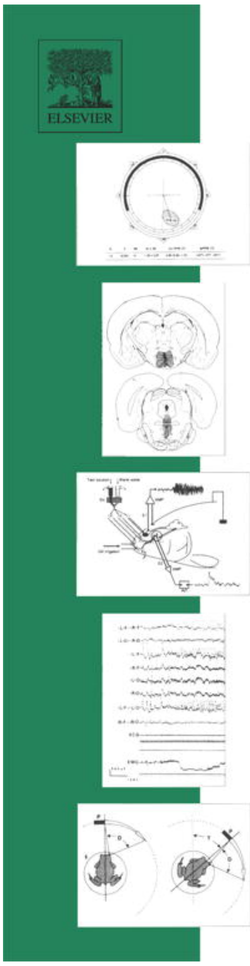

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# Validation of a behavioral observation tool to assess pig welfare

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**Abstract**

Accurately measuring and monitoring of animal behavior is an important factor when assessing on-farm animal welfare. First we developed a feasible and simple method aiming at consistently on-farm measuring of pig's behavior. This test should cover a broad range of welfare-related pig behavior. The reaction towards a novel object, startling, tail and ear biting, play and aggressive behavior, stereotypies, coughing, sneezing, skin lesions, defecation, urination and cleanliness of body and pen are included. The development of accurate measures of on-farm behavior first requires the reliability assessment of the procedure. Therefore, the methodology was tested in a first part by three observers scoring simultaneously and independently pre-defined behavioral characteristics of 108 group-housed fattening pigs. The inter-observer repeatability of the measures was calculated using intraclass correlation coefficients, which ranged from 0.7 to 1.

In a second part, the objective was to validate the behavioral characteristics against salivary cortisol, urinary epinephrine and norepinephrine and production traits. Salivary cortisol concentrations significantly increased in ear-bitten pigs and in pigs with tail lesions. Growth rate significantly dropped when cortisol levels rose. An age effect was also found. The percentage of animals approaching the novel object is positively correlated with the urinary epinephrine concentration. Pigs defecating during the test showed significantly higher epinephrine levels. Urinary norepinephrine concentration decreased significantly with age. Faster growing animals and animals with tail lesions showed significantly higher levels of norepinephrine. Pen dirtiness and number of animals per pen were associated with higher norepinephrine concentrations. Finally, barrows had higher norepinephrine concentrations than sows.


*Keywords:* Pig; On-farm behavior; Stress; Physiology; Welfare assessment; Welfare; Behavior; Cortisol; Catecholamine; Epinephrine; Norepinephrine



## 1. Introduction

Animal welfare is of growing societal and scientific importance. Not only the impact on the animals' wellbeing is important but also animal welfare is inexorably linked with farmers' and suppliers' labor income. Furthermore, implications on food production and product quality must also be considered.

Welfare is a complex construct, combining both subjective and objective aspects of the animal's quality of life. There are many challenging aspects of animal welfare, not the least being the definition and measurement of such an abstract concept [1,2]. The homeostasis approach [3] is used to assess animal welfare in this study. When an animal is challenged by an adverse stimulus, its biological response is either adequate to maintain stability and to cope, or it is inadequate, and the animal does not cope. The definition that supports this approach is 'The welfare of an individual is its state as regards its attempts to cope with the environment' [4]. To cope is to have control of mental and bodily stability [5]. The 'state as regards its attempts to cope' refers to both how much has to be done in order to cope with the environment and the extent to which coping attempts are succeeding. Attempts to cope include the functioning of body repair systems, immunological defenses, physiological stress response and a variety of behavioral responses. Therefore, using such a definition, the risks to the welfare of an animal by an environmental challenge can be assessed at 2 levels: firstly, the magnitude of the behavioral and physiological responses; and secondly, the biological cost of these responses [3,6]. These behavioral and physiological responses include the stress

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response, whereas the biological cost includes adverse effects on the animal's ability to grow, reproduce and remain healthy. This definition has been broadened to incorporate animal emotions [7].

There is no single "golden standard" against which to test any scale. The success and trust of animal welfare schemes relies solely on the validity, reliability and sensitivity of the measurement tool, and to date, no such tools combining several direct and indirect measurements have been subjected to rigorous evaluation [2]. A practical and robust tool must be based on relatively simple observations and records related to aspects of husbandry and welfare so that a skilled and trained researcher should be able to gather this information during a single visit. Although simple, such protocols combining several aspects should provide a detailed and valid picture of the welfare status of commercially kept animals.

