



# Brief Report The Combination of Serum and Oral Fluid Cortisol Levels and Welfare Quality Protocol<sup>®</sup> for Assessment of Pig Welfare on Intensive Farms

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**Abstract:** Animal welfare is important; therefore, veterinarians and other animal welfare experts try to use different tools for pig welfare assessment. Several welfare protocols are available for pig welfare assessment, and one of the most used is Welfare Quality (WQ) protocol<sup>®</sup>. Elevated values of cortisol can be indicative of stress and, therefore, poor welfare. Our aim was to assess the correlation between serum cortisol levels from individual samples and oral fluid cortisol levels in group samples with the grades received for pig welfare using the WQ protocol<sup>®</sup>. Samples were taken at six different commercial pig farms. Animals were divided into age-dependent categories: 5 weeks old (w/o); 7 w/o; 9 w/o; 11 w/o weaners; fatteners; and breeding sows (10 pigs/category). Cortisol was determined in individual sera and group samples of oral fluid (OF), and was compared to values considered to be physiological. Based on WQ protocol<sup>®</sup> answers, five farms' welfare level was deemed acceptable, and one was enhanced. Four out of 29 sera and 5 out of 30 OF samples were considered physiological, while in most other samples it was elevated. The correlation between cortisol levels in sera, OF, and WQ protocol<sup>®</sup> scores was not statistically significant. The cortisol level in OF should be just one of the welfare indicators, i.e., alongside the WQ protocol<sup>®</sup> filled out by a welfare expert.

Keywords: pigs; commercial pig farm; cortisol; oral fluid; Welfare Quality (WQ) protocol<sup>®</sup>; stress

# 1. Introduction

Welfare of farm animals has gained in importance over the last few decades. Therefore, a need for different tools that enable experts to fairly grade welfare has been developed [1–3]. One of the most tested and widely accepted protocols is the Welfare Quality (WQ) protocol<sup>®</sup> for pigs (sows and piglets, growing and finishing pigs) [3], developed in 2009 in Lelystad, the Netherlands. Since then, the WQ protocol<sup>®</sup> value for welfare assessment has been reviewed several times [4–7]. The use of oral fluid (OF) as a sample for a variety of analyses has been gaining in popularity in recent decades [8]. In the detection of various pathogens, OF started receiving more diagnostic importance since 2010 [9], but the same cannot be said for cortisol, which was described as a possible marker of stress and poor pig welfare in the 1990s [10,11].

Cortisol is a steroid hormone normally released into the blood stream in relation to stress factors and low blood glucose levels in most mammals [12]. Cortisol concentrations are linked to circadian rhythm; concentrations are higher late at night and early in the morning, and lower in the afternoon and early night [13]. Serum cortisol levels from the blood stream are connected to the levels in the pig OF, as stated in the study by De Jong et al. [11]. However, their study did not confirm that pigs kept in poorer welfare conditions suffer from higher levels of cortisol compared to pigs housed in enriched pens; rather, the cortisol levels were linked to circadian rhythm and activities of animals kept



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**Copyright:** © 2023 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). in different housing conditions. Between different pig categories from the same farms, weaners tend to have higher cortisol levels than older pigs [14]. Since cortisol is not one of the substances often measured in clinical practice of farm animals, the information on reference values is scarce. Radostitis et al. [15] set the reference value for sera cortisol in pigs, excluding the category, from 27.5 to 31.8 ng/mL. There is more information available on levels of OF cortisol in pigs, but one key element affecting the value is also circadian rhythm, which should be taken into account during sampling [11,13,16]. The salivary cortisol level in pigs is 8.6–13.3% of the serum cortisol concentration [17]. Cook et al. [17] also suggest OF cortisol may be more appropriate as an indicator of stress, because most commercial immunoassay procedures measure bound plus free fractions in blood, whereas OF cortisol is essentially an ultrafiltrate of the free fraction in blood; as such, it is submitted to slower changes after short-lasting stress, i.e., nasal snaring. Physiologically, cortisol levels should fall slightly with age, which was proven in pigs [16], rodents [18], and humans [19]; however, the values and the dynamics of lowering cortisol with age can be severely altered when animals are under stress [11] or in depressed humans [20]. More recently, cortisol in pigs was measured in pig hair, where the concentration is the lowest (approximately 2 ng/g) [21], compared to serum or OF. Wiechers et al. [21] searched for the correlation between welfare and cortisol levels in hair by counting skin lesions in breeding sows. Although sows in loose-housing systems had lower, but not statistically significant, hair cortisol levels than sows in farrowing crates, Wiechers et al. also stated that measuring the level of cortisol has limitations as a single stressor; in this case, skin lesions may be affected by co-existing dominant stressors, such as farrowing or the presence of suckling piglets.

