Universitat Autònoma de Barcelona

INDICADORES DE DOLOR EN CERDAS ALREDEDOR DEL PARTO. EFECTOS DEL MELOXICAM ORAL SOBRE LA TRANSFERENCIA INMUNITARIA, EL BIENESTAR Y LA PRODUCTIVIDAD EN CERDAS Y LECHONES

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Certifican:

Que la memoria titulada **"Indicadores de dolor en cerdas alrededor del parto. Efectos del meloxicam oral sobre la transferencia inmunitaria, el bienestar y la productividad en cerdas y lechones"**, presentada por **Elena Navarro Vives** con la finalidad de optar al Grado de Doctor en Veterinaria, ha sido realizada bajo su dirección y, considerándola finalizada, autorizan su presentación para que sea juzgada por la comisión correspondiente.

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Dr. Xavier Manteca Vilanova

Dra. Eva Mainau Brunso

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"Hasta que no hayas amado a un animal, una parte de tu alma permanecerá dormida."

Anatole France, (1844-1924)

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“Lo esencial es invisible a los ojos.”

El Principito, Antoine de Saint-Exupéry

(1900-1944)

RESUMEN

El parto es un proceso fisiológico doloroso y estresante para las hembras mamíferas. Diversos estudios realizados en la especie porcina evidencian los beneficios de la administración de un antinflamatorio no esteroideo (AINE) alrededor del parto para mejorar el bienestar y la producción de las cerdas. A pesar de esto, se desconocen los efectos de la administración del AINE meloxicam oral alrededor del parto sobre la transferencia inmunitaria de las inmunoglobulinas A (IgA) y las citoquinas. Hasta la fecha, en la especie porcina se han desarrollado varios indicadores de dolor en el parto, pero se desconoce si las expresiones faciales de las cerdas podrían ser un buen indicador de dolor en el parto.

Los objetivos generales de la presente tesis son: (1) evaluar los efectos de la administración de meloxicam oral (Metacam®) al inicio del parto en cerdas sobre el crecimiento de los lechones y la transferencia inmunitaria y (2) desarrollar una escala de dolor basada en las expresiones faciales de las cerdas, usando el parto como modelo de dolor fisiológico.

Se llevaron a cabo dos estudios (uno para cada uno de los objetivos generales). En el primer estudio, se evaluaron 24 cerdas Landrace x Duroc (12 cerdas control y 12 cerdas tratadas con meloxicam oral) el día del parto (día 0). Se obtuvieron muestras de saliva y calostro/leche de todas las cerdas, los días +1, +9 y +20 postparto, se pesaron todos los lechones los días 0, +9 y +20 y se tomaron muestras de sangre a 3-4 lechones de cada cerda los días +1, +9 y +20. En el segundo estudio, se grabaron en video el parto y el día +19 postparto de 21 cerdas Danbred, obteniendo un total de 263 imágenes. A partir de los videos y

las imágenes se estudiaron las expresiones faciales, los indicadores corporales y los cambios posturales realizados por las cerdas.

Los lechones de cerdas tratadas con meloxicam oral y con bajo peso al nacimiento tienden a presentar una mayor ganancia media diaria desde el día +9 hasta el destete en comparación con los lechones de las cerdas control. Los lechones de madres tratadas tienen una concentración más alta de IgA en el suero que los lechones de madres control los días +1 y +9. De la misma forma actuaron las interleucinas IL-4 e IL-2, mostrando una mayor concentración en el suero el día 9 postparto.

Las expresiones faciales en cerdas son un buen indicador de dolor alrededor del parto, y sirven para evaluar la presencia de dolor y para determinar la intensidad de éste. Los indicadores corporales y las expresiones faciales en cerdas muestran una alta correlación alrededor del parto. Cuanto mayor es el dolor sufrido, mayor es la frecuencia de ambos indicadores comportamentales. Antes del destete resultan casi inexistentes y su frecuencia e intensidad aumentan en el momento del parto, siendo máximas en el momento de la expulsión del lechón.

En definitiva, la administración de meloxicam oral a las cerdas al inicio del parto resulta ser beneficioso para los lechones y las expresiones faciales son un buen indicador de dolor alrededor del parto, coincidiendo con los indicadores corporales.

RESUM

El part és un procés fisiològic dolorós i estressant per a les femelles mamíferes. Estudis realitzats a l'espècie porcina evidencien els beneficis de l'administració d'un anti-inflamatori no esteroidal (AINE) al voltant del part per millorar el benestar i la producció de les truges. Tot i això, es desconeixen els efectes de l'administració de l'AINE meloxicam oral al voltant del part sobre la transferència immunitària d'immunoglobulines A (IgA) i les citocines. Fins ara, a l'espècie porcina s'han desenvolupat diversos indicadors de dolor al part, però es desconeix si les expressions facials de les truges podrien ser un bon indicador de dolor al part.

Els objectius generals de la present tesi són: (1) avaluar els efectes de l'administració de meloxicam oral (Metacam®) a l'inici del part en truges sobre el creixement dels garrins i la transferència immunitària i (2) desenvolupar una escala de dolor basada en les expressions facials de les truges, utilitzant el part com a model de dolor fisiològic.

Es van dur a terme dos estudis (un per a cadascun dels objectius generals). En el primer estudi, es van avaluar 24 truges Landrace x Duroc (12 truges control i 12 truges tractades amb meloxicam oral) el dia del part (dia 0). Es van obtenir mostres de saliva i calostre/llet de totes les truges, els dies +1, +9 i +20 postpart, es van pesar tots els garrins els dies 0, +9 i +20 i es van prendre mostres de sang a 3-4 garrins de cada truja els dies +1, +9 i +20. En el segon estudi, es van gravar en vídeo el part i el dia +19 postpart de 21 truges Danbred obtenint un total de 263 imatges. A partir dels vídeos i les imatges es van estudiar les

expressions facials, els indicadors corporals i els canvis posturals realitzats per les truges.

Els garrins de truges tractades amb meloxicam oral i amb un baix pes al naixement tendeixen a presentar un major guany mig diari des del dia +9 fins al deslletament en comparació amb els garrins de les truges control. Els garrins de mares tractades tenen una concentració més alta d'IgA al sèrum que els garrins de mares control els dies +1 i +9. De la mateixa manera van actuar les interleucines IL-4 i IL-2, mostrant una major concentració al sèrum el dia 9 postpart.

Les expressions facials en truges són un bon indicador de dolor al voltant del part, i serveixen per avaluar si hi ha dolor i per determinar-ne la intensitat. Els indicadors corporals i les expressions facials en truges mostren una alta correlació al voltant del part. Com més gran és el dolor patit, més alta és la freqüència dels dos indicadors de comportament. Abans del deslletament, aquets indicadors resulten gairebé inexistents i la seva freqüència i intensitat augmenten en el moment del part, i són màximes en el moment de l'expulsió del garrí.

En definitiva, l'administració de meloxicam oral a les truges a l’inici del part resulta ser beneficiós pels garrins i les expressions facials són un bon indicador de dolor al voltant del part, coincidint amb els indicadors corporals.

ABSTRACT

Parturition is a painful and stressful physiological process for female mammals. Several studies show the benefits of administering a non-steroidal anti-inflammatory drug (NSAID) around farrowing to improve the welfare and production of sows. However, the effects of the NSAID oral meloxicam administration around parturition on immune transfer of immunoglobulin A (IgA) and cytokines are unknown. To date, several indicators of pain at farrowing have been developed in swine, but it is unknown whether the facial expressions of sows could be a good indicator of farrowing pain.

The general objectives of this thesis are: (1) to evaluate the effects of the administration to sows of oral meloxicam (Metacam®) at the beginning of parturition on piglet growth and immune transfer and (2) to develop a pain scale based on facial expressions of sows, using parturition as a physiological pain model.

Two studies were carried out (one for each of the general objectives). In the first study, 24 Landrace x Duroc sows (12 control sows and 12 oral meloxicam-treated sows) were evaluated on the farrowing day (day 0). Saliva and colostrum/milk samples were obtained from all sows on days +1, +9 and +20 postpartum, all piglets were weighed on days 0, +9 and +20 and blood samples were taken from 3-4 piglets from each sow on days +1, +9 and +20. On the second study, 21 Danbred sows were filmed during all farrowing and day +19, obtaining a total of 263 images. Sow's facial expressions, body indicators and postural changes were studied from all the videos and the images obtained.

Piglets from sows treated with oral meloxicam and born with low body weight tended to have a higher average daily gain from day +9 to weaning than piglets from control sows. Piglets from treated sows have a higher serum IgA concentration than piglets from control sows on day+1 and +9. The interleukins IL-4 and IL-2 showed the same pattern, with a higher concentration in the serum on day 9 postpartum.

Facial expressions in sows are a good indicator of pain around farrowing and are useful to assess pain and to determine its intensity. Body indicators and facial expressions in sows show a high correlation around farrowing, and the frequency of both behavioural indicators increases with pain. Prior to weaning, pain indicators are almost non-existent, and their frequency and intensity increase during farrowing, being maximum at the moment of piglet expulsion.

In summary, the administration of oral meloxicam to sows at the beginning of farrowing is beneficial for piglets, and facial expressions are a good pain indicator around farrowing, coinciding with body indicators.

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CAPÍTULO 1. INTRODUCCIÓN GENERAL

Capítulo 1

1.1. Introducción

El dolor causado por el parto se encuentra entre los más intensos que un mamífero puede experimentar a lo largo de su vida. En la especie porcina, el parto y el post parto pueden considerarse momentos críticos, ya que, a pesar de que el parto es un proceso fisiológico, se han estudiado varios problemas productivos que se agravan a raíz de experimentar un parto excesivamente doloroso. Los cambios comportamentales en la cerda son frecuentes, alterando el tiempo que pasa descansando o bien el tiempo que emplea en una postura u otra. Estos cambios comportamentales, en parte, recaen directamente sobre los lechones, pudiéndose reflejar en un peor encalostrado, con una menor transferencia inmunitaria, y/o en un aumento del índice de aplastamiento de lechones.

En diversos estudios se ha demostrado que la administración de antiinflamatorios no esteroideos (AINEs) a las cerdas alrededor del parto alivia parcialmente el dolor sufrido y reduce sus efectos negativos. Por otra parte, se ha visto que la administración de antinflamatorios aumenta la cantidad de inmunoglobulinas G en el suero de los lechones.

1.2. Bienestar animal y percepción del dolor.

La ciencia del bienestar animal estudia el estado físico y mental de los animales, abarcando diversos aspectos importantes tales como el hambre, la sed, el dolor, la enfermedad y el estrés. Cabe destacar que el bienestar animal contempla la expresión de comportamientos propios de la especie, algunos de los cuales son necesarios para la salud física y mental de los animales. Uno de los objetivos principales del bienestar animal es evitar, en la medida de lo posible, que los animales sufran dolor (McLennan y col., 2018). Los procesos dolorosos suelen reducir el confort y causan estrés (Contreras-Aguilar y col., 2019), lo que no solo tiene efectos negativos sobre la salud mental y física de los animales, sino que puede conllevar problemas fisiológicos y productivos en el individuo que lo sufre (Von Borell, 1995).

La Asociación Internacional para el Estudio del Dolor (IASP), define el dolor como una experiencia sensorial y emocional desagradable asociada a un daño tisular real o potencial (Raja y col., 2020). El dolor tiene una función esencial para la supervivencia de los animales, ya que es un indicador de que algo nocivo está afectando al organismo (Gregory, 2004). El dolor se puede clasificar teniendo en cuenta diversos criterios:

1. En función de su localización: dolor somático (dolor bien localizado en piel, huesos o musculatura), dolor visceral (dolor difuso, localizado en órganos internos) y dolor neuropático (dolor causado por una lesión en el sistema nervioso).
2. En función de su naturaleza: dolor físico, provocado por un elemento dañino en contacto con la superficie corporal del animal, o dolor fisiológico, considerado un dolor natural, como es el caso del parto de las hembras mamíferas.
3. En función de la duración y de la inflamación asociada al dolor: dolor nociceptivo (dolor de corta duración, ya que, al cesar el elemento dañino, desaparece), dolor inflamatorio (dolor persistente que surge de un proceso inflamatorio causado por un daño tisular) y dolor crónico (dolor tisular que persiste a pesar de que el daño tisular haya desaparecido) (Herskin y col., 2018).

Está demostrado que un animal puede sentir dolor de forma consciente siempre que se cumplan los siguientes criterios: (1) el cerebro del animal debe tener estructuras análogas a las del cerebro humano, (2) debe poseer receptores sensibles a estímulos tónicos, (3) debe tener vías nerviosas que comuniquen estos receptores con el cerebro y, por último, la aplicación de analgésicos debe disminuir el dolor sufrido (Gregory, 2004). Por lo tanto, al cumplirse todos estos requerimientos en los animales mamíferos, se acepta que estos experimentan el componente afectivo del dolor (Morton y Griffiths, 1985, McLennan y col., 2018).

Los cerdos pueden sufrir dolor debido a varios motivos: durante manejos rutinarios tales como el corte de cola y la castración, cuando están enfermos o lesionados, y durante algunos procesos fisiológicos como sería el parto (Ison y col., 2016).

1.3. El parto, un proceso doloroso

En las mujeres, se ha estudiado y demostrado que el dolor del parto consta de dos elementos, uno sensorial y otro afectivo. En un estudio realizado con 2700 mujeres, el 50% de éstas describió el parto como una experiencia dolorosa o muy dolorosa. El 50% restante describió la experiencia como moderada o levemente dolorosa (Bonica, 1994). De hecho, el dolor del parto está considerado como uno de los dolores más intensos sufridos por una mujer a lo largo de su vida (Melzack, 1975). Se ha descrito que existe una gran similitud entre el dolor sufrido por las mujeres y por los animales en el momento del parto (Morton y Griffiths, 1985). Cabe decir que, como les ocurre a las mujeres, los partos distócicos pueden producir un dolor mucho más intenso que los partos eutócicos. En la especie porcina, se considera que un parto es distócico cuando se prolonga excesivamente en el tiempo y/o cuando es necesaria una extracción asistida de uno o varios lechones (Mee, 2004).

En el caso de partos distócicos en cerdas, Ison y col. (2014) reportó que los ganaderos y veterinarios otorgaban un valor de dolor de 7 y 8 respectivamente (sobre una escala de dolor de 10 donde 0 significaba indoloro y 10 dolor muy intenso), mientras que, en el caso de los partos eutócicos, la puntuación otorgada era de 3,8 y 4,5 respectivamente.

Mediante la combinación de indicadores productivos, fisiológicos y de comportamiento, actualmente podemos evaluar el dolor de las cerdas alrededor del parto (ver punto 1.7.).

1.4. Fisiología del parto en la cerda

A lo largo de la vida de una cerda, el parto es uno de los momentos más agotadores (Vallet y col., 2013). No sólo lo es el parto en sí, sino los días previos a éste. Durante el preparto, las cerdas se encargan de preparar el nido, y esto contribuye al agotamiento de sus reservas de energía (Feyera y col., 2018), lo que provoca que sean más propensas a la fatiga durante el parto (Van Kempen, 2007, Oliveira y col., 2020). En la naturaleza, las cerdas exhiben el comportamiento materno de la construcción de nidos alrededor de las 24 horas antes del inicio del parto, proporcionando comodidad y un ambiente térmico adecuado a los recién nacidos, además de proteger a los lechones contra posibles ataques de depredadores (Jensen, 1986; Wischner y col., 2009). A pesar de llevar miles de años domesticadas, en las granjas las cerdas siguen mostrando una gran motivación por construir su nido en el período anterior al parto, ya sea en condiciones seminaturales, con recursos disponibles para la construcción de nidos (Gustafsson y col., 1999; Jensen, 1986; Stolba y Wood-Gush, 1984) o en ambientes sin estímulos, como es el caso de las jaulas de parto convencionales (Lawrence y col., 1994 y Martins y col., 2021). El estrés causado en el preparto y la falta de estímulos pueden afectar negativamente al rendimiento reproductivo al aumentar la duración del parto y los intervalos entre nacimientos (Oliviero y col., 2008), pudiendo resultar en una mayor incidencia de mortinatos (Gu y col., 2011). Por otro lado, proporcionar materiales para la construcción de nidos se ha asociado con un mejor desarrollo inmunológico y un mayor rendimiento de la camada (Yun y col., 2014, 2015).

El proceso del parto se divide en tres fases distintas:

La primera fase comprende desde el inicio de las contracciones miometriales, que coincide con la dilatación del cérvix y la colocación del feto para su expulsión (Noakes y col., 2001). En las cerdas, la dilatación del cérvix se produce gradualmente durante el último tercio de gestación, acelerándose el proceso justo antes del parto (Taverne, 1992). La preparación del canal del parto se lleva a cabo inicialmente sin contracciones uterinas. Los estrógenos y la relaxina inducen la dilatación del cérvix mediante la modificación de la actividad del colágeno (Taverne, 1992). Las contracciones del miometrio varían entre individuos y, generalmente, su duración, frecuencia e intensidad son más regulares aproximadamente 12 horas antes de la segunda fase. En la primera fase se producen también los movimientos fetales, que se dan debido al incremento de la presión uterina causada por estas contracciones del miometrio (Noakes y col., 2001). Durante esta fase de dilatación, predomina el dolor visceral.

La segunda fase se caracteriza por la aparición de contracciones abdominales y la ruptura del saco alantocoriónico, y finaliza con la expulsión del feto. Esta fase incluye el ensanchamiento final del cuello uterino, que se produce gracias a las contracciones uterinas regulares durante el parto (Taverne, 1992). A medida que avanza el parto, la distensión del canal del parto provoca que la neurohipófisis libere una gran cantidad de oxitocina, y esto, a su vez, acentúa las contracciones miometriales (Noakes y col., 2001). Tanto en las cerdas como en las mujeres, el número de receptores de oxitocina en el útero es muy elevado, lo que explica la gran sensibilidad a la oxitocina exógena en esta etapa, en relación con la respuesta observada durante la gestación (Fuchs, 1987; Gorodeski y col., 1990). En esta fase predomina el dolor somático, que se caracteriza por ser un dolor localizado, que en el parto es debido a la distensión y la tracción sobre las estructuras pélvicas que rodean la vagina y, a su vez, a la distensión del suelo pélvico y el perineo.

La tercera fase incluye la expulsión de la placenta. La cerda presenta gestación múltiple, lo que conlleva la expulsión puntual de membranas fetales al expulsar los lechones, pero solo la expulsión de la última placenta es equivalente a la tercera fase en especies de gestación única (como la mujer). En esta fase, las contracciones del miometrio persisten, disminuyendo en amplitud, pero siendo cada vez más frecuentes y menos regulares (Noakes y col., 2001).

1.5. Factores que afectan al dolor producido por el parto

Hay diversos factores que pueden modificar la intensidad del dolor sufrido en el parto:

1. Paridad: Tradicionalmente se ha pensado que las cerdas primíparas tienen partos más dolorosos que las cerdas multíparas. Esto se debería en parte, a que los partos de las primíparas tienen una mayor duración y que el grado de esfuerzo asociado a éste suele ser mayor que en las multíparas (Noakes y col., 2001). No obstante, recientemente se ha sugerido que, si bien durante la fase inicial del parto las cerdas primíparas experimentan más dolor sensorial que las cerdas multíparas, a medida que avanza el parto estas diferencias serían menos pronunciadas. De hecho, algunos estudios recientes concluyen que las cerdas multíparas experimentan más dolor que las primíparas después del nacimiento del último lechón debido a la actividad uterina. En efecto, parece ser que las contracciones uterinas posparto (cuando el útero se contrae y vuelve al tamaño que tenía antes de la gestación) son más dolorosas para las cerdas multíparas en comparación con las primíparas, debido a la pérdida del tono uterino (Ison y col., 2018).
2. Duración del parto: El tiempo total del parto se considera desde la expulsión del primer lechón hasta la expulsión del último. La duración aproximada de un parto normal es de 2,5-3 horas (Baxter y col., 2018), considerándose que los partos que duran más de 3,5 horas - o más de 5-6 horas en el caso de las cerdas hiperprolíficas (Oliviero, 2010; Yun y col., 2019)- son más dolorosos y problemáticos (Lucia y col., 2002; Borges y col., 2005; Mainau y col., 2016). La duración del parto depende de la raza, de forma que el parto tiende a ser más largo en razas de gestación corta y razas con alta prolificidad que tienen camadas muy grandes y con una mayor proporción de mortinatos debido a asfixias en el canal del parto

(Van Dijk y col., 2005; Van Rens y van der Lende, 2004; Herpin y col., 1996). Recientemente, Thorsen y col. (2017) describió que en cerdas de raza Danbred, un parto normal puede llegar a durar 7 horas. La duración del parto también aumenta en cerdas con lechones de peso elevado al nacimiento (Van Rens y van der Lende, 2004).

3. Distocia: La distocia se puede definir como la dificultad del parto que resulta por un parto excesivamente largo (> 3,5 o > 5-6 horas en función de la raza; Lucia y col., 2002; Borges y col., 2005; Mainau y col., 2016; Oliviero, 2010) y que en ciertos casos puede necesitar de asistencia para extraer uno o varios de los lechones (Mee, 2004). Los partos distócicos se asocian con niveles de dolor inaceptablemente altos. En general, la tasa de distocia es mayor en las cerdas primíparas que en las multíparas (Nix y col., 1998).
4. Inadecuada presentación fetal: El efecto de la presentación anterior o posterior en el progreso del parto es un tema controvertido. En las cerdas, la presentación posterior de los lechones es relativamente común (entre el 25 y el 52%) (Herpin y col., 1996; Jones, 1966; Mainau y col., 2010). Algunos estudios relacionan la presentación posterior de los lechones con una mayor duración de la expulsión que en el caso de los lechones con presentación anterior (Dijk y col., 2005), así como una mayor probabilidad de nacer muertos, probablemente debido a un mayor riesgo de hipoxia en el canal del parto (Herpin y col., 1996). Mainau y col. (2010), relacionó una presentación anterior de los lechones al nacer con un aumento del tiempo total que la cerda permanece tumbada durante el parto, lo cual resulta ventajoso en términos de facilidad de parto.

5. Otros factores: Los defectos físicos en la vulva o el tracto reproductivo de la cerda y las malformaciones en los lechones pueden acabar complicando el parto y hacer que resulte más doloroso.

1.6. La importancia del calostro y la transferencia de inmunidad maternal

El calostro es una secreción mamaria compleja que se produce desde el momento del parto hasta las 24-36 horas posteriores al mismo (Devillers y col., 2004; Theil y col., 2014). Durante este periodo se habla de calostro temprano (0-12h), medio (12-24h) y tardío (24-36h). Desde las 36 horas a los 4 días postparto, se produce la leche transitoria, y posteriormente la leche madura, cuya composición se mantiene constante desde el día 10 hasta el final de la lactación (Hartmann y col., 1984; Csapó y col., 1996). La principal diferencia entre el calostro y la leche es la concentración de inmunoglobulinas maternales y de macronutrientes.

El calostro cumple con 3 funciones clave en los lechones:

1. El calostro es una fuente de energía que favorece la termorregulación y el crecimiento de los animales (Le Dividich y col., 2005; Quesnel y col., 2012; Theil y col., 2012). El nacimiento del lechón causa un descenso brusco de su temperatura corporal (Kammersgaard y col., 2011) y un cambio en el mecanismo de obtención de los nutrientes, pasando de la vía parenteral a través de la placenta a la vía enteral (Siggers y col., 2011). Además, los lechones nacen con un marcado déficit energético y menos del 2% de grasa corporal, y es por ello por lo que tienen grandes problemas para mantener su temperatura corporal (Noblet y col., 1997; Pastorelli y col., 2009; Theil y col., 2014).

Durante las primeras horas de vida, los animales obtienen energía a partir de los depósitos corporales de glucógeno y del calostro ingerido, y esta es una de las razones por las que una ingesta baja de calostro se asocia a altas mortalidades durante los primeros 3 días de vida (Tuchscherer y col., 2000). Cabe destacar que la selección de cerdas hiperprolíficas ha acentuado en gran medida el problema ya que, por un lado, los lechones nacen con pesos inferiores (Foxcroft y col., 2009) y eso limita su capacidad de ingerir calostro (Amdi y col., 2013) y, por otro lado, el número de pezones no aumenta en consonancia con el número de lechones, lo que aumenta la competitividad por ingerir calostro de la madre (Rutherford y col., 2013).

2. El calostro es un elemento clave en el desarrollo del sistema inmune de los lechones. Por un lado, el calostro proporciona inmunoglobulinas maternales, que confieren al lechón protección frente a diversos patógenos. Por otro lado, el calostro posee abundantes células inmunes, las cuales pasan a formar parte de la respuesta adaptativa de los lechones y potencian el desarrollo de su sistema inmune.
3. El calostro proporciona multitud de componentes bioactivos (factores de crecimiento, péptidos, oligosacáridos, hormonas y miRNAs) que favorecen el correcto desarrollo y crecimiento de órganos y tejidos, fundamentalmente del sistema digestivo. Además, el calostro posee relaxina, que es una hormona asociada a un correcto desarrollo del sistema reproductor femenino en la pubertad (Bartol y col., 2017).

La cantidad y composición del calostro depende de multitud de factores propios de la cerda (estado inmunitario, edad, raza, nivel de estrés, factores hormonales, alimentación), de los lechones (viabilidad tras el parto) y del ambiente (temperatura, humedad) (Quesnel y col., 2009; Theily col., 2014). La producción media de calostro puede variar mucho entre unos animales y otros, y se estima que cada cerda produce unos 3,5 - 6 kg/día de calostro (Quesnel y col., 2012; Vadmand y col., 2015). El efecto de la paridad está sujeto a cierta controversia. De forma global se observa que la producción de calostro es menor en cerdas primíparas, aumenta considerablemente en el segundo y tercer parto, y vuelve a disminuir a partir de entonces (Quesnel y col., 2009; Decaluwé y col., 2013; Boonraungrod y col., 2018). A pesar de ello, hay autores que indican que hay un aumento progresivo en la cantidad de calostro en partos sucesivos (Theil y col., 2012).

Una ingesta temprana y suficiente de calostro es crucial para la supervivencia de los lechones (Quesnel y col., 2012; Decaluwé y col., 2014). La cantidad media de calostro que debe ingerir un lechón se ha estimado en 180g/kg/día, lo cual se ha asociado con una reducción significativa del riesgo de mortalidad (Quesnel y col., 2012; Hasan y col., 2019). Además, una correcta ingesta de calostro se asocia a mayores pesos tanto al destete como al final del periodo de engorde (Declerck y col., 2016). La ingesta de calostro se correlaciona negativamente con el tamaño de camada y positivamente con el peso al nacimiento (Amdi y col., 2013). Se estima que el 35% de las cerdas no producen calostro suficiente para todos sus lechones, y a mayor número de lechones por camada, menor es la ingesta de calostro (Quesnel y col., 2012). Por lo tanto, cualquier estrategia de manejo que aumente la producción de calostro en las cerdas tendrá un impacto positivo en la supervivencia de los lechones (Theil y col., 2014).

1.6.1. El calostro como fuente de inmunidad pasiva

Los cerdos tienen una placentación epiteliocorial que es impermeable al paso de inmunoglobulinas y otras macromoléculas, y es por ello que los lechones nacen agammaglobulinémicos (es decir, sin gammaglobulinas o inmunoglobulinas) (Le Dividich y col., 2005; Salmon y col., 2009; Zanello y col., 2013). El calostro es el medio de transferencia de las inmunoglobulinas maternales, las cuales confieren protección al neonato durante sus primeras semanas de vida. Las inmunoglobulinas son absorbidas intactas a través del epitelio intestinal del lechón y pasan a la sangre, siendo éste muy permeable durante las primeras horas de vida y decreciendo la permeabilidad a medida que pasan las horas postparto.

El calostro temprano se produce principalmente antes del parto y contiene hasta un 75% de inmunoglobulina G (IgG) y un 20% de inmunoglobulina A (IgA), que son elementos primordiales de la respuesta inmunitaria humoral (Hurley y Theil, 2011). Después del parto, la concentración de IgG desciende drásticamente, mientras que la reducción de IgA es más gradual a lo largo de la lactación debido a su importancia en la regulación de la microbiota intestinal de los lechones, que es fundamental para la prevención de problemas digestivos (Theil y col., 2014). Una ingesta alta de inmunoglobulinas maternales se correlaciona con un menor número de enfermedades y una mayor supervivencia a lo largo de la etapa productiva (Le Dividich y col., 2005; Devillers y col., 2011). En partos de cerdas hiperprolíficas, alrededor del 20% de los lechones no han mamado pasadas las 6 horas del inicio del parto, lo que supone que un gran número de animales no toman calostro de calidad óptima (Oliviero y Peltoniemi, 2020).

Las inmunoglobulinas circulantes en la sangre de la madre son concentradas en el calostro. Para ello, son capturadas en la glándula mamaria a través de receptores-Fc, que se unen a la cadena pesada de las inmunoglobulinas (Hammer y col., 1978).

La ingesta de inmunoglobulinas por parte de los lechones dependerá fundamentalmente de dos parámetros: la cantidad de calostro ingerido y la concentración de inmunoglobulinas en el calostro. Por lo tanto, todas aquellas estrategias que tengan el objetivo de aumentar el contenido de inmunoglobulinas en el calostro serán beneficiosas para la supervivencia y el bienestar del lechón.

1.6.2. Influencia del calostro en el desarrollo de la inmunidad del lechón

Durante muchos años, el papel de inmunidad celular asociada al calostro ha pasado desapercibido. Sin embargo, el calostro contiene alrededor de 10^6 células / mL, de las cuales hasta un 25% son linfocitos (Le Jan y col., 2018).

