Final report - ViD project

Danish Centre for Animal Welfare

2017

1. Project title:

Administration of oral meloxicam to loose housed sows post-farrowing and the effect on cortisol level
(Dansk titel: Mindre smerterelateret stress ved smertelindring af løse, farende søer)

2. Project leader and project participants (title, name, address, phone, e-mail)**:**

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3. Popular Danish summary (max 250 words)**:**

Faring anses for at forårsage stress og være en smertefuld proces. Sammentrækninger af livmoderen efter faring samt livmodersammentrækninger i forbindelse med diegivning antages at føre til varierende grad af smerte.

Projektet havde til formål at mindske smerterelateret stress (målt som cortisol-niveau) hos løse, farende søer gennem smertelindring af soen umiddelbart efter faring. Endvidere blev det undersøgt, hvorvidt søer med faringsrelaterede problemer havde særlig gavn af smertestillende.

Der indgik i alt 435 løse søer i forsøget fordelt på tre besætninger. Søerne blev inddelt i to grupper og tildelt henholdsvis oral meloxicam og placebo. Fra 152 af disse søer blev der udtaget spytprøver kl 10, 13 og 16, hvori kortisolkoncentrationen blev målt. Ved hver prøvetagning blev soens reaktion på observeren noteret. Under forsøget blev der registreret en række faringsrelaterede risikofaktorer, såsom paritet og fødselshjælp og smerte forud for forsøgsstart blev vurderet ud fra en kort klinisk undersøgelse af søerne.

Der blev fundet en døgnvariation i kortisolkoncentrationen i spyt, hvor niveauet kl. 10 var signifikant højere end kl. 16. Der var ingen signifikant forskel på kortisolkoncentrationen i placebo og meloxicam-gruppen ved de tre udtagningstidspunkter. I de tilfælde hvor søerne var syge og landmanden tildelte behandling ud over forsøgsmedicin, havde søer i placebo-gruppen dog en signifikant højere kortisolkoncentration i spyt sammenlignet med søer i meloxicam-gruppen. Dette tyder på at tildeling af smertestillende i forbindelse med behandling af syge søer er vigtigt i forhold til at reducere stress og ubehag.

4. Popular English summary (max 250 words)**:**

Farrowing is considered a stressful and painful process. Contractions of the uterus after farrowing along with contractions in relation to lactation are assumed to cause pain of varying degrees.

The aim of the study was to reduce pain related stress (as measured by cortisol level) for loose housed farrowing sows by administration of analgesics shortly after farrowing. Moreover, it was investigated whether sows with farrowing related problems had special benefits from analgesic treatment.

A total of 435 loose housed sows from three herds were assigned to treatment with either meloxicam or placebo. From a subsample comprising 152 sows saliva samples were collected at three time points (10am, 1pm and 4pm) and analysed for cortisol concentration. At each sampling the agitation level of the sow was noted. A number of farrowing related risk factors, such as parity and obstetric aid were recorded and pain prior to trial start was evaluated based on a short clinical examination.

A diurnal variation in salivary cortisol concentration with a higher level in the morning (10am) compared to the afternoon (4pm) was found. No difference in salivary cortisol concentration was found when comparing the two treatment groups at the three time points. However, for sows that were diseased and treated by the farmer in addition to trial treatment, sows in the placebo group had a significantly higher salivary cortisol concentration compared to sows in the meloxicam

group. This finding suggests that administration of NSAIDs in connection with disease treatment has the potential to reduce disease related stress and discomfort.

5. Scientific summary of project purpose, methods, most important results and conclusion (max 500 words)**:**

Farrowing is considered a stressful and painful process and involution of the uterus post-farrowing along with lactation related uterine contractions are assumed to cause pain of varying severities.

The purpose of the study was to improve welfare for loose housed sows by reducing pain related stress post-farrowing. The objective was to compare salivary cortisol concentration at three different time points post-farrowing for two groups of loose housed farrowing sows given oral meloxicam and placebo, respectively. Further, farrowing related risk factors were taken into account.

A total of 435 loose housed sows from three Danish herds were randomly assigned to treatment with either a placebo or oral meloxicam in a double blinded study. Treatment was administered for two consecutive days where the first treatment was given as soon as possible after farrowing. From a subsample comprising 152 sows saliva samples were collected at three time points (10am, 1pm and 4pm) and analysed for cortisol concentration. All saliva samples were collected within the effect period of the second treatment. At each sampling the agitation level of the sow was noted. Risk factors related to farrowing, i.e. parity, obstetric aid, disease treatment and anorexia were recorded and pain prior to trial start was evaluated based on a short clinical examination.

