ANIMAL BEHAVIOR AND COGNITION

Investigation of influence of growing pigs' positive affective state on behavioral and physiological parameters using structural equation modeling

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Abstract

The aim of the present study was to investigate whether the primarily positive affective state of fattening pigs influences various behavioral and physiological parameters such as the pigs' playing behavior, way of behaving in behavioral tests, body language signals, or diameter, and astroglia cell numbers of hippocampi, salivary immunoglobulin A (IgA) content, or salivary protein composition. Additionally, the suitability of the variables mentioned was examined to assess the pigs' positive affective state in practice, which still constitutes a latent variable not itself measurable. For this, a dataset including behavioral and physiological data of 60 fattening pigs from 3 different farms with different housing systems was analyzed by the partial least squares structural equation modeling (PLS-SEM) method. A hierarchical component model (HCM) was used including the pigs' positive affective state as a higher-order component (HOC) and the behavioral and physiological parameters as lower-order components (LOC). Playing behavior, body language signals, and behavioral tests were revealed, in this order, to be most influenced by the pigs' positive affective state since these resulted in the corresponding path coefficients (PC) of PC = 0.83, PC = 0.79, and PC = 0.62, respectively. Additionally moderate and weak R^2 -values occurred for the endogenous latent variables playing behavior ($R^2 = 69.8\%$), body language signals ($R^2 = 62.7\%$), and behavioral tests ($R^2 = 39.5\%$). Furthermore, the indicator of the "locomotor play" showed the highest indicator reliability (IR) (IR = 0.85) to estimate the latent variable of pigs' positive affective state. The results of the present study supplement the comprehension and assessment of the pigs' positive affective state in general.

Key words: animal-based indicators, pig, positive emotions, SEM

Introduction

Partial least squares structural equation modeling (PLS-SEM) enables researchers to measure latent variables, which are not directly assessable while measuring them indirectly using indicator variables. Thus, it depicts a statistical method that facilitates the estimation and testing of correlative relationships between dependent and independent variables and the latent structures in between and aims to explain the highest possible variance of latent variables by calculating various algorithms (Hair et al., 2017). SEM is used as a common method in social

science and marketing concerns, though its popularity has also increased in agriculture issues (Lamb et al., 2011; Valente et al., 2013) such as milk production (de los Campos et al., 2006; Heringstad et al., 2009) or animal welfare issues in gallop sports in horses (Müller et al., 2015). In general, animal welfare consists of 3 components: 1) basic health and biologic functioning, 2) natural living, and 3) the animals' affective state (Fraser, 2008). Whereas extensive research in the area of the basic health and biologic functioning and natural living has already been carried out (Blokhuys, 2013), the positive affective state in livestock

Abbreviations

AL	approach latency
AVE	average variance extracted
CR	composite reliability
DC	duration of contact
GPC	general protein content
HAT	human-approach test
HCM	hierarchical component model
HOC	higher-order component
IR	indicator reliability
LOC	lower-order components
NC	number of contacts
NOT	novel-object test
PC	path coefficients
PLS-SEM	partial least squares structural equation modeling
TBI	total band intensity
TNB	total number of bands

particularly still constitutes a latent variable for which there is still no reliable measurement method. For instance, already examined assessment systems such as the “Welfare Quality” protocol have been partially unreliable and strongly influenced by subjective perceptions, especially in terms of the “Qualitative Behavior Assessment” (Temple et al., 2011; Bokkers et al., 2012; Tuytens et al., 2014; Czycholl et al., 2017). However, it is highly relevant to implement reliable measurement methods to assess especially the farm animals’ affective state as there is a consumer demand for higher animal welfare standards (e.g., Bennett et al., 2002; Hobbs et al., 2002) and that animals lead a life worth living (Mellor, 2016). According to literature, the animals’ affective state consists of different emotions such as pleasure, happiness, pain and suffering, and other feelings such as hunger and thirst that are experienced as pleasant or unpleasant (Fraser, 2008). Moreover, the primarily positive affective state presumably includes experienced pleasant emotions such as happiness (Ortony and Turner, 1990; Diener and Lucas, 2000), whereas unpleasant emotions such as fear, pain, or suffering probably indicate the animals’ more negative affective state. However, there is the hypothesis that the positive affective state could influence different behavioral and physiological parameters in livestock, so that it might be possible to make statements about the affective state through the analysis of these possible impacts (e.g., Bosch et al., 2004; Czéh et al., 2006; Siviý et al., 2006) and whether behavioral or physiological parameters could be able to assess the positive affective state.