La respuesta inmune esta mediada por las citoquinas, que son proteínas de bajo peso molecular con múltiples funciones. Las Interleuquinas (IL) son un tipo de citoquinas encargadas de regular las funciones de los linfocitos, tales como la activación, diferenciación, la producción de anticuerpos y la regulación de otras citoquinas y factores, entre otros. La IL-2, producida principalmente por los linfocitos T, induce la proliferación de linfocitos T y B, y la activación de células Natural Killer. La IL-4 desencadena la diferenciación de linfocitos T helper hacia el subconjunto Th2, que están relacionados con las respuestas humorales y antihelmínticas. El interferón Gamma (IFN-Ƴ) activa los macrófagos y provoca la diferenciación de los linfocitos T helper hacia el subconjunto Th1, lo que favorece la respuesta frente a microorganismos intracelulares (Abbas y col., 2018).

Las células inmunitarias cruzan la barrera intestinal del neonato de forma selectiva, llegan a la sangre y se distribuyen a los órganos del sistema inmunitario (Tuboly y col., 1998; Williams y col., 1993; Bandrick y col., 2014). Se desconoce con exactitud los mecanismos involucrados en la transferencia de células linfoides a través del intestino del neonato, aunque algunos estudios sugieren un mecanismo mediado por receptores específicos (Reber y col., 2006). Tras la ingesta de calostro, se observa un aumento de linfocitos γδ en la sangre de los lechones (Bandrick y col., 2014). Estos linfocitos se caracterizan por reconocer antígenos de forma independiente al complejo mayor de histocompatibilidad y se cree que pueden actuar como células presentadoras de antígenos, así como poseer capacidad Natural Killer (Bandes y col., 2005). El rol de la inmunidad celular ha quedado demostrado en estudios de inmunología in vitro, en los cuales los linfocitos y macrófagos sanguíneos de lechones respondían de forma específica frente a ciertos antígenos si sus madres habían sido vacunadas frente a ellos (Donovan y col., 2007; Bandrick y col., 2008). Un estudio de Bandrick y col. (2014) confirmó la capacidad de las células inmunitarias maternas presentes en el calostro de favorecer las respuestas inmunitaria innata y adaptativas en los lechones.

1.7. Indicadores de dolor en el parto de las cerdas.

Los cerdos, al igual que la mayoría de los animales de producción, se caracterizan por mostrar signos de dolor poco aparentes, lo que podría estar relacionado con el hecho de ser animales “presa” en condiciones naturales. (McLennan y col., 2018). Esto hace que identificar el dolor en la especie porcina sea en ocasiones difícil. Idealmente, el dolor en los animales debe estudiarse combinando diferentes tipos de indicadores: productivos, fisiológicos y de comportamiento. Los indicadores productivos posiblemente sean los más fáciles de cuantificar y por lo tanto suelen ser los más utilizados en estudios con animales de producción. Al contrario de lo que se piensa, en muchos casos, el dolor se puede cuantificar a través del estudio de indicadores productivos. Las cerdas que tienen partos muy largos o extremadamente dolorosos suelen tener un comportamiento maternal menos desarrollado (Thorsen y col., 2017). La alteración de la conducta maternal puede resultar en un aumento de la tasa de mortalidad neonatal y en una reducción de los episodios de amamantamiento pudiendo afectar al crecimiento de los lechones. A pesar de ello, los indicadores productivos no son específicos del dolor, ya que hay una gran cantidad de causas que pueden modificarlos (alimentación, instalaciones, manejo…).

Los indicadores fisiológicos carecen de especificidad cuando estudiamos el dolor en el parto. Se ha demostrado que, durante el parto, las cerdas aumentan la frecuencia cardíaca, la frecuencia respiratoria y la temperatura rectal debido al dolor experimentado (Noakes y col., 2001; Österlundh y col., 2002; Mainau y col., 2012). Pero existen otros factores, como el estrés o los cambios hormonales durante el parto, que pueden modificar estos parámetros haciéndolos inespecíficos, y ello puede sesgar la interpretación de los resultados.

Los indicadores de comportamiento son los que se consideran más válidos y específicos para el estudio del dolor en los animales de producción (Viñuela-Fernandez y col., 2007). Además, estos indicadores no son invasivos y no tienen por qué realizarse *in situ*, ya que pueden ser registrados o detectados automáticamente mediante el uso de sensores (Manteuffel, 2019; Zheng y col., 2020). Los indicadores de comportamiento que se utilizan para valorar el dolor causado por el parto en la cerda incluyen cambios de postura y movimientos antiálgicos.

- Cambios de postura. El dolor intenso debido a partos largos y/o distócicos se asocia a un aumento de los cambios de postura de las cerdas durante el parto (Ison y col., 2018; Yun y col., 2019). En consecuencia, el número de episodios de amamantamiento de los lechones disminuye (Baxter y col., 2018), contribuyendo a un aumento de la mortalidad neonatal (Wischner y col., 2010). Por lo contrario, un mayor número de cambios de postura 3 días después del parto se asocia a una mejor recuperación de la cerda, que consume más agua y, en consecuencia, produce más leche para sus lechones (Mainau y col., 2012).

- Movimientos antiálgicos. Los movimientos antiálgicos que muestran una buena correlación con el dolor que experimenta la cerda son: estirar la pata trasera de atrás hacia adelante presionando el abdomen, temblar, encorvar la espalda, dar patadas y mover la cola rápidamente (Ison y col., 2016).

En la última década se ha estudiado un nuevo indicador de comportamiento que resulta muy útil en varias especies: las escalas de dolor basadas en las expresiones faciales de los animales. Hasta la fecha estas escalas se han desarrollado en roedores (Langford y col., 2010; Sotocinal y col., 2011), conejos (Keating y col., 2012), ovejas (McLennan y col., 2016; Guesgen y col., 2016; Häger y col., 2017), caballos (Costa y col., 2014), hurones (Reijgwart y col., 2017) burros (Orth y col., 2020), focas (Macrae y col., 2018) y lechones

(Di Griminiani y col., 2016; Viscardi y col., 2017). Las expresiones faciales han resultado ser una herramienta útil no solo para identificar el dolor, sino también para evaluar su intensidad. Estas escalas de dolor, conocidas como 'Grimace scales' se basan en el estudio para diagnosticar cambios en diversas regiones faciales conocido como Facial Action Coding System (FACS), los cuales fueron descritos por primera vez en humanos por Ekman y Friesen (1978). El desarrollo y la aplicación de las escalas de expresiones faciales requieren la habilidad de identificar cambios en las regiones estudiadas conocidas como 'Facial Action Units' (FAUs) cuando los animales están experimentando dolor. En cada especie, y según su morfología, se pueden analizar y distinguir unas regiones u otras. Hasta la fecha sólo hay dos regiones que todas las especies comparten y que presentan una gran fiabilidad y repetibilidad entre los observadores (Viscardi y col., 2017). Estas regiones faciales son los ojos y el morro, siendo probablemente los ojos la región más fácil de reconocer y evaluar por parte de los evaluadores.

Hasta la fecha las expresiones faciales en cerdas no han sido estudiadas, pudiéndose tratar de una herramienta potencialmente útil para detectar el dolor en estos animales.

1.8. Tratamiento del dolor.

Como hemos comentado, controlar y aliviar el dolor de forma rápida en los animales es algo esencial para lograr que estos vivan bajo condiciones aceptables de bienestar. Cuanto antes administremos el tratamiento, menos tiempo sufrirá el animal. Existen distintos tipos de analgésicos destinados a aliviar el dolor en las cerdas. Los más utilizados son los antiinflamatorios no esteroideos (AINEs), los cuales son capaces de controlar el dolor, reduciendo la inflamación (Short, 1998). Los AINEs actúan inhibiendo la enzima ciclooxigenasa (COX) y previniendo la síntesis de prostaglandinas (Leslie y Petersson-Wolfe, 2012). La COX-1 está presente de forma natural en casi todos los tejidos del organismo y su expresión es particularmente alta en el tracto gastrointestinal, los riñones, las células endoteliales y las plaquetas. La COX-1 actúa principalmente sobre las funciones fisiológicas diarias, evitando efectos como la irritación gastrointestinal, la toxicidad renal y la inhibición de la coagulación sanguínea. La COX-2, por otro lado, es indetectable en condiciones basales en la mayoría de los tejidos y se expresa en respuesta a citoquinas proinflamatorias, promotores tumorales o factores de crecimiento (Gonzalez Perez y col., 2002). Por lo tanto, podemos decir que los AINEs son antiinflamatorios al inhibir la COX-2, pero a su vez pueden producir efectos indeseables en el tracto digestivo, los riñones o el sistema sanguíneo al inhibir la COX-1. La aparición de los efectos indeseables dependerá de la medida en que el AINE seleccionado actúa sobre la COX-1 (Tabla 1).

En la especie porcina es muy común el uso de AINEs como antiinflamatorio, siendo los tres más utilizados el flunixin meglumine, el ketoprofeno y el meloxicam. En las granjas, para tratar el dolor postparto en las cerdas, fundamentalmente se administran el ketoprofeno y el meloxicam (Ison y col., 2014).

Tabla 1: *Principales antiinflamatorios no esteroideos (AINEs) utilizados en porcino y ciclooxigenasa (COX) sobre la que tienen una acción preferente (Clària, 2001; Trimboli y col., 2020).*

Principio activo	**Inhibición COX**
Flunixin Meglumine	COX-1
Meloxicam	COX-2
Ketoprofeno	COX-1

Varios estudios describen que el ketoprofeno es un inhibidor preferente de la COX-1 y su administración en cerdas alrededor del parto reduce significativamente la mortalidad de los lechones (Homedes y col., 2014). Además, acelera la recuperación postparto de la cerda, aumentando la ingestión de alimento y la actividad a las pocas horas de parir (Viitasari y col., 2013; Tummaruk y Sang-Gassanee, 2013).

Por otro lado, el meloxicam es un inhibidor preferente de la COX-2 (Clària, 2001; Beretta y col., 2005) y tiene efectos antinflamatorios, analgésicos y antipiréticos (Clària, 2001; Friton y col., 2006). Una característica deseable del meloxicam es que presenta un perfil más seguro que otros AINEs, ya que inhibe en menor medida la COX-1 y, por lo tanto, tiene pocos los efectos adversos sobre el estómago y los riñones (Pairet y col., 1996; Clària, 2001). El meloxicam es el AINE de elección para los problemas respiratorios en cerdos de engorde (Georgoulakis y col., 2006) y para los problemas no infecciosos del aparato locomotor en todas las fases del ciclo de producción del cerdo, reduciendo notablemente los signos de cojera e inflamación (Friton y col., 2003; Conte y col., 2015). En cuanto a las cerdas, el uso de meloxicam junto con el antibiótico indicado resulta ser un tratamiento muy efectivo para curar el síndrome de mamitis-metritis-agalactia (Hirsch y col., 2003).

El meloxicam, administrado a cerdas sanas, tiene efectos sobre su bienestar, sobre el crecimiento de los lechones y sobre la transferencia de inmunidad. Mainau y col. (2012) y Tenbergen y col. (2014) describieron una mejor recuperación a las pocas horas post parto en las cerdas a las cuales se les había administrado meloxicam en comparación con las cerdas control. Tras dicha administración de meloxicam, Mainau y col. (2012) no encontraron diferencias significativas en la supervivencia de los lechones, pero sí en el crecimiento de los lechones con bajo peso corporal al nacimiento.

Pocos estudios han analizado los efectos de los AINEs sobre la transferencia de inmunidad maternofilial en la especie porcina (Mainau y col., 2016; Ison y col., 2017; Kuller y col., 2021). Estos estudios evalúan la transferencia de inmunidad pasiva a través del calostro y el desarrollo del sistema inmunológico en lechones midiendo la concentración de IgG en el suero de los lechones. Mainau y col. (2016) demostraron que la administración de meloxicam por vía oral al inicio del parto en cerdas multíparas aumentaba la concentración de IgG en el suero de los lechones y mejoraba su crecimiento antes del destete. En cambio, Ison y col., (2017) no encontraron diferencias tras administrar ketoprofeno 90 minutos después de la expulsión del último lechón. El uso de diferentes principios activos y el momento de administración podrían explicar en parte estas discrepancias. Finalmente, Kuller y col. (2021) administraron paracetamol el día del parto encontrando una menor variabilidad individual entre lechones en la concentración de IgG en sangre.

Así pues, hasta la fecha se ha estudiado el efecto de la administración de AINEs alrededor del parto sobre la concentración de IgG en los lechones. A pesar de ello, no hay estudios sobre el efecto de la concentración de IgG en el calostro o la leche de la cerda. Además, se desconoce el efecto de la administración de AINEs alrededor del parto sobre otros factores inmunológicos relevantes del calostro, tales como las IgA o las citoquinas.

1.9. Referencias

Abbas, A.K., Trotta, E., R. Simeonov, D., Marson, A., Bluestone, J.A. 2018. Revisiting IL-2: Biology and therapeutic prospects. Science immunology 3, eaat1482.

Amdi, C., Krogh, U., Flummer, C., Oksbjerg, N., Hansen, C.F., Theil, P.K. 2013. Intrauterine growth restricted piglets defined by their head shape ingest insufficient amounts of colostrum. Journal of Animal Science 91, 5605–5613.

Bandes, M., Willimann, K., Moser, B. 2005. Professional antigen-presentation function by human γδ T cells. Science 309, 264–268.

Bandrick, M., Pieters, M., Pijoan, C., Molitor, T.W. 2008. Passive transfer of maternal Mycoplasma hyopneumoniae-specific cellular immunity to piglets. Clinical and Vaccine Immunology 15, 540–543.

Bandrick, M., Ariza-Nieto, C., Baidoo, S.K., Molitor, T.W. 2014. Colostral antibody-mediated and cell-mediated immunity contributes to innate and antigen-specific immunity in piglets. Developmental & Comparative Immunology 43, 114-120.

Bartol, F.F., Wiley, A.A., George, A.E., Miller, D.J., Bagnell, C.A. 2017. Post-natal reproductive development and the lactocrine hypothesis. Journal of Animal Science 95, 2200–2210.

Baxter, E.M., Andersen, I.L., Edwards, S.A. 2018. Sow welfare in the farrowing crate and alternatives, in: Špinka, M. (Eds.), Advances in pig welfare. Woodhead Publishing, Cambridge. Chapter 2, 27-72.

Beretta, C., Garavaglia, G., Cavalli, M. 2005. COX-1 and COX-2 inhibition in horse blood by phenylbutazone, flunixin, carprofen and meloxicam: an in vitro analysis. Pharmacological research 52, 302-306.

Bonica, J.J., 1994. Textbook of Pain, 5th ed. Churchill Livingstone, London UK.

Boonraungrod, N., Sutthiya, N., Kumwan, P., Tossakui, P., Nuntapaitoon, M., Muns, R., Tummaruk, P. 2018. Control of parturition in swine using PGF2α in combination with carbetocin. Livestock Science 214, 1-8.

Borges, V.F., Bernardi, M.L., Bortolozzo, F.P., Wentz, I. 2005. Risk factors for stillbirth and fetal mummification in four Brazilian swine herds. Preventive Veterinary Medicine 70, 165-176.

Clària, J. 2001. El empleo racional de los nuevos antiinflamatorios. Medicina Integral 38, 167-17.

Conte, S., Bergeron, R., Gonyou, H., Brown, J., Rioja-Lang, F.C., Connor, M.L. 2015. Use of an analgesic to identify pain-related indicators of lameness in sows. Livestock Science 180, 203–208.

Contreras-Aguilar, M.D., Escribano, D., Martínez-Subiela, S., Martínez-Miró, S., Cerón, J.J., Tecles, F. 2018. Changes in alpha-amylase activity, concentration and isoforms in pigs after an experimental acute stress model: an exploratory study. BMC. Veterinary Research 14, 256.

Costa, E.D., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M.C. 2014. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. PLoS ONE 9, e92281.

Csapó, J., Martin, T.G., Csapó-Kiss, Z.S., Házas, Z. 1996. Protein, fats, vitamin and mineral concentrations in porcine colostrum and milk from parturition to 60 days. International Dairy Journal 6, 881–902.

Decaluwé, R., Maes, D., Declerck, I., Cools, A., Wuyts, B., De Smet, S., Janssens, G.P.J. 2013. Changes in back fat thickness during late gestation predict colostrum yield in sows. Animal 7, 1999-2007.

Decaluwé, R., Maes, D., Wuyts, B., Cools, A., Piepers, S., Janssens, G.P.J. 2014. Piglets' colostrum intake associates with daily weight gain and survival until weaning. Livestock Science 162, 185–192.

Declerck, I., Dewulf, J., Sarrazin, S., Maes, D. 2016. Long-term effects of colostrum intake in piglet performance and mortality. Journal of Animal Science 94,1633–1643.

Devillers, N., van Milgen, J., Prunier, A., Le Dividich, J. 2004. Estimation of colostrum intake in the neonatal pig. Animal Science 78, 305–313.

Devillers, N., Le Dividich, J., Prunier, A. 2011. Influence of colostrum intake on piglet survival and immunity. Animal 5, 1605–1612.

Di Giminiani, P., Brierley, V.L.M.H., Scollo, A., Gottardo, F., Malcolm, E.M., Edwards, S.A., Leach, M.C. 2016. The Assessment of Facial Expressions in Piglets Undergoing Tail Docking and Castration: Toward the Development of the Piglet Grimace Scale. Frontiers in Veterinary Science 3, 100.

Donovan, D.C., Reber, A.J., Gabbard, J.D., Aceves-Avila, M., Galland, K.L., Holbert, K.A., Ely, L.O., Hurley, D.J. 2007. Effects of maternal cells transferred with colostrum on cellular responses to pathogen antigens in neonatal calves. American Journal of Veterinary Research 68, 778–782.

Feyera, T., Pedersen, T.F., Krogh, U., Foldager, L., Theil, P.K. 2018. Impact of sow energy status during farrowing on farrowing kinetics, frequency of stillborn piglets, and farrowing assistance. Journal of animal science 96, 2320-2331.

Foxcroft, G.R., Dixon, W.T., Dyck, M.K., Novak, S., Harding, J.C., Almeida, F.C. 2009. Prenatal programming of postnatal development in the pig. Society for Reproduction and Fertililty Supplement 66, 213–231.

Friton, G.M., Philipp, H., Schneider, T., Kleemann, R. 2003. Investigation on the clinical efficacy and safety of meloxicam (Metacam) in the treatment of non-infectious locomotor disorders in pigs. Berliner und Munchener Tierarztliche Wochenschrift 116, 421-426.

Friton, G.M., Schmidt, H., Schrödl, W. 2006. Clinical and anti-inflammatory effects of treating endotoxin-challenged pigs with meloxicam. Veterinary record 159, 552-557.

Fuchs, A.R. 1987. Prostaglandin F2 alpha and oxytocin interactions in ovarian and uterine function. The Journal of Steroid Biochemistry and Molecular Biology 27, 1073–1080.

Georgoulakis, I.E., Petridou, E., Filiousis, G., Alexopoulos, C., Kyriakis, S.C., Papatsas, I. 2006. Meloxicam as adjunctive therapy in treatment and control of porcine respiratory disease complex in growing pigs. Journal of Swine Health and Production 14, 253-257.

González Pérez, R., Poza Guedes, P., Vives, R., Canto, G. 2002. Antinflamatorios inhibidores selectivos de la cicloxigenasa-2 (COX-2). Alergologia e Inmunologia Clinica 17, 247-254.

Gorodeski, G.I., Sheean, L.A., Utian, W.H. 1990. Myometrial oxytocin receptors levels in the pregnant rat higher in distal than in proximal portions of the horn and correlate with disparate oxytocin responsive myometrial contractility in these segments. Endocrinology 127, 1136–1143.

Gregory, N.G. 2004. Physiology and behaviour of animal suffering, third ed. Blackwell Science, Universities Federation for Animal Welfare, Oxford, UK.

Gu, Z., Gao, Y., Lin, B., Zhong, Z., Liu, Z., Wang, C., Li, B. 2011. Impacts of a freedom farrowing pen design on sow behaviours and performance. Preventive Veterinary Medicine 102, 296-303.

Guesgen, M., Beausoleil, N., Leach, M., Minot, E., Stewart, M., Staford, K. 2016. Coding and quantification of a facial expression for pain in lambs. Behavioural Processes 132, 49–56.

Gustafsson, M., Jensen, P., de Jonge, F.H., Illmann, G., Spinka, M. 1999. Maternal behaviour of domestic sows and crosses between domestic sows and wild boar. Applied Animal Behaviour Science 65, 29-42.

Häger, C., Biernot, S., Buettner, M., Glage, S., Keubler, L.M., Held, N., Bleich, E.M., Otto, K., Müller, C.W., Decker, S. 2017. The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. PLoS ONE 12, e0175839.

Hammer, D.K., Mossmann, H. 1978. The importance of membrane receptors in the transfer of immunoglobulins from plasma to the colostrum. Annales de Recherches Veterinaires 9, 229-234.

Hartmann, P.E., McCauley, I., Gooneratne, A., Whitely, J. 1984. Inadequacies of sow lactation: survival of the fittest. In Physiological stategies in lactation (ed. M Peaker, RG Vernon and CH Knight.),301–326. Academic Press, London, UK.

Hasan, S., Saha, S., Junnikkala, S., Orro, T., Peltoniemi, O., Oliviero, C. 2019. Late gestation diet supplementation of resin acid-enriched composition increases sow colostrum immunoglobulin G content, piglet colostrum intake and improve sow gut microbiota. Animal 13, 1599-1606.

Herpin, P., Le Dividich, J., Hulin, J.C., Fillaut, M., De Marco, F., Bertin, R. 1996. Effects of the level of asphyxia during delivery on viability at birth and early postnatal vitality of newborn pigs. Journal of Animal Science 74, 2067–2075.

Herskin, M. S., Di Giminiani, P. 2018. Pain in pigs: characterization, mechanisms and indicators. Advances in pig welfare, 325-355.

Hirsch, A.C., Philipp, H., Kleemann, R. 2003. Investigation on the efficacy of meloxicam in sows with mastitis-metritis-agalactia syndrome. Journal of Veterinary Pharmacology and Therapeutics 26, 355–360.

Homedes, J., Salichs, M., Sabaté, D., Sust, M., Fabre, R. 2014. Effect of ketoprofen on pre-weaning piglet mortality on commercial farms. Veterinary Journal 201, 435–237.

Hurley, W.L., Theil, P.K. 2011. Perspectives on immunoglobulins in colostrum and milk. Nutrients 3, 442–474.

Ison, S.H., Rutherford, K.M.D. 2014. Attitudes of farmers and veterinarians towards pain and the use of pain relief in pigs. Veterinary Journal 202, 622–627.

Ison, S.H., Jarvis, S., Rutherford, K.M.D. 2016. The identification of potential behavioural indicators of pain in periparturient sows. Research in Veterinary Science 109, 114–120.

Ison, S.H. Jarvis, S. Hall, S.A. Ashworth, C.J. Rutherford, K.M.D. 2018. Periparturient behavior and physiology: Further insight into the farrowing process for primiparous and multiparous sows. Frontiers in Veterinary Science 5, 5-7.

Jensen, P. 1986. Observations on the maternal behaviour of free-ranging domestic pigs. Applied Animal Behaviour Science 16, 131-142.

Jones, J.E.T. 1966. Observations on parturition in the sow Part II: The parturient and post-parturient phases. British Veterinary Journal 122, 471–478.

Kammersgaard, T.S., Pedersen, L.J., Jørgensen, E. 2011. Hypothermia in neonatal piglets: interactions and causes of individual differences. Journal of Animal Science 89, 2073–2085.

Keating, S.C.J., Thomas, A.A., Flecknell, P.A., Leach, M.C. 2012. Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses. PLoS ONE 7, e44437.

Kuller, W., Sietsma, S., Hendriksen, S., Sperling, D. 2021. Use of paracetamol in sows around farrowing: effect on health and condition of the sow, piglet mortality, piglet weight and piglet weight gain. Porcine Health Management 7, 1-9.

Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., Lacroix-Fralish, M.L. 2010.

Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7, 447–449.

Lawrence, A.B., Petherick, J.C., McLean, K.A., Deans, L.A., Chirnside, J., Vaughan, A., Clutton, E., Terlouw, E.M.C. 1994. The effect of environment on behaviour, plasma cortisol and prolactin in parturient sows. Applied Animal Behaviour Science 39, 313–330.

Le Dividich, J., Rooke, J.A. Herpin, P. 2005. Nutritional and immunological importance of colostrum for the new-born pig. Journal of Agricultural Science 143, 469–485.

Le Jan, C. 1994. A study by flow cytometry of lymphocytes in sow colostrum. Research in Veterinary Science 57, 300–304.

Leslie, K. E., Petersson-Wolfe, C.S. 2012. Assessment and management of pain in dairy cows with clinical mastitis. Veterinary Clinics: Food Animal Practice 28, 289-305.

Lucia, J.T., Correa, M.N., Deschamps, J.C., Bianchi, I., Donin, M.A., Machado, A.C., Meincke, W., Matheus, J.E.M. 2002. Risk factors for stillbirths in two swine farms in the south of Brazil. Preventive Veterinary Medicine 53, 285–292.

Macrae, A.M., Makowska, I.J., Fraser, D. 2018. Initial evaluation of facial expressions and behaviours of harbour seal pups (Phoca vitulina) in response to tagging and microchipping. Applied Animal Behaviour Science 205, 167–174.

Martins, R., Ribeiro, F., Crone, C., Markiy Odakura, A., Bevilacqua, A., Santos Nieto, V., Felix, G.A., Pereira dos Santos, A., Sousa dos Santos, L., Garofallo Garcia, R., de Castro Lippi, I.C. 2021. Strategic use of straw as environmental enrichment for prepartum sows in farrowing crates. Applied Animal Behaviour Science 234, 105194.

Mainau, E., Dalmau, A., Ruiz-de-la-Torre, J.L., Manteca, X. 2010. A behavioural scale to measure ease of farrowing in sows. Theriogenology 74,1279-1287.

Mainau, E., Ruiz-De-La-Torre, J.L., Dalmau, A., Salleras, J.M., Manteca, X. 2012. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. Animal 6, 494–501.

Mainau, E.; Temple, D.; Manteca, X. 2016. Experimental study on the effect of oral meloxicam administration in sows on pre-weaning mortality and growth and immunoglobulin G transfer to piglets. Preventive Veterinary Medicine 126, 48–52.

Manteuffel, C. 2019. Parturition detection in sows as test case for measuring activity behaviour in farm animals by means of radar sensors. Biosystems Engineering 184, 200-206.

McLennan, K.M., Rebelo, C.J.B., Corke, M.J., Holmes, M.A., Leach, M.C., Constantino-Casas, F. 2016. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Applied Animal Behaviour Science 176, 19–26.

McLennan, K.M. 2018. Why Pain Is Still a Welfare Issue for Farm Animals, and How Facial Expression Could Be the Answer. Agriculture 8, 127.

Mee, J.F. 2004. Managing the dairy cow at calving time. Veterinary Clinics: Food Animal Practice 20, 521–546.

Melzack, R. 1975. The McGill Pain Questionnaire: major properties and scoring methods. Pain 1, 277–299.

Morton, D.B., Griffiths, P.H.M. 1985. Guidelines on the recognition of pain, distress and discomfort in experimental animals and an hypothesis for assessment. Veterinary Record 116, 431–436.

Nix, J.M., Spitzer, J.C., Grimes, L.W., Burns, G.L., Plyler, B.B. 1998. A retrospective analysis of factors contributing to calf mortality and dystocia in beef cattle. Theriogenology 49, 1515–1523.

Noakes, D.E., Parkinson, T.J., England, G.C.W., Arthur, G.H. 2001. Parturition and the care of parturient animals. In: Arthur's Veterinary Reproduction and Obstetrics 8, 155–187.

Noblet, J., Dourmad, J.Y., Etienne, M. Le Dividich, J. 1997. Energy metabolism in pregnant sows and newborn pigs. Journal of Animal Science 75, 2708–2714.

Oliveira, R.A., Neves, J.S., Castro, D.S., Lopes, S.O., Santos, S.L., Silva, S.V.C., Araújo, V.O., Vieira, M.F.A., Muro, B.B.D, Leal, D.F., Camevale, R.F., Almond, G., Garbossa, C.A.P. 2020. Supplying sows energy on the expected day of farrowing improves farrowing kinetics and newborn piglet performance in the first 24 h after birth. Animal 14, 2271-2276.

Oliviero, C., Heinonen, M., Valros, A., Halli, O., Peltoniemi, O.A.T. 2008. Effect of the environment on the physiology of the sow during late pregnancy, farrowing and early lactation. Animal Reproduction Science 105, 365–377.

Oliviero, C. 2010. Successful farrowing in sows. Academic dissertation. (https://helda.helsinki.fi/handle/10138/18971)

Oliviero, C., Peltoniemi, O. 2020. Troubled process of parturition of the domestic pig. In Animal Reproduction in Veterinary Medicine. Intech Open.

Orth, E.K., González, F.J.N., Pastrana, C.I., Berger, J.M., le Jeune, S.S., Davis, E.W. 2020. Development of a donkey grimace scale to recognize pain in donkeys (Equus asinus) post castration. Animals 10, 1411.

Österlundh, I., Hultén, F., Johannisson, A., Magnusson, U. 2002. Sows intramammarily inoculated with Escherichia coli at parturition: I Functional capacity of granulocytes in sows affected or non-affected by clinical mastitis. Veterinary Immunology and Immunopathology 90, 35-44.

Pairet, M., Engelhardt, G. 1996. Distinct isoforms (COX-1 and COX-2) of cyclooxygenase: possible physiological and therapeutic implications. Fundamental & clinical pharmacology 10, 1-15.

Pastorelli, G., Neil, M., Wigren, I. 2009. Body composition and muscle glycogen contents of piglets of sows fed diets differing in fatty acids profile and contents. Livestock Science 123, 329–334.

Quesnel, H., Meunier-Salaün, M.C., Hamard, A., Guillemet, R., Etienne, M., Farmer, C., Dourmad, J.Y., Père, M.C. 2009. Dietary fiber for pregnant sows: influence on sow physiology and performance during lactation. Journal of Animal Science 87, 532–543.

Quesnel, H., Farmer, C., Devillers, N. 2012. Colostrum intake: influence on piglet performance and factors of variation. Livestock Science 146, 105–114.