A diurnal variation in salivary cortisol concentration with a significantly higher level in the morning (10am) compared to the afternoon (4pm) was found ($p<0.001$). No significant difference in salivary cortisol concentration was found when comparing the two treatment groups at the three time points. However, for sows that were diseased and treated by the farmer in addition to trial treatment, sows in the placebo group had a significantly higher salivary cortisol concentration compared to sows in the meloxicam group ($p=0.04$). This finding suggests that administration of NSAIDs in connection with disease treatment is of importance in relation to reduce disease related stress and discomfort.

6. Background for the project:

Parturition is associated with high levels of stress and a painful process in all species (Mainau and Manteca, 2011). In humans, involution of the uterus postpartum can cause pain in the first two to three days after giving birth. Moreover, breastfeeding causes contractions of the uterus which increases the pain (Deussen et al., 2011). Morton and Griffiths (1985) states that conditions causing pain in humans should be assumed to cause pain in animals as well. Therefore, it seems reasonable that involution of the uterus will cause pain in sows post-farrowing and presumably also affect lactation.

Stress or pain in pigs has been associated with increased cortisol level in some studies (Geverink et al., 1999; Smulders et al., 2006). Therefore, it was hypothesized that use of analgesics post-farrowing reduced pain related stress and thereby the cortisol level. However, the importance of taking diurnal variation into account has been stated (Ruis et al., 1997).

Parity and parturition difficulties affect pain caused by parturition (Mainau and Manteca, 2011). This is in accordance with a Danish study performed on crated sows that revealed that the effect of analgesics is influenced by certain risk factors, e.g. parity and obstetric aid (Jensen, 2013).

Therefore, information about relevant risk factors was collected during the ViD project in 2016 (Danish title:*"Lavere pattegrisedødelighed ved smertelindring af løse, farende søer"*). In the ViD project in 2016 pooled saliva samples (three samples per sow were pooled) were analysed. When pooling the samples it was not possible to take into account the sows reaction on the observer at each sampling. Further, it was not possible to compare specific time points (e.g. 10am) between the two treatment groups, thus taking diurnal variation into account. Therefore, analysis of single samples will allow for inclusion of more details.

7. Description of the project purpose, hypotheses and materials and methods:

Purpose, objective and hypothesis

The purpose of this study was to improve welfare for loose housed sows by reducing pain related stress post-farrowing.

The objective was to compare salivary cortisol concentration at three different time points post-farrowing for two groups of loose housed farrowing sows given oral meloxicam and placebo, respectively.

Further, the effect of farrowing related risk factors was taken into account.

It was hypothesised that administration of analgesics post-farrowing would reduce pain related stress and thereby the cortisol level.

Materials and methods

Study design, setting and sample size

The study was a randomized double blinded intervention study with parallel group design with two levels being treatment with oral meloxicam and placebo. The cortisol trial constituted a small part of this larger field trial.

The study protocol was approved by the Danish Medicines Agency prior to trial start.

The study was conducted in the period August to December 2016 on three commercial Danish sow farms (herd A, B and C) with loose housed sows in the farrowing unit. All sows included in the study were loose housed in the farrowing unit from insertion until weaning. Pen design varied between herds and sections, but all pens had solid concrete floor in the resting area and slatted dunging area. Pen size ranged from 6.3 m^2 to 7.7 m^2.

The study was conducted with minimal interference to normal procedures on farm, e.g. normal treatment protocols for diseased sows were followed. Treatment due to disease is denoted 'disease treatment' throughout the report (see also Table 7.1). However, farmers were encouraged to limit the use of analgesics for sows included in the trial when possible.

A total of 435 sows were included in the study (ViD project, 2016). Saliva for cortisol measures was collected from 152 sows. The sample in each herd was 18 sows in herd A, 42 in herd B and 92 in herd C.

Population, blinding and trial treatment

In each of the three herds sows were allocated to the two treatment groups by systematic random sampling based on ear tag numbers. Sows with odd numbers were allocated to the placebo group and sows with even numbers to the meloxicam group. In herd C one batch of sows were allocated to treatment groups by dividing the barn section in two where all sows on the left were given one treatment and all sows on the right the other treatment. The two halves of the section were identical with regard to ventilation, number of pens and pen design, location of alleyways and so forth. This was done due to management constrains.