In the first instance, in behavioral tests, animals might react in certain ways in special test situations, so that their type of behavior, which, for example, seems to express fear (Forkman et al., 2007), could possibly lead to conclusions about their affective state. Moreover, it has been assumed that playing behavior only exists in animals if the animals fare well and feel well (Siviý et al., 2006) and decreases when animals experience negative emotions accompanied by, for example, threats to their fitness (Fagen, 1976; Martin and Caro, 1985; Burghardt, 2005) or adverse environmental conditions (Müller-Schwartz et al., 1982; Siviý and Panksepp, 1985) which could also allow conclusions concerning the affective state. Additionally, the body language signals of animals could also be suitable to indicate their affective state because, for example, the occurrence of curled-up tails in pigs is assumed to be linked to their more positive affective state (Groffen, 2012). Moreover, there are several studies that

refer to transferable similarities between humans and pigs (Lind et al., 2007; Meurens et al., 2012; Bassols et al., 2014), so that it could be reasonable to use physiological information on human depression research to assess livestock’s and in this case pigs’ primarily positive affective state. For instance, due to results from human depression research, it could be conceivable that pigs with a more positive affective state show higher contents of salivary immunoglobulin-A (IgA) (Bosch et al., 2004), larger diameters of hippocampi, and higher numbers of astroglia cells (Czéh et al., 2006) than pigs with a more negative affective state. In the described human depression research studies, humans without depression showed higher values for these physiological parameters than people with depression (Bosch et al., 2004; Czéh et al., 2006).

Thus, in the current study, SEM was used to estimate the complex interactions between the behavioral and physiological latent variables mentioned with regard to the particularly positive affective state in fattening pigs and its influence on these parameters to obtain indicators for a better understanding of the affective state of pigs in general.

Materials and methods

Ethical statement

The authors declare that the experiments were carried out strictly following international animal welfare guidelines. Additionally, the “German Animal Welfare Act” (German designation: TierSchG), the “German Order for the Protection of Animals used for Experimental Purposes and other Scientific Purposes (German designation: TierSchVersV), and the “German Order for the Protection of Production Animals used for Farming Purposes and other Animals kept for the Production of Animal Products” (German designation: TierSchNutztV) were applied. No pain, suffering, or injury was inflicted on the animals during the experiments.

General scheme of a structural equation model (SEM)

An SEM includes 2 different types of variables which represent the relationships between them: these are latent variables, which are not directly measurable, and latent measurement variables, which make an estimation of the former latent variables possible. An SEM is formed by a structural model (endogenous and exogenous latent variables) and at least 2 measurement models (latent measurement variables and their self-characterizing indicators). Endogenous (dependent) latent variables can be influenced by other latent variables, irrelevant of being endogenous or exogenous, although exogenous (independent) latent variables cannot be affected by other latent variables. Thus, the coefficient of determination (R^2), which indicates to which extent the latent variable is explained by other latent variables, is exclusively computed for endogenous latent variables (Figure 1a). The relationships (path coefficients [PC]) between the endogenous and exogenous latent variables indicate the structural model by arrows connected to each other.

Hypotheses of the present SEM

This study examined the hypotheses that the primarily positive affective state of fattening pigs’ influences behavioral and physiological parameters and that behavioral and physiological parameters could be useful to estimate the pigs’ positive affective state. Moreover, the positive affective state of fattening

Figure 1. General scheme of a structural equation model (SEM) (a). IR, indicator reliability; reflective-reflective hierarchical component model (HCM) (b). IR, indicator reliability.

pigs depicts the latent variable, which is not directly measurable, and the behavioral and physiological parameters form the latent measurement variables, which enable an estimation of the pigs' positive affective state. Thus, the positive affective state is an exogenous latent variable and the behavioral and physiological parameters are endogenous latent variables, which are influenced by the pigs' positive affective state.

Hierarchical Component Models (HCM)

The section "General scheme of a structural equation model (SEM)" described first-order models, which contain a single level of constructs. However, in other situations, as in this study, very complex constructs have to be analyzed. Such constructs require an operationalization at a higher abstraction level, so a hierarchical component model (HCM) with 2 levels of abstraction was developed to analyze the construct of this study. The 2 levels of abstraction of an HCM are the higher-order component (HOC), which assesses the more abstract level, and the lower-order components (LOC), which include the subdimensions of the higher component. Further, the pig's affective state depicts the HOC, and the different behavioral and physiological parameters represent the LOC. A reflective-reflective HCM that contained a reflective relationship between the HOC and LOC was used

whereby all constructs of LOC were specified by reflective measurement models. A reflective measurement model means that the indicators represent the underlying construct and that the causality of the construct is directed to the indicators. In order to form the HOC measurement model, all indicators of the LOC are also assigned to the HOC (Hair et al., 2017) (Figure 1b).