Animal welfare is a multidimensional concept and its assessment includes resource-based and animal-based measures such as stereotypic behavior [8–10], tail and ear biting [11–14], aggression [12,15,16], space allowance, etc. The assessment of farm animals' welfare is fraught with difficulty, but is becoming the focus of numerous quality assurance schemes, supported by various commercial companies and supermarket chains. Performance records, behavioral, physiological and clinical parameters can be a good basis for assessing welfare at the animal level [1,10,12,17], but they also indicated the difficult interpretation of these results and the limited value of performance measures for animal welfare [18–20]. However, once a valid animal-based tool is developed, it can be used to determine on-farm risk factors concerning the provision of resources, as well as management, stockmanship and farm characteristics. In Europe, several monitoring systems have been developed such as the Animal Needs Index used in Austria and Germany [21] and the decision-support system in the Netherlands [22,23]. These welfare assessment schemes are mainly based on environmental parameters (e.g. housing criteria supposed to affect welfare) and on selected animal observations. Furthermore, these systems calculate one single score integrating all the different measured factors. This implies the risk to compensate unsatisfactory aspects by others that positively affect animal welfare.

For these reasons, the aim of this study is to develop a feasible, reliable and valid animal-based method, generally based on animal behavior, to assess on-farm animal welfare. Therefore, this study consists of three different stages. The first stage was the development of the behavioral tool to assess on-farm animal welfare based on literature and preceding exploratory work. In the second phase, the feasibility and reliability of this tool was tested. The last stage was seen as a validation phase. In this last stage, the relationship between the behavioral tool, physiological parameters and performance records was investigated to test for validity. The aim was to relate all these different observations into one or more statistical models for evaluating pig welfare at farm level. Validity can be defined as the extent to which a measurement actually measures what it is intended to measure [24].

This study was not meant to be an experimental setup to reveal differences in pig welfare under different housing/management conditions. Therefore no distinction was made between a control group and a test group. All animals were housed under similar housing and management conditions.

Identifying risk factors in different housing systems as related to design criteria, management practice and stockmanship with this method should be another step in the improvement of on-farm animal welfare.

## 2. Material and methods

### 2.1. Inter-observer repeatability

#### 2.1.1. Animals and housing

The study was carried out using 58 female and 50 castrated male Piétrain × Hypor fattening pigs with an average weight of 104 kg and an average age of 26 weeks. The animals were housed in standard fully slatted pens (4 m × 2 m) with a group size of 9 and fed ad libitum. Pens were separated using 99 cm high concrete wall consisting of bars with a width of 12 cm and a 2 cm opening in between.

#### 2.1.2. Behavioral observations

The behavioral test developed consists of a 5-min observation of the animals in their pen. All the observations occurred without entering the pen to minimize the disturbance. The selection of the different behavioral observations was partially based on literature and partially on preceding exploratory work.

- The observation started with gently throwing a plastic yellow ball (21-cm diameter) in the pen. At that moment, the **startling** response was scored 1 when half of the animals in the pen showed vigorous movement when the novel object was introduced or 0 when less than half of the animals reacted. Startling is an indicator of the arousal of the animals, which can be influenced by e.g. environmental enrichment [25] and better attitudinal and behavioral profiles of stockpersons [26].
- The time needed to approach the **novel object** was recorded for each pig that touched the ball within the 5-min limit. The interacting animal was marked using a blue marker spray from outside the pen when physical contact was established with the novel object to distinguish between the interacting and non-interacting animals. This marking procedure only lasted a fraction of a second. Preceding work revealed that this procedure did not affecting the other ongoing behavioral observations.
- **Play behavior** was scored zero or one respectively when this behavior was absent or present. This behavior is defined as pigs shoving, running or scampering [27–29]. It is considered a natural behavior indicating good welfare [12,30]. Pigs running away from the novel object were not considered performing play behavior.
- The presence of **stereotypies** is considered as an evidence of a poor physical and social environment and/or inadequate nutrition [31]. Stereotypies have been described as behaviors that are relatively invariant, regularly repeated and without an obvious function [32]. In this work stereotypic behavior was identified during the 5-min observation period as regularly

repeated, identical movements lasting more than 30 s (examples are sham chewing and repeated head movement).

