

were weighed and fitted with a sterile indwelling jugular catheter (16 gauge, 32 cm long; Cavafix Certo Splittocan, B. Braun Melsungen AG, Melsungen, Germany). Synthetic adrenocorticotrophic hormone (ACTH Fragment 1–24, catalogue no. A 0298, Sigma-Aldrich Chemie GmbH, Buchs, Switzerland) was administered i.v. (16 µg/100 kg BW).

2.2 | Blood sampling and cortisol analysis

Blood was sampled through the jugular catheter every 15 min for 4 hr starting 60 min before and lasting until 180 min after ACTH injection. Blood samples (approximately 10 ml) were immediately transferred into tubes containing Na-EDTA. Samples were immediately put on wet ice until centrifugation (3,000 × g for 20 min at +4°C) for plasma collection and stored at –80°C until analysis. Plasma concentrations of glucose, NEFA, BHB, IGF-1, haptoglobin and serum amyloid A (SAA) were measured in baseline samples as described previously (Zbinden et al., 2016). Plasma cortisol was analysed in all samples during the ACTH test using a radioimmunoassay as described recently by Schwinn, Knight, Bruckmaier, and Gross (2016). The sensitivity of the assay was 0.125 ng/ml. The intra- and interassay CV were 8.5% and 9.1% respectively.

2.3 | Calculations and statistical analysis

Data presented are means ± SEM. Variables derived from the ACTH challenge tests included the baseline cortisol concentration, the total cortisol release from 0 to 180 min after ACTH injection calculated as area under the curve (AUC_t), the AUC above the cortisol baseline (AUC_b), the time and height of the cortisol peak, the difference between peak and basal cortisol concentration, the cortisol concentration at 60 min after the peak and the percental decrease during this interval (Figure 1). The MIXED procedure of SAS (Version 9.4, SAS Institute, Cary, North Carolina, USA) was used to investigate effects of performance (milk yield, feed intake, energy balance, BW) and metabolic status on ACTH test response characteristics (basal cortisol concentration, AUC_t , AUC_b , time and height of the cortisol peak, increase from basal to peak cortisol concentration and decline during 1 hr after the peak) with treatment and time of the ACTH test as class variables. Number of parity was tested as fixed effect in the model, but removed after lacking significance. The MIXED procedure of SAS was used to investigate differences of ACTH test characteristics between treatment groups and times of the performed tests with Bonferroni's correction for multiple testing. Fixed effects were treatment and time of the ACTH test. The individual cow was the repeated subject. The ranking of total cortisol release (AUC_t) following ACTH application in individual cows was additionally compared for the three tests at varying lactational stages. The intraclass correlation coefficient (ICC) was calculated from the covariance parameters of the model output to estimate the repeatability of baseline cortisol concentration, AUC_t , AUC_b , time and height of the cortisol peak after ACTH administration, the difference between peak and basal cortisol concentration, the cortisol concentration at 60 min after the peak and the percental decrease during this interval in the consecutive ACTH tests. Pearson's