Assessment of the SEM

The SmartPLS 3.0 software (Ringle et al., 2015) was used to generate and calculate the SEM. The evaluation was based on a variety of recommended quality criteria by Hair et al. (2017). This evaluation involves a 2-stage process whereby firstly the measurement models are checked, followed by the structural model. Quality criteria of the measurement models included the internal consistency reliability (composite reliability [CR]) and convergent validity (indicator reliability [IR] and average variance extracted [AVE]). For the consistency reliability, the composite reliability considers different loadings of indicators and displays values between 0 and 1, whereas it should assume values between 0.6 and 0.7 in exploratory research studies. For the convergent validity, the indicator reliability assesses how sufficient a latent variable is estimated by an indicator. Thus, indicators with indicator reliabilities between 0.4 and 0.7 should

only be removed from the construct during the assessment process if their removal increases the quality criteria of the model (Hair et al., 2017), whereas indicators with indicator reliabilities below 0.4 should always be removed from the model (Bagozzi et al., 1991; Hair et al., 2011). Further, the elimination of indicators should be carefully considered as they should be retained if they make a corresponding contribution to the content validity of the whole model (Hair et al., 2017). AVE describes the extent to which a latent construct explains the variance of its indicators and AVE should achieve values above or equal to 0.5.

Primary quality criteria of the structural model involve the relevance and statistical significance of path coefficients and the examination of the coefficients of determination (R^2). The path coefficients indicate the relationships between the constructs in the structural model and correspond to the standardized regression coefficients in the regression analysis. Path coefficients show values between -1 and 1 and verify or refute the pre-hypothesized relationships between the constructs in the model. For this, sign and magnitude have to be considered (Henseler et al., 2009). An estimated path coefficient near to +1 represents a strong positive relationship between 2 examined constructs (and vice versa for negative values), which is typically statistically significant. The closer the estimated path coefficient is to 0, the weaker the relationship. Very low values close to 0 are generally not statistically significant (Hair et al., 2017) whereby the statistical significance is tested by the bootstrapping method. The coefficients of determination (R^2) indicate the proportion of the variance of an endogenous construct that is explained by all precursor constructs associated with the endogenous construct. The higher the R^2 is, the better the construct is explained by the latent variables in the structural model, which shows structural path model relationships on the explained construct (Hair et al., 2017). In scientific marketing research, R^2 values of 0.25, 0.50, and 0.75 are assessed as weak, moderate, and substantial, respectively (Henseler et al., 2009; Hair et al., 2011).

Split-half scenario

To further assess the validity of the SEM, the split-half consistency was applied (Martin and Bateson, 2007). After the SEM had been applied to the original dataset, this original dataset was split into 2 halves by using the PROC SURVEYSELECT procedure in SAS 9.4 (SAS® Institute Inc., 2017), to which the SEM was also applied.

Data origin and data structure

The dataset of the present study originated from 60 fattening pigs of 2 batches from 3 different farms with 2 different housing systems in Northern Germany. Each fattening period included about 4 mo. SAS 9.4 (SAS® Institute Inc., 2017) was used for the calculation of descriptive statistics. The dataset ($n = 60$) lasted behavioral data of 2 behavioral tests (human-approach test [HAT] and novel-object test [NOT]), playing behavior (total duration, locomotor play, and social play), body language signals (curled-up, hanging, raised, and wagging tails as well as ears directed forwards, backwards, mixed, and laterally) and physiological data of the saliva regarding its IgA content and protein composition (general protein content [GPC], total number of bands [TNB], and total band intensity [TBI]) as well as physiological data of the diameter and astroglia cell number of the hippocampi. The behavioral tests included parameters of the approach latency (AL), duration of contact (DC), and number of contacts (NC) in each test. AL represented the time in seconds that each pig needed to approach the unknown human (in the

