Universitat Autònoma de Barcelona

FACTORES DE RIESGO ASOCIADOS CON LA MORTALIDAD DE LOS POLLOS DE ENGORDE DURANTE LA PRIMERA SEMANA DE VIDA

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BAJO LA DIRECCIÓN DEL DOCTOR:

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Certifica:

Que la memoria titulada "Factores de riesgo asociados con la mortalidad de los pollos de engorde durante la primera semana de vida", presentada por Marta Yerpes Ron con la finalidad de optar al Grado de Doctor en Veterinaria, ha sido realizada bajo su dirección y, considerándola finalizada, autoriza su presentación para que sea juzgada por la comisión correspondiente.

Y para que conste a los efectos oportunos, firma la presente en Bellaterra a 14 de abril de 2020.

Dr. Xavier Manteca Vilanova

“*The greatness of a nation can be judged by the way its animals are treated.*”

Mahatma Gandhi, (1869-1948)

Agradecimientos

Justo acabando la carrera me decía a mí misma que no asumiría ningún otro proyecto de estudios tan grande como fue en aquel entonces para mí el grado de veterinaria. Lo que no sabía, es que unas semanas después me embarcaría en otro proyecto, si cabe, aún más importante. Fue gracias a Ramón y a su entrevista telefónica lo que me llevo a empezar con todo esto. Y la verdad, es que hasta que no me he puesto a escribir estas líneas no me parecía tan palpable el fin de esta etapa.

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“*A person who never made a mistake never tried anything new*.”

Albert Einstein, (1879-1955)

Resumen

La tasa de mortalidad durante la primera semana de vida es uno de los indicadores más utilizados para evaluar el bienestar de las aves de producción. El objetivo general de la presente tesis doctoral fue estudiar los principales factores que contribuyen a aumentar la mortalidad de los pollitos de engorde durante la primera semana de vida (MPSV). En la planta de incubación la MPSV estuvo relacionada con la temperatura cloacal de los pollitos ($P = 0,0132$), de modo que la MPSV puede aumentar hasta 3 veces cuando la temperatura cloacal está por debajo del rango óptimo (<39,7ºC). La edad de las reproductoras, el sexo de los pollitos, el proceso de incubación, el tiempo de espera en la planta de incubación y el sistema de ventilación de la sala de expedición tuvieron un efecto significativo sobre la temperatura cloacal ($P < 0,05$). Sin embargo, estos factores no tuvieron un efecto directo sobre la MPSV. Durante el transporte se encontró una correlación significativa entre la MSPV, por una parte, y el sexo de los pollitos y la cantidad de pollitos cargados por otra ($P = 0,0082$ y $P = 0,0087$; respectivamente). Los pollitos machos tuvieron una MPSV mayor que los pollitos hembra y cuanto mayor fue la carga de pollitos por viaje mayor fue la MSPV. La humedad relativa (HR) en el vehículo de transporte y la duración del viaje tuvieron un efecto significativo sobre la pérdida de peso de los pollitos ($P = 0,0006$ y $P = 0,0188$; respectivamente). Finalmente, los factores propios de la granja que tuvieron un efecto significativo sobre la MPSV fueron la edad de las reproductoras, el sexo y la estirpe de los pollitos, el tipo de nave, la presencia o no de recuperador en el bebedero, los días de almacenamiento de los huevos, el año de estudio y la estación ($P < 0,05$). Se concluye que la MPSV tiene un marcado carácter multifactorial y, por lo tanto, es necesario identificar en cada caso los factores que tienen un efecto sobre la MPSV para implementar medidas correctoras y mejorar el bienestar de los pollitos.

Resum

La taxa de mortalitat durant la primera setmana de vida és un dels indicadors més utilitzats per avaluar el benestar de les aus de producció. L'objectiu general de la present tesi doctoral va ser estudiar els principals factors que contribueixen a augmentar la mortalitat dels pollets d'engreix durant la primera setmana de vida (MPSV). A la planta d'incubació la MPSV va estar relacionada amb la temperatura cloacal dels pollets (P = 0,0132), de manera que la MSPV pot augmentar fins a 3 vegades quan la temperatura cloacal està per sota del rang òptim (<39,7ºC). L'edat de les reproductores, el sexe dels pollets, el procés d'incubació, el temps d'espera a la planta d'incubació i el sistema de ventilació de la sala d'expedició van tenir un efecte significatiu sobre la temperatura cloacal (P < 0,05). Tanmateix, aquests factors no van tenir un efecte directe sobre la MSPV. Durant el transport es va trobar una correlació significativa entre la MSPV, per una banda, i el sexe dels pollets i la quantitat de pollets carregats per l'altre (P = 0,0082 i P = 0,0087; respectivament). Els pollets mascle van tenir una MSPV major que els pollets femella i com mes gran va ser la carrega de pollets per viatge major va ser la MSPV. La humitat relativa (HR) al vehicle de transport i la duració del viatge van tenir un efecte significatiu sobre la pèrdua de pes dels pollets (P = 0,0006 i P = 0,0188; respectivament). Finalment, els factors propis de la granja que van tenir un efecte significatiu sobre la MSPV van ser l'edat de les reproductores, el sexe i l'estirp dels pollets, el tipus de nau, la presencia o no de recuperador a l'abeurador, els dies d'emmagatzematge dels ous, l'any d'estudi i l'estació (P < 0,05). Es conclou que la MSPV té un marcat caràcter multifactorial i, per tant, cal identificar en cada cas els factors que tenen un efecte sobre la MSPV per implementar mesures correctores i millorar el benestar dels pollets.