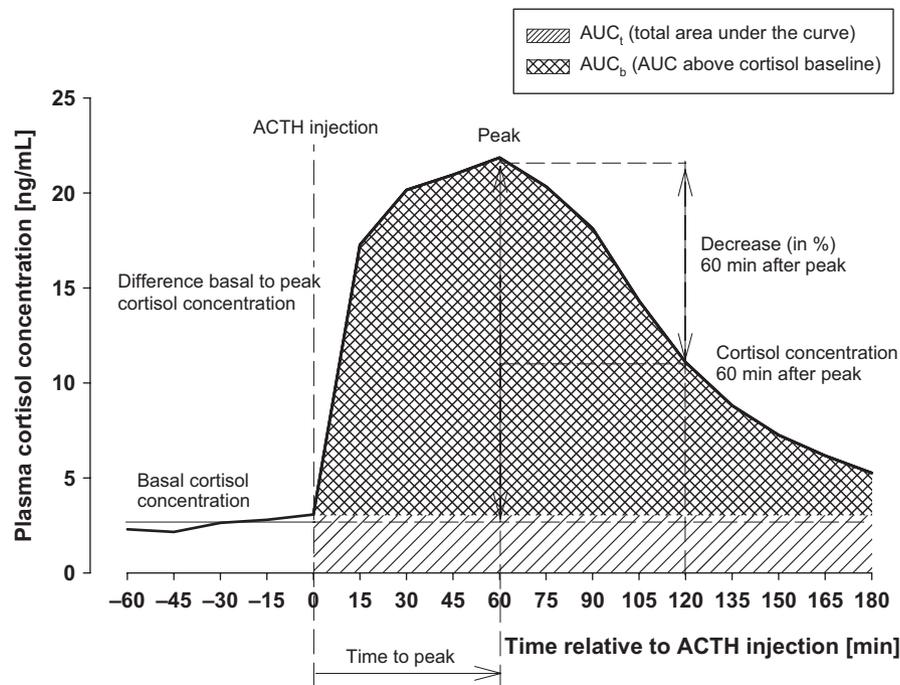


FIGURE 1 Example for the pattern of plasma cortisol concentration in dairy cows following ACTH injection. The figure describes the investigated parameters and terms used in the present study

correlation coefficients were calculated between ACTH challenge test characteristics and performance data (milk yield, milk composition, DMI, EB, development of BW) at the respective tests. Effects were considered to be significant at p -values $< .05$.

3 | RESULTS

3.1 | ACTH challenge test characteristics at different lactational stages

Responses of dairy cows to the ACTH challenges performed at T1, T2, and T3 are shown Table 1. Basal plasma cortisol concentrations were higher at T1 compared with T2 and T3 ($p < .05$, Table 1), while those between T2 and T3 did not differ ($p > .05$). Number of parity had no effect on the investigated parameters. The adrenal cortex sensitivity (expressed as total AUC (AUC_t) of cortisol response after ACTH application) was lowest at T2 compared with T1 and T3 ($p < .05$, Table 1). Similarly, the AUC above the cortisol baseline (AUC_b) tended to be lowest at T2 ($p < .10$) while the AUC_b of the other tests did not differ. The ranking of AUC_t and AUC_b was not repeatable at any time points of the ACTH tests (data not shown). The ICC of AUC_t and AUC_b was 0.08 and 0.11 respectively. Neither cortisol peak values following ACTH administration nor the difference between peak and basal values differed between the ACTH tests performed at T1, T2 and T3 ($p > .05$, Table 1). The peak value and its difference to cortisol baseline concentrations did not differ at the testing time point with a more or less balanced energy supply T1 and at T3 ($p > .05$, Table 1). The time when cortisol concentrations reached a maximum after ACTH application was not different among tests ($p > .05$). The cortisol concentration 60 min after the peak value as well as the percentual decrease of plasma cortisol concentration during this interval were not affected by the time point of the ACTH challenge test ($p > .05$, Table 1).

3.2 | Relationships between ACTH challenge test characteristics, performance and metabolic status

Enhancing the energy deficiency during early lactation by omission of concentrate did not affect baseline cortisol concentrations in plasma ($p > .05$, Table 1). The interaction between treatment and ACTH test was not significant for baseline cortisol concentration ($p = .86$). The adrenal cortex sensitivity (AUC_t from 0 to 180 min after ACTH application) was lower by trend in ZERO at T3 compared to CONC ($p = .07$). The interaction between treatment and ACTH test was not significant for AUC_t ($p = .48$). Omission of concentrate in ZERO resulted in a lower peak cortisol concentration at T2 compared with CONC ($p < .05$). The interaction between treatment and ACTH test was not significant for peak cortisol concentration ($p = .59$). Neither the time of the peak, the difference between baseline and peak values, nor the decline pattern of plasma cortisol concentration after ACTH administration were affected by the feeding level in CONC and ZERO (respective p -values for interactions treatment \times test were 0.22, 0.72 and 0.40).

