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Françoise Pol, Valérie Courboulay, Jean Pierre Cotte, Arnaud Martrenchar, Magali Hay, Pierre Mormède

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Original article

Urinary cortisol as an additional tool to assess the welfare of pregnant sows kept in two types of housing

Françoise Pol[a], Valérie Courboulay[b], Jean-Pierre Cotte[a]*, Arnaud Martrenchar[a], Magali Hay[c], Pierre Mormède[c]

[a] Agence Française de Sécurité Sanitaire des Aliments, BP 53, 22440 Ploufragan, France
[b] Institut Technique du Porc, La Motte au Vicomte, 35650 Le Rheu, France
[c] INSERM, rue Camille Saint Saëns, 33000 Bordeaux, France

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Abstract – The use of urinary cortisol (UC) as an additional tool to evaluate sows welfare was assessed in two experiments. In a preliminary methodological experiment, the kinetics of cortisol excretion in urine was studied during an Adreno Cortico Trophic Hormone (ACTH) challenge test in 10 pregnant sows. In a second experiment, 96 primiparous sows of an experimental unit were assigned to two different housing systems: 48 animals were housed in individual pens (IP) and 48 animals in collective pens (CP) with 6 animals per pen. UC was measured at the beginning and at the end of pregnancy and compared with other welfare indicators such as behaviour or skin damage. In both experiments, UC was measured using a high pressure liquid chromatography assay. In experiment 1, UC was constant on the day before injection of ACTH, with no variations related to circadian rhythm. It began to rise 2 h after the injection, peaked between 2 to 5 h after then returned to the basal concentration on the day after the injection. In experiment 2, UC concentrations were not different between CP- and IP-housed sows but they were higher in sows exhibiting the less stereotypies in comparison with sows exhibiting the most stereotypies. The results of this study suggest that UC is a good indicator of acute stress, more convenient than plasma cortisol measurement since it is a non-invasive method avoiding restraint or catheterisation of sows. They also suggest that UC could also give additional information on the assessment of chronic stress and improve the evaluation of animal welfare if used in conjunction with other welfare indicators.

pig / urinary cortisol / group housing / stress / welfare

Résumé – **Le cortisol urinaire comme critère d'évaluation supplémentaire du bien-être de la truie gravide dans deux systèmes de logement.** L'intérêt du cortisol urinaire (CU) comme critère d'évaluation du bien-être des truies a été testé dans deux expériences. Dans une première expérience méthodologique, la cinétique d'excrétion du CU dans les urines de dix truies gravides a été mesurée suite à un test à l'hormone adrénocorticotrope (ACTH). Dans une deuxième expérience, 96 truies primipares d'une

*Correspondence and reprints
Tel.: (33) 2 96 01 62 22; fax: (33) 2 96 01 62 23; e-mail: jp.cotte@ploufragan.afssa.fr

station expérimentale ont été réparties, pendant la gestation, dans deux systèmes de logement : 48 truies en stalles individuelles (SI) et 48 autres en cases collectives (CC) de 6 animaux. Leur CU a été mesuré en début et fin de gestation puis comparé aux autres critères d'évaluation du bien-être comme le comportement ou les blessures cutanées. Dans chacune des expériences, le CU a été dosé par une méthode de chromatographie liquide haute pression (HPLC). Dans la première expérience, aucun rythme circadien du CU n'a été observé le jour précédant le test à l'ACTH. Le CU a commencé à augmenter deux heures après l'injection d'ACTH, atteint un pic entre 2 et 5 h après puis est revenu à un taux de base le lendemain. Dans la deuxième expérience, le CU des truies logées en SI n'était pas différent de celui des truies logées en CC, mais le CU des truies exprimant le moins de stéréotypies était plus élevé que celui des truies en exprimant le plus. Le résultat de cette étude suggère que le dosage du CU est un bon indicateur de stress aigu, plus facile à mettre en pratique car il n'impose ni prise de sang ni cathétérisation des animaux. D'autre part, le CU est un critère supplémentaire dans l'évaluation du stress chronique et permet d'améliorer l'appréciation du bien-être animal lorsqu'il est associé à d'autres indicateurs.