To ensure a blinded study the sows were divided into a red and a blue group based on ear tag numbers. Sows with even ear tag numbers were allocated to the blue group and given a blue recording sheet. In the same way sows with odd numbers were allocated to the red group and given a red recording sheet. These colours matched the colours of the labels on the two different treatment bottles (meloxicam, blue and placebo, red). Besides the colours, the labels, treatment bottles and oral dosing syringes used for meloxicam and placebo were similar. The research assistants were not aware of the colour blinding code until after the trial ended. Hereby, the study was blinded to both the on-farm staff and the research assistants.

The sows were treated with either oral meloxicam (Metacam oral suspension, 15 mg/ml) or oral placebo for two consecutive days. Placebo was produced by Glostrup Pharmacy and consisted of a solution that matched Metacam in colour, consistency, taste and smell. The pharmacy was also responsible for the blinding code. The first treatment was given as soon as possible after farrowing

and the second treatment was administered 24 hours later. To make sure all sows received the correct dose regardless of appetite administration was directly in the mouth with a dosing syringe.

Only a subsample of sows was used for salivary cortisol measures and they were sampled from two batches in herd A and C and three batches in herd B. Saliva collection was performed by research assistants and because of that only sows that were at a specific point in time in the treatment period at the day of the visit were included in the sample. To ensure the full effect of treatment and avoid interfering with sows right after farrowing saliva samples were not collected until after the second treatment were administered. Further, it was ensured that all samples could be collected before the effect of treatment ceased after 48 hours (Figure 7.1). Saliva was collected at 10 am, 1 pm and 4 pm by use of a cotton swab and analysed for cortisol concentration at Daacro, Saliva lab Trier in Germany. Not all sows could be sampled at the exact time point, but saliva was collected as close to the time point as possible and alternate saliva collection between treatment groups was ensured.

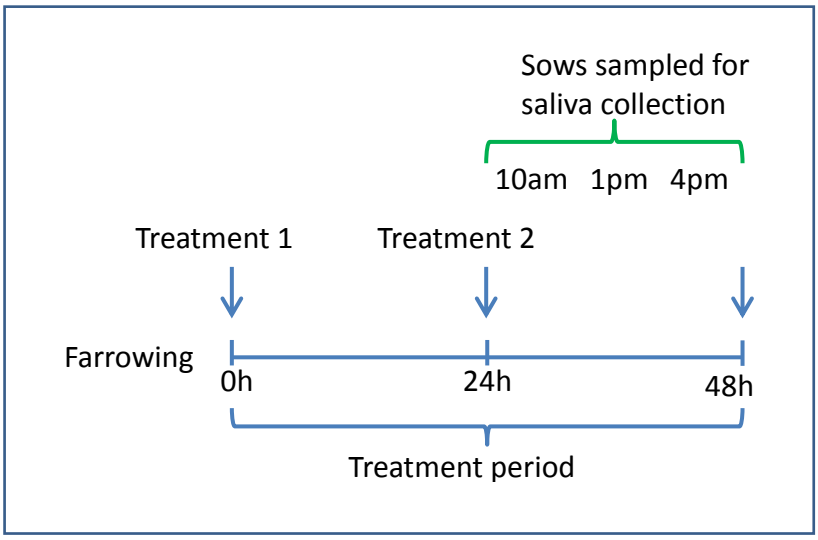

Figure 7.1: *Sampling of sows used for salivary cortisol measures. All sows were within the period from treatment 2 and the end of the treatment period at 48 hours. Saliva was collected at three time points (10am, 1pm and 4pm). Treatments 1 and 2 are the first and second administration of trial treatment.*

Variables

The outcome variable is salivary cortisol concentration (nmol/l) measured on a continuous scale. The variable was log transformed to obtain normality.

Explanatory variables are listed in Table 7.1. Recordings regarding anorexia, disease treatment, parity and obstetric aid were noted on the sow specific sheets by the farm staff. Anorexia was only recorded in herd A and B. Pain prior to trial start was based on a short clinical examination before the trial started. Based on the data from the clinical examination a simple set of rules was used to determine whether the sows should be placed in the pain or no-pain group.