In our study, we wanted to assess the correlation between serum cortisol levels from individual samples and oral fluid cortisol levels in group samples from the same pigs of six different categories from six intensive pig operations with the grades these farms received for animal welfare assessed by veterinarians using the Welfare Quality (WQ) protocol<sup>®</sup>.

# 2. Materials and Methods

# 2.1. Farms and Animals

Samples were taken from six different commercial pig farms. Animals were divided into age-dependent categories at all farms: 5 weeks old (w/o); 7 w/o; 9 w/o; 11 w/o weaners; fatteners; and breeding sows. Farms are of different sizes, from 50 to 3000 breeding sows, but a similar intensive housing system is used on every farm: farrowing crates are used for pregnant sows during the suckling period and piglets are weaned 28 days after farrowing. Piglets always have ad libitum access to water and commercial prestarter feed after 14 days of age. Sows are artificially inseminated 5–7 days after weaning and are kept in individual stands until day 28 after artificial insemination. After a pregnancy check with ultrasound, sows are moved to groups of 10-20 pregnant sows. Sows are fed with commercial feed twice a day, manually or automatically. Weaners are weaned into groups of 20-30 pigs and have ad libitum access to commercial feed. Fatteners are regrouped at 12 weeks of age and are fed with commercial feed twice daily. On Farm 2, fatteners have access to the outdoor part of the building, and the outside part of the pen is fenced and has a combination of solid concrete and a slatted floor. The outdoor access under the same conditions (fenced area, solid concrete, and slatted floor) is also available for fatteners and gestating sows in group pens on Farm 6. Gestating sows have straw in their pens. Farms use commercial vaccines against most common swine pathogens such as porcine circovirus type 2, Erysipelothrix rhusiopathiae, Mycoplasma hyopneumoniae, Escherichia coli, and Clostridium perfringens type C, except Farm 3, where none of the vaccines are used. Pregnant sows are treated against internal and external parasites with the standard application of an antiparasitic 3 weeks before farrowing, except on Farm 3. On Farm 5, the porcine reproductive and respiratory syndrome is confirmed, and gilt acclimatization via natural exposure is used for reduction of clinical cases before entry into the breeding herd.

# 2.2. Sampling

Blood samples were taken as part of regular diagnostics on 6 farms that took part in the Slovenian Target research program (referred to the Slovenian abbreviation of CRP) named CRP V4-1604 (Animal welfare including health of poultry and pigs in conventional and alternative housing systems). In accordance with Directive 2010/63/EU of the European Parliament, the Council on the protection of animals used for scientific purposes, and Slovenian Animal Protection Law (Uradni list RS št. 38/2013 and 21/2018) regarding non-experimental clinical veterinary practices and practices not likely to cause pain, suffering, distress, or lasting harm equivalent to, or higher than, that caused by the introduction of a needle, this study was not considered to be an experiment on animals, and permission to experiment on animals from a competent authority is not required. The supporting document for this study is entitled Resolution: 5-5-2020/3 and was issued by the Committee for Animal Welfare of Veterinary faculty.

All samples were taken from animals that appeared clinically healthy (no visible apathy, locomotory discomfort, diarrhea, or respiratory symptoms). Groups of pigs from different categories were chosen randomly, unless the number of animals was limited by the size of the farm; in this case we chose the only available group of pigs of the same category on the farm. The sampling on farms was always performed in the morning, after the first feeding of the animals. Upon arrival to the facilities, OF samples were always collected before more invasive blood sampling. Three hundred and eighty-three samples were collected overall: 347 individual sera samples and 36 group OF samples. OF was collected by hanging out the cotton ropes provided in the IDEXX Oral Fluid Collection Kit. An Undyed-Cotton 3-Strand Twisted Rope was hung above an open spot in the middle of pens away from feed and drinking water for half an hour. Afterwards, the rope was removed, and OF was squeezed into sterile 50 mL screw-cap plastic containers. Ten individual blood samples were drawn from each group from the anterior vena cava; the number of individual blood samples was less than 10 in some farms, where groups of pigs from the same age were smaller. A group sample of OF was obtained from the same group of pigs from which blood was drawn (Table 1).