HAT) or the novel object (in the NOT) until the snout touched the human or the novel object. DC exposed the accumulated seconds in which the pigs touched the human or the novel object with their snouts. The entire number of snout contacts that occurred during the test phase was indicated by NC. The whole test phase was 3 min and each pig was tested alone in the home pen. The unknown human in the HAT was always a female person who the pigs did not know from daily routine work. A plastic duck presented to the pigs in 3 different sizes (according to the age and the live weight of the animals: 6×7 cm at the beginning of fattening, 12×13 cm at the middle of fattening, and 27×28 cm at the end of fattening) represented the utilized novel objects in the NOT. These ducks showed a yellow body color with a red-colored beak. The behavioral data of the behavioral tests were recorded at the beginning, middle, and at the end of fattening and then summarized for each individual pig. The playing behavior was observed by continuous sampling and involved parameters such as the total duration of playing behavior, locomotor play, and social play in seconds per hour during the daylight of each pig. Locomotor play included play categories as pivoting, hopping, scampering, flopping, rolling, and head-shaking, whereas social play contained non-harmful fighting, chasing, and invitation play. The body language signals represented the prevalence of the curled-up, hanging, raised, and wagging tails as well as ears directed forwards, backwards, mixed, and laterally once an hour during the daylight of each individual pig. The behavioral data of playing behavior and body language signals were recorded for 2 d at the beginning and 2 d at the end of fattening. The hippocampi diameters were measured in millimeters and the astroglia cell number in GFAP pixel intensity due to a semiquantitative evaluation with the Image J software (National Institutes of Health, Bethesda, MD, USA). The IgA content ($\mu\text{g/mL}$) of the pigs' saliva was analyzed with a direct quantitative sandwich-ELISA-Kit for pig-IgA (Celltrend GmbH, Luckenwalde, Germany). According to these results of the IgA content, the 10 pigs with the highest and the 10 with the lowest concentrations per farm were selected (out of a total number of 302 pigs) for the analyses of the structural equation modeling. In human studies, high IgA concentrations could be connected to more positive affective states and low IgA concentrations to more negative affective states (McClelland & Cheriff, 1997). This was carried out in order to achieve the highest variance as possible in the data set. IgA measurements were preferred to, for example, cortisol measurements, as cortisol levels can vary with circadian rhythm, season, and photoperiod or food intake rhythm (Barnett et al., 1981; Mormède, 1988). The general protein content (GPC) ($\mu\text{g/mL}$) were analyzed by colorimetric detection by means of a bicinchoninic acid-based protein assay kit (Pierce BCA Protein Assay Kit, Thermo Fisher Scientific Inc., Waltham, USA) and the TNB and TBI (px) through 1-dimensional sodium dodecyl polyacrylamide gel electrophoresis. All physiological data were collected merely at the end of fattening (Table 1). During the selection process of data, it was considered that each variable contained less than 15% missing values (Hair et al., 2014). The other 2 datasets ($n = 30$; $n = 30$) which were used for the split-half scenario included 30 randomly selected animals from the original dataset, respectively (PROC SURVEYSELECT).

Results

The subsequent sections present the results of the final SEM. An initial SEM was calculated and then checked regarding the quality criteria of measurement respectively structural models which are explained in the "Materials and methods." section

Table 1. Descriptive data of indicators used in the SEM

Related latent variable	Indicator	n	Mean	Median	Minimum	Maximum	Standard deviation
Behavioral tests (endogen)	HAT-AL, s	60	250.90	217.00	2.00	540.00	153.56
	HAT-DC, s	60	138.32	103.50	0.00	419.00	127.26
	HAT-NC, s	60	4.25	4.00	0.00	13.00	2.55
	NOT-AL, s	60	85.07	38.50	5.00	386.00	97.36
	NOT-DC, s	60	179.28	160.00	21.00	459.00	104.24
	NOT-NC, s	60	9.38	9.00	2.00	16.00	2.88
Playing behavior (endogen)	Playing behavior, s/h	60	27.81	20.67	0.00	112.88	26.50
	Locomotor play, s/h	60	8.89	7.50	0.00	42.05	9.37
	Social play, s/h	60	18.88	10.32	0.00	102.88	22.26
Body language signals (endogen)	Curled-up tails (prevalence)	60	0.03	0.03	0.00	0.13	0.03
	Hanging tails (prevalence)	60	0.01	0.00	0.00	0.06	0.02
	Raised tails (prevalence)	60	0.01	0.00	0.00	0.13	0.02
	Wagging tails (prevalence)	60	0.00	0.00	0.00	0.06	0.01
	Ears directed forwards (prevalence)	60	0.03	0.02	0.00	0.19	0.03
	Ears directed backwards (prevalence)	60	0.00	0.00	0.00	0.01	0.00
	Ears directed mixed (prevalence)	60	0.00	0.00	0.00	0.02	0.00
	Ears directed laterally (prevalence)	60	0.02	0.01	0.00	0.09	0.02
Slaughter organs (endogen)	Diameter of hippocampi, mm	60	5.27	5.38	4.25	6.50	0.52
	Astroglia cell numbers (GFAP pixel intensity)	60	175.82	174.50	0.02	0.07	0.01
Saliva (endogen)	IgA, µg/mL	59	125.46	71.33	8.61	992.30	154.19
	GPC, µg/mL	60	1,513.58	1,185.64	341.97	4,563.21	923.17
	TNB	60	11.90	12.00	5.00	17.00	2.20
	TBI, px	60	392.86	381.65	146.31	1,053.33	190.56

To prove the validity of this final SEM, it was also applied to a second dataset (split-half scenario).