Raja, S.N., Carr, D.B., Cohen, M., Finnerup, N.B., Flor, H., Gibson, S., Vader, K. 2020. The revised International Association for the Study of Pain definition of pain: concepts, challenges, and compromises. Pain 161, 1976-1982.

Reber, A.J., Lockwood, A., Hippen, A.R., Hurley, D.J. 2006. Colostrum induced phenotypic and trafficking changes in maternal mononuclear cells in a peripheral blood leukocyte model for study of leukocyte transfer to the neonatal calf. Veterinary Immunology and Immunopathology 109, 139–150.

Reijgwart, M.L., Schoemaker, N.J., Pascuzzo, R., Leach, M.C., Stodel, M., De Nies, L., Hendriksen, C.F.M., Van Der Meer, M., Vinke, C.M., Van Zeeland, Y.R.A. 2017. The composition and initial evaluation of a grimace scale in ferrets after surgical implantation of a telemetry probe. PLoS ONE 12, e0187986.

Rutherford, K.M.D., Baxter, E.M., D'Eath, R.B., Turner, S.P., Arnott, G., Roehe, R., Ask, B., Sandoe, P., Moustsen, V.A., Thorup, F., Edwards, S.A., Berg, P., Lawrence, A.B. 2013. The welfare implications of large litter size in the domestic pig I: biological factors. Animal Welfare Journal 22, 199-218.

Salmon, H., Berri, M., Gerdts, V., Meurens, F. 2009. Humoral and cellular factors of maternal immunity in swine. Developmental and Comparative Immunology 33, 384–393.

Short, C.E. 1998. Fundamentals of pain perception in animals. Applied Animal Behavior Science 59, 125-133.

Siggers, J., Sangild, P.T., Jensen, T.K., Siggers, R.H., Skovgaard, K., Støy, A.C., Jensen, B.B., Thymann, T., Bering, S.B., Boye, M. 2011. Transition from parenteral to enteral nutrition induces immediate diet-dependent gut histological and immunological responses in preterm neonates. American Journal of Physiology and Gastro-intestinal Liver Physiology 301, 435–445.

Sotocinal, S.G., Sorge, R.E., Zaloum, A., Tuttle, A.H., Martin, L.J., Wieskopf, J.S., Mapplebeck, J.C.S., Wei, P., Zhan, S., Zhang, S. 2011. The Rat Grimace Scale: A Partially Automated Method for Quantifying Pain in the Laboratory Rat via Facial Expressions. Molecular Pain 7, 55.

Stolba, A., Wood-Gush, D.G.M. 1984. The identification of behavioural key features and their incorporation into a housing design for pigs. In Annales de Recherches Veterinaires 15, 287-302.

Taverne, M.A.M. 1992. Physiology of parturition. Animal Production Science 28, 433–440.

Tenbergen, R., Friendship, R., Cassar, G., Amezcua, M.R., Haley, D. 2014. Investigation of the use of meloxicam post farrowing for improving sow performance and reducing pain. Journal of Swine Health and Production 22, 10–5.

Theil, P.K., Nielsen, M.O., Sørensen, M.T., Lauridsen, C. 2012. Lactation, milk and suckling. In Nutritional physiology of pigs (ed. KE Bach Knudsen, NJ Kjeldsen, HD Poulsen and BB Jensen), 1–47. Danish Pig Research Centre, Copenhagen, Denmark.

Theil, P. K., Lauridsen, C., Quesnel, H. 2014. Neonatal piglet survival: impact of sow nutrition around parturition on fetal glycogen deposition and production and composition of colostrum and transient milk. Animal 8, 1021-1030.

Thorsen, C.K., Schild, S-L.A., Rangstrup-Christensen, L., Bilde, T., Pedersen, L.J. 2017. The effect of farrowing duration on maternal behavior of hyperprolific sows in organic outdoor production. Livestock Science 204, 92–97.

Tuboly, S., Bernath, S., Glavits, R., Medveczky, I. 1988. Intestinal absorption of colostral lymphoid cells in newborn pigs. Veterinary Immunology and Immunopathology 20, 75–85.

Tuchscherer, M., Puppe, B., Tuchscherer, A., Tiemann, U. 2000. Early identification of neonates at risk: traits of newborn piglets with respect to survival. Theriogenology 54, 371–388.

Tummaruk, P., Sang-Gassanee, K. 2013. Effect of farrowing duration, parity number and the type of anti-inflammatory drug on postparturient disorders in sows: a clinical study. Tropical Animal Health and Production 45, 1071-1077.

Vadmand, C.N., Krogh, U., Hansen, C.F., Theil, P.K. 2015. Impact of sow and litter characteristics on colostrum yield, time for onset of lactation, and milk yieldof sows. Journal of Animal Science 93, 2488–2500.

Vallet, J. L., Miles, J. R., Rempel, L. A. 2013. A simple novel measure of passive transfer of maternal immunoglobulin is predictive of preweaning mortality in piglets. The Veterinary Journal 195, 91-97.

Van Dijk, A.J., van Rens, B.T.T.M., van der Lende, T., Taverne, M.A.M. 2005. Factors affecting duration of the expulsive stage of parturition and piglet birth intervals in sows with uncomplicated, spontaneous farrowings. Theriogenology 64,1573–1590.

Van Kempen, T. 2007. Sports supplements to facilitate parturition and reduce perinatal mortality. Full papers will be published in Recent Advances in Animal Nutrition, Nottingham University Press.

Van Rens, B.T.T.M., van der Lende, T. 2004. Parturition in gilts: duration of farrowing, birth intervals and placenta expulsion in relation to maternal, piglet and placental traits. Theriogenology 62, 331–352.

Viitasaari, E., Hänninen, L., Heinonen, M., Raekallio, M., Orro, T., Peltoniemi, O., Valros, A. 2013. Effects of post-partum administration of ketoprofen on sow health and piglet growth. Veterinary Journal 198,153-157.

Viñuela-Fernandez, I., Jones, E., Welsh, E.M., Fleetwood-Walker, S.M. 2007. Pain mechanisms and their implication for the management of pain in farm and companion animals. Veterinary Journal 174, 227–239.

Viscardi, A.V., Hunniford, M., Lawlis, P., Leach, M., Turner, P.V. 2017. Development of a Piglet Grimace Scale to Evaluate Piglet Pain Using Facial Expressions Following Castration and Tail Docking: A Pilot Study. Frontiers in Veterinary Science 4, 51.

Von Borell, E. 1995. Neuroendocrine integration of stress and significance of stress for the performance of farm animals. Applied Animal Behaviour Science 44, 219-227.

Williams, P.P. 1993. Immunomodulating effects of intestinal maternal colostral leukocytes by neonatal pigs. Canadian Journal of Veterinary Research 57, 1–8.

Wischner, D., Kemper, N., Stamer, E., Hellbruegge, B., Presuhn, U., Krieter, J. 2009. Characterisation of sows' postures and posture changes with regard to crushing piglets. Applied Animal Behaviour Science 119, 49-55.

Wischner, D., Kemper, N., Stamer, E., Hellbrügge, B., Presuhn, U., Krieter, J. 2010. Pre-lying behaviour patterns in confined sows and their effects on crushing of piglets. Applied Animal Behaviour Science 122, 21-27.

Yun, J., Swan, K.M., Farmer, C., Oliviero, C., Peltoniemi, O., Valros, A. 2014. Prepartum nest-building has an impact on postpartum nursing performance and maternal behaviour in early lactating sows. Applied Animal Behaviour Science 160, 31–37.

Yun, J., Valros, A. 2015. Benefits of prepartum nest-building behaviour on parturition and lactation in sows—A review. Asian-Australasian Journal of Animal Sciences 28, 1519-1524.

Yun, J., Han, T., Björkman, S., Nystén, M., Hasan, S., Valros, A., Peltoniemi, O. 2019. Factors affecting piglet mortality during the first 24 h after the onset of parturition in large litters: effects of farrowing housing on behaviour of postpartum sows. Animal 13, 1045-1053.

Zanello, G., Meurens, F., Serreau, D., Chevaleyre, C., Melo, S., Berri, M., Salmon, H. 2013. Effects of dietary yeast strains on immunoglobulin in colostrum and milk of sows. Veterinary Immunology and Immunopathology 152, 20-27.

Zheng, C., Yang, X., Zhu, X., Chen, C., Wang, L., Tu, S., Yang, A., Xue, Y. 2020. Automatic posture change analysis of lactating sows by action localisation and tube optimisation from untrimmed depth videos. Biosystems Engineering 194, 227-250.

CAPÍTULO 2. OBJETIVOS

Capítulo 2

OBJETIVOS

Los objetivos generales de la presente tesis fueron: (1) evaluar los efectos de la administración de meloxicam oral (Metacam®) al inicio del parto en cerdas sobre el crecimiento de los lechones y la transferencia inmunitaria y (2) desarrollar una escala de dolor basada en las expresiones faciales de las cerdas, usando el parto como modelo de dolor fisiológico.

Estos objetivos generales se pueden dividir en los siguientes objetivos específicos:

1- Evaluar los efectos del meloxicam oral sobre el crecimiento de los lechones hasta el destete, teniendo en cuenta su sexo y su peso al nacimiento.
2- Evaluar los efectos del meloxicam oral sobre la transferencia inmunitaria analizando la concentración de inmunoglobulinas (A y G) y citoquinas (IL-2, IL-4 e IFN-Y) en la sangre de los lechones y en la saliva, leche y calostro de las cerdas, teniendo en cuenta su paridad.
3- Desarrollar una escala de dolor basada en las expresiones faciales capaz de valorar la intensidad del dolor alrededor del parto.
4- Evaluar la repetibilidad intra y entre – observadores de la escala de dolor basada en las expresiones faciales en las cerdas, considerando el efecto del género y el conocimiento del sector porcino de los observadores.
5- Correlacionar la escala de dolor basada en las expresiones faciales con los indicadores corporales específicos de dolor a lo largo del parto y el período previo al destete.

6- Evaluar si la escala de dolor basada en las expresiones faciales, los indicadores corporales específicos de dolor y los cambios posturales de la cerda se ven afectados por el momento del parto, la paridad y los eventos de amamantamiento de los lechones.

CAPÍTULO 3. ORAL MELOXICAM ADMINISTRATION IN SOWS AT FARROWING AND ITS EFFECTS ON PIGLET IMMUNITY TRANSFER AND GROWTH

Capítulo 3

ORAL MELOXICAM ADMINISTRATION IN SOWS AT FARROWING AND ITS EFFECTS ON PIGLET IMMUNITY TRANSFER AND GROWTH

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Capítulo 3

ABSTRACT

Many factors can lead to an inadequate development of piglets during their first days of life, including poor maternal behavior, which can be due to pain caused by farrowing, and reduced colostrum ingestion. This study investigates the action of meloxicam administered orally at farrowing on piglet weight gain and immunity transfer. Thirty-five multiparous sows were divided into two groups and treated with 0.4 mg/kg of oral meloxicam (oral meloxicam group; n = 18) or with a mock administration (control group; n = 17). A total of 382 piglets were individually weighed on the farrowing day (day 0), as well as on days +9 and +20. Immunoglobulin G (IgG) and A (IgA) concentrations in piglet serum and in sow's saliva, colostrum and milk were measured. Additionally, Interleukin-2 (IL-2), Interleukin-4 (IL-4) and Interferon gamma (IFN-Y) in serum of piglets and in sow's milk or colostrum were studied. All samples were obtained on days +1, +9, and +20. Piglets from sows in the oral meloxicam group tended to grow faster from day +9 to day +20 than did piglets from control sows (p = 0.059), and this difference was also observed in piglets with low body weight (BW) at birth (p = 0.056). The oral meloxicam group sows tended to increase the colostrum levels of IgA and IgG, as compared with control sows on day +1 (p = 0.068 and p = 0.072, respectively). IgA levels in piglet serum from the oral meloxicam group were significantly higher than in the control group on day +1 and +9 (p = 0.019 and p = 0.011 respectively). Furthermore, IL-2 and IL-4 levels in the serum of piglets from sows in the oral meloxicam group tended to be higher than that in the control group on day +9 (p = 0.078 and 0.056, respectively).

The administration of meloxicam orally at the beginning of farrowing in multiparous sows increased immunoglobin and cytokine concentrations in colostrum, improving both humoral and cellular immune response of piglets. Pre weaning growth of piglets born with a low BW improved in the meloxicam-treated group.

3.1. Introduction

Piglets are born agammaglobulinemic because of the epitheliochorial placentation of swine (1, 2).

An early and sufficient intake of colostrum is crucial for piglet growth and survival (3, 4), as it is the source of energy as well as active and passive immunity. Colostrum is a complex mammary secretion released from the time of farrowing (early-colostrum) to 12 h (mid-colostrum) and up to 36 h post-farrowing (late-colostrum) (5). Early-colostrum is mostly produced before farrowing and contains up to 75% of Immunoglobulin G (IgG) and 20% of Immunoglobulin A (IgA), which are central elements of humoral immune responses. After farrowing, IgG concentration drastically drops, whereas IgA reduction during lactation is more gradual due to its role in the regulation of piglet intestinal microbiota, which is critical for the prevention of digestive problems (5). Colostrum-associated cellular immunity has been overlooked for a long time. It contains around 106 cells/mL, up to 25% of them being lymphocytes (6, 7). Immune responses are orchestrated via complex signaling pathways within cells mediated by cytokines, which are small proteins with a plethora of effects. IL-2, IL-4 and IFN-ϒ are important cytokinemediators of the adaptive immune response, thus their quantification allows for partial characterization of the immune response. IL-2 is mainly produced by T lymphocytes and induces the proliferation of T and B lymphocytes and the activation of Natural Killer cells (NK) (8–10). IL-4 triggers differentiation of T helper lymphocytes toward the Th2 subset, which is related to humoral and anthelmintic responses (8, 10), while IFN-ϒ activates macrophages and elicits the differentiation of T helper lymphocytes toward the Th1 subset, thus favoring cellular responses and boosting protection against intracellular microbes (10).

Non-steroidal anti-inflammatory drugs (NSAIDs) have analgesic, anti-inflammatory, anti-endotoxic and anti-pyretic effects. It has been proven that NSAIDs administered to sows help them recover from a painful situation such as lameness (11) or post-partum dysgalactia syndrome (12, 13). NSAIDs also decrease the mortality rate at weaning in litters from healthy sows (14) and in sows with dysgalactia syndrome (13). However, studies on the effect of NSAIDs, on sow welfare, piglet growth and immunity transfer in healthy sows show discrepancies. Meloxicam administered to healthy sows around farrowing improves post-farrowing sow recovery (15, 16) and enhances piglet growth, especially at weaning (17, 18) and in piglets with low body weight (BW) at birth (18, 19). Nevertheless, other studies administering NSAIDs to healthy sows around farrowing did not find enhanced sow welfare and recovery post-farrowing (20) or improve piglet growth (15, 21).

To our knowledge, only two studies have looked into the effects of NSAIDs administered to sows around farrowing and have assessed passive immunity transfer via colostrum and immune system development in piglets (18, 20). Both studies recorded IgG transfer measured in piglet serum without exploring immunoglobulins in sow colostrum or milk. These studies did not measure other relevant immune factors for piglet growth and survival, such as IgA or cytokines.

Mainau et al. (18) demonstrated that the administration of meloxicam orally at the beginning of farrowing in multiparous sows increased the concentration of IgG in piglet serum and enhanced their pre-weaning growth.

The present study aims to evaluate the effect of meloxicam administered orally to healthy sows at the beginning of farrowing on piglet growth, also including the effect of sex and immune transfer via colostrum of immunoglobulins (A and G) and cytokines (Interleukins IL-2 and IL-4, and Interferon Gamma IFN-Y), taking into account the sow parity effect.

3.2. Material and Methods

The experimental protocol described in this experiment was approved by the Institutional Animal Care and Use Committee of the Universitat Autònoma de Barcelona (CEEAH-1591) and the Generalitat de Catalunya (DMAH-6720). Written informed consent was obtained from the owners for the participation of their animals in this study.

3.2.1. Animals, housing and general management

Sample size was calculated by means of ENE 3.0. The sow was the experimental unit. Based on two previous studies carried out by Mainau et al. (18,19), a reference mean average daily gain (ADG) of 0.2 kg/day was established at sow level for the control group and an expected mean ADG of 0.225 kg/day was considered for the treatment group. An overall standard deviation of 0.025 kg/day (at sow level) was assumed with a power of 80% and confidence level of 95%. A prevision of 17 sows per group was predicted.

The experimental procedure was carried out on a commercial farm (Heura S.L.; Santa Perpètua de Mogoda, Barcelona, Spain), with 9 farrowing barns equipped with an evaporative cooling system each. From December 2017 to March 2018, a total of 35 hybrid (Landrace x Duroc) multiparous sows from 2nd to 7th parturition were randomly selected the day of farrowing. At least 5 replicates with 5 to 10 sows per replicate were studied.

On day 109 of gestation, sows were moved to the farrowing barn and were housed in individual farrowing crates (1.95 × 0.60m) built with steel bars. Farrowing crates were centrally located in farrowing pens (2.40 × 1.80m) with fullymetal-slatted floors for sows and plastic-slatted floors for piglets. A metal pad ensured 36◦C of heat for the piglets during the first week of life, and heat lamps were placed over the metal pad the first day of life. The temperature in the farrowing barn was kept constant at ∼20ºC, and the light was on from 07:00 to 17:00 h every day. Sows were offered 2.6 kg of feed per day, divided into two meals (07:00 and 15:00 h) and water was available ad libitum from drinkers.

Thirty days before farrowing, all sows were vaccinated with Clostridium novyi (2 mLSuiseng®, Hipra SA; Girona, Spain). Sixteen days after farrowing, sows that were not expected to be culled (n = 31 sows) were vaccinated with Parvovirus and Erysipelothrix rhusiopathiae (2 mL Eryseng®parvo, Hipra SA; Girona, Spain) and with Leptospira spp (2 mL Autovacuna®syva, Syva SAU; León, Spain). On day 113 of gestation, farrowing was hormonally induced with 2 mL of Planate® (Cloprostenol 0,092 mg/mL, MSD Animal Health; Friesoythe, Germany) divided into two injections of 1mL (07:00 h and 11:00 h). Only hormonally induced farrowings that started on the morning of day 114 of gestation were included in the study. Lame sows or those with any kind of visible disease symptoms such as mastitis, diarrhea, fever, or respiratory problems were not included in the study.

Treatments and manual interventions during farrowing followed the usual routine of the farm and were performed by the same person. The following treatments were allowed during farrowing and were administered intramuscularly (IM) in the neck. When the time interval between the birth of two piglets exceeded 1 hour and the cervical canal was dilated, 1 mL of Oxytocin (Hormonipra®, HipraSA, Girona, Spain) was injected. When the cervical canal was not sufficiently dilated, sows were treated with 200 mg of Vetrabutine hydrochloride (Monzal®, Boehringer Ingelheim España; SA, Barcelona, Spain). When sows were very nervous around farrowing Carazolol (Suacron®, Divasa Farmavic SA; Barcelona, Spain) or Azaperone (Stressnil®, Janssen Animal Health, Elanco; Brussels, Belgium) were administered.

A total of 382 piglets, identified individually by a numeric ear tag, were included in the study. Piglets were weaned at 21 days of age, according to veterinary recommendations, and moved to another barn of the farm equipped with conditioned infrastructures for very young piglets.

3.2.2. Experimental Procedure

In each replicate, sows were randomly allocated into two homogeneous groups, regarding parity, and treated with either 0.4 mg/kg body weight of meloxicam (Metacam® 15 mg/mL Oral Suspension, Boehringer Ingelheim Vetmedica GmbH; Ingelheim, Rhein, Germany) or a mock administration with an empty syringe. Treatments were administered at the beginning of the farrowing, between the first and the third piglet. If any further anti-inflammatory treatment was required, the sow was excluded from the study.

Litter size was standardized at 11-12 piglets by cross-fostering within 6-8 hours post-farrowing. Cross-fostering was carried out within each treatment. Each treatment was identified with two different colored cards in order to make the treatment blind to farm and laboratory staff.

3.2.3. Data Collection

For each sow, the following parameters were registered during farrowing by direct observations: the duration of farrowing (defined as the period of time between the first and the last piglet born), the condition of each piglet at birth (born alive, stillborn or mummified fetus), the piglet's sex (male or female), the number of treatments and manual interventions during farrowing, and the number of piglets cross-fostered and weaned. The presence of placenta retention was also recorded. During lactation, piglet mortality was registered. The number of sows culled after weaning and the interval between farrowing and the following fertile insemination were recorded.

One and 9 days after farrowing (day +1 and day +9) and the day before weaning (day +20), saliva samples were collected from all sows using Salivette® tubes (Sarstedt; Nümbrecht, Germany). Each tube contained a cotton swab, which was clipped with a Kocher clip, and sows were allowed to chew it for around 1min. Then, the cotton swab was placed in the tube and centrifuged at 6,048 x g for 13min. Saliva samples (approximately 1–2mL per cotton swab) were stored in Eppendorf tubes and frozen at −80°C until analysis. Colostrum and milk samples were collected from all sows on day +1 (colostrum) and on days +9 and +20 (milk). Sows were injected with 0.7mL of Oxytocin IM (Hormonipra®, Hipra SA; Girona, Spain), and 30s later, 2mL of colostrum and milk were collected into sterile tubes. Colostrum and milk samples were immediately frozen at -20ºC until analysis.

Each pig was individually weighed at farrowing (day 0), and on days +9 and +20. One day after farrowing, during one suckling event, 3-4 piglets of each litter were selected for blood sampling. Piglets were chosen so that at least one of them was suckling from the sow's craneal teats, another one from middle teats and yet another one from caudal teats. The same piglets from each litter were blood sampled on days +1, +9 and +20. Blood samples (1-2 mL) were collected into heparinized tubes from the anterior vena cava. Samples were centrifuged for six minutes at 2,058 x g and plasma was stored in Eppendorf tubes at -80ºC until analysis.

All samples were analyzed at the Murcia University Veterinary Hospital Laboratory. Immunoglobulin G (IgG) and A (IgA) concentrations in piglet serum and sow saliva, colostrum and milk were quantified by using two commercially available sandwich ELISAs (IgA and IgG ELISA Quantitation Kit; Bethyl Laboratories; Montgomery, TX, USA). Interleukin-2 (IL-2), Interleukin-4 (IL-4) and Interferon gamma (IFN-ϒ) in piglet serum, sow saliva, milk or colostrum were analyzed using MILLIPEX® MAP Porcine Cytokine/Chemokine Panel Kit, (EMD Millipore; Darmstadt, Germany).

3.2.4. Statistical Analysis

Data were analyzed using the SAS software (SAS Institute Inc.; Cary, NC, USA). The experimental unit for data analysis was the individual sow. All descriptive values in the Results section are shown as the mean ± standard error (SE). Significance was set at $p < 0.05$, and tendency at $p < 0.1$, in all cases.

The Mann-Whitney Wilcoxon test was used to test whether the performance values (other than piglet weight and average daily gain) obtained at the individual sow level were significantly different between treatments.

Normality tests of residuals were performed for each dependent variable. Weight of piglets and ADG (From birth to day+9, from day+9 to weaning and from birth to weaning) were normally distributed without data transformation. A general linear mixed model (proc MIXED) for repeated measures was used. Model included the fixed effects of treatment (control vs. oral meloxicam), day (at birth, day +9 and at weaning), sex (males vs. females) and their pair interactions. Day and piglet within sow were introduced as repeated effects. Weight at birth was introduced as a covariate for the analysis of weight at day +9 and at weaning, and litter size was introduced as a covariate in all the models. The residual maximum likelihood was used as a method of estimation. Differences in least-square means were investigated after using a Tukey adjustment for multiple comparisons. The same models were used to study the performance of piglets categorized by quintiles according to their weight at birth: very light (from 0.670 to 1.294Kg), light (from >1.294 to 1.492Kg), mid (from >1.492 to 1.625Kg), heavy (from >1.625 to 1.878Kg) and very heavy (from >1.878 to 2.427 Kg).

IgG and IgA concentrations in piglet serum, sow saliva and colostrum or milk, IL-2, IL-4 and IFN-Ƴ in colostrum or milk, and IFN-Ƴ in serum of piglets were normally distributed after a log transformation. IL-2 and IL-4 in piglet serum followed a normal distribution without data transformation. Additionally, extreme outliers detected by proc UNIVARIATE box plot procedures were deleted.

Immunity measurements in piglets and sows were analyzed using general linear mixed models (proc MIXED) for repeated measures. Models for immunity sow measurements included the fixed effects of treatment (oral meloxicam vs. control), day (day +1, +9 and +20), parity (from 2^{nd} to 7^{th}) and their pair interactions. Day was introduced as a repeated effect. Models for immunity piglet measurements included the fixed effects of treatment (oral meloxicam vs. control), day (day +1, +9 and +20), sex (male vs. female), the position at the udder (anterior, middle and posterior teats) and their pair interactions. Day and piglet within sow were introduced as repeated effects. The residual maximum likelihood was used as a method of estimation. Differences in least-square means were investigated after using a Tukey adjustment for multiple comparisons. All general linear mixed models included replicate (from 1 to 5) and farrowing barn (from 1 to 9) as random effects.

3.3. Results

3.3.1. Performance parameters and treatment records

Results on performance and treatment records at the individual sow level are summarized in **Table 1.** Both treatment groups (oral meloxicam vs. control) were similar when the experimental procedure started in terms of performance data recorded during farrowing.

Treatment did not have an effect on the time interval between weaning and the following fertile insemination (4.769 ± 0.121 days in oral meloxicam group vs. 7.071 ± 1.811 days in the control group; p = 0.893), or on the number of sows culled after weaning (0.111 ± 0.076 in the oral meloxicam group vs. 0.176 ± 0.095 in the control group; p = 0.608).

Table 1. *Mean, standard error (SE), median (MED) and 95% confidence intervals for median (95% CI) of performance parameters and treatment records studied in the control and oral meloxicam groups during the whole trial period (from farrowing to weaning at 21 days).*

	Control (n = 17 sows)				Oral meloxicam (n = 18 sows)				P-value
Items	**Mean**	**SE**[1]	**MED**	**95%CI**	**Mean**	**SE**	**MED**	**95%CI**	
Parity	4.06	0.441	3	2-7	4.28	0.394	4	2-7	0.582
Piglets born at the moment of the treatment	1.70	0.143	2	1-3	1.78	0.117	2	1-3	0.856
Total duration of farrowing (h)	3.27	0.328	3.47	1.12-5.47	3.31	0.387	3.06	1.38-7.28	0.817
Total piglets born per litter	13.47	0.912	13	6-21	12.73	0.576	13	9-17	0.621
Live born per litter	11.88	0.624	12	6-18	11.36	0.584	11	8-16	0.489
Stillborn per litter	0.94	0.358	1	0-6	0.83	0.259	0.5	0-4	0.986
Mummified fetus per litter	0.65	0.226	0	0-2	0.55	0.217	0	0-3	0.815
Cross-fostered piglets per litter	11.18	0.231	11	10-13	10.67	0.256	10.5	8-12	0.209
Crushing deaths per litter	0.47	0.229	0	0-3	0.17	0.121	0	0-2	0.322
Total liveborn mortality	0.76	0.304	0	0-4	0.61	0.282	0	0-4	0.662
Total weaned piglets	10.41	0.193	10	9-12	10.06	0.338	10	6-12	0.569
Manual intervention per sow	0.47	0.174	0	0-2	0.38	0.230	0	0-4	0.422
Oxytocin treatment per sow	0.24	0.106	0	0-1	0.17	0.900	0	0-1	0.637
Total treatments per sow	0.24	0.106	0	0-1	0.33	0.140	0	0-2	0.731

A total of 35 sows and 354 piglets were included in the study. P-value from Mann-Whitney Wilcoxon test is shown. [1] *SE= standard error.*

Twenty-four piglets died during lactation, which represents 6.28% of mortality. Oral meloxicam treatment of sows did not significantly affect piglet mortality (6.84% from the control group and 5.73% from oral meloxicam group; p = 0.661).

The mean and standard error (SE) of weight at birth, weight on day +9 and weight at weaning are summarized in **Table 2**. Piglet weight at birth, on day +9 and at weaning was not different between the control and the oral meloxicam group. Piglet sex had a significant effect on the weight of the piglets, males being heavier than females at weaning (**Table 1**, **Supplementary Material**).

Average daily gain (ADG) data are also summarized in **Table 2**. Oral meloxicam treatment of sows tended to increase piglet ADG from day +9 to weaning. Piglet sex had a significant effect on the ADG, males growing faster than females from birth to weaning and from day +9 to weaning.

Piglet weights (at birth, on day +9 and at weaning) and ADG (from birth to day +9, from day+9 to weaning and from birth to weaning) were not affected by treatment in piglets born with light, mid, heavy or very heavy weight at birth. Piglets with very light weight at birth tended to have a higher ADG from day+9 to weaning in the oral meloxicam group (267.93 ± 7.793 gr) than in the control group (240.11 ± 9.207 gr) ($p = 0.056$).

Table 2. *Mean and standard error (SE) of the piglet weight at birth, 9 days after farrowing (day+9) and at weaning (day+20) in Kilograms and the Average Daily Gain (ADG) of piglets from birth to day+9 after farrowing, from birth to weaning and from day+9 to weaning in Kilograms per day for 354 piglets regarding treatment (control vs. oral meloxicam).*

	Control		Oral Meloxicam		
	Mean	**SE**	**Mean**	**SE**	**P-value**
Weight at birth (Kg)	1.510	0.065	1.600	0.063	0.996
Weight at day +9 (Kg)	3.556	0.071	3.499	6.538	0.909
Weight at weaning (Kg)	6.479	0.109	6.538	0.103	0.644
ADG from birth to day +9 (Kg/day)	0.218	0.010	0.217	0.010	0.981
ADG from birth to weaning (Kg/day)	0.243	0.006	0.251	0.006	0.275
ADG from day +9 to weaning (Kg/day)	0.261 [b]	0.007	0.275 [a]	0.007	0.059

Different superscripts (a, b) in the same row indicate significant differences within each effect (p < 0.05). Tendency has been shown at p < 0.1.