- The frequency of **urination and defecation** was measured by counting the number of animals showing these behaviors during the behavioral test. These behavioral aspects are considered as indicators of arousal [33]. Defecating can also be seen as an indicator of fear and stress [34,35].
- The number of animals **biting tails** and the number of animals **biting ears** were registered as well as the number of animals with tail or ear lesions. Both ear and tail lesions were scored separately. They were divided into four categories depending on the severity. The first category included those animals without any lesions, category 2 contained animals with only superficial scratches, category 3 animals with deep lesions and bleeding tails or ears and finally category 4 animals with severe lesions where parts of the tail or ears were missing. Besides these tail and ear lesions scores, a **skin lesion** score was also included in the test. This skin lesion score distinguished between animals with less than five lesions (score 1), between five and 20 lesions (score 2) and more than 20 lesions (score 3). The number of animals in each identification class was counted. The decision to score the animal as a whole was linked to the feasibility of the behavioral test. Scoring systems dividing the animal's body in different regions such as used by de Koning [36] would take too much time to score each animal individually. The severity of the skin lesions is correlated to the level of aggression [37]. Physical injuries can affect welfare adversely because they cause pain and suffering, expose sites to infection and indicate that a conflict has occurred.
- Another more direct method to score aggression applied in this study is counting the number of animals showing **aggressive behavior** (i.e. any interaction involving bites, knocks and/or threats, or a combination). A threat is defined as one pig lunging and/or snapping at another without physical contact.
- Several authors refer to **cleanliness** as an important factor in pig housing [38,39]. In this study, a pen was scored as clean when there was a clear separation between defecation and lying area. Pens were mildly dirty when less than half the floor surface is covered with faeces and when defecation and lying areas were not clearly separated. When more than half the floor surface was soiled with faeces the pen was scored as severely dirty. An animal was considered as clean, mildly dirty or severely dirty when respectively less than 20%, between 20% and 50% or more than 50% of the whole body was dirty. We decided to obtain one general score for each animal to ensure that all animals are included and the 5-min duration of the observation period is respected.
- A similar method as described by Ekkel et al. was used for measuring **sneezing and coughing** [40]. The number of animals performing this behavior during the 5-min observation session was registered separately both at pen and compartment level. The frequency of sneezing and coughing could give an indication for lung problems such as pneumonia or pleuritis. Such lesions could be accompanied with pain, influencing animal behavior.
- **Runted pigs** were selected by visually scoring the pen mates' body weight.

To assure the practicality of the behavioral tool, the 5-min time limit was respected. Therefore no time was incorporated to habituate to the observers. The time limit also implicated that time-consuming behavioral measurements such as an open door test or an open field test were excluded from the tool. Besides this, preceding exploratory work revealed the low prevalence of other behavioral characteristics such as lameness. Besides the low prevalence, lameness was excluded from our behavioral test due to the practical difficulty observing lameness on pen level without disturbing the animals. In these circumstances, only gait scores [41] of 3, 4 or 5 can be observed and the prevalence of these gait scores in preceding work was only 0.32%.

To test inter-observer repeatability, three observers scored simultaneously and independently all the behavioral aspects of 12 pens on pen level. The behavioral parameters were recorded using a live observation. There were 9 animals per pen for each pen. The animals were completely unfamiliar with all the observers, but familiar with humans as they were controlled daily by the caretakers.

### 2.2. Linking physiology and behavior

#### 2.2.1. Animals and housing

This part of the study was carried out with 324 female and 299 castrated male Piétrain × Hypor pigs consisting of 155 6-week-old weaned pigs housed in standard pens with partially slatted floors, 136 14-week-old growing pigs housed in standard fully-slatted pens, 131 20-week-old and 201 27-week-old fattening pigs housed in standard fully-slatted pens. Half of the pens were supplied with an iron chain attached to the pen enclosure at eye level. The animals were provided with an ear tag for individual recognition. All the animals were weighed four times (at the age of 10, 14, 20 and 28 weeks) to calculate daily gain. The pig house is divided in physically separated compartments with 12 to 14 pens each, allowing application of biosecurity measurements between age groups. Pens were separated using 99 cm high concrete wall consisting of bars with a width of 12 cm and a 2 cm opening in between.

#### 2.2.2. Observations

All the behavioral observations described above were established at an individual level using the ear tags. The observations were carried out between 08:30 a.m. and 10:00 a.m. by the same observer, excluding a potential observer-effect. The outside temperature and the temperature in the compartment were measured.

#### 2.2.3. Physiological measurement

Cortisol and catecholamines were measured respectively in saliva and urine. Urine and saliva samples were collected from 23 weaning pigs, 41 growing pigs, 36 20-week-old and 69 27-week-old fattening pigs between 10:00 a.m. and 12:00 noon the day following the behavioral observations. The sampled animals were equally distributed over the pens.

*2.2.3.1. Sample collection.* Spontaneously voided urine was collected in a flask. It was acidified using 6 M HCl (1% of urine volume) and frozen at −20 °C until analysis. The saliva samples were obtained allowing the pig to chew on a cotton swab until they were soaked with saliva. This procedure took less than 2 min, thereby minimizing the disturbance of the animals. Saliva was extracted from the swabs by centrifugation at 2000×*g* for 20 min [42] within 2 h after sample collection. Samples were frozen at −20 °C until assay.