At T2 and T3, baseline cortisol concentrations in plasma were higher in cows with higher cortisol peak values after ACTH application, with a higher previous lactation performance, higher current milk yield, higher body weight and higher SCC in milk ($p < .05$, Table 2). Milk composition (fat, protein, lactose, FFA) and plasma metabolites and endocrine factors (glucose, NEFA, BHB, IGF-1, haptoglobin and SAA) did not affect basal plasma cortisol concentration ($p > .05$, Table 2). Furthermore, energy balance and the extent of body weight loss since parturition had no effect on cortisol baseline values ($p > .05$, Table 2).

The adrenal cortex sensitivity (AUC_t) and AUC_b in early lactating cows were positively correlated with baseline cortisol concentration, energy balance and DMI ($p < .05$, Table 2). The cortisol release after ACTH was lower in animals with high plasma concentrations of

TABLE 1 Responses of dairy cows to ACTH challenge tests performed at T1 (in mid to late lactation, prior to drying off), T2 (week 3) and T3 (week 8 after parturition). From week 8 ante partum onwards, animals were divided into two treatment groups based on parity and performance in the previously finished lactation. One group received a sole fresh herbage diet without supplementary concentrate (ZERO, $n = 10$), whereas the second group was fed with concentrate to meet nutrient and energy requirements until week 8 after parturition (CONC, $n = 13$). Data are shown as mean values \pm SEM. Effects of group (CONC, ZERO), time point of the test (T1, T2, T3) and group \times time were considered significant at $p < 0.05$

Parameter	T1			T2			T3			p-values		
	CONC	ZERO	SEM	CONC	ZERO	SEM	CONC	ZERO	SEM	Group	Time	Group \times time
Cortisol baseline concentration (ng/ml)	4.3 \pm 0.6	4.4 \pm 0.8		2.7 \pm 0.6	2.4 \pm 0.4		3.0 \pm 0.4	2.5 \pm 0.3		$p = .54$	$p < .01$	$p = .86$
AUC _t ^a (ng/ml \times 180 min)	201.1 \pm 8.0	196.8 \pm 15.8		174.2 \pm 10.1	166.0 \pm 6.5		210.1 \pm 10.2	182.9 \pm 6.8		$p = .12$	$p < .05$	$p = .48$
AUC _b ^b (ng/ml \times 180 min)	144.9 \pm 11.0	140.2 \pm 13.5		139.0 \pm 11.1	134.9 \pm 8.0		170.5 \pm 11.1	150.5 \pm 6.0		$p = .29$	$p = .09$	$p = .71$
Peak cortisol concentration (ng/ml)	25.7 \pm 1.0	25.8 \pm 2.0		23.3 \pm 1.2	21.8 \pm 0.8		27.1 \pm 1.2	24.5 \pm 1.0		$p = .21$	$p < .05$	$p = .59$
Time to peak (min)	49.6 \pm 6.0	66.0 \pm 6.0		53.1 \pm 3.2	55.5 \pm 5.0		58.8 \pm 4.0	58.5 \pm 5.7		$p = .15$	$p = .66$	$p = .22$
Difference peak-basal cortisol concentration (ng/ml)	21.4 \pm 1.4	21.4 \pm 1.7		20.5 \pm 1.1	19.4 \pm 0.8		24.0 \pm 1.2	22.0 \pm 1.0		$p = .30$	$p < .05$	$p = .72$
Decline of cortisol concentration within 60 min after peak (%)	36 \pm 6	51 \pm 6		45 \pm 4	44 \pm 4		45 \pm 5	48 \pm 7		$p = .21$	$p = .85$	$p = .29$
Cortisol concentration at 60 min after peak (ng/ml)	16.5 \pm 1.7	12.1 \pm 1.1		12.8 \pm 1.1	12.3 \pm 1.0		14.9 \pm 1.3	12.5 \pm 1.7		$p < .05$	$p = .45$	$p = .40$

^aTotal cortisol release from 0 to 180 min after ACTH injection calculated as area under the curve (AUC_t).

^bAUC above the cortisol baseline (AUC_b).