porc / cortisol urinaire / logement en groupe / stress / bien-être

1. INTRODUCTION

Farm animal welfare is usually measured using different indicators. These are mainly body damage, disease, growth, reproductive performance, stress and behavioural abnormalities. Animal observation can provide information on body condition, performance and behaviour. However, stress assessment requires specific evaluation methods. In pigs, stress is usually assessed by the level of plasma cortisol, which reflects the activity of the hypothalamic-pituitary-adrenal (HPA) axis [5, 7, 20]. This measurement can also be done after HPA axis stimulation with exogenous Adreno Cortico Tropic Hormone (ACTH) [18, 26]. However, plasma levels of cortisol vary with circadian rhythm, season and photoperiod or food intake rhythm [4, 24]. Moreover, blood sampling itself causes a rise in cortisol level [21]. Pigs can be fitted with a permanent catheter under general anaesthesia, but this is not possible in normal farming conditions. In other studies in sows [8, 9, 26], salivary cortisol was measured, after the sows were given cotton buds to chew.

Cortisol is mainly excreted in urine and urinary cortisol (UC) has already been used to test the HPA axis in humans [24], bighorn sheep [23], felids [10] and mice [19]. In pigs, UC measured on spontaneously voided urine has been shown to be a good indicator of HPA axis activity [15–17]. Urine collection can be made by a non-invasive method and so may be stress-free for the animal. The aim of our study was therefore to assess the use of UC as an additional indicator of pig welfare, as related to housing conditions.

In a preliminary experiment, the kinetics of UC excretion was measured in pregnant sows under routine husbandry conditions and after an ACTH challenge test. The aim of the experiment was to look for any variation in UC secretion correlated with circadian rhythm and to estimate the lag time between activation of the adrenal cortex by an ACTH challenge and UC secretion.

In a second experiment the validity of UC level as an indicator of sow welfare in two types of housing (individual or collective pens) was assessed in comparison with other usual welfare indicators such as behaviour and skin damage. Since it is currently debated in European regulation projects whether the housing of pregnant sows in individual stalls should be phased out, it could be useful to have another reliable welfare indicator.

2. MATERIALS AND METHODS

2.1. Animals and housing

For the kinetics of UC after an ACTH challenge test, 20 pregnant multiparous sows (Large White × Landrace; weights range: 250–270 kg) from an experimental pig farm were used in a three-day study (day–1, day 0 and day+1). The sows were at weeks 6 ($n = 8$), 9 ($n = 4$) or 12 ($n = 8$) of pregnancy and were kept in girth tethers. They were fed twice a day at 7:30 and 17:00 with a standard mixed diet (2.8 kg/day). Lights were on between 6:30 and 18:00.

For the assessment of UC as a welfare indicator in two different housing conditions, 96 nulliparous gilts of four successive batches of 24 animals from the experimental pig farm of Romillé (France) were studied during pregnancy and the farrowing period. At the beginning of the experiment, all the animals were housed in collective pens (CP) measuring 4.3 m × 3 m, with 6 animals per pen which were surrounded by two concrete walls plus two partitions made of metal bars, spaced 20 cm apart, running vertically. Six metal troughs were located at the bottom of a wall and separated by partial stalls made of metal bars running horizontally, which allowed the sows to be separated from each other during feeding. The temperature inside the barns ranged from 18 to 24 °C. The floor was made of solid concrete and was totally slatted.

Six days before artificial insemination (day–6), one half of the sows (12 animals of each batch) remained in CP as described above and the other half was moved to individual pens (IP). IP measured 2.25 m × 0.65 m (1.46 m^2/sow). In front of each pen there was a metal trough on the floor measuring 0.3 m × 0.65 m. Each side was made of five metal bars spaced 20 cm apart, running horizontally. In IP, sows could have contact with their neighbours through the bars.

The two types of housing systems were located in the same building where temperature, lights, food, floor and husbandry were similar. Ambient temperature remained within 18 to 24 °C. Lights were on between 7:00 and 17:30. Food was delivered twice a day at 7:00 and 13:00, giving a total of 2.8 kg of standard soup per sow. The total amount of daily-distributed water was 18 L per animal. The floor was made of solid concrete and was totally slatted.

According to routine production conditions, animals were introduced into conventional individual farrowing crates, located in the same building as the pregnancy room, one week before the expected date of parturition (day+108 of gestation).