*2.2.3.2. Hormonal analysis.* The cortisol samples were analyzed using the DSL-10-67100 ACTIVE® Cortisol Enzyme Immunoassay Kit (Diagnostic Systems Laboratories, Inc.). This kit uses specific rabbit anti-cortisol antibody and does not require prior sample extraction of saliva. Cross-reactivity to other naturally occurring steroids is low. The endogenous steroid demonstrating significant cross-reactivity is 11-deoxycortisol (5.7%). The cross-reactivity with cortisone was 7.0%. The intra-assay variability was calculated from replicate analyses ($n=12$). The coefficient of variation was <5% for cortisol concentrations between 0.47 and 4.09 µg/dl. The inter-assay variability was calculated from replicate analyses ($n=13$) of quality controls. The coefficients of variation were 7.2%, 2.8% and 3.8% at concentrations of 0.5, 1.51 and 4.12 µg/dl, respectively. The test kit is characterized by a recovery of more than 94%.

Urinary-free catecholamines (norepinephrine (NE) and epinephrine (EPI)) were assayed using an ion-exchange purification procedure followed by a liquid chromatography with electrochemical detection, as previously described by Hay and Mormède [43]. Briefly, the urine samples were loaded on cationic columns and catecholamines were eluted using boric acid. The eluates were assayed using HPLC with electrochemical detection with an oxidizing potential of +0.65 V. The intra- and interassay CV were 7.0% and 7.1% for NE and 6.5% and 11.6% for EPI, respectively.

Creatinine levels were determined using a colorimetric reaction (Jaffe reaction) with alkaline picrate measured kinetically at 490 nm, without a pretreatment step (Creatinine Kinetic Method, BIOLABO SA, Maizy, France).

### 2.3. Data analysis

Statistical analyses were carried out using the statistical package SAS 9.1 (SAS Institute Inc., USA). To examine the assumption of normality, the Shapiro–Wilk procedure was used [44]. Logarithmic transformation was used when a normal distribution or the homogeneity of variances could not be assumed on raw data. All the dependent variables presented conformed to a normal distribution. The inter-observer repeatability for each subcategory of the behavioral test was calculated on pen level using the intraclass correlation coefficients (ICC) [45–47], which is determined by the covariance parameter estimates of the fitted general linear mixed model with random intercept and pen introduced as a random factor.

The independence of observation between pens was investigated according to the following procedure. The effect of pen on the mean value of the normal distributed physiological parameters was tested by means of an analysis of variance using the GLM procedure with each normal distributed physiological parameter introduced as a dependant variable and pen as an explanatory class variable. To test the effect of pen on the variance of the physiological parameters, a confidence interval around the variance of each pen was calculated. No differences in the mean and variance could be detected between the different pens concerning the observed physiological parameters.

Analyses of variance of the normally distributed physiological parameters, with animal as experimental unit, were performed using the GLM procedure to link physiology with behavioral, clinical and performance parameters. The model statement included all the significant behavioral parameters as general fixed effects and interactions between these variables if $P<0.05$. The class variable indicating the weight of the animals was also included to correct for the difference in weight. Environmental parameters which can influence animal behavior such as temperature and the availability of chains are also taken into account if $P<0.05$. All the statistical analysis to link physiology with behavioral, clinical and performance parameters, were performed with data collected on an individual level. The means of transformed data were retransformed, and the standard errors were calculated using the delta method [48].

## 3. Results

### 3.1. Behavioral characteristics

The actual values of the investigated behavioral parameters are represented in Tables 1 and 2. The average number of animals per pen was 10.92 with a standard deviation of 2.10. It has to be noted that play behavior, stereotypies, the worst category of ear lesions and severe dirty pens were not observed in this study.

Table 1
Frequency (%) of the observed behavioral parameters and number of observed animals ($n=623$) during a 5-min observation period

| Behavioral aspect | Frequency |
|---|---|
| Startling behavior | 28.62 |
| Play behavior | 0 |
| Stereotypies | 0 |
| Urination | 1.95 |
| Defecation | 0.65 |
| Tail biting victim | 1.30 |
| Ear biting victim | 7.48 |
| Tail lesion category 2 | 2.93 |
| Tail lesion category 3 | 1.95 |
| Tail lesion category 4 | 0.33 |
| Ear lesion category 2 | 2.11 |
| Ear lesion category 3 | 0.81 |
| Ear lesion category 4 | 0 |
| Skin lesion score 2 | 0.16 |
| Skin lesion score 3 | 10.57 |
| Aggressive behavior | 0.16 |
| Animal housed in a mildly dirty pens | 10.24 |
| Animal housed in a severely dirty pens | 0 |
| Coughing | 3.58 |
| Sneezing | 2.60 |
| Runted animals | 0.33 |