NEFA, BHB and with higher contents of fat and free fatty acids in milk ($p < .05$, Table 2). The cortisol peak height after ACTH administration was concomitantly higher in cows with a more positive energy balance, higher DMI and lower plasma concentrations of NEFA and BHB ($p < .05$, Table 2). Time when cortisol concentration reached its peak after ACTH application was only related to energy balance and plasma concentrations of NEFA (Table 2).

4 | DISCUSSION

4.1 | Application of ACTH challenge tests in dairy cows

In early lactating dairy cows, Beerda, Kornalijnslijper, van der Werf, Noordhuizen-Stassen, and Hopster (2004) and Trevisi et al. (2013) found that the extent of the postpartal negative energy balance is linked to the adrenocortical reactivity. In a previous study (Gross, Wellnitz, & Bruckmaier, 2015), cortisol release during an intramammary lipopolysaccharide challenge was associated with the concomitant metabolic state, whereas short-term provoked metabolic changes (hypoglycemia, hyperketonemia, hyperinsulinemic-euglycemia) alone did not affect the pattern of cortisol release. In contrast to earlier studies that performed ACTH challenge tests only once (e.g., Van Reenen et al., 2005; Weiss et al., 2004), we further investigated changes in ACTH challenge test characteristics (i.e., total capacity of cortisol release, peak height and time of peak, etc.) in terms of repeatability in dairy cows changing their endocrine and metabolic status including observations on the changes in the ranking of cortisol response between animals. In order to clarify the question whether HPA-axis response to exogenous ACTH could be indicative for characterization of the metabolic adaptation to a negative energy balance, we studied the impact of performance level and enhanced metabolic load in early lactation on the adrenocortical responsiveness.

4.2 | ACTH challenge test characteristics at different physiological stages and levels of performance

The activity in the HPA axis in lactating animals was shown to be different from non-lactating growing animals (Beerda et al., 2004; Munksgaard et al., 2006). Lay et al. (1996) repeatedly tested ACTH injections in pregnant Brahman heifers at 20-day intervals and found no alterations in adrenal response due to serial ACTH challenges. It can be assumed that the observations of Lay et al. (1996) apply to animals not changing their physiological status in the short term. Similarly Scheidegger et al. (2016) attested the ACTH challenge test a high repeatability in horses not changing their physiological status in the short and long term. However, in the present study, adrenal responsiveness was not repeatable and the ranking of individual animals' cortisol response characteristics (AUC_t, AUC_b, peak height, time of peak, decline pattern, etc.) was not consistent between the successive ACTH tests. This finding might be attributed to the fact that dairy cows in the present study were exposed to the burden imposed by the tremendous metabolic and endocrine changes during

TABLE 2 Relationships between performance [milk yield, milk composition, somatic cell count (SCC) in milk, DMI, energy balance, BW loss since parturition], metabolic [plasma concentrations of glucose, NEFA, BHB, serum amyloid A (SAA), haptoglobin], endocrine (baseline cortisol concentration, IGF-1) and ACTH-induced cortisol response characteristics [height and time to cortisol peak, area under the curve (AUC) above the baseline cortisol concentration (AUC_b) and total AUC (AUC_t)] in early lactation dairy cows. Data represent Pearson's correlation coefficients of data obtained at the ACTH challenge tests in week 3 and 8 p.p. Significant correlation coefficients ($p < .05$) are highlighted in bold type