Initial SEM

The initial SEM estimated 5 relationships between 6 latent variables, divided into 5 endogenous latent variables (LOC) and 1 exogenous latent variable (HOC). Thus, 5 arrows connected 1 exogenous latent variable with 5 endogenous latent variables, which served as latent measurement variables associated with 23 indicators (Figure 2).

Final SEM

In the final SEM, 3 relationships between 4 latent variables remained (3 endogenous latent variables [LOC] and 1 exogenous latent variable [HOC]). According to the quality criteria of the indicator reliability, 17 of the initial 23 indicators were removed from the model during the assessment process, as their loadings, which represent the magnitude of indicator reliability, showed values below 0.4 or did not achieve statistical significance (Hair et al., 2017). The endogenous measurement latent variable slaughter organs, respectively saliva and their remaining associated indicators “astroglia cell numbers” respectively “total number of bands” were in case of becoming a so-called single-item also removed from the SEM as the use of single-items is risky, because it generally reduces reliability and negatively impacts prediction validity of the SEM (Hair et al., 2017) (Figure 3).

Assessment of the measurement models (final SEM)

The SEM measurement models were checked for reliability and validity based on the test criteria composite reliability, indicator

reliability, and AVE, which are explained in the “Materials and methods” section. After calculating the first PLS-algorithm, all indicators with loadings below 0.4 or without statistically significance were removed from the initial model (Hair et al., 2017). For behavioral tests, the associated indicator “NOT-approach latency” showed higher indicator reliability (IR = 0.80) than the indicator “HAT-approach latency” (IR = 0.78). Thus, “NOT-approach latency” appeared to be better than “HAT-approach latency” to estimate behavioral tests whereby it should also be noted that both tests measure 2 things: attraction or fear of novelty and attraction or fear of human. For playing behavior, the indicator “locomotor play” (IR = 0.93) estimated the latent variable more sufficiently than the indicator “total play” (IR = 0.84). The relating indicators “curled-up tails” and “ears directed laterally” of the body language signals indicated similar indicator reliabilities (IR = 0.90 and IR = 0.90, respectively), which means that these 2 estimate the endogenous latent variable body language signals equally well. For the pig’s positive affective state, “locomotor play” (IR = 0.85) was the most appropriate indicator to estimate its latent variable, followed by “curled-up tails” (IR = 0.71) and “ears directed laterally” (IR = 0.71), “total play” (IR = 0.59), “NOT-approach latency” (IR = 0.51), and “HAT-approach latency” (IR = 0.48) (Table 2).

As a next step, the reliability of the measurement models was examined using the composite reliability. The measurement models behavioral tests had the lowest composite reliability (composite reliability [CR] = 0.77), whereas playing behavior and body language signals had the highest composite reliabilities (CR = 0.88 and CR = 0.89, respectively). The composite reliability of the pigs’ positive affective state (CR = 0.81) was in between the abovementioned values. As the composite reliability was above

Figure 2. Initial SEM. Circles indicate latent variables; rectangles represent indicators; arrows connecting circles represent relationships between latent variables (path coefficients); arrows between circles and rectangles indicate indicator reliabilities.

Figure 3. Final SEM. Circles indicate latent variables; rectangles represent indicators; arrows connecting circles represent relationships between latent variables (path coefficients); arrows between circles and rectangles indicate indicator reliabilities; numbers in circles represent the coefficient of determination.

0.6 for all measurement models, all measurement models are proven to be reliable (Table 2).

The test criterion AVE was used to examine the validity of the measurement models. For all measurement models apart from pigs' positive affective state, the AVE was above 0.5 (behavioral tests: AVE = 0.63; playing behavior: AVE = 0.79; body language signals: AVE = 0.81); this proves the validity of all these measurement models (Henseler et al., 2009). The value

for the pig's affective state (AVE = 0.43) is the lowest of all the measurement models and was close to the set threshold (Table 2).

Assessment of the structural model (final SEM)

The evaluation of the structural model revealed that all relationships between the exogenous and endogenous latent variables were statistically significant ($P < 0.05$). Playing behavior was the parameter most influenced by the pigs' positive affective state

Table 2. Indicator reliabilities (IR), coefficients of determination (R^2), composite reliabilities (CR), and average variance extracted (AVE) of the final SEM, split-half consistency SEM₁, and split-half consistency SEM₂