3.3.2. Immunoglobulins G and A concentrations in saliva and colostrum or milk of sows and in piglet serum.

Immunoglobulin G and A (IgG and IgA) concentrations in saliva, colostrum or milk of sows and in piglet serum by treatment effect on days +1, +9, and +20 after farrowing are shown in **Figure 1**.

IgG levels in sow saliva (μg/mL) were affected by day after farrowing (day +1: 44.89 ± 4.486; day +9: 9.08 ± 1.810 and day +20: 3.28 ± 0.475; $p < 0.001$ in all pair comparisons), but were not affected by treatment ($p = 0.547$) irrespectively of the day studied.

IgA levels in sow saliva (μg/mL) were affected by day after farrowing (day +1: 239.64 ± 21.202, day +9: 118.477 ± 12.267 and day +20: 60.81 ± 7.295; $p < 0.001$ in all pair comparisons), but were not significantly affected by treatment; ($p = 0.704$) irrespective of the day studied. IgG levels in colostrum or milk of sows (mg/mL) were affected by day after farrowing and were higher on day +1 (24.48 ± 1.484) than on days +9 (3.75 ± 0.953) and +20 (1.77 ± 0.298) ($p < 0.001$ in both cases). IgG levels in colostrum from the sows treated with oral meloxicam tended to be higher than in the control group on day +1 ($p = 0.072$). However, on days +9 and +20, IgG concentration in sow milk was not affected by treatment.

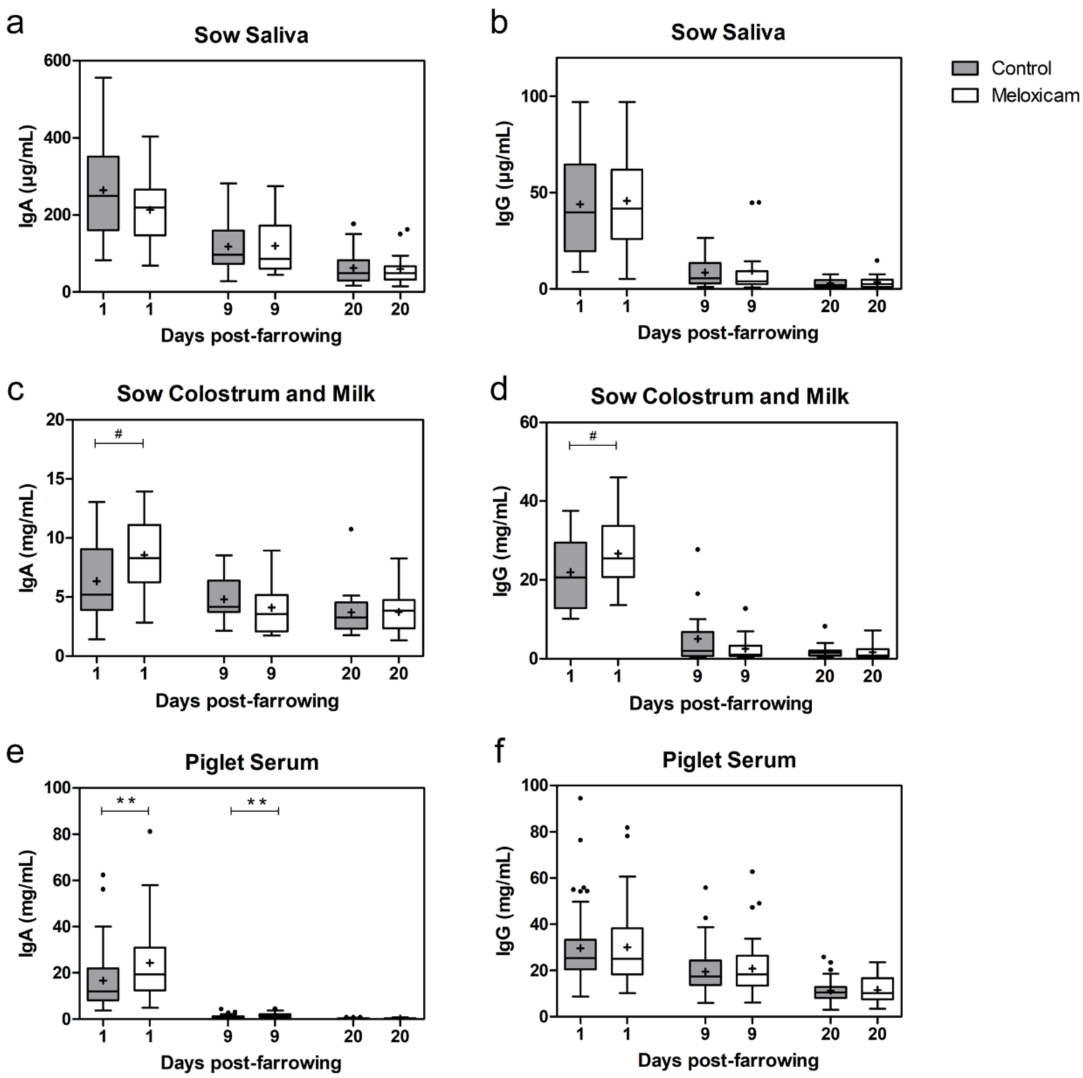
IgA levels in colostrum or milk of sows (mg/mL) were affected by day after farrowing and were higher on day +1 (7.48 ± 0.577) than on days +9 (4.41 ± 0.347; $p < 0.001$) and +20 (3.72 ± 0.315; $p < 0.001$). IgA concentration on day +9 and +20 were similar ($p = 0.246$). Furthermore, IgA levels in colostrum of sows treated with oral meloxicam tended to be higher than in the control group on day +1 ($p = 0.068$), but on day +9 and +20, IgA levels in sow milk were not affected by treatment.

IgA and IgG concentrations in saliva and in colostrum or milk were not affected by parity (saliva: $p = 0.290$ and $p = 0.192$, respectively, and colostrum or milk: $p = 0.127$ and $p = 0.232$). The interaction between treatment and parity was not significant (saliva IgA $p = 0.113$; IgG $p = 0.925$ and colostrum or milk IgA $p = 0.239$; IgG $p = 0.112$).

IgG levels in piglet serum (mg/mL) were affected by day after farrowing (day +1: 29.93 ± 1.377; day +9: 20.26 ± 0.935 and day +20: 11.48 ± 0.466; $p < 0.001$ in all pair comparisons). IgG levels in piglet serum were not significantly affected by treatment ($p = 0.963$), sex ($p = 0.189$) or piglet position at the udder ($p = 0.811$) irrespective of the day studied.

IgA levels in piglet serum (mg/mL) were affected by day after farrowing (day +1: 20.63 ± 1.314, day +9: 1.36 ± 0.080 and day +20: 0.27 ± 0.018 $p < 0.001$ in all pair comparisons), and there was an interaction between treatment and sampling day ($p = 0.020$). IgA levels in piglet serum from sows treated with oral meloxicam were significantly higher than in piglets from the control group on day +1 ($p = 0.019$) and day +9 ($p = 0.011$). However, on day +20, IgA level in piglet serum was not significantly affected by treatment (p=0.943). IgA levels in piglet serum were not significantly affected by sex (7.79 ± 0.944 in females vs. 7.89 ± 1.001 in males; p = 0.633) or by piglet position at the udder (anterior teats: 7.67 ± 1.048, middle teats: 7.17 ± 1.328 and posterior teats: 9.07 ± 1.448, p = 0.725) irrespective of the day studied.

Figure 1: *IgA and IgG at days +1, +9, and +20 after farrowing regarding treatment received by sows (control vs. oral meloxicam) in sow saliva (A,B), sow colostrum and milk (C,D) and piglet serum (E,F). Significant differences were established at p < 0.01(**) and tendency was set at p < 0.1(#). Boxes represent the interquartile range (IQ = Q3-Q1), horizontal lines inside the boxes represent the median and the cross (+) represents the mean values of the data. Whisker bars were calculated from the IQ (Upper: Q3 + 1.5 × IQ; lower: Q1 – 1.5 × IQ), and reflect the variability of the data outside Q1 and Q3. Points outside the box-and-whiskers plot represent extreme values of the population.*



3.3.3. Concentration of cytokines (IL-2, IL-4 and IFN-Ƴ) in colostrum or milk of sows and in piglet serum.

Concentration of interleukins (IL-2 and IL-4) and interferon gamma (IFN-Ƴ) in colostrum or milk of sows and in piglet serum by treatment effect on days +1, +9, and +20 after farrowing are

shown in **Figure 2**.

IL-2, IL-4 and IFN-Ƴ concentration in colostrum or milk of sows (ng/mL) were affected by day after farrowing ($p < 0.001$ in all cases). IL-2, IL-4 and IFN-

ϒ in sow colostrum on day +1 (IL-2: 1.51 ± 0.166; IL-4: 10.12 ± 1.289; IFN-ϒ: 62.99 ± 5.505) showed higher concentrations than in sow milk on day +9 (IL-2: 0.50 ± 0.079; IL-4: 2.60 ± 0.540; IFN-ϒ: 24.31 ± 3.827) and on day +20 (IL-2: 0.73 ± 0.089; IL-4: 4.02 ± 0.641; IFN-ϒ: 41.86 ± 4.585), whereas concentrations on day +20 were higher than on day +9.

IL-2, IL-4 and IFN-ϒ concentrations in colostrum or milk of sows were not significantly affected by treatment (p = 0.206, 0.142 and 0.322 respectively).

IL-2, IL-4 and IFN-ϒ concentrations in colostrum or milk of sows were affected by parity (p = 0.010, p < 0.001 and p = 0.008 respectively). The general pattern was that sows in their second parity showed lower levels of cytokines than did older sows (3 parturitions or more). Specifically, IL-2 levels in colostrum or milk of sows in their second parity were lower than those in sows in their fourth (p = 0.016) and fifth parity (p = 0.023) and tended to be lower than in sows in their seventh parity (p = 0.098). IL-4 levels in colostrum or milk of sows in their second parity were lower than those in sows in their third (p = 0.005), fourth (p = 0.001), fifth (p = 0.001), sixth (p = 0.035) and seventh parity (p = 0.002). IFN-ϒ levels in colostrum or milk of sows in their second parity were lower than those in sows in their fourth parity (p = 0.008) and tended to be lower than in sows in their sixth parity (p = 0.070).

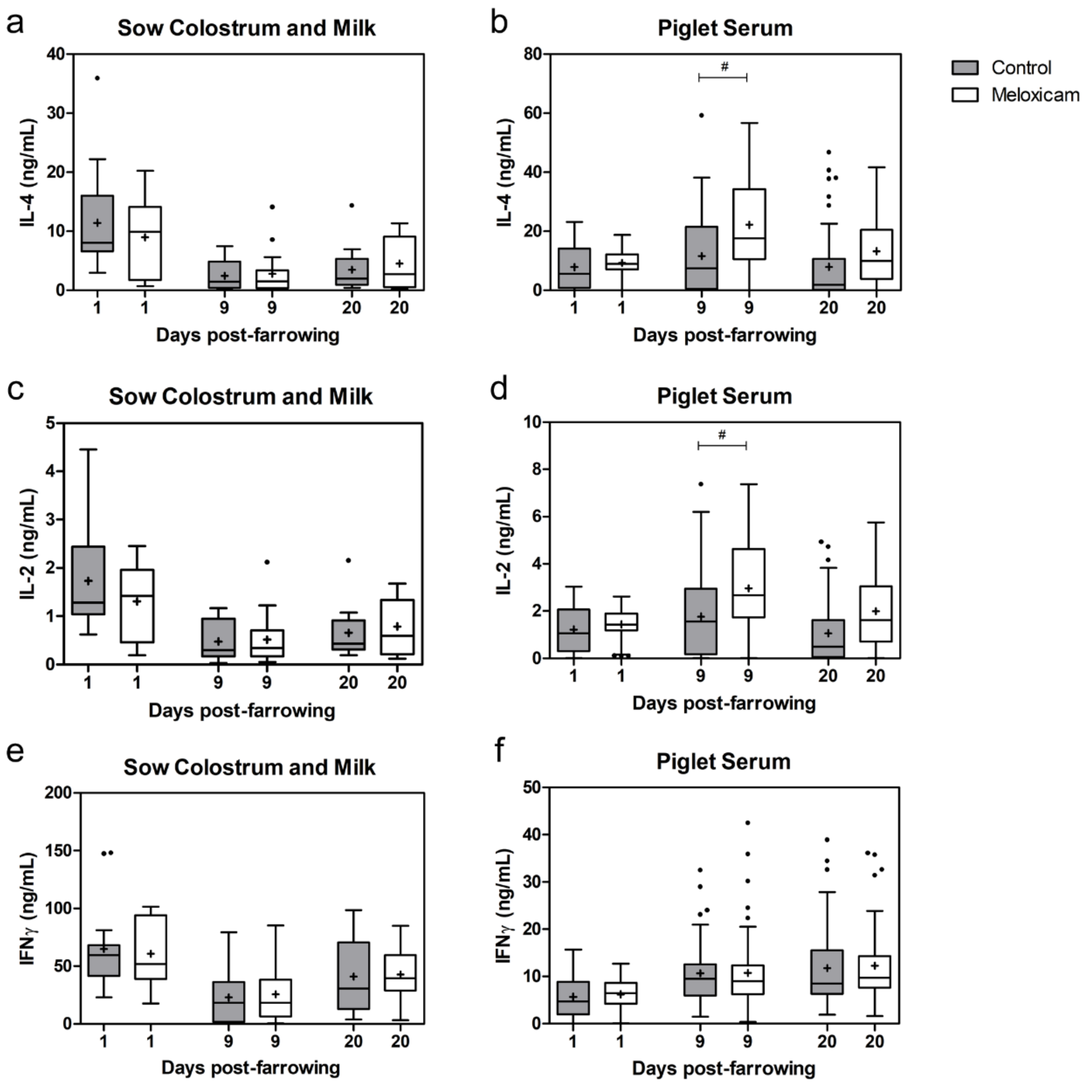
IL-2 and IL-4 levels in piglet serum (ng/mL) were affected by day after farrowing, and were higher on day +9 (IL-2: 2.40 ± 0.173; IL-4: 17.15 ± 1.442) than those on days +1 (IL-2: 1.33 ± 0.070; IL-4: 8.53 ± 0.526; p < 0.001 in both cases) and +20 (IL-2: 1.55 ± 0.145; IL-4: 10.67 ± 1.156; p < 0.001 and p = 0.001, respectively).

IL-2 and IL-4 levels in piglet serum were affected by treatment, and tended to be higher in the oral meloxicam group than those in the control group on day +9 ($p = 0.078$ and $p = 0.056$, respectively). IL-2 and IL-4 levels in piglet serum were not significantly affected by sex of piglets ($p = 0.596$ and $p = 0.868$, respectively) or by piglet position at the udder ($p = 0.888$ and $p = 0.715$, respectively) irrespective of the day studied.

IFN-ϒ levels in piglet serum (ng/mL) were affected by day after farrowing, and were lower on day +1 (6.00 ± 0.334) than those on day +9 (10.74 ± 0.654) and day +20 (12.07 ± 0.758) ($p < 0.001$ in all cases).

IFN-ϒ levels in piglet serum were not significantly affected by treatment ($p = 0.409$), sex of piglets ($p = 0.858$), or piglet position at the udder ($p = 0.320$), irrespective of the day studied.

***Figure 2:*|** *Concentration of IL-4, IL-2, and IFN-ϒ at days +1, +9, and +20 after farrowing regarding treatment received by sows (control vs. oral meloxicam) in sow colostrum and milk (a, c, e) and piglet serum (b, d, f). Tendency differences were established at $p < 0.1$(#). Boxes represent the interquartile range (IQ = Q3 – Q1), horizontal lines inside the boxes represent the median and the cross (+) represents the mean values of the data. Whisker bars were calculated from the IQ (Upper: Q3 + 1.5 × IQ; lower: Q1 - 1.5 × IQ) and reflect the variability of the data outside Q1 and Q3. Points outside the box-and whiskers plot represent extreme values of the population.*



3.4. Discussion

In the present study, both treatment groups (oral meloxicam and control) were well-matched in terms of performance variables recorded during farrowing. Early administration of oral meloxicam treatment did not negatively affect total piglets born alive per litter, total duration of farrowing, treatments administered during farrowing (such as oxytocin) or the number of manual interventions during farrowing. Hence, it appears that the use of oral meloxicam during parturition (more specifically between the first and the third piglet born) did not interfere with the progression of the birth process.

3.4.1. Piglet mortality and growth

In agreement with other authors that studied the effect of NSAIDs around farrowing (17, 18, 20, 21), oral meloxicam administered to healthy sows did not show an effect on preweaning piglet mortality. On the contrary, Homedes et al. (14), in a large-scale study on commercial farms with a high incidence of pre-weaning mortality (±10%), showed lower pre-weaning piglet mortality after ketoprofen administration to sows within 12 h after farrowing. Homedes et al. (14) explained such an effect due to higher milk production by the sow ketoprofen treatment group. We assume that a larger sample size enrolling different farms with high pre-weaning mortality would be needed to observe differences in piglet mortality (piglet mortality in our study was 6.8%).

Piglet weights at birth were similar (16, 17) or slightly higher (3, 22, 23) than values reported in other studies. The administration of oral meloxicam at the beginning of farrowing tended to enhance the ADG of piglets from day +9 to weaning, and particularly for the lightest piglets. A similar effect was described by Mainau et al., in two studies (18, 19), treating the sows around farrowing with injectable and with oral meloxicam. Tenbergen et al. (17), injected meloxicam intramuscularly within 12 h of farrowing and found that piglet ADG tended to be higher for piglets from the meloxicam group sows than for control piglets in medium-sized litters (11–13 piglets). Ketoprofen is another AINE used in pig production, but Viitasaari et al. (21) and Ison et al. (15), who both injected sows with ketoprofen during farrowing, did not find that it had any effect on piglet average daily gain to weaning. These discrepancies in the effects of NSAIDs administered to healthy sows around farrowing on piglet growth could be due to different factors such as the active principle administered. Meloxicam is a selective COX-2 inhibitor and may be a more specific treatment for inflammation caused by farrowing than a non-selective COX inhibitor, like ketoprofen (24). The time of administration is another important factor to take into consideration. Studies administering meloxicam at the beginning of farrowing (17–19) show the effect on weaning weights of piglets and ADG. Thus, the active ingredient administered (preferably a selective COX-2 inhibitor) and the administration time (as soon as possible after farrowing starts) are presumably important factors to improve piglet growth and weight at weaning.

3.4.2. Transfer of passive and active immunity

Colostrum intake is crucial for development of piglet immunity. In this study, and in accordance with normal colostrum and milk immunoglobulin kinetics (5), sow colostrum and milk IgG and IgA showed an abrupt and steady decrease respectively (**Figures 1C, 1D**). Interestingly, colostrum immunoglobulin content on day +1 was higher in the oral meloxicam group then in the control group. The difference between groups was more pronounced in IgA than in IgG, which could be explained by the switch between the IgG/IgA ratio after farrowing (5). One hundred percent of colostrum IgG and 40% of colostrum IgA come from sow blood via an Ig receptor, whereas up to 60% of IgA is directly synthesized in the mammary gland (1). Our data support the local role of oral meloxicam in the mammary gland, which likely decreases local inflammation, thus favoring both immunoglobulin recruitment from plasma and local production of IgA in plasma cells (1). Indeed, in vitro studies developed in cattle have shown the anti-inflammatory effect of meloxicam in mammary epithelial cells (25). Furthermore, mastitis in cows has been associated with reduced pre-weaning immunity, growth, and health of the offspring (26, 27), so the anti-inflammatory effect elicited by meloxicam treatment is presumed to have the opposite effect.

In comparison with blood sampling, saliva sampling is generally considered to be a non-invasive and stress-free methodology (28). IgG levels in sow saliva are directly proportional to the levels in sow serum, whereas IgA in saliva is mostly produced locally, so IgA levels are highly variable in response to environmental factors such as stress and oral infections (29). In our study, saliva IgG levels, a marker of plasma IgG levels, showed no differences between groups, which probably rules out a systemic effect of oral meloxicam administration on the Ig increase in piglets from treated sows.

IgA and IgG concentration in piglet serum during lactation is the result of intake of immunoglobulins from colostrum. The quick drop of IgA and the slow drop of IgG in piglet serum is likely explained by the different half-lives of these immunoglobulins in serum, being 6 days for IgA and 21 days for IgG (30). IgA concentration was higher in piglet serum in the oral meloxicam group on days +1 and +9. Interestingly, diarrhea of newborn piglets is one of the biggest health issues in pig production, and increased IgA levels could play a major role in preventing these problems by their protective effect on the intestinal mucosa (1). Mainau et al. (18) found that the administration of meloxicam orally at the beginning of farrowing (on average, when 2.6 piglets had already been born) increased the concentration of IgG in the serum of piglets. In the present study, sows were treated early at the beginning of farrowing, when early colostrum (with the highest IgG levels) has already been produced and thus the influence of treatment on the IgG serum levels of piglets fed with this colostrum was lower.

Nevertheless, weaker piglets and those born later during parturition are known to suffer from delayed and reduced colostrum intake (31). These animals have lower survival and growth rates, which may be improved by treatment with meloxicam, as shown by our results with piglets born with a very light weight at birth, as well as by other studies (31). These differences could be explained by a higher IgG and IgA immunity transfer in the treatment group in these weaker animals, which are likely to consume a smaller quantity of early-colostrum and a larger proportion of mid- and late-colostrum. Unfortunately, in this study a low percentage of piglets with low BW at birth were blood sampled, thus hampering a proper analysis of their serum IgG and IgA levels.

Regarding colostrum and milk cytokines, higher levels of IL-2, IL-4, and IFN-ϒ were found on day +1, which is likely to be related to pain and to contamination of the reproductive tract induced by farrowing. Milk cytokine levels moderately decreased between day +1 and +9 and increased again between day +9 and +20, likely in response to the vaccination given to sows on day +16. Cytokines and lymphoid cells have been demonstrated to cross the intestinal barrier of newborn piglets (32–34). In piglet serum, cytokine levels measured on day +1 are expected to be the result of both colostrum-derived cytokines and cytokines produced by the piglets. In contrast, taking into consideration the short half-life of these cytokines (minutes for IL-2 and IL-4 and a few hours for IFN-ϒ) and the loss of piglet intestinal permeability, cytokine levels on days +9 and +20 reflect only the activity of the piglet immune system. Higher concentrations of all cytokines were found on day +9, likely due to the immune challenge elicited by tail docking (in both sexes) and castration (in males), which were performed on their second day of life. Interestingly, higher IL-4 and IL- 2 levels were measured in piglets from the meloxicam treated group on days day +9 (**Figures 2B, 2D**). Increased secretion of IL-4 and IL-2 in piglets has been related to better Th2 and Th1 immune responses, respectively (8). Moreover, IL-4 induces antibody production and tissue repair, whereas IL-2 plays a major role in the activation of NK-cells and the generation of effector and memory cells (9, 10). This positive influence of colostrum on the immune system development could be related to the transfer of colostrum-associated immune cells, which are absorbed selectively in the newborn gut, although the precise mechanisms remain unclear (1). Therefore, it could be hypothesized that meloxicam treatment around farrowing had an impact on the concentration of immune cells in colostrum, but further studies are needed to investigate this hypothesis.

This study was developed on a commercial farm with really good sanitary and husbandry conditions. Further research is required to determine if these positive results on piglet welfare can be even more pronounced by studding a larger set of commercial farms with higher mortality rates and lower growth rates during lactation. In summary, the results of this study show that early administration of oral meloxicam improves some aspects of piglet performance and welfare. Further research is needed to study whether these effects are also observed in primiparous sows or could be improved by administering meloxicam before the onset of farrowing.

3.5. Conclusions

The administration of meloxicam orally at the beginning of farrowing in multiparous sows increased the concentration of immunoglobins and cytokines in sow colostrum and improved both humoral and cellular immune response in piglets. Pre-weaning growth of piglets, especially in piglets born with low BW, tended to be higher in the meloxicam-treated group than that in the control group.

Ethics Statement

The experimental protocol described in this experiment was approved by the Institutional Animal Care and Use Committee of the Universitat Autònoma de Barcelona (CEEAH-1591) and the Generalitat de Catalunya (DMAH-6720). Written informed consent was obtained from the owners for the participation of their animals in this study.

Conflict of interests

The authors declare that this study received funding from Boehringer Ingelheim Vetmedica GmbH. The funder was not involved in the study design, collection, analysis, interpretation of data and the writing of this article.

Author contributions

EN: data acquisition, structurization, and interpretation, drafting of the manuscript and final approval of the version to be published. EM: study concept and design, data acquisition, data analysis and interpretation, drafting of the manuscript and final approval of the version to be published. RM: immunology data analysis and interpretation, preparation of figures, and drafting of the manuscript and final approval of the version to be published. DT and MS: data acquisition and final approval of the version to be published. XM: study concept and design, drafting of the manuscript and final approval of the version to be published. All authors contributed to the article and approved the submitted version.

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3.6. References

1. Salmon H, Berri M, Gerdts V, Meurens F. Humoral and cellular factors of maternal immunity in swine. Dev Comp Immunol (2009) 33:384–393. doi:10.1016/j.dci.2008.07.007
2. Zanello G, Meurens F, Serreau D, Chevaleyre C, Melo S, Berri M, et al. Effects of dietary yeast strains on immunoglobulin in colostrum and milk of sows. Vet Immunol Immunopathol (2013) 152:20–27. doi:10.1016/j.vetimm.2012.09.023
3. Decaluwé R, Maes D, Wuyts B, Cools A, Piepers S, Janssens GPJ. Piglets' colostrum intake associates with daily weight gain and survival until weaning. Livest Sci (2014) 162:185–192. doi:10.1016/j.livsci.2014.01.024
4. Quesnel H, Farmer C, Devillers N. Colostrum intake: Influence on piglet performance and factors of variation. Livest Sci (2012) 146:105–114. doi:10.1016/j.livsci.2012.03.010
5. Theil PK, Lauridsen C, Quesnel H. Neonatal piglet survival: Impact of sow nutrition around parturition on fetal glycogen deposition and production and composition of colostrum and transient milk. Animal (2014) 8:1021–1030. doi:10.1017/S1751731114000950

6. Evans PA, Newby TJ, Stokes CR, Bourne FJ. A study of cells in the mammary secretions of sows. Vet Immunol Immunopathol (1982) 3:515–527. doi:10.1016/0165-2427(82)90017-4

7. Le Jan C. A study by flow cytometry of lymphocytes in sow colostrum. Res Vet Sci (1994) 57:300–304. doi:10.1016/0034-5288(94)90121-X

8. Junttila IS. Tuning the cytokine responses: An update on interleukin (IL)-4 and IL-13 receptor complexes. Front Immunol (2018) 9: doi:10.3389/fimmu.2018.00888

9. Abbas AK, Trotta E, Simeonov D, Marson A, Bluestone JA. Revisiting IL-2: Biology and therapeutic prospects. Sci Immunol (2018) 25:3;6. doi: 10.1126/sciimmunol.aat1482

10. Kumar V, Abbas AK, Aster J.C. Robbins & Cotran Pathologic Basis of Disease, 10th Edition. El Sevier (2020), Philadelphia, PA.

11. Conte S, Bergeron R, Gonyou H, Brown J, Rioja-Lang FC, Connor ML, et al. Use of an analgesic to identify pain-related indicators of lameness in sows. Livest Sci (2015) 180:203–208. doi:10.1016/j.livsci.2015.08.009

12. Hirsch AC, Philipp H, Kleemann R. Investigation on the efficacy of meloxicam in sows with mastitis-metritis-agalactia syndrome. J Vet Pharmacol Ther (2003) 26:355–360. doi:10.1046/j.1365-2885.2003.00524.x

13. Sabaté D, Salichs M, Bosch J, Ramó P, Homedes J. Efficacy of ketoprofen in the reduction of pre-weaning piglet mortality associated with sub-clinical forms of post-partum dysgalactia syndrome in sows. Pig J (2012) 67:19–23.

14. Homedes J, Salichs M, Sabaté D, Sust M, Fabre R. Effect of ketoprofen on

pre-weaning piglet mortality on commercial farms. Vet J (2014) 201:435–437. doi:10.1016/j.tvjl.2014.05.038

15. Ison SH, Jarvis S, Rutherford KMD. The identification of potential behavioural indicators of pain in periparturient sows. Res Vet Sci (2016) 109:114–120. doi:10.1016/j.rvsc.2016.10.002

16. Schinckel AP, Cabrera R, Boyd RD, Jungst S, Booher C, Johnston M, et al. Impact of Birth and Body Weight at Twenty Days on the Post-weaning Growth of Pigs with Different Weaning Management. Prof Anim Sci (2007) 23:197–210. doi:10.15232/S1080-7446(15)30965-7

17. Tenbergen R, Friendship R, Cassar G, Amezcua MR, Haley D. Investigation of the use of meloxicam post farrowing for improving sow performance and reducing pain. J Swine Heal Prod (2014) 22:10–15.