2.2. Procedure

In the ACTH challenge test, all spontaneously voided urine was collected on day–1 from 6:30 to 18:00 in order to detect any circadian rhythm and on day 0 from 6:30 to 18:00 and on day+1 from 6:30 to 12:00 to estimate the lag time between ACTH injection and UC secretion. On day 0, at 9:00, 10 animals at week 6, 9 or 12 of pregnancy were injected with 1 mg of synthetic ACTH (100 IU of Synacthene® Retard, Ciba-Geigy Laboratories, Rueil-Malmaison, France) into the neck muscles. As previously shown by Hay et al. [17], UC excretion is not influenced by gestation stages. So in the following results, data were pooled between animals. The slow release formulation, which induces a higher cortisol secretion, was used in order to get a more pronounced stimulation. Ten other animals used as negative controls received 1 mL of saline.

In the experiment comparing two different housing conditions, UC was measured in spontaneously voided urine, at day–6 and day+108 of gestation. At the same dates, for another experiment not shown here, blood samples were collected from the jugular vein of the animals, snared by the snout, just before their transfer into gestation and farrowing rooms respectively. The animals were also weighed and their backfat thickness was measured. At both times, urine was collected just before the transfer and/or manipulations of animals (giving basal concentrations of UC) and four hours later, giving the concentrations of UC after the stress of transfer and/or manipulations. At day–6 and day+108, all body lesions such as scratches and bursitis of the hock were counted and scored.

Behaviour was studied by the scan sampling method [22] on five occasions: at day–12, day+7, day+43, day+98 and at day+109 of pregnancy.

2.3. Analyses

2.3.1. Urinary analysis

To collect urine samples, operators were observing the sows. As soon as one of the animals spontaneously urinated, an operator collected the urine in a flask. The first spurt of urine was discarded to avoid any bacterial contamination of the sample. Aliquots were acidified with 6 M HCl (1% of urine volume) and frozen.

UC was assayed using a solid phase extraction procedure followed by high pressure liquid chromatography (HPLC) with UV absorbance detection (254 nm), as previously described [15], for all samples in ACTH challenge and for samples of batches 2 and 3 in housing comparison.

A difference in urine concentration between samples can strongly influence the level of urinary cortisol. To account for this bias, cortisol concentrations were divided by urinary creatinine concentrations. Creatinine levels were determined by spectrophotometry (Ektachem Kodack DT II, Johnson & Johnson Clinical Diagnostics, Les Ulis, France). The results of cortisol levels were expressed as the UC/creatinine ratio in μg/g.

2.3.2. Behavioural observations

For comparison of two housing conditions, an observer, slowly walking along the alley behind the sows, recorded the postures and activities of each gilt every 3 min during 90 min starting after the morning food distribution. Each posture and activity was exclusive and recorded as present or absent. The postures recorded were: standing, sitting, lying (lateral lying, i.e. lying on their flank with all four legs stretched out, was also recorded). The number of posture changes were counted. The activities recorded were: feeding (eating and drinking), moving (walking or running, possible only in CP), exploring (sniffing part of the pen or crate, rooting, sniffing or licking the floor), exhibiting stereotypies (chewing with an empty mouth, licking the empty trough, barbiting, teeth grinding, suction), performing social interactions (agonistic interaction with contact, non-agonistic interaction with contact) and remaining inactive (not performing any other categorised activity).

2.3.3. Clinical examination

These observations were conducted in the experiment of comparison of housing conditions only. The body was divided into five regions: head, body, legs, udder and vulva. In each region, countable scratches were scored from 1 (non-extensive lesion) to 10 (serious lesion) and non-countable scratches were scored from 5 (non-extensive lesions) to 15 (serious lesions). All scores were summarised per animal. Bursitis of the hock was scored from 1 (weak bursitis) to 10 (deep bursitis). Scores at day–6 and day+108 were compared and sows were classified into three categories: reduced, stable and increased lesion count.

2.3.4. Relation between UC and other measured variables

In order to determine if there is a correlation between the UC level and the time the sows spent exhibiting stereotypies, sows were classified into two categories: sows exhibiting the most stereotypies (upper quartile at day+98) and sows exhibiting the least stereotypies (lower quartile at day+98) and their UC concentrations were compared. Additionally, correlation between the time spent exhibiting stereotyped behaviour and UC was determined for the whole sample.