Latent variable	Indicators	Final SEM				Split-half consistency SEM ₁				Split-half consistency SEM ₂			
		IR	CR	AVE	R^2	IR	CR	AVE	R^2	IR	CR	AVE	R^2
Behavioral tests (endogenous)	HAT-approach latency	0.78	0.77	0.63	0.39	0.82	0.71	0.55	0.39	0.87	0.82	0.69	0.53
	NOT-approach latency	0.80				0.65				0.80			
Playing behavior (endogenous)	Total play	0.84	0.88	0.79	0.69	0.91	0.92	0.85	0.77	0.76	0.84	0.72	0.69
	Locomotor play	0.93				0.93				0.93			
Body language signals (endogenous)	Curled-up tails	0.90	0.89	0.81	0.62	0.85	0.86	0.76	0.60	0.88	0.87	0.78	0.61
	Ears directed laterally	0.90				0.89				0.88			
Pigs' positive affective state (exogenous)	HAT-approach latency	0.48	0.81	0.43	—	0.52	0.82	0.44	—	0.66	0.82	0.44	—
	NOT-approach latency	0.51				0.39				0.54			
	Total play	0.59				0.77				0.49			
	Locomotor play	0.85				0.85				0.86			
	Curled-up tails	0.71				0.62				0.68			
	Ears directed laterally	0.71				0.73				0.69			

by indicating a path coefficient of 0.83. After that, body language signals were more influenced by the pigs' positive affective state than behavioral tests ($PC = 0.79$ and $PC = 0.62$, respectively).

The R^2 values of the structural model were assessed in the following step. Here, the structural model explained 39.5% of the variance in behavioral tests, 62.7% of the variance in body language signals, and 69.8% of the variance in playing behavior. Therefore, the structural model best explains the variance of playing behavior, followed by the variance of body language signals and the variance of behavioral tests (Table 2).

Assessment of the split-half scenario SEM

Also, the split-half scenario SEM₁ and split-half scenario SEM₂ were checked for reliability and validity. As shown in Table 2, the test criteria of the split-half scenario SEMs indicated similar tendencies as the results of the final SEM. The relationships (path coefficients) between the exogenous and endogenous latent variables were statistically significant as well ($P < 0.05$).

Discussion

Assessment of the measurement models (final SEM)

Concerning indicator reliability, 6 of the initial 23 indicators were considered reliable for the estimation of the respective associated latent variables. The high loadings (above 0.7) of the indicators of the endogenous latent variables indicate that they all seem to be well suited to measure their latent variable. The indicator reliabilities of the exogenous latent variable pigs' positive affective state indicated values below 0.7 for the indicators "total play," "NOT-approach latency," and "HAT-approach latency." Nonetheless, these indicators were not removed from the model, as indicators with indicator reliabilities between 0.4 and 0.7 should only be removed from the construct if their removal increases the quality criteria of the model (Hair et al., 2017). Further, the elimination of indicators should be carefully considered as they should be retained if they make a corresponding contribution to the content validity of the whole model (Hair et al., 2017), which was the case in this

study. However, according to the highest indicator reliability, the indicator "locomotor play" seems to be most appropriate to measure the latent variable of the pigs' positive affective state, followed by the indicators "curled-up tails" and "ears directed laterally," "total play," "NOT-approach latency," and "HAT-approach latency."

These results seem to be realistic, as the occurrence of playing behavior revealed to be suitable to show a more positive affective state of the examined pigs. Also found in other studies, playing behavior has been assumed to exist if animals feel positive emotions (Siviy et al., 2006) and decreases when animals are supposed to be in more negative affective states accompanied by, for example, threats to their fitness (Fagen, 1976; Martin and Caro, 1985; Burghardt, 2005) or adverse environmental conditions (Müller-Schwartz et al., 1982; Siviy and Panksepp, 1985). The fact that the indicator "locomotor play," according to the indicator reliabilities, seems to be the most suitable for estimating the pigs' positive affective state, could be related to the fact that the opportunity to perform locomotor play improves neuromuscular development, motor performance (Byers, 1977), and cardiovascular fitness (Bekoff, 1988; Byers and Walker, 1995) resulting in enhanced health of the pigs. This possibly points to increased welfare including experienced positive emotions as basic health and biologic functioning depicts one of the most important parts of animal welfare (Fraser, 2008) and affirms the presumably described good suitability of "locomotor play" to estimate the pigs' positive affective state. However, the indicator "total play," which consists of the locomotor play and social play of the examined pigs, indicated lower indicator reliability than "locomotor play." This could be explained by the controversially discussed literature regarding the occurrence of social play as an indication of an exclusively positive affective state. Hausberger et al. (2012) linked the significantly increased occurrence of social play in captive horses to poor welfare and, therefore, to a more negative affective state. Thus, there is no definite evidence that social play indicates an exclusively more positive affective state in the fattening pigs examined, even though the transferability from horses to pigs could be questionable, although both are gregarious species which need social contacts. In addition, the

transferability must be considered with caution, as the horses in this study were kept isolated, which could have been the reason for less well-being and thus expression of social play, and on the other hand, the pigs in this study were kept in groups. However, it might explain the lower indicator reliability of “total play” compared with “locomotor play.” Besides, it should be considered that the social play measured in this study included “non-harmful fighting,” which can nonetheless be related to minor fights that could have a negative value for the affective state. Nevertheless, both indicators of playing behavior can be concluded as suitable indicators to estimate the latent variable of the pigs’ positive affective state.