18. Mainau E, Temple D, Manteca X. Experimental study on the effect of oral meloxicam administration in sows on pre-weaning mortality and growth and immunoglobulin G transfer to piglets. Prev Vet Med (2016) doi:10.1016/j.prevetmed.2016.01.032

19. Mainau E, Ruiz-De-La-Torre JL, Dalmau A, Salleras JM, Manteca X. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. Animal (2012) 6:494–501. doi:10.1017/S1751731111001790

20. Ison SH, Jarvis S, Ashworth CJ, Rutherford KMD. The effect of post-farrowing ketoprofen on sow feed intake, nursing behaviour and piglet performance. Livest Sci (2017) 202:115–123. doi:10.1016/j.livsci.2017.06.001

21. Viitasaari E, Hänninen L, Heinonen M, Raekallio M, Orro T, Peltoniemi O, et al. Effects of post-partum administration of ketoprofen on sow health and piglet growth. Vet J (2013) 198:153–157. doi:10.1016/j.tvjl.2013.06.013

22. Roelofs S, Godding L, de Haan JR, van der Staay FJ, Nordquist RE. Effects of parity and litter size on cortisol measures in commercially housed sows and their offspring. Physiol Behav (2019) 201:83–90. doi:10.1016/j.physbeh.2018.12.014

23. Udomchanya J, Suwannutsiri A, Sripantabut K, Pruchayakul P, Juthamanee P, Nuntapaitoon M, et al. Association between the incidence of stillbirths and expulsion interval, piglet birth weight, litter size and carbetocin administration in hyper-prolific sows. Livest Sci (2019) 227:128–134. doi:10.1016/j.livsci.2019.07.013

24. Ison SH, Jarvis S, Hall SA, Ashworth CJ, Rutherford KMD. Periparturient behavior and physiology: Further insight into the farrowing process for primiparous and multiparous sows. Front Vet Sci (2018) 5: doi:10.3389/fvets.2018.00122

25. Caldeira MO, Bruckmaier RM, Wellnitz O. Meloxicam affects the inflammatory responses of bovine mammary epithelial cells. J Dairy Sci (2019) 102:10277–10290. doi:10.3168/jds.2019-16630

26. Heravi Moussavi A, Danesh Mesgaran M, Gilbert RO. Effect of mastitis during the first lactation on production and reproduction performance of Holstein cows. Trop Anim Health Prod (2012) 44:1567–1573. doi:10.1007/s11250-012-0107-3

27. Ferdowsi Nia E, Nikkhah A, Rahmani HR, Alikhani M, Mohammad Alipour M, Ghorbani GR. Increased colostral somatic cell counts reduce pre-

weaning calf immunity, health and growth. J Anim Physiol Anim Nutr (Berl) (2010) 94:628–634. doi:10.1111/j.1439-0396.2009.00948.x

28. Escribano D, Gutiérrez AM, Martínez Subiela S, Tecles F, Cerón JJ. Validation of three commercially available immunoassays for quantification of IgA, IgG, and IgM in porcine saliva samples. Res Vet Sci (2012) 93:682–687. doi:10.1016/j.rvsc.2011.09.018

29. Muneta Y, Yoshikawa T, Minagawa Y, Shibahara T, Maeda R, Omata Y. Salivary IgA as a useful non-invasive marker for restraint stress in pigs. J Vet Med Sci (2010) 72:1295–1300. doi:10.1292/jvms.10-0009

30. Curtis J, Bourne FJ. Half-lives of immunoglobulins IgG, IgA and IgM in the serum of new-born pigs. Immunology (1973) 24:147–55.

31. Devillers N, Le Dividich J, Prunier A. Influence of colostrum intake on piglet survival and immunity. Animal (2011) 5:1605–1612. doi:10.1017/S175173111100067X

32. Elahi S, Thompson DR, Van Kessel J, Babiuk LA, Gerdts V. Protective role of passively transferred maternal cytokines against Bordetella pertussis infection in newborn piglets. Infect Immun (2017) 85:1–16. doi:10.1128/IAI.01063-16

33. Bandrick M, Theis K, Molitor TW. Maternal immunity enhances Mycoplasma hyopneumoniae vaccination induced cell-mediated immune responses in piglets. BMC Vet Res (2014) 10: doi:10.1186/1746-6148-10-124

34. Perrine Susan. 基因的改变NIH Public Access. Bone (2005) 23:1–7. doi:10.1038/jid.2014.371

3.7. Supplementary Material

Table 1. *Mean and standard error (SE) of the piglet weight at birth, 9 days after farrowing (day+9) and at weaning (day +20) in Kilograms and the Average Daily Gain (ADG) of piglets from birth to day+9 after farrowing, from birth to weaning and from day+9 to weaning in grams per day for 354 piglets regarding piglet sex (females vs. males) effects.*

	Male		**Female**		
	Mean	**SE**	**Mean**	**SE**	**P-value**
Weight at birth (Kg)	1.583	0.046	1.527	0.047	0.566
Weight at day +9 (Kg)	3.533	0.061	3.523	0.063	0.944
Weight at weaning (Kg)	6.601[a]	0.099	6.415[b]	0.103	0.016
ADG from birth to day +9 (Kg/day)	0.220	0.008	0.215	0.009	0.388
ADG from birth to weaning (Kg/day)	0.253[a]	0.005	0.241[b]	0.006	0.024
ADG from day +9 to weaning (Kg/day)	0.277[a]	0.007	0.260[b]	0.007	0.005

Different superscripts (a, b) in the same column indicate significant differences within each effect ($p < 0.05$). Tendency has been shown at $p < 0.1$.

CAPÍTULO 4. DEVELOPMENT OF A FACIAL EXPRESSION SCALE USING FARROWING AS A MODEL OF PAIN IN SOWS

Capítulo 4

DEVELOPMENT OF A FACIAL EXPRESSION SCALE USING FARROWING AS A MODEL OF PAIN IN SOWS

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Capítulo 4

SIMPLE SUMMARY

Pain evaluation using non-invasive indicators can be difficult in production animals. Some years ago, a group of scientists realized that it was possible to evaluate pain by just considering animals' facial expressions. All animals have their own facial expressions, and the animal´s expressiveness affects how difficult it is to find facial zones to evaluate pain. Until today, facial expressions have never been studied in sows. Our group decided to use farrowing (sow parturition) as a pain model to evaluate the sows' facial expressions. Five different facial expression zones were found in our study: Tension above eyes, Snout angle, Neck tension, Temporal tension and ear position, and Cheek tension. The five zones were studied and evaluated by eight observers after a training session, obtaining good reliability results, especially on Tension above eyes, Snout angle, and Neck tension. These good results suggest that the sow facial expression scale could be a good non-invasive indicator to evaluate pain in sows.

ABSTRACT

Changes in facial expression have been shown to be a useful tool to assess pain severity in humans and animals, but facial scales have not yet been developed for all species. A facial expression scale in sows was developed using farrowing as a pain model. Five potential facial zones were identified: (i) Tension above eyes, (ii) Snout angle, (iii) Neck tension, (iv) Temporal tension and ear position (v), and Cheek tension. Facial zones were examined through 263 images of a total of 21 sows at farrowing, characterizing moments of non-pain (19 days post-farrowing; score 0), moderate pain (time interval between the delivery of two consecutive piglets; score 1) and severe pain (during active piglet delivery; score 2). Images were evaluated by a “Silver Standard” observer with experience in sows’ facial expressions, and by a group of eight animal welfare scientists, without experience in it, but who received a one-hour training session on how to assess pain in sows’ faces. Intra- and inter-observer reliability of the facial expression ranged from moderate to very good for all facial expression zones, with Tension above eyes, Snout angle, and Neck tension showing the highest reliability. In conclusion, monitoring facial expressions seems to be a useful tool to assess pain caused by farrowing.

4.1. Introduction

Pain is a sensory and emotional experience that has significant effects on animal welfare, leading to a negative production impact [1]. The measurement of pain in animals is very complex, and it continues to be a critical issue in veterinary care and biomedical research [2].

Almost two hundred years ago, Darwin had already described the non-human animals' capacity for expressing emotions such as pain through facial expression [3]. In the last decade, facial expressions have been studied rigorously in several animal species including rats, mice, rabbits, ferrets, horses, sheep, piglets, and sals [4–14]. Facial expressions have been shown to be a good pain indicator in most species, allowing one not only to detect pain, but also to grade its severity after a noxious stimulus [4–10,12–14]. Grimace scales are based on the Facial Action Coding System (FACS), which were initially developed by Ekman and Friesen [15]. The development of facial expression scales requires the ability to identify the change of specific facial action units (FAUs) when animals are in pain. At least three FAUs have been described for each pain scale and all scales have demonstrated high inter-observer reliability among observers, proving their accuracy and feasibility [6]. Pain scores assigned by observers to painful conditions in farm animals are usually influenced by gender, age, and/or profession of the observers [16,17]. Di Giminiani et al. [8] stated that the level of pig knowledge of the observers was not correlated to the pain scores assigned in the facial scale in piglets. Despite this, to our knowledge, the effects of the observer's gender and age on the inter-observer reliability test performed in facial expression scales have not been previously studied.

Many of the facial scales studied in different species showed that orbital tightening is one of the easiest regions for observers to recognize and use to evaluate pain [4,5,8–10,12], focusing on eye aperture.

Pigs are a prey species, so, although it has not been proven, it seems likely that they would tend to not express pain or weakness, making pain recognition and evaluation incredibly difficult [1]. For this reason, limited pain indicators have been documented to evaluate whether an animal is suffering from pain in a specific moment [18]. The parturition in sows is associated with increased plasma cortisol concentrations, C-reactive protein, and Haptoglobin, which could be indicative of pain [19]. So far, effective and non-invasive pain indicators in sows during farrowing are based on behavioral and corporal indicators such as legging, pawing, arching, trembling, or tail tickling [19,20].

As mentioned before, piglet facial scales have been studied, and they seem to be a very good tool to assess pain, but until now, no facial scales have been developed in sows.

Farm animals can suffer from pain throughout their lives. All mammalian female farm animals give birth at least once in their lives, and it is often without the provision of either anesthesia or analgesia during the process [21]. As happens in other species, it has been demonstrated that farrowing itself is a painful moment for the sows [19, 21,22], and a difficult farrowing may affect sow behavior through exhaustion, sickness, or pain [23], which can develop into deficient maternal behavior.

The aims of this study were to (i) develop a facial expression scale based on FAUs using farrowing as a model of pain, thus avoiding producing unnecessary pain, and (ii) evaluate the effect of the observers' sex and knowledge of the pig production sector on their rating behavior. The grading scale for pain provided herein might represent a substantial advance in pain recognition and management in sows and may become a fast, easy-to-learn, and highly reproducible tool for farrowing sows.

4.2. Materials and Methods

4.2.1. Animals, Housing, and General Management

The experimental procedure was carried out on a commercial farm (Casa-Ramona; Sora, Barcelona, Spain) from April to July 2018. A total of twenty-one Danbred sows, seven gilts and fourteen small-sized multiparous sows (from 2nd to 4th parity), were randomly selected on the day of parturition. A total of six different weekly study replicates with one to five sows in each replicate were studied. Sows that farrowed at night, sows that showed signs of lesions, illness, or lameness, and sows that had a poor body condition (score < 2 on a five-point scale) were not included in the study.

On Day 109 of gestation, sows were moved to the farrowing room and were allocated into farrowing crates (1.95 m × 0.60 m) built with steel bars, which were centrally positioned in the farrowing pens (2.40 m × 1.80 m). Farrowing pens had a fully metal-slatted floor for sows and plastic-slatted floor for piglets. All farrowing pens had a metal heat pad at 36 °C and a supplemented heat lamp for the piglets during the first week of life.

The temperature in the farrowing room was kept constant, approximately at 22 °C, and light was on from 7:00 h to 18:00 h every day of the week. Sows were offered three meals per day (7:00 h, 13:00 h, and 17:00 h) and water was available ad libitum from drinking.

On Day 116 of gestation, at 7:00 h, farrowing was hormonally induced with 1mL of Dalmazin® in multiparous sows (D Cloprostenol 0.075 mg/mL, FatroIberica; Barcelona, Spain).

Treatments and manual interventions during farrowing followed the usual routine of the farm and were performed by the same person. When needed, intramuscular (IM) treatments were injected in the neck. When the interval time between two piglets exceeded 1 h and the cervical canal was dilated, 1 mL of Cabetocin (Decomoton®, Calier S.A.; Les Franqueses del Vallés, Barcelona, Spain) was injected. When the cervical canal was not sufficiently dilated, sows were treated with 200 mg of Vetrabutine Hydrochloride (Monzal®, Boehringer Ingelheim España, S.A.; Barcelona, Spain). When sows were very nervous to farrow, 5 mL of Azaperone (Stressnil®, Janssen Animal Health, Elanco; Brussels, Belgium) were administered.

Litter size was standardized by cross-fostering within 8 h post-farrowing, and piglets were weaned at twenty-one days old, according to veterinary recommendations.

4.2.2. Experimental Procedure

It was necessary to see the sow’s face throughout the parturition without interference of the feeder. A vertical metal barrier (50 cm × 30 cm × 2 cm) was installed in the cranial part of the crate (in front of the sow’s feeder). Thus, the sow could not put her head under the feeder, and a complete vision of the sow’s face was guaranteed during parturition. One action camera (SK8 HD 4K; SK8 Urban, Spain) per sow was installed at 120 cm above the floor when the sow was lying down. The camera was focused on the sow’s face and its angle allowed us to record the rest of the body and the movements of the sow. When needed, an LED light was added to produce clearer and better-quality images. Video recordings were obtained from the beginning of the farrowing (before the first piglet´s expulsion) until the last piglet was born. If the sow was uncomfortable with the vertical metal barrier installed in front of the feeder, it was excluded from the study.

On Day 19 post-farrowing, sows were recorded again for 2 h between 8:30 h and 12:30 h, avoiding meals and farmer management, with the aim of obtaining control images. It was assumed that after more than two weeks of farrowing, there was no pain associated with farrowing, or its intensity was minimal.

For each sow, the following parameters were registered during farrowing by direct observations: the duration of farrowing, defined as the period of time between the first and the last piglet born; the time of expulsion of the piglet; the condition of each piglet at birth (born alive, stillborn, or mummified fetuses); and the number of treatments and manual interventions during farrowing.

4.2.3. Collection, Selection, and Processing of Images

One observer visualized all of the videos (partum and post-partum) and selected images in three different moments, always when the sow was lying laterally, and its face was fully visible:

- Pre-weaning (indicative of painless; Score 0). Images were chosen every 15–20 min.
- Interval time between two piglets' expulsion (indicative of moderate pain; Score 1). One image was chosen in each interval time.
- Expulsion of the piglets (indicative of severe pain; Score 2). One image was chosen from within the 30 s prior to each piglet expulsion.

A total of 268 images were obtained (78 pre-weaning images; 91 images between two piglets' expulsion; and 99 images before the expulsion of the piglet).

Once all of the images were collected, they were cropped so only the faces of the sows were visible, to guarantee blinding by not revealing the rest of the sows' body.

4.2.4. Development of the Facial Expression Scale

4.2.4.1. Obtention of the Facial Expression Scale

A total of 268 images was assessed by the same observer (hereafter, the Silver Standard; SS). Images were randomly mixed and analyzed independently and blindly by the SS. For each image, SS determined five potential FAUs based on previous grimace score studies [4,5,8,11], which showed clear differences among the three moments studied. The five potential FAUs noticed in sows were: Tension above eyes, Snout angle, Neck tension, Temporal tension and ear position, and Cheek tension. Descriptions of the three moments of pain (score 0 = painless; score 1 = moderate pain; and score 2 = severe pain) of each FAU are explained in the Results section (**Figure 1**). The SS scored each FAU of all of the images using four categories: 0 (painless), 1 (moderate pain), 2 (severe pain), and IDK (I do not know or I do not feel confident assigning a degree-of-pain score).

4.2.4.2. Inter- and Intra-Observer Reliability

A group of eight observers working as scientific researchers in the field of animal behavior and welfare at the Universitat Autònoma de Barcelona, without experience in studying animal facial expressions, were selected. Four observers (one man and three women) usually work in pig behavior, while the other four observers (one man and three women) work in other species such as cattle, companion animals, and zoo animals. The age range of the observers was between 28 and 37 years old. They were trained for 45 min by the SS concerning the physical differences among the three painful moments of the five FAUs in sows. The SS and observers evaluated 15 images together to standardize their assessment criteria. After that training, observers had to evaluate the five FAUs of 60 selected images for 45 s per image. Sixty images were randomly chosen from the 268 selected images: 18 images from the pre-weaning moment (score 0), 20 images from between two piglets' expulsion (score 1), and 22 images from the moment of the piglet expulsion (score 2). Twelve of the 60 images were repeated twice to study intra-observer reliability. Observers were blind to the moment when images were taken, and they evaluated each FAU with the same four-point system used by the SS: 0 (painless), 1 (moderate pain), 2 (severe pain) or IDK (I do not know or I do not feel confident assigning a degree-of-pain score).

4.2.5. Statistical Analysis

Data were analyzed using the Statistical Analysis System (SAS 9.4 software, SAS institute Inc.; Cary, NC, USA). The significance level was established at $p < 0.05$. Descriptive values are given as mean ± SE.

Cohen's Kappa coefficient was used to test the reliability between the SS assessment of the five FAUs and the moment when each image was obtained (pre-weaning, interval time between two piglets´ expulsion, or expulsion of the piglets). In addition, Spearman Correlation (r) was performed among the five FAUs (taking into account the SS´s assessment of 268 images).

Cohen's Kappa coefficient (κ) was used to test intra- and inter-observer reliability. Inter-observer reliability was carried out by comparing the SS with the eight trained observers. Intra-observer reliability was accomplished by comparing repeated images per observer. The reliability among the following scores was analyzed: score 0 (painless), score 1 (moderate pain), score 2 (severe pain), and IDK (I do not know, introduced as a missing value). The level of reliability was categorized as follows: poor reliability ($\kappa < 0.20$), fair reliability ($0.20 \geq \kappa < 0.40$), moderate reliability ($0.40 \geq \kappa < 0.60$), good reliability ($0.60 \geq \kappa < 0.80$), and very good reliability ($0.80 \geq \kappa < 1.00$) [24].

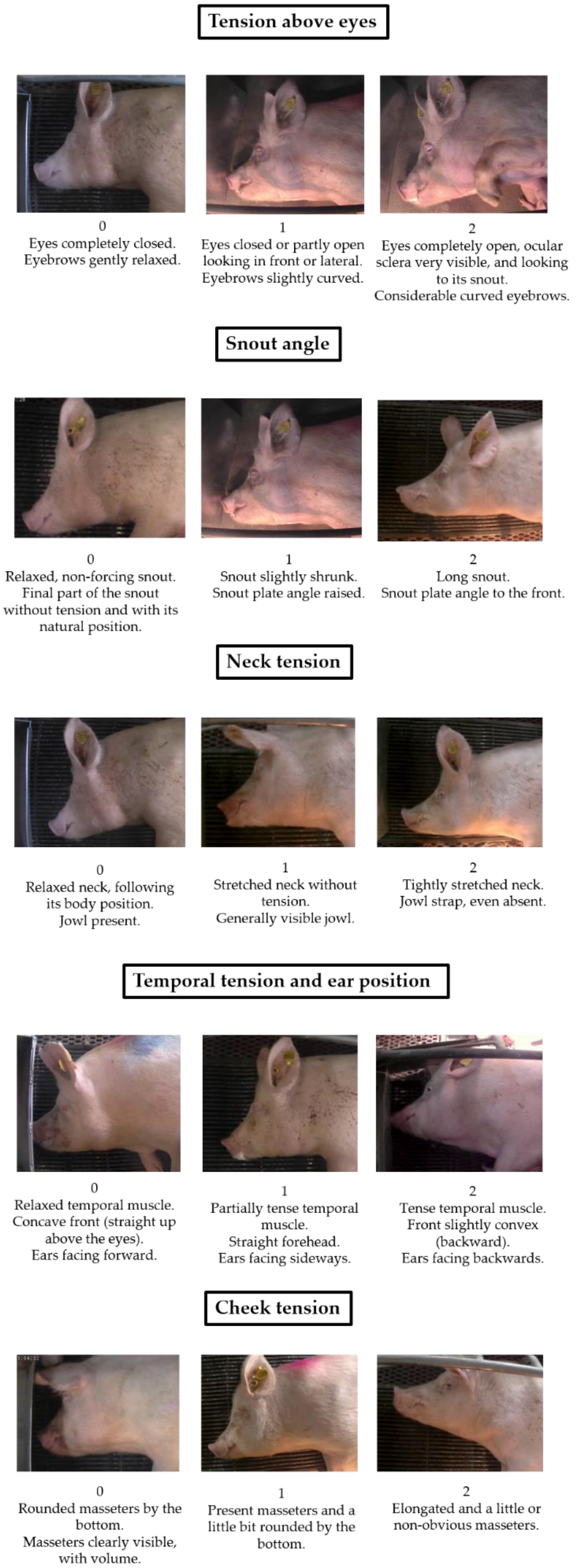
In order to explore the effects of observer gender and their experience in pig behavior on the pain scores given in the evaluation session, two statistical analyses were performed. Firstly, for each FAU assessed in the evaluation session (scored as 0, 1 or 2), data were expressed as the number of FAUs with a score of 1 or 2 out of the total number of images assessed. Data were analyzed using generalized linear mixed models (GLIMMIX procedure) followed by a binary distribution. The model included the fixed effect of observer gender (women vs. men), observer experience in pig behavior (yes vs. no), and their interaction effect. The image was the experimental unit. The LSMEANS adjusted to Tukey's honestly significant difference was used as a test of comparisons. Secondly, differences between inter- and intra-observer reliability means from the eight trained observers were evaluated using the univariate ANOVA test (PROC ANOVA). The fixed effects studied were observer gender (women vs. men), observer experience in pig behavior (yes vs. no), and the FAU assessed (from 1 to 5) and their interactions effects.

4.3. Results

4.3.1. Obtention of the Facial Expression Scale

Figure 1 shows the sow grimace scale with the five potential FAUs indicative of pain (Tension above eyes, Snout angle, Neck tension, Temporal tension and ear position, and Cheek tension) and the description of the three moments of pain (score 0 = painless; score 1 = moderate pain; and score 2 = severe pain) of each FAU. To determine which moment of pain the sow is in, at least one of the descriptions of each area must be observed clearly.

Figure 1. *Sow grimace scale, with descriptions for each of the five facial action units (FAUs) employed: Tension above eyes, Snout angle, Neck tension, Temporal tension and ear position, and Cheek tension. FAUs are scored based on a three-grade scale: (score 0 = painless; score 1 = moderate pain and score 2 = severe pain).*


Tension above eyes
0
Eyes completely closed.
Eyebrows gently relaxed.
1
Eyes closed or partly open looking in front or lateral.
Eyebrows slightly curved.
2
Eyes completely open, ocular sclera very visible, and looking to its snout.
Considerable curved eyebrows.
Snout angle
0
Relaxed, non-forcing snout.
Final part of the snout without tension and with its natural position.
1
Snout slightly shrunk.
Snout plate angle raised.
2
Long snout.
Snout plate angle to the front.
Neck tension
0
Relaxed neck, following its body position.
Jowl present.
1
Stretched neck without tension.
Generally visible jowl.
2
Tightly stretched neck.
Jowl strap, even absent.
Temporal tension and ear position
0
Relaxed temporal muscle.
Concave front (straight up above the eyes).
Ears facing forward.
1
Partially tense temporal muscle.
Straight forehead.
Ears facing sideways.
2
Tense temporal muscle.
Front slightly convex (backward).
Ears facing backwards.
Cheek tension
0
Rounded masseters by the bottom.
Masseters clearly visible, with volume.
1
Present masseters and a little bit rounded by the bottom.
2
Elongated and a little or non-obvious masseters.

High reliability was obtained between the SS assessment of the five FAUs and the moment when each image was obtained (pre-weaning, interval time between two piglets' expulsion or piglet expulsion). Very good reliability was obtained for Tension above eyes and Snout angle FAUs, and good reliability was obtained for Neck tension, Temporal tension and ear position, and Cheek tension FAUs (**Table 1**).

Table 1. *Percentage of reliability and Kappa coefficient between images and the silver standard evaluation in each FAU of 268 sow face images: 78 images from before weaning (score 0), 91 images from the interval time between two piglets' expulsion (score 1), and 99 images from piglet expulsion (score 2). The silver standard evaluation was defined as follows: painless moment (score 0), moderate painful moment (score 1), severe painful moment (score 2), and I do not know (IDK).*

Image Moment	Silver Standard Evaluation	Tension Above Eyes	Snout Angle	Neck Tension	Temporal Tension and Ear Position	Cheek Tension
Before Weaning (score 0)	0	89.74	88.46	73.08	64.10	69.23
	1	10.26	11.54	19.23	34.62	16.67
	2	0.00	0.00	0.00	0.00	0.00
	IDK	0.00	0.00	7.69	1.28	14.10
The Interval Time between Two Piglets' Expulsion (score 1)	0	0.00	1.10	2.20	6.59	3.29
	1	93.41	89.01	74.73	86.82	70.33
	2	6.59	9.89	13.19	6.59	8.80
	IDK	0.00	0.00	9.89	16.67	17.58
Piglet Expulsion (score 2)	0	1.01	0.00	1.01	3.03	1.01
	1	2.02	7.07	13.13	24.24	19.19
	2	96.97	92.93	78.79	72.73	67.68
	IDK	0.00	0.00	7.07	0.00	12.12
Cohen's Kappa Coefficient		0.90 ***	0.85 ***	0.74 ***	0.63 ***	0.71 ***

*** *p-value < 0.0001.*

Studying the Spearman Correlations among the five FAUs (taking into account the SS assessment of 268 images), the highest positive correlations were found between Tension above eyes and Snout angle FAUs (r = 0.91), Tension above eyes and Neck tension FAUs (r = 0.87), Snout angle and Neck tension FAUs (r = 0.85), and Tension above eyes and Cheek tension FAUs (r = 0.84). All of the other FAUs showed positive correlations among each other (from r = 0.60 to r = 0.79) with $p < 0.0001$ in all pair correlations.

4.3.2. Inter- and Intra-Observer Reliability

Inter-observer reliability is summarized in **Table 2**. Good reliability was obtained for the Tension above eyes FAU. Moderate reliability was observed for the other FAUs studied.

Table 2. *Inter-observer reliability (Kappa Coefficient) between the individual and mean evaluation of eight observers (from 01 to 08) and the Silver Standard of the five facial action units (FAUs) of 60 sow face images.*

	Observers								
FAU	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**Mean**
Tension above Eyes	0.55	0.65	0.70	0.60	0.56	0.73	0.48	0.75	0.63
Snout Angle	0.24	0.55	0.33	0.50	0.33	0.37	0.27	0.31	0.36
Neck Tension	0.41	0.36	0.43	0.37	0.48	0.46	0.57	0.53	0.45
Temporal Tension and Ear Position	0.30	0.50	0.33	0.23	0.29	0.37	0.43	0.41	0.36
Cheek Tension	0.25	0.41	0.62	0.58	0.39	0.48	0.32	0.32	0.42

$p < 0.0005$ in all cases.

Table 3 shows the intra-observer reliability of each FAU, analyzing 12 sow face images repeated twice during the 60-image evaluation. Neck tension was the FAU where the observers coincided in more cases with their own results, showing the best reliability. Tension above eyes and Snout angle FAUs also had good reliability. The Cheek tension FAU obtained a moderate reliability score and Temporal tension and ear position FAUs obtained a fair one.

Table 3. *Individual and mean intra-observer reliability (Kappa Coefficient) of eight observers (from 01 to 08) for each facial action unit (FAU) of 12 sow face images repeated twice each during the 60-images evaluation.*

	Observers								
Pain Indicators	**01**	**02**	**03**	**04**	**05**	**06**	**07**	**08**	**Mean**
Tension above Eyes	0.09	0.71 ***	0.67 **	0.68 **	0.40 ***	0.89 ***	0.87 ***	0.65 ***	0.62
Snout Angle	0.73 ***	0.96 ***	0.81 ***	0.24 **	0.73 ***	0.77 ***	0.31 **	0.27 *	0.60
Neck Tension	0.22 *	0.87 ***	1 ***	0.40 *	0.87 ***	0.87 ***	0.75 ***	0.91 ***	0.74
Temporal Tension and Ear Position	0.07	0.91 ***	0.09	0.56 **	0.18	0.06	0.38 **	0.34 **	0.32
Cheek Tension	0.19 *	0.63 **	1 ***	0.26 **	0.40 ***	0.70 **	0.76 ***	0.21 *	0.52

*** $p < 0.0005$, ** $p < 0.005$, * $p < 0.05$.

4.3.3. Effect of the Observer Gender and Experience in Pig Behavior in Assessing Pain at Farrowing

Pain scores given by trained observers during the 60-image evaluation differed by gender.

Women scored a higher percentage of images as severe and moderate pain than did men in the Cheek tension FAU (severe pain: 51.9% vs. 45.5%, p = 0.0287 and moderate pain: 70.3% vs. 59.5%, p = 0.0302). Similarly, women scored a higher percentage of images as severe pain than did men in the Snout Angle FAU (47.9% vs. 27.7%, respectively, p = 0.0001) and Neck tension FAU (44.39% vs. 37.8%, respectively, p = 0.0392). However, pain scores given to the

Tension above eyes and Temporal tension and ear position FAUs were not different by gender.

Pain scores given by trained observers were not affected by their experience in pig behavior or by the interaction effect of experience in pig behavior by gender.

The inter-observer reliability mean was affected by observer gender, as women observers obtained higher reliability with the SS (k = 0.47 ± 0.025) than did men (k = 0.38 ± 0.037) (p = 0.0318).

The inter-observer reliability mean was also affected by the FAUs, Tension above eyes (k = 0.63 ± 0.034) being the FAU with the best reliability with the SS, followed by Neck tension (k = 0.45 ± 0.026), Cheek tension (k = 0.42 ± 0.046), Snout Angle (k = 0.36 ± 0.038), and Temporal tension and ear position (k = 0.36 ± 0.031) (p = 0.0001).

Observer experience in pig behavior had no influence in the inter-observer reliability mean (k = 0.45 ± 0.039 from observers working with pigs vs. k = 0.44 ± 0.029 from observers working with other species, p = 0.8309).

The intra-observer reliability mean was also affected by observer gender. Women observers obtained higher reliability within images assessed twice (k = 0.62 ± 0.0524) than did men (k = 0.39 ± 0.0923) (p = 0.0254).

The intra-observer reliability was not affected by observer experience in pig behavior (k = 0.57 ± 0.0764 from observers working with pigs vs. k = 0.55 ± 0.0593 from observers working with other species, p = 0.8821).