Parameter	Baseline cortisol	AUC_t	AUC_b	Peak height	Time to peak
Baseline cortisol	–	0.32 ($p < .01$)	0.37 ($p < .01$)	0.34 ($p < .01$)	0.15 ($p = .19$)
Peak height	0.34 ($p < .01$)	0.90 ($p < .0001$)	0.64 ($p < .0001$)	–	0.13 ($p = .29$)
Previous lactation milk yield	0.42 ($p < .01$)	0.04 ($p = .78$)	–0.20 ($p = .18$)	0.07 ($p = .63$)	0.03 ($p = .86$)
Current milk yield	0.29 ($p < .05$)	0.19 ($p = .21$)	0.02 ($p = .89$)	0.27 ($p = .07$)	–0.11 ($p = .45$)
Milk fat	0.05 ($p = .76$)	–0.41 ($p < .01$)	–0.43 ($p < .01$)	–0.44 ($p < .01$)	–0.05 ($p = .74$)
Milk protein	0.04 ($p = .80$)	–0.15 ($p = .33$)	–0.17 ($p = .28$)	–0.23 ($p = .14$)	0.16 ($p = .32$)
Milk lactose	–0.18 ($p = .26$)	0.15 ($p = .33$)	0.25 ($p = .11$)	0.13 ($p = .40$)	–0.09 ($p = .56$)
Milk FFA	0.01 ($p = .94$)	–0.44 ($p < .01$)	–0.44 ($p < .01$)	–0.45 ($p < .01$)	–0.13 ($p = .42$)
Milk SCC	0.39 ($p < .05$)	0.02 ($p = .89$)	–0.20 ($p = .21$)	0.05 ($p = .76$)	0.02 ($p = .90$)
Glucose	–0.08 ($p = .60$)	0.22 ($p = .13$)	0.26 ($p = .08$)	0.28 ($p = .06$)	–0.15 ($p = .34$)
NEFA	0.05 ($p = .75$)	–0.47 ($p < .01$)	–0.48 ($p < .001$)	–0.41 ($p < .01$)	–0.29 ($p < .05$)
BHB	0.03 ($p = .82$)	–0.44 ($p < .01$)	–0.44 ($p < .01$)	–0.43 ($p < .01$)	0.05 ($p = .76$)
IGF-1	–0.15 ($p = .33$)	0.23 ($p = .12$)	0.31 ($p < .05$)	0.21 ($p = .16$)	0.22 ($p = .13$)
Haptoglobin	0.25 ($p = .12$)	–0.09 ($p = .57$)	–0.22 ($p = .17$)	–0.13 ($p = .43$)	–0.16 ($p = .34$)
SAA	–0.10 ($p = .49$)	–0.19 ($p = .21$)	–0.13 ($p = .41$)	–0.23 ($p = .14$)	0.01 ($p = .95$)
Energy balance	0.07 ($p = .65$)	0.48 ($p < .001$)	0.43 ($p < .01$)	0.44 ($p < .01$)	0.31 ($p < .05$)
DMI	0.16 ($p = .30$)	0.37 ($p < .05$)	0.27 ($p = .07$)	0.41 ($p < .01$)	–0.01 ($p = .95$)
Body weight	0.32 ($p < .05$)	0.22 ($p = .15$)	0.03 ($p = .87$)	0.24 ($p = .12$)	–0.07 ($p = .64$)
BW loss since parturition	0.04 ($p = .77$)	–0.10 ($p = .53$)	–0.12 ($p = .44$)	–0.14 ($p = .36$)	0.12 ($p = .43$)

the transition from late lactation and subsequent dry period with a positive energy balance to a marked energy deficiency at the onset of lactation. Although metabolic load was enhanced by omitting concentrate supplementation in one group of the present study, changes in adrenal cortex reactivity were not significantly affected by concentrate supplementation. In contrast, Trevisi et al. (2013) could prove that cows with a more severe inflammatory status and higher metabolic load showed a lower response to an ACTH challenge performed at day 35 of lactation.

Results of the present study showed that cows with a higher performance after parturition, that is current milk production at the respective time of the ACTH challenge test and previous lactation yield, had higher cortisol baseline concentrations and higher cortisol peak values. In agreement with our findings, high milk yield and the concomitant large energy deficit were associated with decreased adrenocortical reactivity, that is lower cortisol responses after ACTH administration as described earlier (Beerda et al., 2004; Shayanfar, Head, Wilcox, & Thatcher, 1975; Van der Kolk, Breukink, Wensing, & Mol, 1991; Van der Kolk, Fouché, Gross, Gerber, & Bruckmaier, 2016). Although animals of the present study were ACTH challenged at different lactational stages, the decline pattern (rate of decline and recovery of baseline cortisol concentration after peak) was not affected by neither lactational nor metabolic state. Furthermore, variation in recovery between individuals likely contributed to the low repeatability (as indicated by low ICC) while differences in clearance of cortisol

due to lactational stage had been shown to be of minor importance (Dunlap et al., 1981; Mormède et al., 2007).