Table 2
Mean, standard deviation (S.D.) of the percentage of animals approaching the novel object, individual growth rates and number of observed animals (*n*)

| Behavioral aspect | Mean | S.D. | *n* |
|---|---|---|---|
| Percentage of animals approaching the novel object | 65.918 | 20.702 | 623 |
| Growth rate kg/day from week 6 to week 20 | 0.675 | 0.083 | 591 |
| Growth rate kg/day from week 20 until slaughter | 0.691 | 0.166 | 589 |

### *3.2. Inter-observer repeatability*

In general all the individual aspects scored using the behavioral test have a high to very high inter-observer repeatability, i.e. an ICC ranging from 0.7 to 1. The time to approach the novel object are highly repeatable with an ICC higher than 0.99. All the other ICCs were equal to one except those shown in Table 3.

### *3.3. Linking physiology and behavior*

No differences in the mean and variance could be detected between the different pens concerning the observed physiological parameters, indicating that the physiological observations on animal level are not affected by the pen in which the animals are housed.

Table 3
ICCs (intraclass correlation coefficients) for the different behavioral subcategories of the test

| Behavioral aspect | ICC | Behavioral aspect | ICC |
|---|---|---|---|
| Defecation | 0.90 | Mildly dirty animals | 0.85 |
| Tail biting | 0.84 | Coughing in the pen | 0.71 |
| Ear biting | 0.83 | Coughing in the compartment | 0.74 |
| Skin lesion score 2 | 0.70 | Sneezing in the compartment | 0.72 |

Table 4
Statistical model for pig salivary cortisol linked to behavioral parameters, with superscripts a and b significantly different at $P<0.05$ within each parameter

$Y$=Log10 (cortisol concentration (ng/ml)+0.01)

| Parameter | Estimate |
|---|---|
| Intercept | 0.107 |
| Growth rate kg/day (from week 6 to week 20) | −0.982 |
| No tail lesions | −0.525[a] |
| Tail lesions (category 2, 3 or 4) | 0[b] |
| Ear biting victim | 1.255[a] |
| No ear biting victim | 0[b] |
| Weaners (6 weeks old) | 0.234[a] |
| Growers (14 weeks old) | −0.296[b] |
| Fattening pigs (20 weeks old) | 0.216[a] |
| Fattening pigs (27 weeks old) | 0[a] |

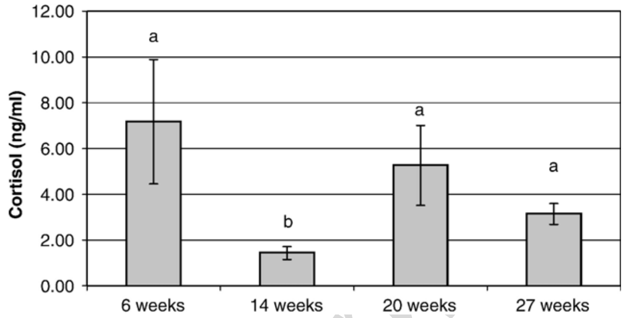


Fig. 1. Least squares means (±S.E.M.) of pig salivary cortisol levels at different ages, with a and b significantly different at $P<0.05$.

#### *3.3.1. Cortisol*

The four significant factors in predicting pig salivary cortisol levels are shown in Table 4. This model is characterized by a *P*-value of 0.0003 and an $r^2$ of 0.18. Firstly slower growing animals were characterized by higher cortisol concentrations. Secondly the presence of tail lesions had a negative effect on the level of cortisol. Thirdly animals which were bitten in the ears during the 5-min test show higher salivary cortisol levels. Finally the animal's age had a significant effect on the cortisol levels. This age effect is shown in Fig. 1.

#### *3.3.2. Epinephrine*

Table 5 contains all the significant factors influencing the urinary EPI concentration. The model to estimate urinary EPI concentrations is characterized by a *P*-value of <0.0001 and an $r^2$ of 0.22. The first factor was the percentage of animals approaching the novel object which is positively linked to the EPI concentration. The second factor was defecation. Animals who defecated during the 5-min test were characterized by a higher urinary EPI concentration than animals not showing this behavior. The effect of age was the last factor. The growers and

Table 5
Statistical model for pig urinary epinephrine linked to behavioral parameters, with superscripts a and b significantly different at $P<0.05$ within each parameter