Table 7.1: *Explanatory variables.*

Explanatory variable	Method
Sow id	The ear tag number was noted on the sow sheet.
Herd	The CHR number was noted on the sow sheet. The three herds were denoted A, B and C.
Anorexia	Anorexia was defined as: Not emptying the trough or does not get up on its own at feeding time. A date was noted on the sow sheet. The variable was dichotomized (y/n).
Disease treatment	Date and type of disease and treatment was noted on the sow sheet. Treatment included antibiotic, oxytocin and analgesic. The variable was dichotomized (treatment/no treatment).
Parity	Parity was noted. The variable was categorised into parity 1, 2, 3 and 4+. The grouping of higher parity sows was done to avoid too few sows in each category.
Obstetric aid	Obstetric aid was defined by vaginal exploration. It was noted on the sow sheet. The variable was dichotomized (y/n).
Pain prior to trial start	Pain prior to trial start was based on a short clinical examination before the trial started. The variable was dichotomized (y/n).
Agitation	Assessed at each saliva sampling. The categories were calm, slightly agitated and agitated
Sampling time	Saliva sampling took place at 10am, 1pm and 4pm.

Statistics

Statistical analyses were performed in R version 3.3.1.

The distribution of explanatory variables for included sows in the two treatment groups, i.e. parity, obstetric aid, pain prior to trial start, disease treatment and agitation will be presented in different ways depending on the type of variable. Parity will be presented as mean, minimum and maximum and for the remaining variables the prevalence will be calculated.

Salivary cortisol concentration in each treatment group at each time point will be presented as n, mean, median, maximum and minimum and first and third quartile. In addition, salivary cortisol concentration in each treatment group for explanatory variables will be presented by n, mean, median, maximum and minimum.

Cortisol concentration was log transformed to obtain normality before performing analytical statistics. A linear mixed model was used to analyse the data. Variables with a p-value ≤ 0.2 when tested individually were included in the model. Model reduction was based on backward elimination where AIC was used for exclusion of variables. In the reduced model interactions between fixed effects were included one by one and evaluated based on AIC. All excluded variables were tested for significance in the final model. Sow id was used as random effect and treatment group was kept in the model due to the study design.

Variables changing the estimate at least 20% when included in the final model were considered confounders. They were not included in the final model, but assessed by chi-square tests.

8. Overview of project results (including how the results contribute to fulfilling the purpose of the study)**:**

Population

Anorexia was only recorded on two farms and therefore excluded due to too many missing values. Further, it was decided to exclude all sows treated with additional analgesics (other than trial treatment) to avoid analgesic treated sows in the placebo group and thereby confusion with the effect of trial meloxicam. Number of sows treated with additional analgesic in the placebo and meloxicam group was 6 (3.9%) and 7 (4.6%), respectively (p=0.96). Based on this, 13 sows were excluded and the number of sows reduced to 139 ending up with the population shown in Table 8.1. The number of salivary cortisol samples was 411 out of 417 possible samples due to six missing values.

Table 8.1. *Characteristics for included sows in the two treatment groups. For parity mean, min and max is given. Categorical variables are displayed by n (%). A total of 139 sows were included.*n is number of observations*

		Placebo (n=71)	**Oral meloxicam (n=68)**	
	n (total)	Mean (min-max)	Mean (min-max)	P-value
Parity	132	3.0 (1 - 8)	3.1 (1-8)	0.75
		n (%)	n (%)	P-value
Obstetric aid	139	13 (9.4)	18 (12.9)	0.34
Pain prior to trial start	132	9 (6.8)	3 (2.3)	0.16
Disease treatment	139	21 (15.1)	22 (15.8)	0.86
Agitation*				0.99
Score 0	282	144 (37.9)	138 (36.3)	
Score 1	79	40 (10.5)	39 (10.3)	
Score 2	19	10 (2.6)	9 (2.4)	

Descriptive statistics

The salivary cortisol concentration for individual sows varied between time points in both treatment groups (Figure 8.1).