In the same way, CP-housed sows were divided into two classes on the basis of the lesion scores at day+108: high score (upper quartile) and low score of lesions (lower quartile). The UC concentrations of each class were compared.

2.4. Statistical analyses

In kinetics of UC after an ACTH challenge test, the means of UC were calculated per sow for each day (and per half day for day–1 and day+1) and for each 3 hours period of the days. The means fit Gaussian distributions. Means of UC among sows from different days were compared with a paired t-test and means of UC between treatments were compared with a two-group t-test at day–1 and day 0.

At day–1, UC values were arbitrarily pooled per sow from 6:30 to 8:30 (around first meal), from 11:00 to 13:00 (between meals) and from 16:00 to 18:00 (around second meal) in an attempt to detect a circadian rhythm of cortisol excretion (NB: in Fig. 1, UC are pooled on 3 hours periods for 10 sows).

In the experiment of housing conditions, for the comparison of data between treatments, the experimental unit was chosen to be the sow for IP-housed sows and the pen for CP-housed sows. Data or log-transformed data which fit a Gaussian distribution were determined by analysis of variance using an analysis of repeated measures where appropriate. The batch effect was included in the model as an independent variable.

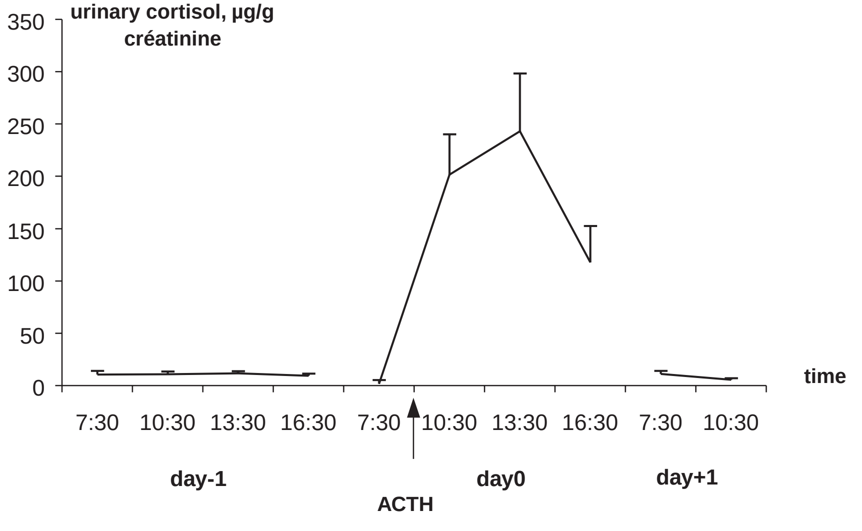

Figure. 1. Mean concentration of urinary cortisol during 3 hour-periods, expressed as a function of creatinine concentration in 10 sows submitted to an ACTH challenge test.

For behavioural observations, each activity and posture was expressed as the percentage of observations per pig or per pen during 90 min. Each 90 min of observation was considered to be one statistical unit. Comparison between treatments was made as described above. Some behaviours were exhibited by few animals, so the corresponding data did not fit a Gaussian distribution. These data were converted into dichotomous variables (0 for "always no" and 1 in the other cases). Lesion score data were divided into three classes as indicated above. They were converted into dichotomous variables (0 for "absence within the class" and 1 in other cases). Binary variables were included in a logistic regression model where the batch effect was treated as a fixed effect.

For investigation of possible correlation between UC and stereotypies and lesions, log-transformed data were determined by analysis of variance. Housing and batch effect were included in the model as independent variables. Correlations were determined on log-transformed data using the Systat procedure with Pearson coefficient.

All data were expressed as means by treatment and standard error of the mean (SEM). All statistical tests were carried out using the SYSTAT package [28]. Significance was $P < 0.05$.

3. RESULTS

3.1. Kinetics of UC after an ACTH challenge test

At day–1, no circadian rhythm could be detected in UC (10.7 ± 3.9, 13.1 ± 2.8 and 10.5 ± 2.5 µg/g, around the first meal, between meals and around the second meal, respectively).