$Y$=Log10 (epinephrine concentration ng/(mg creatinine)+0.01)

| Parameter | Estimate |
|---|---|
| Intercept | 0.543 |
| Percentage of animals per pen approaching the novel object | 0.347 |
| Animal defecates in 5-min interval | 0[b] |
| Animal does not defecate in 5-min interval | −0.402[a] |
| Weaners (6 weeks old) | 0.335[a] |
| Growers (14 weeks old) | 0.013[b] |
| Fattening pigs (20 weeks old) | 0.304[a] |
| Fattening pigs (27 weeks old) | 0[b] |

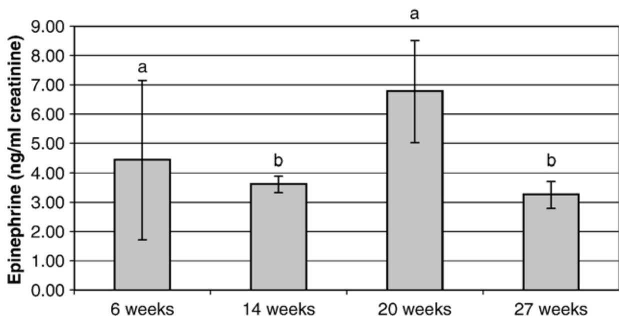


Fig. 2. Least squares means (±S.E.M.) of pig urinary epinephrine levels at different age, with a and b significantly different at $P<0.05$.

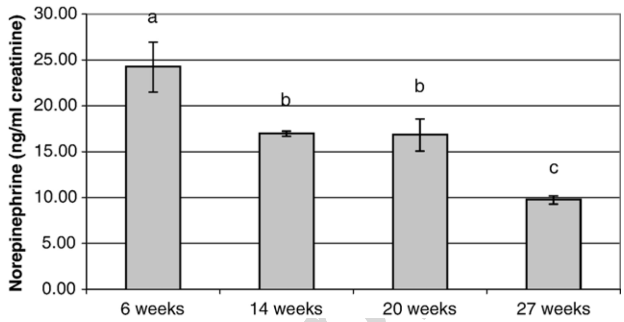


Fig. 3. Least squares means (±S.E.M.) of pig urinary norepinephrine levels at different age, with a, b and c significantly different at $P<0.05$.

27-week-old fattening pigs showed significant lower EPI levels than animals of the other two age classes (Fig. 2).

### 3.3.3. Norepinephrine

Urinary NE was significantly affected by the factors shown in Table 6. The model is characterized by a $P$-value $<0.0001$ and an $r^2$ of 0.37. Firstly, the NE concentration dropped when the number of animals per pen increased. Secondly, animals with higher growth rate between an age of 20 weeks and slaughter were characterized by higher urinary NE concentrations. Thirdly clean pens were associated with lower NE concentrations. Fourthly, animals without any tail lesions had lower urinary NE concentrations. Fifthly, runted animals were characterized by higher levels of NE. Sixthly, NE concentrations in sows were significantly lower compared to barrows. Finally, the age of the animals had an effect on the urinary NE concentration (Fig. 3).

Table 6
Statistical model for pig urinary norepinephrine linked to behavioral parameters, with superscripts a, b and c significantly different at $P<0.05$ within each parameter

| $Y$=Log10 (norepinephrine concentration ng/(mg creatinine)+0.01) | |
|---|---|
| Parameter | Estimate |
| Intercept | 1.947 |
| Number of animals per pen | −0.043 |
| Growth rate between an age of 20 weeks and slaughter (kg/day) | 0.593 |
| Clean pens | $-0.1795^{a}$ |
| Mildly or severely dirty pens | $0^{b}$ |
| No tail lesions | $-0.403^{a}$ |
| Tail lesions (category 2, 3 or 4) | $0^{b}$ |
| Non-runted animals | $-0.559^{a}$ |
| Runted animals | $0^{b}$ |
| Sow | $-0.115^{a}$ |
| Barrow | $0^{b}$ |
| Weaners (6 weeks old) | $0.749^{a}$ |
| Growers (14 weeks old) | $0.447^{b}$ |
| Fattening pigs (20 weeks old) | $0.4627^{b}$ |
| Fattening pigs (27 weeks old) | $0^{c}$ |

## 4. Discussion

### 4.1. Inter-observer repeatability

The first aim of this study was the development of a feasible and reliable multifactorial test to characterize on-farm pig behavior. Animal behavior is regarded as one of the best indicators of animal welfare [49]. It is considered as a valid indicator through which the animal expresses its basic needs, deficits and contentment in a given housing system [50]. Concerning the measures of on-farm animal behavior, reliability and feasibility are two important features conditioning their inclusion in a welfare index. As behavioral observations are time consuming, they are rarely used as part of a welfare index. Hence, the developed test, covering a broad range of behavioral characteristics is a new step forward. A well considered selection of behavioral parameters was chosen, based on literature and previous exploratory work.