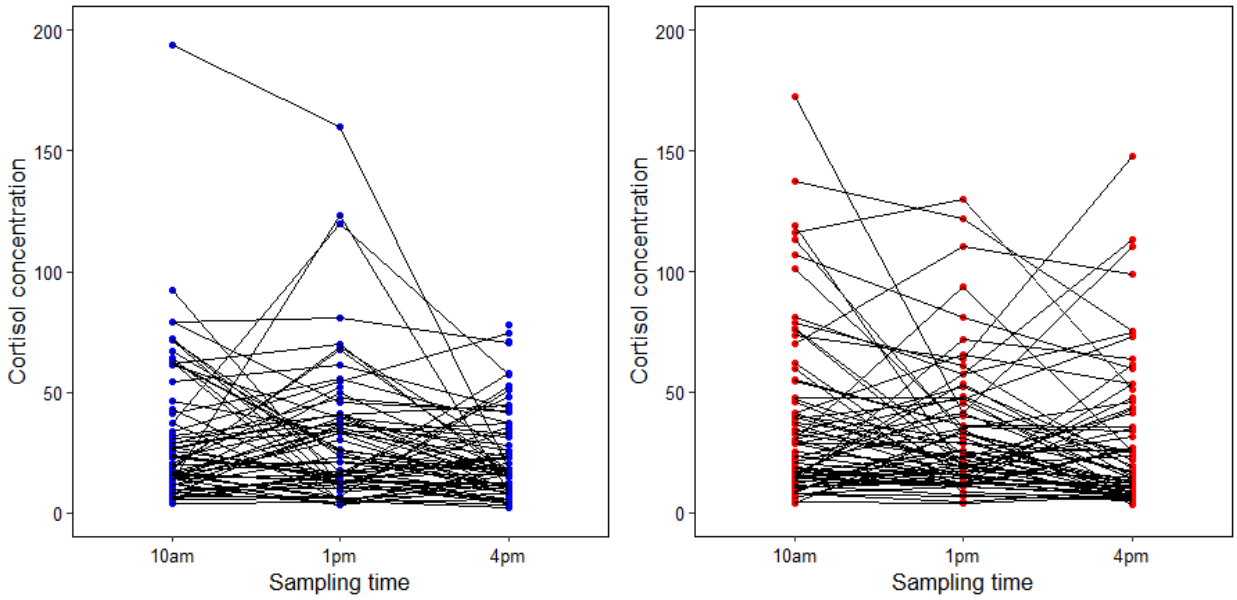

Figure 8.1: Salivary *cortisol concentrations for individual sows in the meloxicam group (blue) and placebo group (red). Measures from individual sows are connected by a line.*

For each time point variation in salivary cortisol concentration between sows was seen in both treatment groups (Table 8.2). The greatest variation was at 10am for the meloxicam group ranging from a minimum of 4.1 to a maximum of 193.8. The lowest range was seen at 4pm for the meloxicam group with a minimum of 2.0 to a maximum of 77.9. For each time point some variation between treatment groups was present although this was not consistent. At 10 am the median for the meloxicam group was lower (22.8) than the median for the placebo group (28.6) while at 1pm medians in the meloxicam group and placebo group were more similar being 23.3 and 23.9, respectively. At 4 pm the median was higher in the meloxicam group (17.9) than in the placebo group (14.9). Further, some variation between time points was seen. At 4 pm medians and means for both the placebo group and the meloxicam group were lower than medians and means for both treatment groups at 10am and 1pm.

Table 8.2. *Salivary cortisol concentration presented by n, median, min, max, mean, 1. quartile and 3. quartile at each time point (10am, 1pm and 4pm) for the two treatment groups.*

		Cortisol concentration						
Time	**Group**	**n**	**Min**	**1. quartile**	**Median**	**Mean**	**3. quartile**	**Max**
10am	Meloxicam	68	4.1	13.8	22.8	32.8	43.6	193.8
	Placebo	71	4.1	14.9	28.6	39.1	47.6	172.5
	All	139	4.1	14.2	24.1	36.0	47.2	193.8
1pm	Meloxicam	66	3.2	12.9	23.3	31.8	40.5	160.0
	Placebo	71	4.2	15.1	23.9	32.8	41.4	129.8
	All	137	3.2	13.2	23.6	32.3	41.1	160.0
4pm	Meloxicam	66	2.0	11.1	17.9	24.2	33.5	77.9
	Placebo	69	3.4	8.6	14.9	28.9	41.2	147.7
	All	135	2.0	9.2	17.4	26.6	35.3	147.7

When comparing the salivary cortisol concentration in the two treatment groups across time points for the different herds no significant differences were seen (Table 8.3). Likewise, no significant difference was found for parities, pain prior to trial start and agitation. There was a significant difference in salivary cortisol concentration between treatment groups ($p=0.004$) for sows that received treatment due to disease ('Dis. treat.') and a tendency ($p=0.07$) for sows where obstetric aid were performed. For both variables the mean and median cortisol concentration was higher in the placebo group compared to the meloxicam group.