4.3 | Relationships between ACTH challenge test characteristics, feeding level and metabolic status

In growing beef cattle, Ward, Henricks, Jenkins, and Bridges (1992) observed that feeding and fasting induced changes in the activity of the HPA axis. However, in lactating cows, total energy intake obviously did not affect plasma concentrations of cortisol, ACTH or the reactivity at the adrenal and pituitary level (Fisher et al., 2002; Sejrsen, Huber, & Tucker, 1983). Simonetta, Walker, and McMillen (1991) observed a diurnal variation in plasma cortisol concentration in ewes fed only once daily, but not in ewes fed several times per day. While we did not observe changes of cortisol baseline concentrations in cows without supplementary concentrate compared with supplemented animals, Ward et al. (1992) and Samuelsson, Uvnäs-Moberg, Gorewit, and Svennersten-Sjaunja (1996) found increased cortisol concentrations after fasting beef cattle and dairy cows respectively. Fasting, however, can be considered as an acute form of feed deprivation, while cows of the present study could freely access roughage and omission of concentrate therefore resulted in a reduced dietary energy density.

Gwazdauskas, Keys, and McGilliard (1986) observed lower responses to ACTH challenge in dairy cows of similar physiological stage

when grass-legume silage was fed along with corn silage than solely corn silage feeding. Smith, Hansel, and Coppock (1975) reported that cows fed corn silage showed reduced adrenal capability to respond to exogenous ACTH compared to grass silage fed cows. In the present study, the cows that were not fed concentrate tended to have a lower adrenal responsiveness. However, it is not clear to which extent the dietary composition itself (herbage ad libitum with/without supplementary concentrate) of the present study contributed to the adrenal cortex reactivity or if effects are more due to the changed energy supply affecting the metabolic status of the animal. Dynamics in body weight loss since parturition were not affecting the present results, while cows with elevated concentrations of NEFA and BHB released less cortisol after ACTH injection confirming the results of Trevisi et al. (2013). Similarly to our findings, Beerda et al. (2004) suggested that overfeeding during the dry period as risk factor contributing to the severity of the NEB had relatively small effects on the increases in pituitary (re)activity and decreases in adrenocortical reactivity in comparison with changes related to the stage of lactation. Cows in the present study with a higher feed and thus energy intake showed a higher release of cortisol following ACTH injection, but no differences in baseline cortisol concentrations. In the study of Beerda et al. (2004), effects of milk production level on HPA function were relatively small, and baseline cortisol levels were unaffected. But as individual animals' ranking of the cortisol response was not consistent between time points of the ACTH tests and feeding levels, an a priori characterization of the HPA axis' response does not allow to predicting metabolic adaptation to metabolic stress in early lactation.

5 | CONCLUSIONS

The adrenal cortex reactivity was shown to be lower in dairy cows experiencing a higher metabolic load during the negative energy balance in early lactation but scarcely affected during aggravated imbalances in energy supply by omission of concentrate. Responses of the HPA axis to ACTH challenge tests at different lactational stages were not repeatable in our study, that is, the differences of the endocrine and metabolic adaptation had a greater influence on the adrenal cortex responsiveness than the inherent individual genetic disposition. Hence, an a priori characterization of the adrenal cortex reactivity might not allow to predicting metabolic adaptation to metabolic stress in early lactation. In dairy cows with rapidly changing physiological status as present during early lactation, we could not perform a pro- or retrospective extrapolation of only once-obtained ACTH test responses onto other lactational stages. Referring to the present results, conclusions from ACTH challenges on the physiological status apply only to the very narrow timeframe of the respective test.

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