Farm No.	Pig Category	No. of Individual Blood Samples *	No. of Group OF Samples	Total No. of Samples Collected Per Farm
	5 w/o weaners	10	1	
	7 w/o weaners	10	1	
Γ 1	9 w/o weaners	10	1	( (
Farm 1	11 w/o weaners	10	1	66
	fatteners	10	1	
	breeding sows	10	1	
	5 w/o weaners	10	1	
	7 w/o weaners	10	1	
E O	9 w/o weaners	10	1	
Farm 2	11 w/o weaners	10	1	66
	fatteners	10	1	
	breeding sows	10	1	
	5 w/o weaners	10	1	
	7 w/o weaners	10	1	
<b>F</b> 2	9 w/o weaners	10	1	()
Farm 3	11 w/o weaners	8	1	63
	fatteners	9	1	
	breeding sows	10	1	

Table 1. Number of individual blood samples and group OF samples collected on each farm.

Farm No.	Pig Category	No. of Individual Blood Samples *	No. of Group OF Samples	Total No. of Samples Collected Per Farm
	5 w/o weaners	10	1	
	7 w/o weaners	10	1	
<b>F</b> (	9 w/o weaners	10	1	
Farm 4	11 w/o weaners	10	1	66
	fatteners	10	1	
	breeding sows	10	1	
	5 w/o weaners	10	1	
	7 w/o weaners	10	1	
	9 w/o weaners	10	1	
Farm 5	11 w/o weaners	10	1	66
	fatteners	10	1	
	breeding sows	10	1	
	5 w/o weaners	6	1	
	7 w/o weaners	8	1	
F (	9 w/o weaners	10	1	-
Farm 6	11 w/o weaners	10	1	56
	fatteners	6	1	
	breeding sows	10	1	
total		347	36	383

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Table 1. Cont.
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\* Number of individual blood samples drawn is less than 10 on smaller farms, where some groups of pig categories were smaller.

Samples were transported to the laboratory in a refrigerated box at 4 °C. Sera were centrifuged for 10 min at  $3000 \times g$  after formation of coagula. OF samples were centrifuged for 10 min at  $2000 \times g$ . All samples were stored individually in 20 mL sterile cryotubes at -70 °C.

# 2.3. Cortisol Level Determination

Serum cortisol concentrations were determined by a Demeditec solid phase ELISA kit (DEH 3388) while cortisol concentration in OF was performed by a Demeditec cortisol free in saliva ELISA kit (DES6611) following the original users' manual. The microtiter wells of the kits are coated with an anti-cortisol antibody. The unknown amount of cortisol in the sample competes with cortisol horseradish peroxidase conjugate for binding to the coated antibody. The absorbance was measured with a Multiskan FC microtiter plate photometer (Thermo Fisher Scientific, Waltham, MA, USA) at 450 nm. The calibration curve was derived based on standards. The amount of bound conjugate is inversely proportional to the concentration of cortisol in the sample. The detectable range is between 10 and 800 ng/mL for serum cortisol and 0.1 and 30 ng/mL for OF cortisol. Intra- and interassay coefficients of variations were 8.12% and 11.80%, respectively, for serum cortisol assay, and 10.28% and 12.36% for OF cortisol assay.

#### 2.4. Welfare Assessment

Animal welfare was assessed using the survey and observation points described in the publicly available Welfare Quality assessment protocol for pigs (sows and piglets, growing and finishing pigs) [3]. Welfare assessment by the WQ protocol<sup>®</sup> is based on 4 general welfare (WP) principles: good feed (WP 1), good housing (WP 2), good health (WP 3), and appropriate behavior (WP 4), which cover the following 12 welfare criteria: absence of prolonged hunger, absence of prolonged thirst, comfort around resting, thermal comfort, ease of movement, absence of injuries, absence of disease, absence of pain induced by managemental procedures, expression of social behaviors, expression of other behaviors, good human–animal relationship, and positive emotional state. Based on information

gained by the assessors (veterinarians), every WP is rated from 0 to 100 and, based on the combination of all them, each farm can be categorized into four welfare statuses: excellent (>80), enhanced (55–80), acceptable (20–55), not classified (<20), from the best to the worst result. The welfare assessors were always the same team of three veterinarians. Prior to the first welfare assessment, the team went through the protocol and used it for welfare assessment on a random farm for training and synchronization of observations and measurements. The assessors were independent, and no one from the team was a regular health care provider for the farms in the study.