Table 8.3. *Salivary cortisol concentration in each treatment group for explanatory variables presented by n (number of samples), mean, median, min and max. A total of 411 cortisol samples were included. *Pain prior to trial start.*

	Cortisol concentration										
	Placebo (n=211)					Oral meloxicam (n=200)					P-
Variable	**N**	**Mean**	**Median**	**Min**	**Max**	**n**	**Mean**	**Median**	**Min**	**Max**	**value**
Herd											
A	24	40.9	23.6	6.4	137.3	21	26.1	14.2	4.6	120.1	0.2
B	50	28.4	19.2	4.4	113.5	45	32.7	17.7	3.3	193.8	0.9
C	137	34.3	25.2	3.4	172.5	134	29.2	22.8	2.0	123.1	0.4
Parity											
Parity 1	51	28.6	18.5	4.2	129.8	38	33.4	28.0	3.3	92.1	0.1
Parity 2	51	37.4	21.1	4.4	137.3	48	30.3	23.8	3.2	123.1	0.5
Parity 3	39	35.8	22.8	5.7	118.9	24	37.0	16.6	4.0	193.8	0.4
Parity 4+	68	32.9	26.2	3.4	172.5	72	26.5	17.7	2.0	120.1	0.2
Obs. aid	39	38.8	33.6	7.5	147.7	54	29.4	22.0	2.0	92.1	0.07
Pain*	27	38.7	28.6	4.4	129.8	8/9	45.6	37.5	15.9	81.0	0.5
Dis. treat.	63	42.6	35.6	6.6	172.5	66	28.7	20.5	2.0	123.1	0.004
Agitation											
Score 0	144	32.9	19.8	4.1	172.5	137	28.7	19.8	3.2	193.8	0.5
Score 1	40	30.0	22.2	3.4	118.9	39	31.0	24.1	2.0	81.0	0.7
Score 2	10	53.1	34.3	7.8	137.3	9	31.7	16.9	7.4	68.8	0.2

Analytical statistics

The final model included treatment group, disease treatment, sampling time and the interaction between treatment group and disease treatment (Table 7.4). The salivary cortisol concentration was not significantly different in the two treatment groups. Likewise, no difference in salivary cortisol concentration was found between sows given disease treatment and sows were no disease treatment was administered. However, when disease treatment was administered sows in the placebo group had a significantly higher salivary cortisol concentration (p=0.036) compared to sows in the meloxicam group. Based on the model the salivary cortisol concentration (inverse log) was 38.47 nmol/l and 23.57 nmol/l, respectively. A graphical illustration of this interaction is shown in Figure 8.2. Salivary cortisol concentration at 4 pm was significantly lower (p<0.001) than the reference at 10. The salivary cortisol concentration (inverse log) was 17.46 nmol/l at 4 pm for sows in the meloxicam group not given disease treatment compared to 24.29 nmol/l at 10 am.

Table 8.4: *Results of the final model describing the explanatory variables of the log transformed salivary cortisol concentration.*

Variable	**Estimate**	**SE**	**P-value**
(Intercept)	3.19	0.12	-
Placebo	-0.07	0.15	0.64
Disease treatment, yes	-0.03	0.19	0.89
Sampling at 1 pm	-0.05	0.08	0.57
Sampling at 4 pm	-0.33	0.08	< 0.001
Placebo : Disease treatment, yes	0.56	0.26	0.036

Several excluded variables (obstetric aid, parity, herd and pain prior to trial start) were found to confound the variables disease treatment and treatment group (estimate change > 20%) in the final model when assessed one by one. However, none of the excluded variables were significant and due to the limited dataset the confounders were not included. The association between confounding variables (based on estimate changes) are shown in Table 8.5.

Table 8.5: *Associations based on a chi-square test between explanatory variables with estimate changes > 20 % in the final model. Explanatory variable 1 is the variable included in the final model. Explanatory variable 2 changed the estimate > 20% when included in the final model.*

Explanatory variable 1	Explanatory variable 2	P-value
Disease treatment	Pain	0,10
	Obstetric aid	< 0.001
	Parity	< 0.001
	Herd	< 0.001
Treatment group	Pain	0,003