Particularly the indicator “curled-up tails” of the body language signals seems to be realistic to measure the pigs’ positive affective state, as according to literature pigs curl up their tails in positive situations such as satisfying their essential needs for feeding and drinking (Cabanac, 1992; Carver, 2001; Rolls, 2005; Burgdorf and Panksepp, 2006), rewarding events (Goursot et al., 2018), or being housed in enriched environments (Groffen, 2012). Kiley-Worthington (1975) interpreted the appearance of curled-up tails in pigs even as health indicators. Thus, it seems acceptable that curled-up tails could be suitable to estimate the pigs’ positive affective state because satisfying essential needs, rewarding events, enriched environments, or good health presumably induce positive emotions and, therefore, a positive affective state in pigs. As mentioned above, good health and biologic functioning represent one of the most important parts of animal welfare. The given opportunity to perform natural living in enriched environments probably also indicates good welfare. In consequence, the pigs’ positive affective state as natural living constitutes an important part of good welfare as well (Fraser, 2008). Further, it has to be taken into account that all parts of good welfare must be valued equally and none is more important than the other (Fraser, 2008). According to the calculated indicator reliabilities, the ears directed laterally also seem to be suitable to estimate the pigs’ positive affective state almost as well as the curled-up tails. However, regarding this, further research is needed because it is imaginable that the ears directed laterally of the present study could be related to both a passive ear posture (ears hanging down, relaxed) or a change in ear postures. Further, Reimert et al. (2013) observed more ear posture changes in pigs in aversive situations such as social isolation combined with negative, unpredictable interventions, which could be an indication of a more negative affective state. In opposite to this, higher amounts of passive ear postures in sheep occurred more in positive than in negative situations (Reefmann et al., 2009). Thus, it is difficult to conclude whether the ears directed laterally in the present study estimate an exclusively positive affective state since due to the snapshot of the scan-sampling method, it cannot be unequivocally evaluated whether the ears directed laterally in this study are related to ear posture changes or rather passive ear postures. Both, presumably, could be able to indicate negative respectively positive affective states.

The indicators “HAT-approach latency” and “NOT-approach latency” of the behavioral tests could be realistic to assess the pigs’ affective state. According to Brown et al. (2009), quicker latencies to approach novel stimuli, such as unknown humans or novel objects, are associated with less fearful animals. Therefore, it could be imaginable that quicker approach latencies indicate pigs that possess a more positive affective state due to less fearfulness. However, quicker approach latencies could also be a reason for a stronger motivation to explore novel stimuli (Stolba and Wood-Gush, 1980) related to boredom in the housing environment. This probably indicates pigs with a more negative affective state, since then the pigs are possibly not

able to perform their natural living, which depicts an important part of good welfare (Fraser, 2008), as mentioned above. Based on these results and also due to the controversially discussed literature concerning the ears directed laterally as well as social play, it could also be assumed that the exogenous latent variable pigs’ positive affective state corresponds more to the general affective state than to the primarily positive one. Additionally, it seems quite understandable that according to the higher indicator reliability, the indicator “NOT-approach latency” could be more appropriate to estimate the pigs’ positive affective state than the indicator “HAT-approach latency” as the pigs’ behavior in human-approach tests can be influenced by their previous experiences with humans. There are studies that demonstrate that negative handling leads to more avoidance responses (Carreras et al., 2017) and Brajon et al. (2015) refer that the affective state of pigs can be affected by human handling. Hence, it could be conceivable that the pigs’ behavior in the NOT is better suited than the pigs’ behavior in the also but less suitable HAT to estimate the pigs’ positive affective state. However, it cannot be excluded that a habituation effect to the human in the HAT and novel object in the NOT could occur within the fattening period (Krugmann et al., 2019), which can influence the summarized results. This hypothesis is supported by the fact that Mieloch et al. (2020) detected different influencing effects on HAT and NOT and moreover by the study of Grimberg-Henrici et al. (2017) who proved that subtle changes in test settings of behavioral tests change the measured concepts of behavior. Due to this, in preliminary studies, individual models were carried out for each recording (beginning and end of fattening) and at each time the behavioral tests had the lowest rates and less influence in the affective state of the pigs (beginning: behavioral tests $R^2 = 0.048$; play behavior $R^2 = 0.712$; body language signals $R^2 = 0.778$; end of fattening: behavioral tests $R^2 = 0.117$; play behavior $R^2 = 0.668$; body language signals $R^2 = 0.684$). This shows that the habituation effect can be neglected in the present study. Presumably, in further studies, it would be useful to include further indicators of behavioral tests in the dataset, for instance, related observations between quick approach latencies or avoidance reactions, which could provide more detailed information on whether the pigs show quick approach latencies due to less fear or a high motivation to explore unknown humans or novel objects.