#### 2.5. Statistic Analyses

Statistical analyses were performed using SPSS ver. 25 software (IBM, Chicago, IL, USA). The distribution of the data was evaluated using Shapiro and Wilk's test. The statistical significance of serum cortisol concentration of each animal category between the farms was evaluated using one-way ANOVA with the Bonferroni post-hoc test. A detailed description of how to assess WP values, total scores, and statistical evaluation of the results obtained with the WQ protocol is described in detail in the protocol [3]. The correlation between cortisol in serum and OF was estimated with Spearman, Pearson, and Kendall correlation tests. Statistical significance was defined as p < 0.05. The welfare score for farms was estimated based on the predetermined weight system in the WQ protocol<sup>®</sup>.

#### 3. Results

Cortisol levels were detected in each individual sera sample and the mean value was calculated and applied for the pig category on a farm. The lowest serum cortisol value was detected in 11 w/o weaners from Farm 5 (23.77 ng/mL), whereas the highest serum cortisol was detected in 5 w/o weaners from Farm 4 (68.52 ng/mL); the highest value was also the only value that was statistically significantly differentiated from the cluster of other pigs of the same age in other farms (p < 0.05). Cortisol levels detected in OF were like those in serum, but 5–20-fold lower. All WPs were graded from 0–100; only WP4 on Farm 4 was graded bellow 20 (the worst). Final welfare scores of six conventional farms were "acceptable", and Farm 1 was scored "enhanced" (detailed data with the questionnaires are listed in Table A1 in the Appendix A). All the data are presented in Table 2.

Table 2. Cortisol levels in sera and OF, and welfare scores assigned by the WQ Protocol<sup>®</sup>.

Farm No.	Pig Category	Cortisol, Serum [ng/mL] *	Cortisol, OF [ng/mL]	Serum:OF Ratio [%]	WP 1 **	WP 2 **	WP 3 **	WP 4 **	Welfare Score <sup>a</sup>
	5 w/o weaners	49.93	4.62	9.25					
	7 w/o weaners	51.56	4.68	9.08					
г 1	9 w/o weaners	39.74	4.01	10.09					
Farm 1	11 w/o weaners	53.67	2.26	4.21					
	fatteners	43.64	2.75	6.30	57.0	24.6	27.3	63.9	enhanced
	breeding sows	34.07	2.93	8.60					
	5 w/o weaners	44.99	4.79	10.65					
	7 w/o weaners	47.71	6.21	13.80					
г о	9 w/o weaners	53.77	4.54	8.44					
Farm 2	11 w/o weaners	55.73	3.74	6.71					
	fatteners	48.85	6.10	12.49	96.8	26.0	29.3	26.6	acceptable
	breeding sows	29.93	6.16	20.58					-
	5 w/o weaners	38.29	3.84	10.03					
	7 w/o weaners	38.94	5.6	14.38					
Farm 3	9 w/o weaners	47.28	2.89	6.11					
	11 w/o weaners	56.93	4.41	7.75					
	fatteners	44.43	4.61	10.38	41.3	25.1	50.5	37.4	acceptable
	breeding sows	35.15	4.19	11.92					-

Farm No.	Pig Category	Cortisol, Serum [ng/mL] *	Cortisol, OF [ng/mL]	Serum:OF Ratio [%]	WP 1 **	WP 2 **	WP 3 **	WP 4 **	Welfare Score <sup>a</sup>
	5 w/o weaners	68.52 ***	9.65	14.08					
	7 w/o weaners	36.01	7.48	20.77					
Г 4	9 w/o weaners	43.24	5.51	12.74					
Farm 4	11 w/o weaners	40.87	4.51	11.03					
	fatteners	41.02	5.54	13.51	81.0	22.5	32.1	11.3	acceptable
	breeding sows	31.96	1.71	5.35					
	5 w/o weaners	47.15	6.29	13.17					
	7 w/o weaners	31.47	3.33	10.58					
т <b>г</b>	9 w/o weaners	34.84	4.81	13.81					
Farm 5	11 w/o weaners	23.77	3.91	16.44					
	fatteners	44.47	4.14	9.31	87.5	24.8	20.2	24.2	acceptable
	breeding sows	32.74	2.99	9.13					-
	5 w/o weaners	29.30	2.79	9.52					
Farm 6	7 w/o weaners	46.08	5.11	9.02					
	9 w/o weaners	37.31	4.67	7.99					
	11 w/o weaners	55.67	5.49	9.86					
	fatteners	43.23	4.48	10.36	61.3	25.4	34.7	49.6	acceptable
	breeding sows	27.79	4.03	14.50					-

Table 2. Cont.