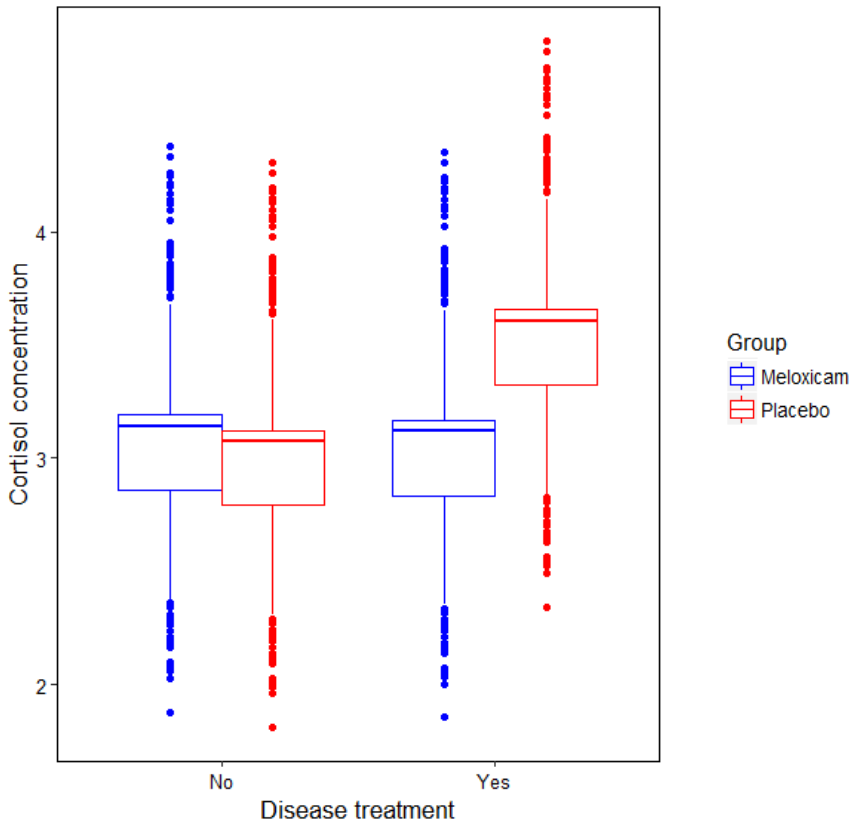

Figure 8.2: *Predicted cortisol concentration (log transformed) for the meloxicam group (blue) and placebo group (red) with and without administration of disease treatment.*

9. Discussion of the project results:

Bias and study limitation

In herd C one batch of sows was not assigned to treatment groups by systematic random sampling based on ear tag numbers, but all sows in the left side of the barn section was given one treatment and all sows in the right side the other treatment. However sows were randomly placed in the pens and the two halves of the barn were identical with regard to ventilation, number of pens and pen design, location of alleyways and so forth. Therefore, this is not expected to influence the result.

Sampling of cortisol sows was limited to the days where the research assistants were on farm which means that sows farrowing very early or very late were often not included. Although this applied to both treatment groups and therefore is not expected to influence the results, an even greater variation in cortisol concentration within each treatment group could have been expected.

Saliva samples could not be collected from all sows at the exact time point, but alternate sampling between treatment groups was ensured. However, this might result in slight changes in cortisol concentrations, which could influence the result regarding the diurnal variation. However, this is not expected to affect the result when comparing the two treatment groups because the groups are evenly affected.

The cortisol test was used as a measure of pain and stress, but since other factors can influence the cortisol level there is a risk of misclassification. Further, the accuracy of the cortisol test might affect the cortisol concentration. However, it can be assumed that the same measurement errors were introduced to both treatment groups.

Incorrect registrations of risk factors on the recording sheets, e.g. parity and disease treatment could lead to misclassification bias. In most cases, these errors will probably be random and evenly distributed between the two treatment groups.

Differences in management procedures between employees and farms together with general differences when handling the animals were not included in this study, but are likely to influence cortisol level. Potential differences regarding handling and other procedures among employees in the same herd are not expected to affect the results due to blinding and random sampling.

Several variables excluded from the model seemed to confound the variables disease treatment and treatment group in the final model. Pain prior to trial start seemed to confound both variables, but only pain prior to trial start and treatment group was significant when a Chi-square test was performed. Pain prior to trial start was not evenly distributed between the two treatment groups and only three sows had pain prior to trial start in the meloxicam group compared to nine in the placebo group. Therefore, the two treatment groups will not be evenly affected. A larger sample size would probably have resulted in an even distribution of explanatory variables in the two treatment groups. The frequency of sows that needed to be treated due to disease varied greatly between farms which is assumed to account for the confounding effect of herd. Further, disease treatment was more frequent for higher parity sows and for sows where obstetric aid was performed. Therefore, some of the effect of disease treatment on salivary cortisol concentration is explained by these variables.

Interpretation and generalisation

A diurnal variation with a higher cortisol concentration in the morning compared to the afternoon is well known (Ruis et al., 1997; Jarvis et al., 2006) and was also confirmed by this study.