After the ACTH challenge test at day 0, UC increased significantly (Tab. I and Fig. 1). It peaked on average 3 h 40 min after the injection of ACTH (from 2 h to 5 h) and returned to the basal concentration between 9 and 24 h after injection (Fig. 1). Indeed at day+1, am UC (from 6:30 to 12:00), was the same as am UC at day–1. In control sows, UC did not differ between day–1 and day 0 (Tab. I).

3.2. Assessment of UC as a welfare indicator in two different housing conditions

3.2.1. UC concentrations

UC basal concentrations at day–6 and day+108 were not significantly different among IP-housed sows and CP-housed sows (Tab. II). Moreover UC did not increase significantly after manipulations (weighing and blood sampling) at day–6.

Table I. Mean (± SEM, $n = 10$ by treatment) concentrations of urinary cortisol expressed as a function of creatinine concentration (UC in µg of cortisol / g creatinine) in sows subjected to an ACTH challenge test (at day 0) and in control sows.

Day of injection	ACTH	Control
Day–1	10.3 ± 1.8	9.3 ± 0.8
Day–1 morning	10.7 ± 2.2	
Day 0	141.1 ± 38.8[a]	9.0 ± 1.7
Day+1 morning	8.0 ± 1.9	

[a] Significantly different from control ($P < 0.001$).

Table II. Mean concentrations of urinary cortisol expressed as a function of creatinine concentration (UC in µg of cortisol / g creatinine) in sows kept in individual pens (IP) or in collective pens (CP) at day–6 and day+108 of gestation.

	Day-6		Day+108	
	IP	CP	IP	CP
n	23	4	21	4
Before transfer[a]	20.6 ± 2.9	14.0 ± 0.7	16.3 ± 2.1	19.7 ± 4.0
After transfer	19.7 ± 3.9	14.1 ± 2.0	23.1 ± 6.0	15.2 ± 1.3

[a] Transfer in individual pen at day–6 and in farrowing crates at day+108.

3.2.2. Behavioural observations

At day–12, no significant difference in the different postures and activities and number of posture changing was found between the two types of housing except for the standing posture, which was more prevalent in IP-housed sows compared to CP-housed sows (52.9 vs. 49.9%, $P = 0.036$). During pregnancy, CP-housed sows spent less time sitting than IP-housed sows (7.3 vs. 7.7%, $P = 0.016$ at day+43) and more time laterally lying (10.5 vs. 3.7%, $P < 0.001$ at day+43 and 16.5 vs. 9.9%, $P = 0.019$ at day+98). They also spent more time performing social interactions (5.2 vs. 1.8%, $P = 0.002$ at day+7, 7.0 vs. 2.5%, $P = 0.001$ at day+43 and 4.3 vs. 1.5%, $P = 0.001$ at day+98 for CP- and IP-housed sows, respectively). Among these behaviours, non-aggressive behaviours were the most prevalent. IP-housed sows spent more time performing stereotypies than CP-housed sows (30.7 vs. 18.3%, $P = 0.009$ at day+7 and 31.7 vs. 21.4%, $P = 0.081$ at day+43). Vacuum chewing was the most prevalent stereotypy.

At day+109, after the move into farrowing crates, there was no difference among sows housed in both types of pens.

3.2.3. Clinical examinations

There was a higher degradation of the state of the skin in CP-housed sows when compared to IP-housed sows, especially on the body region (68.8% of IP– vs. 42.1% of CP-, $P = 0.002$, had a improved state and 18.8% of IP– vs. 36.8% of CP, $P = 0.028$, had a damaged state between day–6 and day+108). No significant difference was found as regards bursitis of the hock and the other regions of the body.

3.2.4. Relation between UC and other measured variables

Basal UC at day+98 tended to be higher in sows with a low level of stereotypies (9% or less observations, $n = 14$) when compared to sows with a high level of stereotypies (35% or more, $n = 12$) (17.9 ± 3.7 vs. 12.1 ± 3.1 µg/g, respectively, $P = 0.051$). However, no correlation was found between basal UC and the frequency of stereotypies.

At day+108, CP-housed sows with high injury scores ($n = 8$) had the same basal UC as CP-housed sows with low injury scores ($n = 8$) (15.3 ± 13.7 vs. 20.88 ± 8.84 µg/g, respectively).