\* Cortisol value is a mean value of 10 individual sera samples from the group of pigs. All individual sera values are presented in Table A2 in the Appendix A. \*\* WP can be presented as the formula-calculated numeric value only for the category of fatteners. \*\*\* p < 0.05 for a significant difference from the same age category on other farms. <sup>a</sup> End welfare score is calculated only from the scores based on fatteners. WP—welfare principle. **bold** values are those within the reference range [27.5–31.8 ng/mL] in serum "Reprinted/adapted with permission from Ref. [15]. 2000, Radostitis"; reference values for OF are based on the proposal of 8.6–13.3% of value of cortisol in serum "Reprinted/adapted with permission from Ref. [17]. 1996, Cook"; setting the lowest and highest limits for OF at 2.37 and 4.23 ng/mL, respectively.

The correlation between cortisol levels in serum, OF, and WPs was not confirmed as being statistically significant (p < 0.05) for any of the WPs. The only notable exception was the correlation between the average serum cortisol value and WP2 estimated with the Pearson correlation coefficient (p = 0.049); the value was borderline, and the correlation was not confirmed with any of the other tests.

#### 4. Discussion

Cortisol is a hormone with wide-ranging affinity, although it is most well-known as a substance related to stress, especially in human-medicine-based literature. We aimed to assess the correlation between cortisol levels in serum and OF, and welfare, assessed with one of the tools for welfare evaluation. We evaluated the welfare situation on six conventional pig farms, where the living conditions for pigs are expected to be the furthest from the pigs' natural habitat. The total score was "acceptable" on all but one farm, where the score was enhanced". Although all farms were far from the "excellent" score, which was not expected in the beginning, it is positive that they obviously meet, at least, some satisfactory welfare standards for pigs. Therefore, it was also not surprising that there were almost no significantly different values from the baseline result of all the categories on a single farm, or between them; all rearing systems are similar regarding the welfare scored by the WQ protocol<sup>®</sup>. Our values were within the range of the results of the study of De Jong et al. [13], where authors undertook a detailed study assessing OF cortisol in enriched and barren environments. De Jong et al. [13] measured the highest OF cortisol level in 15 w/o pigs (8.0  $\pm$  2 ng/mL), whereas the highest value in this study was obtained in 5 w/o weaners on Farm 4 (9.65 ng/mL). This group also had the highest absolute mean sera cortisol levels, which were differentiated statistically from all other groups. A comparison of our sera values to those published in previous studies [15,17] suggests our results may be an indicator of abnormalities; the majority of our mean sera values (32 out of 36; 88.89%) and our OF values (27 out of 36; 75%) fell above or below the proposed physiological limit of 27.5–31.8 ng/mL [15]. This finding poses a significant question—is this the consequence