Concerning the inter-observer reliability, all parameters scored using the described method have high intraclass correlation coefficients, despite being observed simultaneously. Subcategories with the lowest inter-observer repeatability are the behavioral aspects lasting only for short moments (coughing, sneezing, ear biting and tail biting), probably because of easily missing the event. One could consider extending the time needed to score all the behavioral aspects. Although this would probably improve the inter-observer repeatability of the test, this would also deteriorate the feasibility of the test, which might be unacceptable in the application to assess on-farm animal welfare.

It has to be noted that the parameters such as play behavior, stereotypies, ear lesions of category 4 and severely dirty pens were not observed in this study. Although there was a perfect agreement throughout the three observers concerning these four parameters in the preliminary phase, the reliability of observing these parameters should be interpreted with care. Further investigation is recommended. It is thought that the detection of stereotypies and play behavior is more time consuming than the 5-min time limit used in our study.

### *4.2. Linking hormonal data with behavioral related scores*

The second objective was to investigate the validity of this behavioral tool to score animal welfare. Validity can be defined as the extent to which a measurement actually measures what it is intended to measure [24]. Therefore the link between the behavioral tool and performance records, hormonal and clinical parameters was investigated.

The HPA axis and the SNS are markedly activated during stress, resulting in the release of glucocorticoids and catecholamines, respectively. Extensive work has been done on the relation between acute environmental and psychological challenges in farm animals and levels of these hormones [51]. However, little is known about the long-term effect of stressors on the neuroendocrine activity.

It is thought that behavioral parameters are likely to be dependent on their penmates' behavior. But the behavioral parameters in this study are included in the statistical analyses as explanatory variables. Very little is known about the dependence of physiological levels of cortisol and catecholamines with those of penmates. In this study, no significant difference could be detected concerning the mean and variance of the normally distributed physiological parameters between the different pens. Therefore the physiological measures are independent of the pen in which animals are housed in this study.

#### *4.2.1. Salivary cortisol*

Increased exposure to stress can induce long-term changes in the regulation of the HPA axis. Repeated exposure to stressors can produce increases in adrenocortical function, as evidenced by increased basal plasma corticosteroid concentrations or increased adrenal weight [52–55]. It is generally accepted that social stress can cause an activation of the HPA axis in pigs [56–59]. An increase in salivary cortisol levels was associated with recipients of ear biting and animals with tail lesions in this study. Tail and ear biting behavior is considered as an abnormal behavior in pigs and the welfare of the recipient may also be poor [13,60]. The higher levels of cortisol found in animals with tail lesions and in ear-bitten animals confirm an activation of the general adaptation syndrome [61]. Hunter et al. [62] found that pigs with bitten tails had significantly higher levels of ear damage than pigs with unbitten tail. In due course the biting can become more severe resulting in wounds and hemorrhage [63]. In severe cases, the wounded animal gradually gives up its resistance and its effort to flee. It becomes apathetic, lies down much of the time, seldom changes position, and reacts only slightly to being bitten. The constantly moist tail attracts bacteria leading to inflammation [63]. Besides the pain and the clinical symptoms involved for the victim, this biting behavior increases the disturbance among a pen-group. Hansen et al. [64] made clear that there is a connection between tail biting and aggression.

Animals with higher cortisol levels were characterized by a lower average daily gain. A chronic elevation of corticosteroids is associated with depressed growth. Corticosteroids decrease amino acid incorporation into body tissues and mobilize energy reserves [65]. Cortisol also favors the accretion of fat at the expense of proteins [66] leading to a depressed growth.

Finally a significant age effect was observed. Cortisol levels in weaning pigs aged 14 weeks were significantly higher than the levels in the other age groups. De Jong et al. [67] did not find any differences between pigs of 9, 11, 13, 17, 19 and 22 weeks old. They reported a sudden significant increase of salivary cortisol at an age of 15 weeks and between 15 and 22 weeks of age the levels decreased. To conclude whether or not these results are contradictory, animals should have been sampled during more different stages of growth.

#### *4.2.2. Urinary catecholamines*

Urinary catecholamines in other species than humans have received less attention [43]. Catecholamines released by the SNS increase the use of energy stores [68] and exert anabolic effects on protein metabolism [69].