4.4. Discussion

The present study´s results suggest that the proposed facial expression scale is a useful tool to assess pain in sows around farrowing. It has been demonstrated that a sow's facial expressions change according to pain intensity and these expressions have been classified into three degrees of pain intensity: absence of pain, moderate pain, and severe pain. In fact, a substantial or near perfect reliability indicates that more than 80% of the images were scored as severe pain when they were obtained during piglet expulsion, and as moderate pain during the interval time between two piglets' expulsion. These results are in agreement with Ison et al. [20], who were able to evaluate the intensity of pain around farrowing through the presence and frequency of corporal pain indicators in sows. They found that most of the corporal pain indicators were observed during farrowing, and considerably increased during piglet expulsion.

Five FAUs were identified by the SS, four of them have already been described in other species [4–14]. It should be noted that our study shares four out of five FAUs with the piglet grimace scale [8] (Tension above eyes, Snout angle, Cheek tension, and Temporal tension and ears), but has different FAUs compared to facial expression scales performed in other species [4–7,9–14]. The present work identified Neck Tension as a new facial expression zone never before described in animals. In previous studies of other species, almost all pictures were taken from the animals' front, allowing the investigators to evaluate their whole faces. In our case, it was not possible to take an image from this angle, because the sows were lying laterally throughout almost all of the parturition. Therefore, we were able to evaluate the neck position. In addition, it could be that, for some investigators, the neck is not considered as a facial indicator. The neck is extended from the head, and its position can influence other FAUs expression, so it seems to be a good indicator that gives us valuable information. In fact, Häger et al. [14] described the head position in sheep as a pain indicator jointly with ear position. Head position is closely, though not completely, related to the neck position. There is a clear difference between the three neck pain descriptions, obtaining good reliability with the intensity of pain suffered by the sow. Based on the results of the present work, Neck Tension could be considered for study in other animal species as a potential facial expression zone indicative of pain.

The SS found that there is good or very good reliability in all FAUs studied, correlating the facial images with the painful moment that the sows are feeling. As shown in other studies [4,5,8–10,12], the Tension above eyes FAU (similar to the “Orbital Tightening” in other studies) showed the highest reliability in the sow’s facial expression scale. Tension above eyes not only evaluates the eye opening (orbital tightening), but also the eyebrow and its expression. In fact, we found a completely different pattern between Tension above eyes in sows at farrowing and the Orbital Tightening of the rest of the studies. The other animals´ facial scales [4–14] describe that the more pain the animal is suffering, the more tightly it closes its eyes; however, sows open them completely, partially showing the sclera. Di Giminiani´s [8] piglet study is the only other study that also described the eyebrow changes, agreeing with us that it becomes more curved when stronger pain was experienced by the animal.

As in other facial expression studies [8,9], inter- and intra-observer reliability analyses demonstrated good reliability with the SS and among themselves in the FAUs evaluation. Cheek tension and Tension above eyes were the best FAUs to assess pain in sows, as they were highly reproducible and highly predictive of pain status. Tension above eyes was a very good pain indicator in the rest of the facial expression studies [4–14]. Cheek tension was also a good FAU in other species such as sheep [9], mouse [4], rabbit [10], and ferret [13], where it is easy to observe cheek bulges when they are in pain. However, rat [5] and piglet [8] grimace scales also consider Cheek tension as a good pain indicator, but its interpretation is totally different. In both species, as with sows, bulging [5,8] occurs naturally when they eat or when they are relaxed, and this characteristic actually diminishes when the animals are in pain, flattening the cheek appearance. Temporal tension and ear position was the most imprecise FAU in sows, probably due to the ear size of sows and/or their position when sows were lying laterally (because they could cover parts of the face). In contrast, Ear position was a very good indicator in other species' facial scales, being considered to be one of the best FAUs to evaluate pain in sheep [9,11], cats [25], rats [5], and mice [4]. There is even a horse grimace scale study [12] where all of the observers were able to evaluate horses' ears.

There was a clear observer gender effect. As was seen in another study based on corporal pain indicators [16], women assessed significantly higher pain scores than did men when the sow was suffering, especially evaluating the moments with more intense pain, related to the piglet expulsion event. It could be because women commonly show a greater empathy towards human and non-human animals [16,26,27].

A lack of correlation was found between the observers' swine production knowledge and the scores assigned to each FAU. Similar results were found in a previous study of facial piglet expression [8]. This expands the scale´s validity and its field of application. A short teaching period for all observers was enough to assure unified grading of facial expression changes and on the pain intensity suffered by the sow. Indeed, additional training is expected to ensure very good pain assessment.

Further studies are needed to validate this facial expression scale of pain during farrowing in sows. It could be carried out by correlating the sows' facial expressions with the corporal pain indicators described by Ison et al. [20], which showed higher prevalence when the sow experienced stronger pain. Another way to validate the scale could be by non-steroidal anti-inflammatory drugs (NSAID) administration, comparing eutocic and dystocic farrowing, or comparing physiological indicators indicative of pain.

4.5. Conclusions

In conclusion, this is the first facial scale for pain assessment in sows during farrowing. This scale is accurate and easy to learn and could be easily applied in the field by veterinarians and farmers to detect animal suffering and improve animal welfare. Additionally, the reproducibility of the technique opens a broad field of pain research associated with several processes in swine.

Author Contributions

E.N.: Study concept and design, data acquisition, images selection and evaluation, results interpretation, and drafting of the manuscript. E.M.: Study concept and design, data analysis and interpretation, drafting of the manuscript, and final approval of the version to be published. X.M.: Study concept and design, drafting of the manuscript, and final approval of the version to be published. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest

The authors declare no conflict of interest. The funder was not involved in the study design, collection, analysis, interpretation of data, or the writing of this article.

4.6. References

1. McLennan, K.M. Why Pain Is Still a Welfare Issue for Farm Animals, and How Facial Expression Could Be the Answer. *Agriculture* **2018**, *8*, 127, doi:10.3390/agriculture8080127.
2. Mogil, J.S.; Pang, D.S.; Dutra, G.G.S.; Chambers, C.T. The development and use of facial grimace scales for pain measurement in animals. *Neurosci. Biobehav. Rev.* **2020**, *116*, 480–493, doi:10.1016/j.neubiorev.2020.07.013.
3. Crowley, T. The Expression of the Emotions in Man and Animals. *Philos. Stud.* **1957**, *7*, 237, doi:10.5840/philstudies1957703.
4. Langford, D.J.; Bailey, A.L.; Chanda, M.L.; Clarke, S.E.; Drummond, T.E.; Echols, S.; Glick, S.; Ingrao, J.; Klassen-Ross, T.; Lacroix-Fralish, M.L.; et al. Coding of facial expressions of pain in the laboratory mouse. *Nat. Methods* **2010**, *7*, 447–449.
5. Sotocinal, S.G.; Sorge, R.E.; Zaloum, A.; Tuttle, A.H.; Martin, L.J.; Wieskopf, J.S.; Mapplebeck, J.C.S.; Wei, P.; Zhan, S.; Zhang, S.; et al. The Rat Grimace Scale: A Partially Automated Method for Quantifying Pain in the Laboratory Rat via Facial Expressions. *Mol. Pain* **2011**, *7*, 55, doi:10.1186/1744-8069-7-55.
6. Viscardi, A.V.; Hunniford, M.; Lawlis, P.; Leach, M.; Turner, P.V. Development of a Piglet Grimace Scale to Evaluate Piglet Pain Using Facial Expressions Following Castration and Tail Docking: A Pilot Study. *Front. Vet. Sci.* **2017**, *4*, 51, doi:10.3389/fvets.2017.00051.
7. Macrae, A.M.; Makowska, I.J.; Fraser, D. Initial evaluation of facial expressions and behaviours of harbour seal pups (*Phoca vitulina*) in

response to tagging and microchipping. *Appl. Anim. Behav. Sci.* **2018**, *205*, 167–174, doi:10.1016/j.applanim.2018.05.001.

8. Di Giminiani, P.; Brierley, V.L.M.H.; Scollo, A.; Gottardo, F.; Malcolm, E.M.; Edwards, S.A.; Leach, M.C. The Assessment of Facial Expressions in Piglets Undergoing Tail Docking and Castration: Toward the Development of the Piglet Grimace Scale. *Front. Vet. Sci.* **2016**, *3*, 100, doi:10.3389/fvets.2016.00100.
9. McLennan, K.M.; Rebelo, C.J.B.; Corke, M.J.; Holmes, M.A.; Leach, M.C.; Constantino-Casas, F. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. *Appl. Anim. Behav. Sci.* **2016**, *176*, 19–26.
10. Keating, S.C.J.; Thomas, A.A.; Flecknell, P.A.; Leach, M.C. Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses. *PLoS ONE* **2012**, *7*, e44437, doi:10.1371/journal.pone.0044437.
11. Guesgen, M.; Beausoleil, N.; Leach, M.; Minot, E.; Stewart, M.; Stafford, K. Coding and quantification of a facial expression for pain in lambs. *Behav. Process.* **2016**, *132*, 49–56.
12. Costa, E.D.; Minero, M.; Lebelt, D.; Stucke, D.; Canali, E.; Leach, M.C. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. *PLoS ONE* **2014**, *9*, e92281, doi:10.1371/journal.pone.0092281.
13. Reijgwart, M.L.; Schoemaker, N.J.; Pascuzzo, R.; Leach, M.C.; Stodel, M.; De Nies, L.; Hendriksen, C.F.M.; Van Der Meer, M.; Vinke, C.M.; Van Zeeland, Y.R.A. The composition and initial evaluation of a grimace scale in

ferrets after surgical implantation of a telemetry probe. *PLoS ONE* **2017**, *12*, e0187986, doi:10.1371/journal.pone.0187986.

14. Häger, C.; Biernot, S.; Buettner, M.; Glage, S.; Keubler, L.M.; Held, N.; Bleich, E.M.; Otto, K.; Müller, C.W.; Decker, S.; et al. The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. *PLoS ONE* **2017**, *12*, e0175839, doi:10.1371/journal.pone.0175839.
15. Ekman, P.; Friesen, W.V. Facial action coding system: investigator's guide. Consulting Psychologists Press: Palo Alto, CA, USA, 1978;
16. Huxley, J.N.; Whay, H.R. Current attitudes of cattle practitioners to pain and the use of analgesics in cattle. *Vet. Rec.* **2006**, *159*, 662–668.
17. Ison, S.H.; Rutherford, K.M.D. Attitudes of farmers and veterinarians towards pain and the use of pain relief in pigs. *Vet. J.* **2014**, *202*, 622–627.
18. Lascelles, B.D.X.; Brown, D.C.; Conzemius, M.G.; Gill, M.; Oshinsky, M.L.; Sharkey, M. Measurement of chronic pain in companion animals: Discussions from the Pain in Animals Workshop (PAW) 2017. *Vet. J.* **2019**, *250*, 71–78.
19. Mainau, E.; Manteca, X. Pain and discomfort caused by parturition in cows and sows. *Appl. Anim. Behav. Sci.* **2011**, *135*, 241–251, doi:10.1016/j.applanim.2011.10.020.
20. Ison, S.H.; Jarvis, S.; Rutherford, K.M.D. The identification of potential behavioural indicators of pain in periparturient sows. *Res. Vet. Sci.* **2016**, *109*, 114–120.
21. Ison, S.H.; Jarvis, S.; Hall, S.A.; Ashworth, C.J.; Rutherford, K.M.D. Periparturient behavior and physiology: Further insight into the farrowing

process for primiparous and multiparous sows. *Front. Vet. Sci.* **2018**, *5*, 5, doi:10.3389/fvets.2018.00122.

22. Mainau, E.; Temple, D.; Manteca, X. Experimental study on the effect of oral meloxicam administration in sows on pre-weaning mortality and growth and immunoglobulin G transfer to piglets. *Prev. Vet. Med.* **2016**, *126,* 48-52 doi:10.1016/j.prevetmed.2016.01.032.

23. Thorsen, C.K.; Schild, S.-L.A.; Rangstrup-Christensen, L.; Bilde, T.; Pedersen, L.J. The effect of farrowing duration on maternal behavior of hyperprolific sows in organic outdoor production. *Livest. Sci.* **2017**, *204*, 92–97.
24. Altman, D.G. *Practical statistics for medical research*; Chapman and Hall: London, UK, 1991; pp. 403–405, ISBN 9780429258589.
25. Holden, E.; Calvo, G.; Collins, M.; Bell, A.; Reid, J.; Scott, E.M.; Nolan, A.M. Evaluation of facial expression in acute pain in cats. *J. Small Anim. Pract.* **2014**, *55*, 615–621.
26. Graça, J.; Calheiros, M.M.; Oliveira, A.; Milfont, T.L. Why are women less likely to support animal exploitation than men? The mediating roles of social dominance orientation and empathy. *Pers. Individ. Differ.* **2018**, *129*, 66–69.
27. Duarte, M.I.F.; Raposo, M.L.B.; Rodrigues, P.J.F. da S.F.; Branco, M.C. Measuring empathy in medical students, gender differences and level of medical education: An identification of a taxonomy of students. *Investigación en Educación Médica* **2016**, *5*, 253–260.

CAPÍTULO 5. CORRELATION BETWEEN BODY PAIN INDICATORS AND FACIAL EXPRESSION SCALE IN SOWS AROUND FARROWING AND THE EFFECT ON PARITY, FARROWING MOMENT AND SUCKLING EVENTS.

Capítulo 5

CORRELATION BETWEEN BODY PAIN INDICATORS AND FACIAL EXPRESSION SCALE IN SOWS AROUND FARROWING AND THE EFFECT ON PARITY, FARROWING MOMENT AND SUCKLING EVENTS.

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Keywords: Behaviour, pig, pain, facial expressions, farrowing

Capítulo 5

HIGHLIGHTS

-Body pain indicators are positively correlated with facial expression scale in sows around farrowing.

-Primiparous sows perform more Body Pain Indicators per minute than do multiparous sows around farrowing.

-Multiparous sows are more active around farrowing, carrying out more postural changes per minute than primiparous ones.

-Temporal tension and ear position was the only Facial Action Unit that showed differences between multiparous and primiparous sows, multiparous sows being the ones that showed higher pain expression, particularly during the time interval between the delivery of two consecutive piglets.

ABSTRACT

It is widely accepted that parturition is painful. However, there are few studies investigating pain-specific behaviours associated with the progression of farrowing in sows. The objectives of this study were: (1) to assess if behavioural pain indicators are affected by farrowing moment, parity and suckling events, and (2) to determine the relationship between the Facial Expression Scale in sows and five Body Pain Indicators (BPIs) around farrowing. Five multiparous and five primiparous Dandred sows were studied. Sows were continuously recorded throughout farrowing and on Day 19 post-farrowing for two hours. One observer continuously recorded 56 hours of video and a total of 122 facial images selected from these videos in three different phases of farrowing: i) during the intervals between the expulsion of two consecutive piglets (n= 43 images), ii) at the delivery of piglets (n = 41 images), and iii) 19 days after farrowing, which was used as control (n = 38 images). Five facial action units (FAUs) and five BPIs were assessed, and all them showed significant differences between the three farrowing moments. All were rare or absent post-farrowing, and almost all of them were more frequent during farrowing (especially at the moment of delivery). Back arching was the BPI with the highest correlation with the different five FAUs, and Tension above eyes was the FAU with the highest correlation with four of the five BPIs. We conclude that both indicators lead to the same interpretation: sows experience more pain during farrowing than during the third week post-farrowing and piglet expulsion is the most painful moment of farrowing.

5.1. Introduction

Animal pain is a current problem in farm animals, which receives increased attention from scientists, who are focusing on how to assess, control and minimize it (McLennan, 2018; Mogil et al., 2020). Pain in pigs can be caused by multiple factors, including some farm routine procedures such as castration and tail docking (Di Giminiani et al., 2016 and Viscardi et al., 2017) as well as farrowing is also a (Jarvis et al., 1997). In particular, births associated with difficult parturitions or dystocia may cause unacceptable levels of pain in the mother (Mainau and Manteca, 2011). It has been studied that the administration of non-steroidal anti-inflammatory drugs (NSAIDs), after farrowing, enhances sow recovery, making them more active post-farrowing (Mainau et al., 2012; Viitasaari et al., 2013) and this increases piglet immunity (Mainau et al., 2016; Navarro et al., 2021).

Objective measurements of pain severity can be difficult, and it remains a critical issue in veterinary and biomedical research (Mogil et al., 2020). Pain assessment in animals tends to use three approaches based on the measurement of general indexes, physiological indicators and behavioural indicators (Weary et al., 2006). Both general indexes and physiological indicators are often unspecific, which means they are often sensitive to other states of an animal rather than to pain (such as stress, illness, fear…) (Hansen, 1997). Therefore, pain assessment based on behaviour has received increasing attention and is the most commonly used parameter to assess pain in farm animals (Viñuela-Fernandez et al., 2007). Several behavioural studies are making pain measurement more consistent and accurate (Graham and Hampshire, 2016).

The assessment of spontaneous pain-related behaviour (spontaneously occurring behaviours arising or increasing in the context of painful conditions) is one of the most promising methods for assessing pain in pigs (Ison et al., 2016). Pain-specific behaviours have been studied in pigs after castration (tail wagging; Hay et al., 2003), tail docking (tail wagging and jamming), teeth clipping (teeth champing), and ear notching (head shaking) (Noonan et al., 1994).Recently, facial expressions in piglets have been studied, resulting in being a good tool to evaluate when they are suffering pain due to castration or tail docking (di Giminiani et al., 2016; Viscardi et al., 2017).

Regarding pain at farrowing in sows, Ison et al.2016 described potential behavioural pain indicators, including pulling the back leg forward, trembling, back arching, pawing and tail flicking. There are also studies that describe postural changes of the sow during farrowing (Ison et al., 2016; Mainau et al., 2010; Wischner et al., 2009; Zhang et al., 2020). In fact, pain can modify sows' postural changes, which can lead to a higher risk for piglet crushing (Wischner et al., 2010, 2009). Recently, Navarro et al. (2020) published a facial expression scale in sows, in which five different facial action units were found (FAUs) in the sow's face that determine if an animal is experimenting pain at certain moment during farrowing.

There are some factors that can modify the incidence of pain indicators around farrowing such as duration of farrowing, sows' parity and the different moments around farrowing. In fact, longer parturitions are considered to be more painful and problematic than shorter ones (Borges et al.,2005). Around farrowing, multiparous sows show a higher number of pain-specific behaviours than do primiparous sows, but the latter performed more postural changes than did multiparous sows (Ison et al., 2018). Navarro et al. (2020) described that sows showed more facial expressions of pain during piglet expulsions than in the moments between piglet expulsion or in the post-farrowing moments, where the pain facial expressions were almost absent. To our knowledge, other factors such as the effect of suckling events on pain experienced by sows around farrowing are not documented. The aims of this study are/were: 1) to assess if behavioural pain indicators (both facial expression scale and body indicators) are affected by farrowing moment, parity and suckling events, and 2) to determine the relationship between the facial expression scale and body indicators around farrowing. We expected that all of these behavioural indicators studied will change similarly during farrowing, and consequently can be a good tool to assess pain in peri-parturient sows.

For this study, behavioural pain indicators described by Ison et al. (2016) will be referenced as body pain indicators (BPIs), because both, facial and body indicators, are considered behavioural indicators.

5.2. Material and methods

The experimental protocol described in this study was approved by the Institutional Animal Care and Use Committee of the Universitat Autònoma de Barcelona (CEEA-5377) and by the Generalitat de Catalunya (Protocol Number 11385).

5.2.1. Animals, housing and general management

The experimental procedure was carried out on a commercial farm (Casa Ramona, Sora, Barcelona, Spain) from May to June 2018. A total of ten Danbred sows, five primiparous and five small-sized multiparous sows in their second and third parity, were randomly selected the day of parturition. Selected animals were free of disease symptoms such as lameness, diarrhoea, fever, mastitis or respiratory problems.

On Day 109 of gestation, sows were moved to the farrowing room and were allocated in farrowing crates (1.95 x 0.60 m) built with steel bars, which were centrally positioned in farrowing pens (2.40 x 1.80 m). Farrowing pens were built with metal-slatted floor for sows and plastic-slatted floor for piglets. Farrowing pens had a metal heat pad at 36ºC and a heat lamp for the piglets during their first seven days of life. The temperature in the farrowing room was kept constant at approximately at 22ºC, and light was on from 7:00 to 18:00 every day of the week. Three meals per day were offered to the sows and water was available *ad libitum* from drinkers.

On Day 116 of gestation, farrowing was hormonally induced in multiparous sows, with 1ml of Dalmazin® (D-Cloprostenol 0.075 mg/ml, FatroIberica; Barcelona, Spain). Piglets were weaned at 21 days of age and moved to another barn of the farm equipped with conditioned infrastructures for very young piglets.

5.2.2. Experimental procedure

Throughout farrowing (from before the first piglet expulsion until the last piglet was born) sows were recorded with an action camera (SK8 Urban, SK8 HD 4K; Spain), which was installed on the crate, at 120 cm above the floor when the sow was lying down. The camera was focused on the sow's face and its angle allowed for the recording of the rest of the body and the movements of the sow. It was necessary to guarantee a complete vision of the sow's face and body. A vertical metal barrier (50 cm x 30 cm x 2 cm) was installed in the cranial part of the crate (in front of the sow's feeder), to prevent the sow from putting her head under the feeder. If the sow was uncomfortable with the vertical metal barrier installed in front of the feeder, it was excluded from the study.

Nineteen days post-farrowing (two days pre-weaning), sows were recorded again for 2h, between 8:30h and 12:30h, avoiding meals and farm management with the aim of obtaining videos as control moments. It was assumed that after more than two weeks of farrowing, there was no pain associated with this, or its intensity was minimal.

For each sow, the duration of farrowing, defined as the period of time between the first and the last piglet born, and the time of each piglet expulsion were registered by direct observations.

5.2.3. Images selection and evaluation

A total of 122 images were collected, selected and evaluated by a Silver Standard (SS), which visualized all of the videos (partum and pre-weaning) and selected images at three different moments, following the same methodology described in Navarro et al. (2020). The images just showed the sows faces, to guarantee blinding by not revealing the rest of the sow's body. The three different moments selected were:

- Pre-weaning (indicative of pain-free; Score 0; n = 38 images). Images were chosen every 15–20 min.

- Inter-expulsion moment, described as the interval between two piglets' expulsion (indicative of moderate pain; Score 1; n= 43 images). One image was chosen at each interval.
- Expulsion of the piglets (indicative of severe pain; Score 2; n = 41 images). One image was chosen within the 30 seconds prior to each piglet expulsion.

The following five FAUs in sows were studied (based on Navarro et al., 2020): Tension above eyes, Snout angle, Neck tension, Temporal tension and ear position, and Cheek tension. The SS scored all FAUs in each image and assigned a degree of pain: 0 (pain-free), 1 (moderate pain), 2 (severe pain), and DK ("Don't know", meaning "I do not know or I do not feel confident assigning a pain score"). Additionally, a Total Facial Index (TFI) was calculated as the sum of the 5 FAUs, obtaining a value that ranged from 0 (pain-free) to 10 (maximum degree of pain) and attributes a global facial expression score to each image.

5.2.4. Video analysis

All videos (including farrowing and pre-weaning) from the same sows studied were observed continuously by the SS. Four different items were noted every ten seconds in the three above-mentioned moments (pre-weaning or score 0; inter-expulsion moment or score 1 and the expulsion of the piglets or score 2):

- The number of Body Pain Indicators (BPIs) (based on Ison et al., 2016):

  - Leg: The back leg pushes towards the abdomen.
  - Paw: Front leg is strongly pulled forward.
  - Arch: Back arching in lateral lying position, one or both sets of legs are pushed away from the body and/or inwards towards the centre.

- Tremble: Visible shaking of the sow's body.

- Tail tickling: Tail is moving intensely in all directions.

- The sow's posture: Lying laterally (L. LAT), Lying on the sternum and abdomen (L. ST), Standing (ST)or Sitting (SIT).

- The number of the sow's postural changes.

- If one or more piglets were suckling (Yes or No).

5.2.5. Statistical Analysis

All statistical analyses were performed using IBM SPSS 19.0 for Windows (IBM Corp.; Armonk, NY, USA). Graphs were produced with Prism 8.0.2 (GraphPad Software). BPIs were analysed as a number of events each 10 seconds and represented as a number of events per minute. FAUs were calculated as the mean value of the pain score (0, 1, 2) assessed in the pictures in the three farrowing moments. Additionally, total BPIs (as the addition of the 5 BPIs) and a TFI (as the addition of the 5 FAUs and scored from 0 to 10) were also analysed. For BPIs and FAUs, general lineal models (GLM) were used to assess the effect of different fixed factors, such as the farrowing moment (Pre-weaning; Inter-expulsion moment; Piglet expulsion), parity effect (Primiparous vs Multiparous) and suckling effect (Piglets suckling vs. No piglets suckling). When GLM showed statistically significant differences, Duncan *post hoc* was employed. Each BPI and FAU was correlated amongst themselves with Spearman's rank correlation coefficient (ρ). The interpretation of this ρ coefficient value was as follows: negligible ($\rho < 0.20$), weak ($0.21 \geq \rho < 0.40$), moderate ($0.41 \geq \rho < 0.60$), strong ($0.61 \geq \rho < 0.80$) and very strong ($0.81 \geq \rho < 1.0$). Sow posture (L.LAT, L.ST, ST, SIT) and postural changes (Yes vs. No) were analysed based on the parity and the farrowing moment. Assessment of the association between these discrete variables was carried out using Pearson's Chi-square test or alternatively Likelihood ratio and Fisher's exact test when needed. When this test showed statistically significant differences, standardized residuals *post hoc* were used after Bonferroni adjustment of the alpha value.

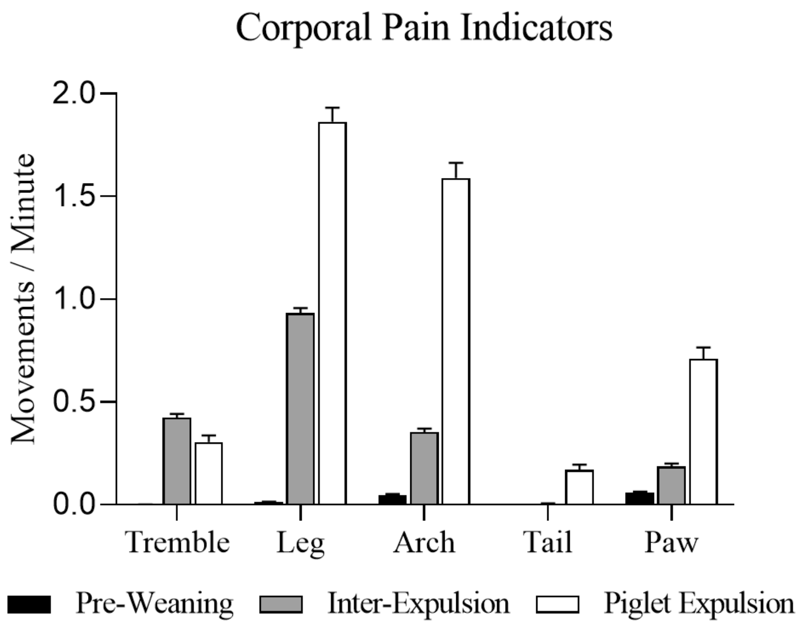
5.3. Results

5.3.1. Body pain indicators incidence

The incidence of total BPIs was significantly different between the three different farrowing moments ($p < 0.001$). Sows exhibited a higher number of BPIs per minute during the piglet expulsion moment (mean = 4.62 BPI/min), followed by the inter-expulsion moment (mean = 1.92 BPI/min). Both farrowing moments showed a higher number of BPIs than the pre-weaning one, where the BPIs incidence per minute was infrequent (mean = 0.12 BPI/min). Additionally, there was a parity effect, as multiparous sows showed fewer BPIs than did primiparous ones during pre-weaning (primiparous: 0.18 BPI/min *vs* multiparous: 0.06 BPI/min; $p < 0.001$) and inter-expulsion moments (primiparous: 2.88 BPI/min *vs* multiparous: 1.02 BPI/min; $p < 0.001$). No differences based on parity effect were found during piglet expulsion (primiparous: 4.68 BPI/min *vs* multiparous: 4.56 BPI/min; $p = 0.035$). Suckling did not affect the incidence of total BPIs ($p = 0.285$).

Individual BPIs are shown in **Figure 1**. The five BPIs showed significant differences between the three farrowing moments ($p < 0.001$). Incidences of leg, paw and arch movements were higher during piglet expulsion, followed by the inter-expulsion moment. At the pre-weaning moment, they were much more infrequent. The Tremble BPI followed the opposite pattern during farrowing, the movements being higher at inter-expulsion moments. Interestingly, only two out of the ten sows showed this body indicator. Tail BPI was only present during piglet expulsion (**Figure 1**).