of a lack of studies on the reference values of cortisol in serum in pigs of different ages? Among other factors, cortisol levels are significantly age-dependent [11,16], which can also be partially observed in our results. For example, sows had the lowest average serum cortisol values on five out of six farms, compared to the five other categories, but statistically the value did not significantly differ from that of other age categories. In the case of cortisol levels in OF, the situation was unclear, with no statistically significant differences between age groups or even no visible pattern that would indicate the lowering of cortisol levels in OF with age. It is necessary that age is considered when cortisol is measured and, for future research, it would be useful to set the reference values accordingly. Other factors should be investigated in detail, including some additional testing for the setting of modern standard reference physiological values for sera cortisol in pigs, depending on category, farming system, breed, feeding regime, etc. Nonetheless, the cortisol values in both sera and OF were above the reference limit in most of our cases. This was not entirely unexpected. Pigs in intensive farm units are known to be kept according to only the minimal standards. Although all our farms were ranked as at least "acceptable" the by WQ protocol<sup>®</sup>, this is closer to being rated as "not classified" (in other words "unacceptable") than "excellent". There is thus huge room for improvement in welfare on these farms. Another important fact in cortisol level determination in sera is that blood drawing in field conditions is always stressful for pigs. For example, Cook et al. [17] collected blood via a permanent ear catheter and snared the pigs between both samplings (5 min window). Snaring resulted in an almost two-fold rise in serum in the first 5 min, whereas levels of OF stayed almost the same. Furthermore, the peak of cortisol was the highest 10 min after snaring; at that time, the relative cortisol values in serum and OF evened out. OF was mentioned as a possible sample for welfare assessment, although the results were not always very informative on their own [10-14]. Considering the OF sample is easy to collect, especially on conventional farms where pigs see ropes for chewing as a good enrichment material, we may suggest this approach for sample collection for cortisol testing. Collecting OF is less stressful and less costly for analysis of the whole group of pigs, and, due to physiological characteristics of cortisol in OF versus the serum, OF is strongly favored as the sample of choice for welfare estimation in field conditions. However, for OF cortisol levels to be useful as one of the parameters for determination of pig welfare, the physiological reference values should be set first for pigs of different ages. With the current version of the WQ protocol<sup>®</sup>, WPs can only be presented numerically for fatteners, which severely limits the estimation of the correlation between cortisol values in serum and OF and WP results. The three methods used for determination of the correlation did not show a significant correlation between cortisol values and welfare scores calculated with the protocol. There was a slight correlation between WP2 and serum cortisol with the Pearson test (p = 0.049), but this was not confirmed with other tests. Due to a very limited amount of data for comparison (six farms, sera, OF, only fatteners) and the obtained correlation coefficients, this study does not offer any results that indicate linear or monotonic correlation relationships. Another issue arising in this study that needs to be addressed is the usefulness of the WQ Protocol® itself. It was found to be very useful for testing these six farms. As it was previously used by different researchers [4,6,7], it is possible to find some reference points in the assessment of WPs. However, we encountered a problem when assessing welfare on farms of a smaller size. Moreover, one of the shortfalls is the lack of a weight-based scoring system for numeric evaluation of welfare in pig categories other than fatteners. Thus, a significant portion of the data cannot be compared using statistical methods of stronger significance. At present, no tool is available for completely objective and fast evaluation of animal welfare, although we found the WQ protocol to be helpful and useful. In our opinion, the WQ protocol in the hands of seasoned experts is more useful for welfare assessment than measuring cortisol, given that cortisol is one indicator of stress, but not the sole indicator, and that it has less of a pathological role than an essential physiological role.

# 5. Conclusions

Serum samples are not considered useful for welfare assessment via cortisol, especially in field conditions. Cortisol levels in OF can be a useful tool for welfare assessment, because the method of acquiring the sample is almost entirely stress-free. As several factors affect the cortisol levels, the value in OF should be just one of the welfare indicators. This approach can be used in combination with, or as an addition to, the WQ protocol<sup>®</sup> filled out by a welfare expert.

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**Institutional Review Board Statement:** Blood samples were taken as part of regular diagnostics on six farms participating in the study. In accordance with Directive 2010/63/EU of the European Parliament, the Council on the protection of animals used for scientific purposes, and Slovenian Animal Protection Law (Uradni list RS št. 38/2013 and 21/2018) regarding non-experimental clinical veterinary practices and practices not likely to cause pain, suffering distress or lasting harm equivalent to, or higher than, that caused by the introduction of a needle, this study was not considered to be an experiment on animals and any consent by a national ethics committee was deemed unnecessary. Verbal and written consent for participation in the study was obtained from farm owners/farm managers; this is stated in the document Resolution: 5-5-2020/3 issued by the Committee for Animal Welfare of Veterinary faculty.

Data Availability Statement: Not applicable.

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**Conflicts of Interest:** The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

#### Appendix A

Table A1. Sera cortisol levels of individual pigs [ng/mL].