No difference in cortisol concentration was found in the two treatment groups, even though the diurnal pattern was taken into consideration. Although cortisol level has been associated with pain or stress in some studies (Geverink et al., 1999; Smulders et al., 2006) it is debatable and several factors can influence the cortisol level.

Geverink et al. (1999) showed that shot biopsies (a method used to obtain samples of muscle tissue) in pigs resulted in a short-term rise in cortisol concentration. Another study was unable to show a difference in urinary cortisol between castrated piglets and controls (Hay et al., 2003). However, the first urine sample was not collected until 2.5 to 4 hours after castration and the authors concluded that a rise in cortisol following castration would either be minor or take place shortly after castration. Based on that, it seems that the stressor or pain has to be more constant or chronic to result in a long term rise in cortisol level. Therefore, measuring cortisol at only three time points may not be sufficient if short term rises are to be measured.

Some sows in the study probably experienced more chronic pain based on the clinical examination prior to trial start, although pain for the majority of sows was most likely caused by involution of the uterus and uterine contractions during lactation. Pain related to involution of the uterus varies between sows and not all sows will experience pain. Uterine contractions during lactation are considered to lead to increased pain, although the contractions will only be short term. Therefore, it seems likely that mainly short term rises in cortisol was present and more frequent saliva sampling would have been necessary to measure the possible short term rises.

Further, it was uncertain to what degree the different sows experienced pain and it is likely that variation was present, with sows in both groups being without pain. Sows without pain would probably not have any effect of meloxicam and therefore the cortisol level would be the same for these sows regardless of treatment group. A high number of sows experiencing no or limited pain would therefore erase the effect of meloxicam and thereby the difference in cortisol between the two groups.

A higher cortisol concentration was found for diseased sows given treatment without administration of meloxicam. This finding is supported by several studies where disease or inflammation causes an increase in cortisol concentration. Wang et al. (2006) showed that intramammary infusion of lipopolysaccharide resulted in an increase in serum cortisol concentration in lactating sows. Likewise, pigs infected with *Actinobacillus pleuropneumoniae* experienced a rise in serum cortisol compared to controls (Balaji et al., 2002). Löfstedt et al. (1983) showed an increase in cortisol 72h and 7 days after *Escherichia coli* induced mastitis in susceptible sows.

Based on the above mentioned studies, the lower cortisol concentration for diseased sows in the meloxicam group is probably due to the anti-inflammatory effect of NSAIDs, which at the same time reduces pain and discomfort in relation to disease further leading to reduced stress level. This emphasizes the importance of use of NSAIDs as part of disease treatment.

The findings of this study also suggest that cortisol level can be used to assess longer lasting moderate to severe stressors resulting in a more persistent cortisol rise, while short term discomfort probably needs cortisol measures at shorter intervals.

10. Conclusions and perspectives (including suggestions for follow up projects)**:**

Conclusion

A diurnal variation with higher cortisol concentrations in the morning compared to the afternoon was found.

No significant difference in salivary cortisol concentration was found between the two treatment groups at the three time points. However, when disease was present and disease treatment administered, sows in the placebo group had a significantly higher salivary cortisol concentration compared to sows in the meloxicam group. This finding suggest that use of NSAIDs in addition to treatment with antibiotics and other medicaments for diseased sows is of importance in relation to reduce stress and discomfort.

When comparing the results of the current study with the results from the ViD project of 2016, use of NSAIDs for both diseased and non-diseased sows experiencing discomfort seems advantageous. In the ViD project from 2016 significantly fewer sows had anorexia in the meloxicam group compared to the placebo group ($p=0.004$). All sows treated due to disease were excluded from the data analyses and therefore disease was not the cause of anorexia. More likely, anorexia was caused by pain which was emphasized by a tendency towards a higher risk of anorexia for sows where obstetric aid was performed ($RR=5.28$; $p=0.076$). Obstetric aid probably led to pain and discomfort. Therefore, the lower occurrence of anorexia in the meloxicam group was likely due to the pain reducing effect of meloxicam. However more research will be needed to fully clarify when the use of NSAIDs are beneficial.

11. Description of how the results from the project have been or will be published:

This project is an add-on to the ViD project from 2016 which was presented by a poster at the ViD conference in 2016. Further, the results were presented by a poster at SVEPM 2017, Inverness.

It is expected that the results of this project and the results from the ViD project in 2016 will be published in an international publication.

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