Figure 1: *Individual body pain indicators per minute, expressed as Mean and Standard deviation (SD). Bars represent the three farrowing moments: Pre-weaning, inter-expulsion moment and during piglet expulsion. A total of 10 sows and 56 hours of videos were studied. Statistically significant differences (p < 0.001) between the three farrowing moments were found in the five body pain indicators.*



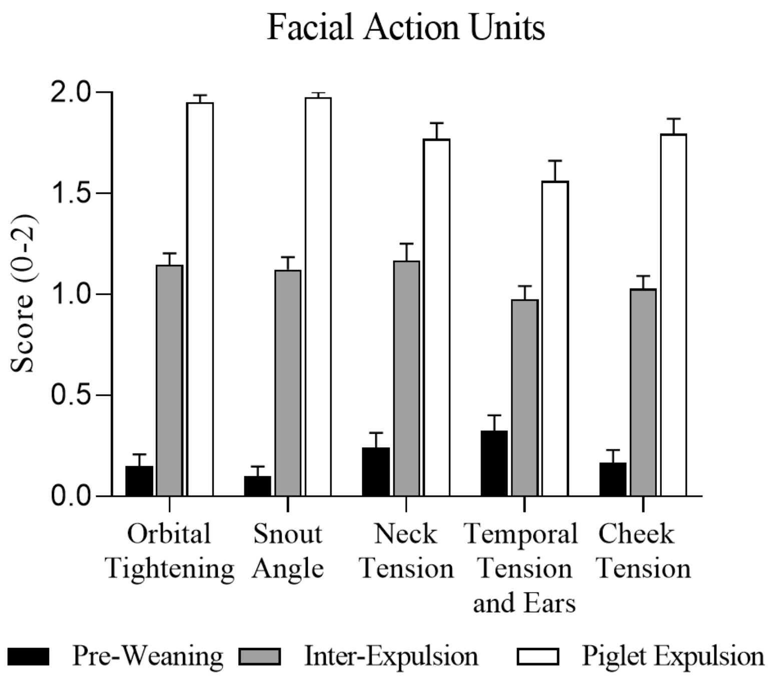
Parity had a statistically significant effect on four BPIs ($p < 0.001$). Primiparous sows showed a higher incidence of these BPIs per minute than did multiparous ones: tremble (Pre-weaning: 0.003*vs* 0.000 BPI/min; Inter-expulsion: 0.860 *vs* 0.037 BPI/min; Piglet expulsion: 0.542 *vs* 0.051 BPI/min), leg (Pre-weaning: 0.024 *vs* 0.004 BPI/min; Inter-expulsion: 1.411 *vs* 0.520 BPI/min; Piglet expulsion: 1.633 *vs* 2.110 BPI/min), tail (Pre-weaning: 0.000 *vs* 0.000 BPI/min; Inter-expulsion: 0.006 *vs* 0.006 BPI/min; Piglet expulsion: 0.151 *vs* 0.191 BPI/min) and paw (Pre-weaning: 0.077 *vs* 0.041 BPI/min; Inter-expulsion: 0.235 *vs* 0.144 BPI/min; Piglet expulsion: 0.920 *vs* 0.485 BPI/min) movements. However, arch movement showed no differences (p = 0.121) between multiparous and primiparous sows (Pre-weaning: 0.076 *vs* 0.025 BPI/min; Inter-expulsion: 0.385 *vs* 0.327 BPI/min; Piglet expulsion: 1.469 *vs* 1.721 BPI/min). Total BPI was statistically significant only in the pre-weaning moment, with a higher number of BPIs when piglets were suckling *versus* no piglets suckling (Pre-weaning: 0.18 *vs* 0.06 BPI/min, p < 0.001; Inter-expulsion: 1.98 *vs* 1.56 BPI/min, p = 0.936; Piglet expulsion: 4.62 *vs* 4.74 BPI/min, p = 0.761).

5.3.2. Facial Action units

When the five FAUs were analysed globally, TFI showed statistically significant differences when the farrowing moment was studied (p < 0.001). The highest pain scores were noted during piglet expulsion (TFI = 8.97), followed by the inter-expulsion moment (TFI = 5.26). The lowest pain scores were significantly reported at the pre-weaning moment (TFI = 0.94). Parity had no statistically significant effects on FAUs (p = 0.813).

Individual FAUs are shown in **Figure 2**. Each individual FAU showed differences between the three farrowing moments studied (p < 0,001). During the piglet expulsion moment, sows showed a higher pain score than during the inter-expulsion moment, and that moment, in turn, showed higher scores than the pre-weaning moment (**Figure 2**). Temporal tension and ears position FAUs showed a statistically significant parity effect (p = 0.048), as multiparous facial expressions received higher pain scores than did primiparous ones, especially at the inter-expulsion moment. Instead, there were not statistically significant changes between primiparous and multiparous sows analysing Orbital tightening (p = 0.612), Snout angle (p = 0.944), Neck tension (p = 0.636) and Cheek tension (p = 0.456).

Figure 2: *Sows' Facial Action Units expressed in pain intensity punctuation (0-2) per image represented as Mean and Standard deviation (SD). Bars express the three farrowing moments: Pre-weaning, inter-expulsion moment and during piglet expulsion. A total of 10 sows and 56 hours of videos were studied. Statistically significant differences (p < 0.001) were found in the five Facial Action Units when the farrowing moment was studied.*



5.3.3. Correlation between body pain indicators and facial expressions

Spearman correlations among the five BPIs and the five FAUs showed that Arch was the BPI with the highest positive correlation with the five different FAUs (**Table 1**). Individually, strong positive correlations were found between Arch and Tension above eyes, Arch and Snout angle and Arch and Cheek tension. Paw and Leg BPIs showed a moderate correlation considering the five FAUs means. The correlation obtained by Tail was weak and the Tremble one was negligible (**Table 1**).

Spearman correlations means of the total BPI and each individual FAU showed differences between each FAU. Tension above eyes, Snout angle and Cheek tension showed a strong correlation with total BPI (**Table 1**). Instead, Neck tension and Temporal tension and ear position showed a weak-to-moderate correlation with the total BPIs (**Table 1**). As Tremble BPI was only present in two out of the ten sows studied, the correlation between the TFI and the "total BPI without Tremble" was also documented. Overall, a strong correlation between TFI and total BPIs was obtained, with a moderate improvement when excluding Tremble BPI.

Table 1: *Spearman correlation between the five Facial Action Units studied and the five body pain indicators, including the Total mean and the Total mean without Tremble*

Facial Action Units	Body pain indicators						
	Tremble	**Leg**	**Arch**	**Tail**	**Paw**	**Total BPI**	**Total BPI without Tremble**
Tension above eyes	0.141	0.520***	0.737***	0.383***	0.591***	0.771***	0.799***
Snout angle	0.141	0.445***	0.709***	0.311***	0.590***	0.718***	0.746***
Neck tension	0.108	0.397***	0.578***	0.252**	0.449***	0.568***	0.596***
Temporal tension and ear position	0.060	0.367***	0.543***	0.246**	0.396***	0.515***	0.573***
Cheek tension	0.151	0.508***	0.622***	0.319**	0.532***	0.711***	0.735***
Total Facial Index[1]	0.116	0.529***	0.704***	0.332**	0.558***	0.722***	0.763***

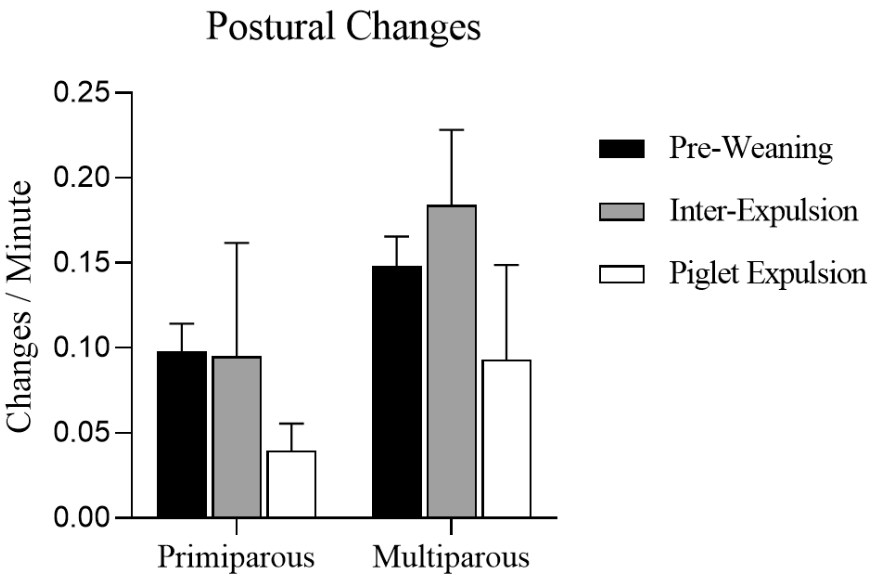
****p < 0.001, ** p < 0.01*

[1] *Total Facial Index* = the sum of the 5 Facial Action Units (ranging from 0 -painless- to 10 -maximum degree of pain).

5.3.4. Activity around farrowing

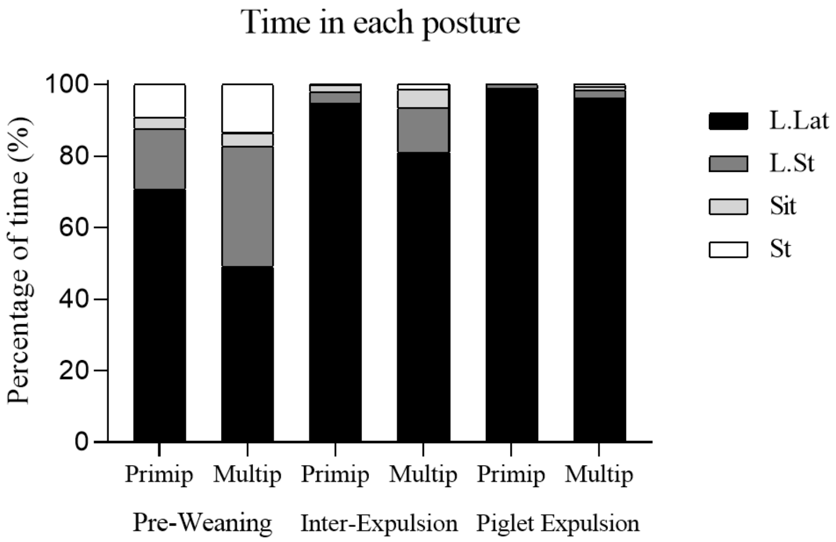
Statistically significant differences in postural changes around farrowing in sows were found according to the moment studied (p = 0.001). Sows performed more postural changes in the inter-expulsion moment and in the pre-weaning one than in the piglet expulsion moment (p < 0.001 and p = 0.001, respectively) (**Figure 3**). Sow postural changes around farrowing showed statistically significant differences according to parity, as multiparous sows performed more postural changes per minute than did primiparous ones (p < 0.001) (**Figure 3**).

Figure 3: *Sows' Postural changes per minute, expressed as Mean and Standard deviation (SD) according to parity. Bars express the three farrowing moments: Pre-weaning, between two piglets' expulsion and during piglet expulsion. Statistically significant differences (p < 0.001) were found when parity was studied.*



The percentage of time spent by sows in each posture was different between the three farrowing moments in both primiparous and multiparous animals ($p < 0.001$) (**Figure 4**). Lying Lateral (L.LAT) posture was the most common posture in the three different moments, being much more frequent during farrowing, as compared with the pre-weaning moment. Accordingly, sows spent more time Lying Sternal (L.ST) and Standing (ST) during the pre-weaning moment. Considering parity effect, primiparous sows spent more time in the L.LAT position than did multiparous sows, and multiparous sows spent more time in the L.ST position than did primiparous ones.

***Figure 4**: Percentage of total farrowing time spent in each posture (Lying Laterally, Lying Sternal, Sitting and Standing). Bars express the three farrowing moments: Pre-weaning, between two piglets' expulsion and during piglet expulsion, and differentiates between multiparous and primiparous sows. Statistically significant differences (p < 0.001) were found when the farrowing moment was studied.*



5.4. Discussion

5.4.1. Body pain indicators incidence considering parturition, piglets-suckling effect and farrowing moment.

Four of the five BPIs studied showed the same pattern that Ison et al. (2016) described: arching back, tail flicking and pulling the back leg forward were much more frequent during farrowing, especially during piglet expulsion. In agreement with Ison et al. (2016), tail flicking appears during piglet expulsion, being almost inexistent during the inter-expulsion moment and after the farrowing process. Tail flicking is strongly associated with imminent birth, and it can be associated with localized somatic pain from pressure of the newborn piglet on the pelvic structures surrounding the vagina (Ison et al., 2016). Alternatively, tail flicking can be related to a physiological act associated with piglet expulsion (Ison et al., 2016), otherwise, we should see this indicator during all of the farrowing, where it is clearly seen that sows suffer pain.

In the present study, trembling shows great individual variability, as only two out of the ten sows studied performed it. Ison et al. (2016), described trembling as a very common BPI during all of farrowing and argued that it is associated with pain, inflammation and fatigue as parturition progresses. We agree that trembling is indicative of pain and inflammation. In fact, Jinhyeon Yun et al. (2017), assessing pain and stress in sows in an experimental procedure (insertion of an intravenous catheter), correlated higher levels of plasmatic cortisol with trembling behaviour. But, in our study, trembling does not appear as an indicator of fatigue as parturition progresses, because the two affected sows start trembling almost at the beginning of parturition. Overall, we conclude that not all sows that are suffering during farrowing show the trembling indicator, but if trembling appears, it is indicative of pain and inflammation.

Ison et al. (2018) studied the parity differences during the farrowing moment, showing that primiparous sows exhibited fewer back arches and fewer back-leg forward behaviours than did multiparous sows. In this study, we found statistically significant differences during farrowing in the opposite sense. Primiparous sows performed more BPIs per minute than did multiparous sows, especially in the inter-expulsion moment. These results agree with the fact that primiparous sows generally have more painful parturitions than do multiparous ones, associated with the lack of experience of the dams at first parturition and the novelty of the pain suffered (Mainau and Manteca, 2011). Management factors or the methodology of behavioural observations can partially explain these differences.

The only BPI that showed no differences between parities was arching the back. Arching can be related to uterine contractions trying to adopt a posture that facilitates piglet expulsion. It has been documented that multiparous sows can experience more pain from uterine activity than do primiparous ones, due to the loss in uterine tone (Ison et al., 2018).

The Suckling effect was statistically significant only in the pre-weaning moment, showing more BPIs when piglets were suckling. Intact teeth of piglets can hurt the teats of sows, which cause avoidance behaviours of sows (Fu et al., 2019). Avoidance behaviour indicates that the sow attempted to avoid piglets' contact with the udders because of discomfort and frustration (D'Eath et al., 2014). That effect is not observed during farrowing, presumably for at least two reasons. Firstly, the discomfort produced by piglet suckling can be lower at the beginning of lactation than at the end. Secondly, the discomfort produced by piglet suckling can be masked by the pain suffered by the farrowing.

5.4.2. Facial expressions and their correlation with the BPIs

FAUs herein studied were developed in a previous study by Navarro et al. (2020), and the five of them showed good or very good reliability among different observers. In the present study, all FAUs showed marked differences between the farrowing moments studied, and these results paralleled those obtained in the analysis of BPIs. Maximum pain scores were found during piglet-expulsion moments, whereas the inter-expulsion moment was associated with moderate FAU pain scores, irrespective of the parity studied.

Focusing on the five FAUs Spearman Correlations means, Tension above eyes, Snout angle and Cheek tension were the FAUs with a stronger correlation with four of the BPIs (Leg, Paw, Tail and Arch). In Navarro et al. (2020), these FAUs were also the ones with higher reliability between the observer and the farrowing moment. That positive correlation confirms the usefulness of the FAUs pain scale and highlights them as a reliable and easy-learning tool that could be potentially applied in commercial farms. Parity effect showed moderate differences between BPIs and FAUs results. A lack of differences between primiparous and multiparous can stem from the lower number of pictures analysed, which limits the statistical analyses. Further studies applying the facial expression scale in a broader number of animals may help to clarify this point.

When studying the five BPIs and correlating them with the five different FAUs, Arch was the BPI with the highest correlation with the five different FAUs. A strong correlation was found with Tension above eyes, Snout angle and Cheek tension. Supporting our observation, Grégoire et al. (2013), described the posture "Arched back" to be a body indicator in lame sows, describing intense pain in them. Paw and Leg BPIs showed a moderate correlation taking into account the mean of the five FAUs because it could be, as happened in the Ison et al. (2016) study, both BPIs were present during all parturition, showing a higher incidence at piglet expulsion, but looks to be not different enough to show strong correlations. Tremble correlation was negligible, which could be due to the high individual variability in trembling BPI, which consequently shows a low correlation.

5.4.3. Sows' postures, postural changes and time spent in each posture.

Results related to postural changes and total time spent in each posture agree with previous research in crated sows (Mainau et al., 2009; Mainau et al., 2010; Melišová et al., 2014; Ison et al., 2016). Overall, the number of postural changes and time spent lying laterally increases on the day of parturition, as compared with the days after it. Lying laterally during the day of farrowing is associated with physical exhaustion after birth (Nicolaisen et al., 2019), being higher in primiparous sows. In the present study, we are able to identify that postural changes were mainly performed at the inter-expulsion moment instead of the expulsion moment, being higher in multiparous sows. In addition, lying sternal and standing behaviour appeared more frequently during the pre-weaning days, as compared to the farrowing day. Several days after farrowing, lying sternal is associated with sows often awake, attentive and able to interact with their piglets (Nicolaisen et al., 2019). Alternatively, sows lying sternal (without showing teats for piglet suckling) can be associated with an avoidance behaviour due to pain or discomfort when piglets are suckling (Fu et al., 2019). That avoidance behaviour is supported in our study by the increased BPIs in the pre-weaning moment that sows showed when piglets were suckling.

5.5. Conclusion

Sows' BPIs are positively correlated with FAUs in sows around farrowing. A higher prevalence of both behavioural indicators is present when the sows are experiencing a stronger pain, during the farrowing moment (especially at the expulsion piglet moment), than when they do not (pre-weaning). Future steps should include fully validating both behavioural pain indicators by the administration of non-steroidal anti-inflammatory drug (NSAID) at farrowing or by comparing eutocic and dystocic farrowing.

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Conflicts of Interest

The authors declare no conflict of interest. The funder was not involved in the study design, collection, analysis, interpretation of data or the writing of this article.

5.6. References

Borges, V.F., Bernardi, M.L., Bortolozzo, F.P., Wentz I., 2005. Risk factors for stillbirth and fetal mummification in four Brazilian swine herds. Prev. Vet. Med. 70. p. 165-76. doi:https://doi.org/10.1016/j.prevetmed.2005.03.003.

Di Giminiani, P., Brierley V.L.M.H., Scollo A., 2016. The assessment of facial expressions in piglets undergoing tail docking and castration: Toward the development of the Piglet Grimace Scale. Front. Vet. Sci. 3(NOV). doi:10.3389/fvets.2016.00100.

Fu, L., Zhou,B., Li, H., Liang,T., Chu, Q., Schinckel, A.P., Li, Y., Xu., F., 2019 Effects of tail docking and/or teeth clipping on behavior, lesions, and physiological indicators of sows and their piglets. Anim. Sci. Jour. 90 (9). P. 1320-1332. doi:https://doi.org/10.1111/asj.13275.

Graham, D.M., Hampshire, V., 2016. Methods for measuring pain in laboratory animals. Lab. Anim. (NY). 45(3):99-101. doi:10.1038/laban.962.

Grégoire, J., Bergeron R., d'Allaire, S., Meunier-Salaün, M.C. Devillers, N., 2013. Assessment of lameness in sows using gait, footprints, postural behaviour and foot lesion analysis. Animal 7, 1163-1173. doi: https://doi.org/10.1017/S1751731113000098

Hansen, B. 1997. Through a glass darkly: using behavior to assess pain. In:Seminars in Veterinary Medicine and Surgery (Small Animal), p. 61–74. doi: 10.1016/s1096-2867(97)80003-5.

Hay, M., Vulin, A., Génin, S., Sales, P., Prunier, A., 2003. Assessment of pain induced by castration in piglets: behavioral and physiological responses over the subsequent 5 days. Appl. Anim. Behav. Sci. 82:201–18. doi:10.1016/ S0168-1591(03)00059-5.

Ison, S.H, Jarvis, S., Rutherford, K.M.D., 2016 The identification of potential behavioural indicators of pain in periparturient sows. Res. Vet. Sci. 109:114-120. doi:10.1016/j.rvsc.2016.10.002.

Ison, S.H., Clutton, R.E., Di Giminiani, P., Rutherford, K.M., 2016. A review of pain assessment in pigs. Front. in Vet. Sci. 3:108. doi: 10.3389/fvets.2016.00108.

Ison, S.H., Jarvis, S., Hall, S.A., Ashworth, C.J., Rutherford, K.M.D., 2018 Periparturient behavior and physiology: Further insight into the farrowing process for primiparous and multiparous sows. Front. Vet. Sci. 2018, 5, 5, doi:10.3389/fvets.2018.00122.

Jinhyeon, Y., Björkman, S., Pöytäkangas, M., Peltoniemi, O., 2017. The effects of ovarian biopsy and blood sampling methods on salivary cortisol and behaviour in sows. Res. Vet. Sci. 114. doi: 10.1016/j.rvsc.2017.03.004.

Jarvis, S., McLean, K.A., Chirnside, J., Deans, L.A., Calvert, S.K., Molony, V., Lawrence, A.B., 1997. Opioid-mediated changes in nociceptive threshold during pregnancy and parturition in the sow. Pain. 72(1-2):153-159. doi:10.1016/S0304-3959(97)00027-4.

Mainau, E., Dalmau, A., Ruiz-de-la-Torre, J.L., Manteca, X., 2009. Validation of an automatic system to detect position changes in puerperal sows. Appl. Anim. Behav. Sci. 121(2):96-102. doi:10.1016/j.applanim.2009.09.005.

Mainau, E., Dalmau, A., Ruiz-de-la-Torre, J.L., Manteca, X., 2010. A behavioural scale to measure ease of farrowing in sows. Theriogenology. 74(7):1279-1287. doi:10.1016/j.theriogenology.2010.05.034.

Mainau, E., Manteca, X.. 2011. Pain and discomfort caused by parturition in cows and sows. Appl. Anim. Behav. Sci. 135(3):241-251. doi:10.1016/j.applanim.2011.10.020.

Mainau, E., Ruiz-De-La-Torre, J.L., Dalmau, A., Salleras, J.M., Manteca, X., 2012. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. Animal. 6(3):494-501. doi:10.1017/S1751731111001790.

Mainau, E., Temple, D., Manteca, X., 2016. Experimental study on the effect of oral meloxicam administration in sows on pre-weaning mortality and growth and immunoglobulin G transfer to piglets. Prev. Vet. Med. doi:10.1016/j.prevetmed.2016.01.032.

McLennan, K.M., 2018. Why pain is still a welfare issue for farm animals, and how facial expression could be the answer. Agric. 8(8). doi:10.3390/agriculture8080127.

Melišová, M., Illmann, G., Chaloupková, H., Bozdechová, B., 2014. Sow postural changes, responsiveness to piglet screams, and their impact on piglet mortality in pens and crates. Anim. Sci. Jour. 92: 3064-3072. doi: 10.2527/jas.2013-7340.

Mogil, J.S., Pang, D.S.J., Silva Dutra, G.G., Chambers, C.T., 2020. The development and use of facial grimace scales for pain measurement in animals. Neurosci. Biobehav. Rev. 116(April):480-493. doi:10.1016/j.neubiorev.2020.07.013.

Navarro, E., Mainau, E., Manteca, X., 2020. Development of a Facial Expression Scale Using Farrowing as a Model of Pain in Sows. Animals. 10, 2113; doi:10.3390/ani10112113.

Navarro, E., Mainau, E., de Miguel , R., Temple, D., Salas, M., Manteca, X., 2021. Oral Meloxicam administration in sows at farrowing and its effects on piglet immunity transfer and growth. Front. Vet. Sci. 8 doi: 10.3389/fvets.2021.574250.

Nicolaisen, T., Lühken, E., Volkmann, N., Rohn, K., Kemper, N., Michaela, F., 2019. The effect of sows' and piglets' behaviour on piglet crushing patterns in two different farrowing pen systems. Animals. 9(8):538. doi: 10.3390/ani9080538.

Noonan, G., Rand, J., Priest, J., Ainscow, J., Blackshaw, J., 1994. Behavioural observations of piglets undergoing tail docking, teeth clipping and ear notching. Appl. Anim. Behav. Sci. 39:203–13. doi:10.1016/0168-1591(94) 90156-2.

Van Dijk, A.J., van Rens, B.T.T.M., van der Lende, T., Taverne, M.A.M., 2005. Factors affecting duration of the expulsive stage of parturition and piglet birth intervals in sows with uncomplicated, spontaneous farrowings. Theriogenology 64,1573–1590. doi:10.1016/j.theriogenology.2005.03.017.

Viitasaari, E., Hänninen, L., Heinonen, M., Raekallio, M., Orro, T., Peltoniemi, O., Valros, A., 2013. Effects of post-partum administration of ketoprofen on sow health and piglet growth. Vet J. 198(1):153-157. doi:10.1016/j.tvjl.2013.06.013.

Viñuela-Fernandez, I., Jones, E., Welsh, E.M., Fleetwood-Walker, S.M., 2007. Pain mechanisms and their implication for the management of pain in farm and companion animals. Vet. Jour. 174, 227–239. doi:https://doi.org/10.1016/j.tvjl.2007.02.002

Viscardi, A.V., Hunniford, M., Lawlis, P., Leach, M., Turner, P.V., 2017. Development of a piglet grimace scale to evaluate piglet pain using facial expressions following castration and tail docking: A pilot study. Front. Vet. Sci. 4. doi:10.3389/fvets.2017.00051.

Weary, D.M., Niel, L., Flower, F.C., Fraser, D., 2006. Identifying and preventing pain in animals. Appl. Anim. Behav. Sci.100, 64–76. doi:https://doi.org/10.1016/j.applanim.2006.04.013.

Wischner, D., Kemper, N., Stamer, E., Hellbruegge, B., Presuhn, U., Krieter, J., 2009. Characterisation of sows' postures and posture changes with regard to crushing piglets. Appl. Anim. Behav. Sci. 119(1-2):49-55. doi:10.1016/j.applanim.2009.03.002.

Wischner, D., Kemper, N., Stamer, E., Hellbrügge, B., Presuhn, U., Krieter, J., 2010. Pre-lying behaviour patterns in confined sows and their effects on crushing of piglets. Appl Anim. Behav. Sci. 122(1):21-27. doi:10.1016/j.applanim.2009.11.010.

Zheng, C., Yang, X., Zhu, X., Chen, C., Wang, L., Tu, S., Yang, A., Xue, Y., 2020. Automatic posture change analysis of lactating sows by action localisation and tube optimisation from untrimmed depth videos. Biosyst. Eng. 194:227-250. doi:10.1016/j.biosystemseng.2020.04.005.

Capítulo 6

CAPÍTULO 6. DISCUSIÓN GENERAL

Capítulo 6

6.1. Introducción

Los dos estudios que forman parte de esta tesis abarcan diversos aspectos relacionados con el dolor sufrido por las cerdas en el momento del parto. En el primer estudio se analizaron los efectos de la administración del AINE meloxicam oral sobre el crecimiento de los lechones a lo largo de la lactación y sobre la transferencia de inmunidad maternofilial. En el segundo estudio se desarrollaron y analizaron los indicadores faciales, corporales y posturales de las cerdas alrededor del parto.

La discusión se divide en cuatro partes: (1) efectos del meloxicam oral administrado en cerdas durante el parto, (2) expresiones faciales en cerdas alrededor del parto como indicador de dolor, (3) limitaciones de los estudios de la presente tesis y (4) aspectos no resueltos a lo largo de esta tesis y nuevos frentes para continuar la investigación.

6.2. Efectos del meloxicam oral administrado en cerdas durante el parto.

6.2.1. Crecimiento de los lechones al destete

Los efectos del meloxicam oral fueron beneficiosos en cuanto al crecimiento de los lechones con bajo peso corporal al nacimiento, mostrando una tendencia desde el día +9 postparto hasta el destete. El meloxicam ayuda a disminuir la inflamación y probablemente también disminuye el dolor de la glándula mamaria, facilitando que la cerda pase más tiempo tumbada en decúbito lateral, lo que permite que los lechones se amamanten durante periodos de tiempo más largos. Los lechones más pequeños son los que se benefician en mayor medida, ya que suelen ser los que tienen más dificultad para acceder a las glándulas mamarias y muchas veces tienen que esperar a que los más grandes se hayan saciado. Nuestros resultados coinciden con otros estudios que administraron meloxicam alrededor del parto en cerdas y obtuvieron resultados significativos o tendencias en el mismo sentido (Mainau y col., 2012; Tenbergen y col., 2014; Mainau y col., 2016). Por otro lado, Viitasaari y col. (2013) y Ison y col. (2016), no encontraron diferencias sobre el crecimiento de los lechones de cerdas tratadas con el AINE ketoprofeno alrededor del parto.

Estas discrepancias entre estudios podrían ser debidas al diferente tipo de principio activo utilizado (meloxicam vs. ketoprofeno). El meloxicam inhibe preferentemente la actividad COX-2, mientras que el ketoprofeno inhibe preferentemente la actividad COX-1 (Trimboli y col., 2020). En consecuencia, el meloxicam podría ser más eficaz a la hora de reducir la inflamación resultante del parto.

Otros factores tales como la dosificación y el modo de administración del principio activo, así como el momento de administración relativo al parto, podrían explicar parte de la variabilidad de los resultados obtenidos sobre los crecimientos de los lechones de cerdas tratadas con AINEs alrededor del parto. Con relación al momento de administración relativo al parto, cabe recordar que cuando se administra un AINE antes de una condición o enfermedad dolorosa específica (en lugar de administrarlo durante o después del momento doloroso), los animales regresan a un estado fisiológico normal más rápidamente (Anderson y Muir, 2005). A pesar de esto, se ha demostrado que la administración de flunixin meglumine (que es un AINE que inhibe preferentemente la actividad de la COX-1) alrededor del parto en vacuno, puede provocar efectos secundarios importantes, tales como un mayor riesgo de retención de placenta, metritis o incremento de la tasa de desecho (Newby y col., 2017). En consecuencia, es necesario ser cautelosos en la administración temprana de AINEs alrededor del parto. En nuestro estudio, el meloxicam se administró a las cerdas cuando el parto ya se había iniciado (cuando habían sido expulsados entre uno y tres lechones). El número de lechones nacidos vivos no se vio afectado por el tratamiento, ni tampoco se observó un aumento en el número de casos de retención placentaria. Así pues, aunque este no fue el objetivo del presente estudio, el meloxicam oral no parece tener efectos adversos en el desarrollo normal del parto en las cerdas.