	5 w/o Weaners	7 w/o Weaners	9 w/o Weaners	11 w/o Weaners	Fatteners	Breeding Sows
	31.27	44.68	39.21	46.39	49.20	46.46
	34.55	40.37	23.41	56.12	40.68	30.53
	36.34	61.63	24.59	59.97	36.49	36.39
	75.36	35.49	31.66	46.22	31.24	15.28
F 1	43.60	57.05	26.39	63.68	43.79	40.98
Farm 1	41.78	70.60	44.98	50.77	32.10	40.16
	54.60	54.19	56.22	50.93	52.50	29.03
	58.77	56.50	55.76	66.15	47.20	40.06
	57.74	69.69	43.78	44.75	58.07	37.33
	65.23	25.40	51.42	51.76	45.06	24.52

	5 w/o Weaners	7 w/o Weaners	9 w/o Weaners	11 w/o Weaners	Fatteners	Breeding Sow
	47.04	62.64	57.38	50.33	73.75	10.00
	33.38	39.51	48.91	64.63	50.28	14.80
	51.54	39.58	38.56	49.09	32.76	46.70
	37.37	35.85	38.78	39.03	41.97	47.91
F 0	53.59	65.02	63.33	62.75	58.20	43.54
Farm 2	75.37	44.43	52.93	35.84	87.81	23.80
	50.41	23.58	56.38	62.41	43.89	28.60
	67.06	69.62	49.08	47.15	23.12	10.00
	62.62	74.22	25.83	35.00	43.34	10.00
	78.89	83.28	18.76	30.92	39.17	63.98
	42.61	26.79	55.19	53.08	21.32	31.38
	40.75	21.66	54.40	50.69	31.68	40.03
	29.54	31.17	35.65	51.96	48.40	63.63
	45.28	10.41	51.02	60.68	38.43	19.23
	38.44	32.44	54.15	47.98	49.13	27.32
Farm 3	45.08	43.03	36.90	48.18	49.66	33.06
	22.97	55.27	45.72	85.90	62.03	26.37
	15.83	57.73	31.73	56.99	63.18	51.18
	45.93	43.77	53.90		36.06	32.15
	56.46	67.18	54.17			27.13
	53.08	19.89	49.03	26.44	18.61	23.95
	50.69	12.28	20.34	16.35	47.64	36.80
	51.96	16.03	38.59	25.56	29.94	32.87
	60.68	36.83	31.39	48.12	52.10	35.37
	47.98	20.72	42.88	41.24	38.33	25.37
Farm 4	48.18	54.52	38.80	36.01	71.27	32.24
	85.90	54.00	53.32	53.76	58.30	36.17
	56.99	58.14	52.27	59.15	43.54	28.28
	83.86	10.00	81.37	52.42	14.20	28.04
	145.85	77.70	24.36	49.67	36.25	40.56
	28.25	32.64	14.61	10	27.97	20.07
	16.22	15.06	38.29	10	47.57	34.38
	41.13	39.91	38.34	10	37.32	20.36
	38.10	10.36	17.40	29.59	31.74	29.87
	91.90	40.59	19.48	20.13	35.81	33.41
Farm 5	50.75	29.37	51.52	24.90	48.40	41.86
	46.97	34.02	26.75	17.80	38.58	46.68
	55.49	39.22	51.37	36.24	51.44	24.23
	52.88	36.50	56.88	33.78	53.27	44.15
	49.83	37.03	33.80	45.27	72.61	32.38
	26.91	34.35	33.90	24.22	45.70	11.57
	38.81	32.89	33.66	68.29	49.71	17.22
	32.83	35.89	10.00	44.94	33.22	26.18
	32.53	31.37	43.31	41.94	26.22	22.67
<b>-</b>	25.28	54.88	50.39	62.49	52.44	35.11
Farm 6	19.41	58.28	29.88	49.06	52.10	31.91
		70.23	55.83	64.47		32.21
		50.75	25.91	80.38		42.11
			32.22	45.66		48.90
			58.00	75.20		10.00

Table A1. Cont.

	Score WP1	Score WP2	Score WP3	Score WP4	No. of WP > 10	No. of WP > 20	No. of WP > 55	No. of WP > 80	Final Score
Farm 1	57.0	24.6	27.3	63.9	4	4	2	0	enhanced
Farm 2	96.8	26.0	29.3	26.6	4	4	1	1	acceptable
Farm 3	41.3	25.1	50.5	37.4	4	4	0	0	acceptable
Farm 4	81.0	22.5	32.1	11.3	4	3	1	1	acceptable
Farm 5	87.5	24.8	20.2	24.2	4	4	1	0	acceptable
Farm 6	61.3	25.4	34.7	49.6	4	4	1	0	acceptable

Table A2. Scoring of WP 1-4 and determination of final welfare score.

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