4. DISCUSSION

The experiment of ACTH challenge test showed that UC is responsive to exogenous ACTH. Two to 5 h after an ACTH injection, UC rise could be measured in urine and the basal concentration increased by at least a factor 10. It has already been shown that, 30 min after an intravenous ACTH injection, basal plasma cortisol level is multiplied by 2.3 [25], 3 [14] or 5 [30] and that 45 min after an ACTH injection [25], salivary cortisol rise by 130%. In urine, the rise may be greater because UC has accumulated before urination. The rise may also last longer because ACTH was injected into the muscle as a long-lasting formulation. Twenty-four hours after ACTH injection, UC returned to the basal concentration, suggesting that the stimulation was over. It should be noted that in agreement with the results of Parrott et al. [25], who demonstrated that the cortisol response was independent of animal weight, the dose of ACTH was not adjusted to the body weight of the sow.

No variation was detected in UC secretion between the three-day periods around and between meals. Hay et al. [17] showed diurnal changes in UC excretion in sows fed once a day; in their study, UC peaked in the early morning (around feeding) and declined afterwards; the sows had lights all day long. The sows in our experiment had lights all day long too but they were fed twice a day, in the morning and in the evening. The difference of daily feeding regimens or the lack of nocturnal samples may be the reasons why we did not observe such a fluctuation. We cannot conclude on the influence of photoperiod, showed by Barnett et al. [4] in plasma corticosteroid concentrations maybe in relation with the season of the experiments.

This experiment suggests that in practice, sampling urine around the morning feeding time should be used to detect individual differences, and a rise of urinary cortisol concentration could be detected in a single urine sample collected 4 hours after a stress.

In the second experiment, IP-housed sows more frequently exhibited stereotypies than CP-housed sows at day+7 and day+43. Previous works [1, 9, 31] have already provided evidence that IP-housed sows performed more stereotypies than CP-housed sows and that vacuum chewing was the most prevalent stereotypy observed in IP-housed sows. Stereotypies are generally considered to be related to a welfare deficit [12, 27]. CP-housed sows showed more lateral lying than IP-housed sows in the second half of pregnancy. This may suggest that this posture was no longer possible for pregnant sows in 65 cm-wide pens, and this impossibility may induce chronic stress in IP-housed sows.

UC concentrations (standardised by urinary creatinine) were not different between CP– and IP-housed sows.

UC tended to be higher in sows exhibiting a low level of stereotypy. Other studies [13, 29] showed that animals which expressed stereotypies had the same or a reduced plasma cortisol level as compared to others kept in the same environment, and that stereotypies could be a means of coping with the environment. Our study agrees with these results.

At the end of pregnancy, body lesions observed in CP-housed sows were probably the result of aggressive behaviours among sows. Aggressive behaviours are more acute when pigs have just been grouped. This has been related to the establishment of hierarchy [3]. Nevertheless, some studies have reported that CP-housed sows were still fighting for food or resting place [2, 11] after the establishment of hierarchy (after 10 days). Our study tends to confirm these reports and we can put forward the hypothesis that fights, which occurred throughout gestation, result in some form of acute stress. However, basal UC concentration does not confirm this hypothesis.

This second experiment has highlighted some doubt concerning the influence of previous experience on sow behaviours. At day+109, when sows were in farrowing accommodation, no differences were observed in their behaviours, suggesting that previous housing had no influence on their behaviour. Beattie et al. [6] already compared the behaviour and reproduction performance of sows reared in enriched gestating pens or barren gestating stalls which were moved into the same conventional farrowing crates a few days before the expected date of parturition. They found that sows exhibited different behaviours (such as exploring only) during the first hour following movement. These results and ours suggest that the influence of previous housing accommodation has only a short term influence.

This study confirms that hypothalamic-pituitary-adrenal axis function can be monitored by urinary cortisol (UC) analyses in pigs in routine conditions or after functional tests (ACTH challenge). UC concentrations are not influenced by rapid changes in hormone secretion because urine accumulates over several hours. Furthermore, urine collection, which causes minimal trouble to the animals, is a non-invasive method which is easy to practice in farms and avoids catheterisation.

UC seems to be a reliable indicator of chronic stress, because it is related to the expression of stereotypies. UC can also be a reliable indicator of acute stress, raising significantly after an ACTH challenge. If used in conjunction with other welfare indicators, this measure may improve our global assessment of animal welfare.

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