*4.2.2.1. Urinary epinephrine.* Hay et al. [70] demonstrated a significant increase in urinary EPI concentrations in pregnant sows at the end of the dark period and around feeding. A period of high activity is likely to be responsible for the increase in catecholamine excretion. Our research demonstrated significant higher EPI levels when more animals approached the novel object. A higher approach rate could be related to higher activity levels with the higher EPI concentration as a result.

Measures such as increased defecation are indicators of porcine arousal [33] and stress or fear [34,35]. The original view proposed by Hall [71] is that the fear response of an animal exposed to a novel, potentially dangerous environment is characterized by a high defecation rate caused by an activation of the autonomic nervous system. In rodents, this original view has evolved in the sense that a low ambulation also appeared as a main fear response of animals exposed to novelty [71–74]. Genetically homogenous groups of rodents are characterized by a negative correlation between defecation scores and locomotive activity in a novel environment (open field) [75]. Désautés et al. [76] however associated high levels of locomotive activity with high defecation scores in Meishan pigs in a novel environment. The higher levels of EPI found in defecating pigs support the original view proposed by Hall [71]. Several studies have shown defecation increases by increasing the aversiveness of the situation [77–79].

Up till now, very little data are available concerning the age effect on basal catecholamine concentrations in pigs. Hay et al. [80] found lower urinary EPI concentrations in 11- and 19-day-old pigs compared to 5-day-old pigs. This research demonstrates significantly higher urinary EPI concentrations in pigs aged 6 and 20 weeks compared to 14- and 27-week-old animals. Further research is needed to explain this age effect.

*4.2.2.2. Urinary norepinephrine.* It has been suggested that differences in body posture and physical activity account for most of the diurnal changes in catecholamine secretion [81]. A similar influence of meal distribution on the pattern of diurnal activity has been reported by others [82]. These periods of high activity are likely to be responsible for the elevated catecholamine excretion. The cleanliness of the pens, tail lesions, the number of animals per pen, the growth rate and whether or not animals are runted, are parameters that can influence the animal's activity level. NE is highly related to thermoregulation [80]. A reduction in NE concentration with age can be expected since heat production per kg body weight decreases with age. The cleanliness of the pens can affect thermoregulation which can explain the relation between NE concentration and cleanliness of the pen. Von Borell and Van den Weghe [38] indicated the importance of avoiding discomfort through wet and soiled lying areas. Dirty pens increase the incidence of leg injuries and decrease the hygiene status [83]. Dirty pens appear when temperature or ventilation are suboptimal [83–85] resulting in a disturbed resting behavior and more aggressive behavior. This behavioral disturbance could result in higher levels of NE.

Tail biting is one of the most important welfare reducing factors in modern pig production [13]. Tail biting increases the disturbance among a pen-group which leads to unrest, irritability, overexcitement and increased activity which makes the problem worse [11]. Again the increased activity can lead to higher NE levels [70].

Runted animals mostly are diseased animals or chronically stressed animals. The higher basal levels of NE could be an indication of the chronically stressed state of these animals.

In this study, all animals are housed under commercial conditions, the available space decreases with an increasing number of animals per pen. This could lead to a diminished possibility to perform active behavior and lower NE levels and thereby a lower degree of animal welfare. Although some past research focused on the link between the activity and physiological parameters (e.g. [25,70]), further behavioral research should follow with long term observation of the activity level of the animals to certify the positive effect of physical activity on the catecholamine level in pigs.

Foury et al. [86] found no significant sex influence neither on the concentration of urinary cortisol, EPI nor NE. This study can confirm these results concerning cortisol and EPI, however NE concentrations in castrated male pigs are significantly higher than in sows.

Cortisol and catecholamines both influencing the physiological processes involved in growth. Cortisol favors the accretion of lipids in fat at the expense of proteins from muscle and other tissues [66]. Activation of β-adrenergic receptors increases mobilization of fat and reduces protein catabolism, mostly via $\beta_2$-type receptors [69]. A direct action of EPI, a potent $\beta_2$ agonist, in these processes should therefore reduce the fat content of the carcass and increase the yield of muscle. The exact role of NE in the process of growth remains unknown.

## 5. Implications

The present data confirm that stress hormones from the adrenal cortex (cortisol) and the sympathetic nervous system (catecholamines) are linked with behavioral aspects related to animal welfare. Investigating on-farm animal welfare in commercial pig farms is often difficult due to the limited available time and the genetic, behavioral and physiological diversity of the animals. A simple though reliable behavioral test as being developed in this study is an innovative step for future on-farm animal welfare assessment. The statistically inferred variables of the basic model were very well explained by information from the literature. This test, in combination with clinical, physiological and performance parameters can be used to determine on-farm risk factors concerning management, farm characteristics, stockmanship and the provision of resources leading to a further improvement of on-farm animal welfare.

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