All values were above 0.6 for composite reliability, which indicates reliability in general (Henseler et al., 2009). Thus, all 4 latent variables were sufficiently measured by their respective indicators. Three out of the 4 measurement models reached the recommended threshold for AVE, which states that AVE should be above 0.5 (Henseler et al., 2009). The AVE for the exogenous latent variable pigs’ positive affective state was 0.43, which is close to the mentioned threshold. An explanation for the lower AVE could be the lower indicator reliabilities of the indicators “HAT-approach latency” ($IR = 0.48$), “NOT-approach latency” ($IR = 0.51$), and “total play” ($IR = 0.59$). Therefore, it could be possible that the pigs’ positive affective state is not sufficiently estimated by these indicators, which could also indicate an insufficient estimation of the whole exogenous latent variable of the pigs’ positive affective state. This, however, seems obvious because this study is a first approximation, which does not exist in the form yet and which lays the foundation for making the pigs’ affective state almost measurable and understandable in the future. Conclusively, as mentioned above, additional indicators are necessary to complement the estimation of the pigs’ positive affective state sufficiently. Also, Ringle (2004) advises modifying the measurement model when there are deficiencies in discriminant validity, indicated by AVE values below 0.5.

Assessment of the structural model (final SEM)

All 3 relationships between the exogenous and the endogenous latent variables showed statistical significance according to the bootstrapping method. Thus, the pigs' positive affective state influenced all 3 endogenous latent variables significantly. Moreover, the highest path coefficient between the pigs' positive affective state and playing behavior indicated that this was the most influenced parameter ($PC = 0.83$), followed by the body language signals ($PC = 0.79$) and behavioral tests ($PC = 0.62$). This is in line with expectations since also in previous literature playing behavior has been suggested as suitable to indicate good welfare and a positive affective state (Fagen, 1976; Lawrence, 1987; Boissy et al., 2007). The results of path coefficients also show that the body language signals such as curled-up tails respectively ears directed laterally as well as the pigs' type of behavior in the behavioral tests are influenced by the pigs' positive affective state. Hence, these results also suggest that playing behavior, the body language signals mentioned, and behavioral tests could be potential indicators of a realistic assessment of the pigs' positive affective state in practice. All other parameters that were eliminated in the quality assessment of the SEM, therefore, do not appear to be significantly influenced by the pigs' positive affective state and, therefore, do not appear to be suitable for capturing the pigs' positive affective state in practice. This seems to be comprehensible especially with regard to the elimination of the salivary parameters during the quality assessment of the model, because it is conceivable that the saliva of the pigs may be affected by many factors such as different ingredients in the feed, and especially the salivary IgA-content may rather be influenced by the health status of pigs and not by their positive affective state.

Concerning the coefficients of determination (R^2), the model explained moderate R^2 -values for the endogenous latent variables playing behavior (69.8%) and body language signals (62.7%) and an almost moderate R^2 -value for the endogenous latent variable behavioral tests (39.5%) (Henseiler et al., 2009; Hair et al., 2011). This proves that the pigs' positive affective state explains the variance of playing behavior and body language signals slightly better than the variance of the pigs' behavior in the behavioral tests and could imply that the former could be preferable to assess the pigs' positive affective state in practice. Additionally, it could be conceivable that behavioral tests are also influenced by the animals' personality traits (Boivin et al., 1992) and not only by the affective state. However, further indicators should be tested in the future and researched in other animal species to obtain a more detailed understanding of the affective state.

Comparison of the final SEM with the split-half scenario SEM

Since the results of the split-half scenario SEMs indicated similar tendencies of test criteria for reliability and validity as the final SEM does (Table 2), the model quality was assessed as preserved. This confirms the overall validity of the final SEM that was used to pursue the research issue of this study.

Concluding statement

The current study is one of the first research studies using the PLS-SEM method for the assessment of the pigs' particularly positive affective state and its possible influence on various behavioral and physiological parameters. This method seems to be suitable to analyze the influence of the pigs' positive affective state on behavioral and physiological parameters and to assess

the pigs' positive affective state in practice. However, this study which contributes to the understanding of the affective state in pigs, in general, depicts a first approximation to estimate the pigs' positive affective state using the PLS-SEM method.

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Conflict of interest

The authors declare that they have no competing interests.

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