6.2.2. Transferencia de inmunidad maternofilial.

El consumo temprano de calostro es imprescindible para la supervivencia de los lechones (Hurley y Theil, 2011). El calostro contiene una alta concentración de inmunoglobulinas, que van disminuyendo a medida que pasan las horas después del parto (Theil y col., 2014). La administración de meloxicam oral resultó en una mayor concentración de inmunoglobulinas (IgG e IgA) en el calostro el día posterior al parto. El hecho de que el aumento de las IgA fuera más pronunciado que el de las IgG puede deberse a dos factores. En primer lugar, después del parto se produce un descenso brusco de la concentración de IgG en el calostro, mientras que el descenso de IgA es más paulatino debido a su papel de regulación sobre la microbiota intestinal (Theil y col., 2014). En segundo lugar, un notable porcentaje de IgA se sintetiza directamente en la glándula mamaria, mientras que las IgG se encuentran en la sangre (Hammer, 1978). Nuestros datos sugieren que la administración de meloxicam oral durante el parto ayuda a reducir la inflamación de la glándula mamaria, favoreciendo tanto la síntesis local de inmunoglobulinas como su transporte desde la sangre. En cuanto a las muestras de saliva, no se obtuvieron diferencias significativas entre tratamientos. La concentración de IgG en la saliva está directamente relacionada con la concentración de IgG en el suero (Datos propios sin publicar). Así pues, el hecho de que no se observe un aumento de la concentración de IgG en saliva nos permite afirmar que el aumento de IgG en la glándula mamaria es debido a un efecto local del fármaco y no a un aumento sistémico de las concentraciones de IgG.

Los lechones de cerdas tratadas con meloxicam oral mostraron una mayor cantidad de IgA (y una tendencia a un aumento de las IgG) en suero, al menos durante los primeros 9 días postparto en comparación con los lechones de cerdas control. De forma similar, Mainau y col. (2016), observó que la administración de meloxicam oral al principio del parto (cuando habían nacido una media de 4 lechones) aumentaba la concentración de IgG en el suero de los lechones.

En el primer día postparto, de forma natural, las interleucinas IL-4 e IL-2, están más elevadas en el suero de los lechones, y van decreciendo con el paso de los días. En los lechones de madres tratadas con meloxicam, ambas interleucinas tendieron a ser más elevadas el día +9 en comparación con los lechones de madres control. Esto podría traducirse en una mayor respuesta inmunitaria de los lechones, lo cual sería beneficioso en la defensa del organismo frente a agentes infecciosos.

Finalmente, es relevante el hecho de que uno de los factores que influye sobre la transferencia de inmunidad maternal a los lechones es el manejo de las adopciones postparto. En nuestro estudio, las adopciones se realizaron durante las primeras 8 horas de vida. Según Brandrick y col. (2011) la transferencia de inmunidad celular proporcionada por el calostro sólo es óptima si los lechones permanecen al menos 12 horas con su madre biológica. Así pues, se podría esperar que los resultados obtenidos en nuestro estudio se viesen potenciados realizando las adopciones a partir de las 12 horas después del nacimiento.

6.3. Expresiones faciales en cerdas alrededor del parto como indicador de dolor.

Tal y como se ha demostrado en otros estudios, las expresiones faciales resultan ser un buen indicador para detectar dolor en varias especies (Mogil y col., 2020). En el caso de las cerdas, en esta tesis se demuestra que con las expresiones faciales se pueden diferenciar tres grados de dolor distintos alrededor del parto, siendo la expulsión del lechón la fase más dolorosa y la fase previa al destete la menos dolorosa.

6.3.1. Indicadores comportamentales de dolor y correlaciones entre ellos

La escala de dolor basada en las expresiones faciales de las cerdas alrededor del parto que se ha desarrollado en esta tesis tiene en cuenta cinco regiones faciales: ojos, hocico, maseteros, zona temporal y orejas y cuello. Cuatro de las cinco regiones se utilizan para evaluar el dolor en otras especies. La región del cuello, en cambio, no había sido descrita previamente en otras especies, probablemente debido a que la mayoría de las escalas faciales evalúan la cabeza del animal en una posición frontal. En el caso de nuestro estudio vimos interesante incluir la región del cuello ya que, en función de su posición, la expresión facial cambia.

Se han detectado cinco indicadores comportamentales de dolor alrededor del parto: estirar la pata trasera presionando el abdomen, arquear la espalda, empujar las patas delanteras hacia delante, temblar y mover la cola (basado en Ison y col., 2016). Cuatro de éstos (estirar la pata trasera presionando el abdomen, arquear la espalda, empujar las patas delanteras hacia delante y mover la cola) se manifestaron tal como fue descrito por Ison y col. (2016). Estos cuatro indicadores corporales de dolor estudiados fueron los que se observaron más frecuentemente, y se observaron más a menudo durante el parto que durante la fase previa al destete, siendo su frecuencia máxima en el momento de la expulsión de los lechones. En nuestro estudio, el indicador corporal 'temblar' sólo se vio en dos de los diez animales observados. Ison y col. (2016) lo describió como un indicador eficaz para determinar si un animal estaba sufriendo dolor. En nuestro caso, "temblar" aparece como un indicador inequívoco de dolor, pero hay mucha variabilidad individual en su expresión. Así pues, a diferencia de los resultados obtenidos por Ison y col. (2016), donde la mayoría de las cerdas presentaban este indicador, en nuestro estudio podemos afirmar que el hecho de que una cerda no muestre el comportamiento "temblar" alrededor del parto, no significa que no esté sufriendo dolor.

Los resultados basados en las expresiones faciales se correlacionaron con los indicadores corporales de dolor descritos por Ison y col. (2016). En este estudio, también se pudo comprobar que cuanto mayor es el dolor sufrido por la cerda, la frecuencia de indicadores corporales aumenta y las expresiones faciales de las cerdas expresan su grado máximo de intensidad de dolor. En ambos casos, el momento de expulsión del lechón resulta ser el más doloroso. La región facial que presentó una mayor fiabilidad de detección según el 'silver standard' (el observador que analizó todas las imágenes y valoró la intensidad del dolor sufrido por las cerdas) fue la tensión ocular. Esto también ocurre en muchas de las especies ya descritas (Langford y col., 2010; Sotocinal y col., 2011; Keating y col., 2012, 2016; Di Griminiani y col., 2016; Häger y col., 2017; Costa y col., 2014; Orth y col., 2020). Por otro lado, podemos considerar la tensión ocular como un buen indicador de dolor ya que cuando esta se manifestó con mayor intensidad, coincidió con el aumento de la frecuencia de los cuatro indicadores corporales de dolor anteriormente mencionados (estirar la pata trasera de atrás hacia adelante presionando el abdomen, arquear la espalda, empujar las patas delanteras hacia delante y mover la cola rápidamente).

Según las repetibilidades intra e inter observadores, se vio que no sólo era fácil de detectar los cambios oculares, sino también la región del masetero. Así pues, ambas zonas (la tensión ocular y la región del masetero) son las más sencillas a la hora de detectar los cambios faciales debidos al dolor. Los maseteros también se categorizan como una región fácil de determinar en otras especies como las ovejas (McLennan y col., 2016), los ratones (Langford y col., 2010), los conejos (Keating y col., 2012) y los hurones (Reijgward y col., 2017), pero a diferencia de lo que sucede en las cerdas (cuanto más dolor, mayor es el estiramiento de los maseteros), al resto de especies se les abultan los maseteros al sentir dolor. Cabe decir que, en las ratas y los lechones, el dolor evidenciado por los maseteros se evalúa de la misma forma que en las cerdas, abultándose cuando los animales están relajados y estirándose cuando sufren dolor

(Sotocinal y col., 2011; Di Giminiani y col., 2016). En cuanto correlacionamos este indicador con los indicadores corporales, vimos que la correlación era elevada con los cuatro indicadores corporales más frecuentes, lo cual corroboró que los maseteros resultan ser una región fiable para valorar el dolor en las cerdas alrededor del parto.

El morro fue la tercera región facial que mostró una gran correlación con los cuatro indicadores corporales más frecuentes. Además, coincide que estas tres regiones con correlaciones más elevadas con los indicadores corporales son las que tuvieron mayor repetibilidad entre observadores. Esta correlación confirma que las expresiones faciales son un buen indicador de dolor para las cerdas en el parto.

En cuanto a los indicadores corporales, el arqueamiento de la espalda descrito por Grégoire y col. (2013) como un indicador capaz de reflejar mucho dolor, fue el indicador corporal con mayor correlación con las cinco regiones faciales estudiadas a lo largo del parto, especialmente en el momento de la expulsión de los lechones. Lo siguieron, en el mismo sentido, el movimiento de la cola y la presión de la pata trasera hacia el abdomen. El movimiento de las patas delanteras mostró una correlación moderada con la media de las cinco regiones faciales. Esto probablemente se deba a que, como describió previamente Ison y col. (2016), este indicador se ve de forma más continua a lo largo de todo el parto, mostrando una mayor incidencia en el momento de la expulsión de los lechones. El movimiento de la cola y el indicador temblar mostraron una correlación muy baja, lo que se debe probablemente a que como Ison y col. (2016) describió, el movimiento de la cola se da únicamente durante la expulsión del lechón. Por otra parte, el indicador temblar, a diferencia de los resultados obtenidos por Ison y col. (2016), mostró una gran variabilidad individual en nuestro estudio, ya que sólo dos de las diez cerdas lo presentaron. Coincidiendo con Jinhyeon Yun y col. (2017), que relacionaron el temblar con el aumento de cortisol en sangre, consideramos que es un indicador que muestra dolor e inflamación, y por lo tanto podemos decir que, si una cerda lo presenta, está sufriendo dolor, pero el hecho de no manifestarlo no es indicativo de ausencia de éste.

6.3.2. Cambios posturales y efecto del amamantamiento

Como se ha descrito en otros estudios (Mainau y col., 2009; Mainau y col., 2010; Melišová y col., 2014; Ison y col., 2016), el número de cambios posturales en la cerda aumenta el día del parto, al igual que lo hace el tiempo que permanece tumbada lateralmente, si lo comparamos con los días postparto. La postura latero-lateral está asociada al cansancio sufrido debido al parto (Nicolaisen y col., 2019). En nuestro estudio, cabe decir que el mayor número de cambios posturales tuvo lugar entre las expulsiones de lechones, ya que durante la expulsión, las cerdas estaban en postura latero-lateral. En el día previo al destete se vio una mayor frecuencia de las posturas esternal y de pie. La postura esternal se relaciona con una mayor facilidad para interaccionar con los lechones (Nicolaisen y col., 2019). Además, con esa postura las cerdas evitan que los lechones accedan a las mamas y les provoquen dolor (Fu y col., 2019).

Tras estudiar el efecto del amamantamiento, se vieron diferencias en la fase previa al destete, registrándose una mayor incidencia de indicadores corporales en el momento en el que los lechones estaban mamando. Esto se debe probablemente, a que, conforme pasan los días, el dolor asociado al parto que podría diluir el dolor del amamantamiento, va disminuyendo. Por otra parte, a medida que los lechones crecen, la succión es más fuerte y los dientes de estos son más grandes, lo cual puede causar pequeñas heridas en las mamas de las cerdas y causarles mayor dolor (Fu y col., 2019). Cabe decir que, de forma natural, durante los días previos al destete, las cerdas suelen tender a expresar comportamientos para evitar que los lechones mamen durante mucho tiempo (D'Eath y col., 2014; Fu y col., 2019), lo cual nos indica que el dolor sufrido por el amamantamiento al final de la lactación, es algo frecuente y común.

6.3.3. Efecto de la paridad sobre el dolor en el parto

El dolor en el parto en función de la paridad es un tema controvertido. Estudiando los cambios posturales, se detectó que las cerdas multíparas llevan a cabo un mayor número de cambios por minuto durante el día del parto que las primíparas. En cambio, analizando los indicadores corporales, se vio que las cerdas primíparas mostraban un mayor número de indicadores corporales por minuto que las multíparas, sobre todo en los momentos entre la expulsión de los lechones. Este hecho corrobora la revisión realizada por Mainau y Manteca (2011), la cual describe que el parto es más doloroso para las primíparas debido a la falta de experiencia y a la novedad del dolor sufrido. Ison y col. (2018) describió que las cerdas primíparas suelen experimentar más dolor durante el momento del parto debido a la mayor dilatación del canal del parto. Por otro lado, tras la expulsión de los lechones, cuando comienza la involución uterina, las multíparas pueden llegar a sufrir un dolor más intenso debido a la perdida de tono uterino. En nuestro estudio, el arqueamiento de espalda fue el único indicador corporal de dolor que no mostró ningún tipo de diferencia entre las cerdas primíparas y las multíparas. Es probable que el arqueamiento esté asociado a las contracciones abdominales que facilitan la expulsión de los lechones, y que éstas sean experimentadas con la misma intensidad por las cerdas multíparas como para las primíparas.

6.4. Limitaciones de los estudios de la presente tesis

A continuación, se describen las principales limitaciones encontradas a lo largo de esta tesis.

6.4.1. **Con relación al estudio de la inmunidad**

-Bajo número de animales muestreados. Hemos visto tendencias en ciertos resultados, como la ganancia media diaria. Las tendencias obtenidas probablemente se deban a la falta de individuos muestreados. Con un mayor número de individuos podríamos confirmar si el resultado es o no es significativo y por lo tanto definir si el uso de meloxicam oral es una buena herramienta para aumentar la ganancia media diaria de los lechones en una granja.

- Muestreo de animales de mayor tamaño al nacimiento. El muestreo de los animales fue consensuado, escogiendo animales que se amamantaban de las mamas craneales, de las mediales y de las caudales. Con este muestreo evitábamos seleccionar animales excesivamente pequeños, por la dificultad y el riesgo asociado al hecho de obtener sangre de animales de menor tamaño. Al no tener muestras de sangre de un número representativo de animales pequeños para poder compararlos entre ellos, no hemos podido evaluar en qué dirección hubiesen ido los valores de Inmunoglobulinas en suero. Esperaríamos resultados muy interesantes al comparar tratamientos por tamaños, ya que los lechones más pequeños, aparte de tener más dificultad para acceder a las mamas (Deen y col., 2004), suelen ingerir el calostro más tarde (Deen y col., 2004). En los lechones más pequeños, esperaríamos una mayor cantidad de IgAs, ya que la cantidad que se produce en sangre ya habría llegado al calostro y lo más probable es que los niveles de IgA en sangre fuesen superiores en los animales de este tamaño, por el simple hecho de amamantarse más tarde.

-El orden de nacimiento de los lechones también es una variable a tener en cuenta, ya que los primeros en nacer tienen acceso a un calostro con mayor concentración de inmunoglobulinas, especialmente de IgG, de forma más sencilla y durante mayor tiempo (Deen y col., 2004; Devillers y col., 2011). Sería interesante estudiar la concentración de inmunoglobulinas e interleucinas teniendo en cuenta esta variable, ya que en nuestro estudio no registramos en qué orden nacieron los individuos muestreados.

- Posible escasez de transferencia de inmunidad celular debido a que las adopciones se realizaron demasiado pronto. Según Brandrick y col. (2011) la transferencia inmunitaria proporcionada por el calostro es óptima si permitimos que los lechones permanezcan al menos 12 horas con su madre biológica. En nuestro estudio las adopciones tuvieron lugar dentro de las primeras 8 horas de vida. Es probable que la concentración de interleucinas hubiera sido mayor si hubiésemos dejado a los lechones permanecer más tiempo con sus madres biológicas.

-El estudio se realizó en una granja bajo condiciones óptimas de higiene y manejo. Es probable que, en granjas con un peor estado sanitario, se produzca un mayor número de muertes previas al destete o crecimientos más dispares. Podríamos encontrar más diferencias entre los grupos tratamiento y control, ya que hemos visto que el meloxicam oral mejora el crecimiento de los lechones más pequeños. Este hecho probablemente se pueda extrapolar al crecimiento de lechones que no están en granjas con condiciones óptimas o en granjas en los que los programas vacunales de las madres no sean tan completos y por lo tanto el sistema inmunitario de sus lechones esté más limitado.

6.4.2. Con relación a los estudios de indicadores comportamentales

-El ángulo de la imagen utilizado limita la visión total de la cara de las cerdas ya que únicamente tenemos una perspectiva lateral. En el resto de las especies estudiadas hasta la fecha, las imágenes tomadas son frontales, y por lo tanto suelen tener acceso a un mayor número de expresiones faciales. Cabe decir que algunos estudios sobre expresiones faciales describen la sujeción de los animales en el momento de realizar la fotografía, produciendo un estrés adicional al animal y conllevando que los animales inmovilizados presenten expresiones de mayor dolor que los animales que no son inmovilizados (Guesgen y col., 2016; Senko y col., 2017; Mogil y col., 2020). En el caso del parto, es imposible obtener imágenes frontales sin molestar a los animales, ya que como hemos visto, pasan la mayoría del parto en postura latero-lateral y con la cara en contacto con el suelo.

-En cuanto al estudio comparativo de expresiones faciales e indicadores corporales, el número de animales estudiados por paridad es limitado. Por otro lado, la inclusión de un mayor número de cerdas nos facilitaría determinar el porcentaje de animales que muestran el indicador de temblar, ya que en este caso han sido muy pocos los individuos que lo presentaban.

6.5. Aspectos no resueltos durante la tesis y nuevos frentes para continuar la investigación

Como se ha comentado anteriormente, a lo largo de la tesis hemos encontrado ciertas limitaciones, pero éstas nos aportan ideas para proponer nuevos retos y líneas de investigación en el ámbito del dolor alrededor del parto en cerdas.

Se propone administrar el meloxicam a cerdas alrededor del parto y muestrear a los lechones más pequeños de la camada. Se espera que el aumento de la transferencia inmunitaria en animales pequeños sea claramente decisivo para su crecimiento y supervivencia. En la presente tesis, se ha evidenciado que los lechones de cerdas tratadas con meloxicam alrededor del parto presentan una mayor cantidad de inmunoglobulinas en sangre que los lechones procedentes de madres no tratadas. Así pues, se propone ver la eficacia de una vacuna con transferencia maternofilial junto con la administración del meloxicam, y valorar si la transferencia inmunitaria es mayor, siempre dejando que los lechones permanezcan con su madre biológica al menos 12 horas.

Otro campo de estudio por explorar es el efecto de la administración de AINEs sobre la transferencia inmunitaria maternofilial en otras especies (por ejemplo, vacuno u ovino). La transferencia de inmunidad mediante el calostro es esencial para casi todos los mamíferos; por lo tanto, poder confirmar que la administración de un AINE la mejora supone asegurar que el futuro productivo de los animales será mejor y por lo tanto repercutirá en el bienestar de los animales y en la economía del ganadero.

Se propone extrapolar la escala de dolor basada en el parto a otros procesos dolorosos tales como las cojeras. Esto nos daría una visión más amplia de la expresión facial de las cerdas ya que podríamos evitar el limitante que tenemos en el parto acerca del ángulo de la imagen, tomando imágenes con una visión frontal y pudiendo encontrar así, probablemente, alguna otra expresión relevante de dolor.

La expresión facial de las cerdas adultas y de los cerdos de engorde es bastante similar y, por lo tanto, consideraríamos muy interesante extrapolar la escala de dolor que hemos desarrollado a otras fases de producción del cerdo, con el fin de poder enseñar a los ganaderos los cambios de expresión que realizan los animales en el momento que están experimentando un dolor severo. De esta forma, el dolor podría tratarse de una manera temprana y sin necesidad de buscar indicadores fisiológicos, los cuales, generalmente tardan más tiempo en manifestarse.

Por último, sería importante validar la escala de expresiones faciales indicativa de dolor. Fruto de los datos obtenidos en la presente tesis, actualmente se está analizando estadísticamente el efecto del meloxicam oral alrededor del parto sobre las expresiones faciales en cerdas. También sería interesante evaluar el efecto del meloxicam administrado a las cerdas durante la lactación con el fin de evaluar los indicadores comportamentales y ver cuál es el efecto del amamantamiento. Aplicando el meloxicam podríamos ver si la inflamación de la glándula mamaria disminuye. Si es así, esperaríamos que el tiempo que las cerdas permanecen en decúbito lateral aumentase, y en el caso de hacerlo, los lechones tendrían un mejor amamantamiento y consecuentemente un mayor crecimiento, lo cual repercutiría de forma directa en la economía de los ganaderos.

6.6. Referencias.

Anderson, D.E., Muir, W.W. 2005. Pain management in ruminants. Veterinary Clinics: Food Animal Practice 21, 19-31.

Costa, E.D., Minero, M., Lebelt, D., Stucke, D., Canali, E., Leach, M.C. 2014. Development of the Horse Grimace Scale (HGS) as a Pain Assessment Tool in Horses Undergoing Routine Castration. PLoS ONE 9, e92281.

D'Eath, R.B., Arnott, G., Turner, S.P., Jensen, T., Lahrmann, H.P., Busch, M. E., Sandøe, P. 2014. Injurious tail biting in pigs: how can it be controlled in existing systems without tail docking?. Animal 8, 1479-1497.

Di Giminiani, P., Brierley, V.L.M.H., Scollo, A., Gottardo, F., Malcolm, E.M., Edwards, S.A., Leach, M.C. 2016. The Assessment of Facial Expressions in Piglets Undergoing Tail Docking and Castration: Toward the Development of the Piglet Grimace Scale. Frontiers in Veterinary Science 3, 100.

Fu, L. L., Zhou, B., Li, H.Z., Liang, T.T., Chu, Q.P., Schinckel, A.P., Xu, F.L. 2019. Effects of tail docking and/or teeth clipping on behavior, lesions, and physiological indicators of sows and their piglets. Animal Science Journal 90, 1320-1332.

Grégoire, J., Bergeron, R., d'Allaire, S., Meunier-Salaün, M.C. Devillers, N. 2013. Assessment of lameness in sows using gait, footprints, postural behaviour and foot lesion analysis. Animal 7, 1163-1173.

Häger, C., Biernot, S., Buettner, M., Glage, S., Keubler, L.M., Held, N., Bleich, E.M., Otto, K., Müller, C.W., Decker, S. 2017. The Sheep Grimace Scale as an indicator of post-operative distress and pain in laboratory sheep. PLoS ONE 12, e0175839.

Hammer, D.K., Mossmann, H. 1978. The importance of membrane receptors in the transfer of immunoglobulins from plasma to the colostrum. Annales de Recherches Veterinaires 9, 229-234.

Hurley, W.L., Theil, P.K. 2011. Perspectives on immunoglobulins in colostrum and milk. Nutrients 3, 442–474.

Ison, S.H., Jarvis, S., Rutherford, K.M.D. 2016. The identification of potential behavioural indicators of pain in periparturient sows. Research in Veterinary Science 109, 114–120.

Keating, S.C.J., Thomas, A.A., Flecknell, P.A., Leach, M.C. 2012. Evaluation of EMLA Cream for Preventing Pain during Tattooing of Rabbits: Changes in Physiological, Behavioural and Facial Expression Responses. PLoS ONE 7, e44437.

Langford, D.J., Bailey, A.L., Chanda, M.L., Clarke, S.E., Drummond, T.E., Echols, S., Glick, S., Ingrao, J., Klassen-Ross, T., Lacroix-Fralish, M.L. 2010. Coding of facial expressions of pain in the laboratory mouse. Nature Methods 7, 447–449.

Mainau, E., Dalmau, A., Ruiz-de-la-Torre, J.L., Manteca, X. 2009. Validation of an automatic system to detect position changes in puerperal sows. Applied Animal Behaviour Science 121, 96-102.

Mainau, E., Dalmau, A., Ruiz-de-la-Torre, J.L., Manteca, X. 2010. A behavioural scale to measure ease of farrowing in sows. Theriogenology 74, 1279-1287.

Mainau, E., Manteca, X.. 2011. Pain and discomfort caused by parturition in cows and sows. Applied Animal Behaviour Science 135, 241-251.

Mainau, E., Ruiz-De-La-Torre, J.L., Dalmau, A., Salleras, J.M., Manteca, X. 2012. Effects of meloxicam (Metacam®) on post-farrowing sow behaviour and piglet performance. Animal 6, 494–501.

Mainau, E.; Temple, D.; Manteca, X. 2016. Experimental study on the effect of oral meloxicam administration in sows on pre-weaning mortality and growth and immunoglobulin G transfer to piglets. Preventive Veterinary Medicine 126, 48–52.

McLennan, K.M., Rebelo, C.J.B., Corke, M.J., Holmes, M.A., Leach, M.C., Constantino-Casas, F. 2016. Development of a facial expression scale using footrot and mastitis as models of pain in sheep. Applied Animal Behaviour Science 176, 19–26.

Melišová, M., Illmann, G., Chaloupková, H., Bozděchová, B. 2014. Sow postural changes, responsiveness to piglet screams, and their impact on piglet mortality in pens and crates. Journal of animal science 92, 3064-3072.

Mogil J.S., Pang, S.J.D., Dutra, G.G.S., Chambers, C.T. 2020. The development and use of facial grimace scales for pain measurement in animals. Neuroscience and Biobehavioral Reviews 116, 480-493.

Newby, N.C., Leslie, K.E., Dingwell, H.D.P., Kelton, D.F., Weary, D.M., Neuder, L., Millman, S.T., Duffield, T.F. 2017. The effects of periparturient administration of flunixin meglumine on the health and production of dairy cattle. Journal of Dairy Science 100, 582–587.

Nicolaisen, T., Lühken, E., Volkmann, N., Rohn, K., Kemper, N., Fels, M. 2019. The effect of sows' and piglets' behaviour on piglet crushing patterns in two different farrowing pen systems. Animals 9, 538.

Orth, E.K., González, F.J.N., Pastrana, C.I., Berger, J.M., le Jeune, S.S., Davis, E.W. 2020. Development of a donkey grimace scale to recognize pain in donkeys (Equus asinus) post castration. Animals 10, 1411.

Reijgwart, M.L., Schoemaker, N.J., Pascuzzo, R., Leach, M.C., Stodel, M., De Nies, L., Hendriksen, C.F.M., Van Der Meer, M., Vinke, C.M., Van Zeeland, Y.R.A. 2017. The composition and initial evaluation of a grimace scale in ferrets after surgical implantation of a telemetry probe. PLoS ONE 12, e0187986.

Sotocinal, S.G., Sorge, R.E., Zaloum, A., Tuttle, A.H., Martin, L.J., Wieskopf, J.S., Mapplebeck, J.C.S., Wei, P., Zhan, S., Zhang, S. 2011. The Rat Grimace

Scale: A Partially Automated Method for Quantifying Pain in the Laboratory Rat via Facial Expressions. Molecular Pain 7, 55.

Tenbergen, R., Friendship, R., Cassar, G., Amezcua, M.R., Haley, D. 2014. Investigation of the use of meloxicam post farrowing for improving sow performance and reducing pain. Journal of Swine Health and Production 22, 10–5.

Theil, P.K., Lauridsen, C., Quesnel, H. 2014. Neonatal piglet survival: impact of sow nutrition around parturition on fetal glycogen deposition and production and composition of colostrum and transient milk. Animal 8, 1021-1030.

Trimboli, F., Ragusa, M., Piras, C., Lopreiato, V., Britti, D. 2020. Outcomes from Experimental Testing of Nonsteroidal Anti-Inflammatory Drug (NSAID) Administration during the Transition Period of Dairy Cows. Animals 10, 1832.

Viitasaari, E., Hänninen, L., Heinonen, M., Raekallio, M., Orro, T., Peltoniemi, O., Valros, A. 2013. Effects of post-partum administration of ketoprofen on sow health and piglet growth. Veterinary Journal 198, 153-157.

Yun, J., Björkman, S., Pöytäkangas, M., Peltoniemi, O. 2017. The effects of ovarian biopsy and blood sampling methods on salivary cortisol and behaviour in sows. Research in Veterinary Science 114, 80-85.

CAPÍTULO 7. CONCLUSIONES

Capítulo 7

Las conclusiones de la presente tesis son las siguientes:

1. La administración de meloxicam oral al inicio del parto tiende a aumentar el peso de los lechones desde el día 9 postparto hasta el destete, siendo mayor esta tendencia en los lechones con bajo peso corporal al nacimiento. Los lechones machos presentan un mayor crecimiento medio diario respecto a las hembras desde el nacimiento hasta el destete.

2. La administración de meloxicam oral al inicio del parto tiende a aumentar la concentración de inmunoglobulinas en el calostro de las cerdas, resultando en una mayor respuesta humoral y celular de los lechones. La concentración de inmunoglobulinas no se ve afectado por la paridad, al contrario de lo que pasa con las citoquinas, las cuales se ven aumentadas en las cerdas de tres partos en adelante.

3. Se ha desarrollado una escala de dolor basada en las expresiones faciales de las cerdas alrededor del parto. La escala tiene en cuenta cinco regiones faciales (ojos, hocico, maseteros, región temporal y orejas y cuello) y tres intensidades de dolor (previo al destete, intervalo entre la expulsión de dos lechones y expulsión de un lechón).

4. La escala de dolor resulta ser un indicador sencillo de aprender y de valorar para personas del sector porcino y personas ajenas a éste, siendo

las mujeres más sensibles que los hombres a la hora de cuantificar el dolor.

5. La escala de dolor basada en las expresiones faciales de las cerdas esta correlacionada con los indicadores corporales de dolor alrededor del parto. La prevalencia de ambos indicadores aumenta durante el parto, cuando las cerdas experimentan un dolor más severo, siendo máxima en el momento de la expulsión de los lechones.

6. Las cerdas primíparas muestran un mayor número de indicadores corporales de dolor por minuto que las cerdas multíparas a lo largo del parto, especialmente en el momento de la expulsión de los lechones. Por otro lado, las cerdas multíparas llevan a cabo un mayor número de cambios posturales por minuto a lo largo del parto. Este hecho nos lleva a afirmar que generalmente, las cerdas primíparas experimentan mayor dolor que las multíparas en el momento del parto.

7. El efecto de los episodios de amamantamiento aumenta la frecuencia de los indicadores corporales de dolor de la cerda en la fase previa al destete.

"I think using animals for food is an ethical thing to do, but we've got to do it right. We've got to give those animals a decent life and we've got to give them a painless death. We owe the animal respect."

– Temple Grandin