Summary

The mortality rate during the first week of life is one of the most widely used indicators to assess animal welfare in poultry production. The general objective of this thesis was to study the main factors contributing to the mortality of broiler chicks during the first week of life (FWM). In the hatchery the FWM was related to the cloacal temperature of the chicks ($P = 0.0132$) and FWM can increase up to 3 times when the cloacal temperature is below the optimal range (<39.7°C). The age of the breeders, the sex of the chicks, the incubation process, the waiting time in the hatchery and the ventilation system in the expedition room all had a significant effect on the cloacal temperature ($P < 0.05$). However, these factors did not have a direct effect on FWM. During transport, a significant correlation was found between FWM on the one hand and the gender of the chicks and the number of chicks loaded on the other hand ($P = 0.0082$ and $P = 0.0087$; respectively). Male chicks had a higher FWM than female chicks and the greater the quantity of chicks loaded per journey the higher the FWM. The relative humidity (RH) in the transport vehicle and the duration of the journey had a significant effect on chick weight loss ($P = 0.0006$ and $P = 0.0188$; respectively). Finally, farm-specific factors that had a significant effect on FWM were breeder age, chick gender, breed, type of house, presence or absence of drinker drip cup, days of egg storage, year of study and season ($P < 0.05$). It is concluded that FWM has a multifactorial nature and it is therefore necessary to identify the factors that have an effect on FWM in order to implement corrective measures and improve the welfare of the chickens.

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"*Creativity is intelligence having fun.*"

A. Einstein

1.1. Introducción

En todo el mundo hay actualmente unos 30.000 millones de animales de producción si se consideran únicamente las 5 especies principales no acuáticas (vacas, cerdos, aves, ovejas y cabras); esto supone aproximadamente 4 animales de producción por cada ser humano. De este total de animales de producción, la inmensa mayoría está constituida por gallinas y pollos de engorde, con un total aproximado de 24.000 millones de individuos (FAO STAT, 2018). Como reflejan los números, la producción global avícola se realiza a escala masiva con decenas de miles de millones de pollos y otras especies de aves de corral criadas cada año.

Dentro de la producción avícola deben diferenciarse dos sectores: el sector de producción de carne y el sector de producción de huevos. En el caso de los pollos de engorde, España es el segundo productor europeo de carne de pollo por detrás del Reino Unido. En el año 2019, según las estadísticas del Ministerio de Agricultura, Pesca y Alimentación, se produjeron 628,4 millones de pollitos destinados a la producción de carne y 37,9 millones de pollitas destinadas a la producción de huevos. Para permitir este aumento de la producción todo el proceso de cría y engorde se ha automatizado e intensificado en gran medida. Paralelamente a todo este crecimiento y desarrollo, en los países industrializados el bienestar animal se ha convertido en uno de los temas más importantes de la ganadería. La preocupación pública por lo que respecta a las condiciones en las que se mantiene a los animales de producción, ha llevado a la necesidad de desarrollar métodos para verificar las normas mínimas de bienestar animal. A modo de ejemplo, esta preocupación queda reflejada en el Eurobarómetro realizado en 2015, según el cual el 94% de los 27.000 ciudadanos encuestados pertenecientes a los estados miembros de la Unión Europea (UE) indicaron que es importante proteger el bienestar de los animales de granja. Este porcentaje varió entre el 86 y 99% dependiendo del país de la UE que se considere (EC, 2015).

1.2. Bienestar animal y su relación con la avicultura

Concepto de bienestar animal

Existen numerosas definiciones de bienestar animal. No obstante, todos los autores coinciden en que el bienestar animal no es sinónimo de salud física o ausencia de lesiones y enfermedades (Laywel, 2006), sino que el bienestar animal incluye, además de la salud física, el estado emocional y el comportamiento de los animales.

El bienestar animal es un atributo del individuo en un momento dado o a lo largo de un período de tiempo. Puede medirse y puede variar desde muy deficiente a muy satisfactorio, y está estrechamente relacionado con la adaptación del animal a su entorno (Broom, 2011). Además, debe tenerse en cuenta que se trata de un concepto multidimensional (Mason y Mendl 1993; Fraser, 1995) que incluye diferentes aspectos relacionados con la salud física, las emociones y el comportamiento (Mellor, 2016). Este aspecto multidimensional aparece recogido en el concepto de las 5 libertades, compilado por primera vez en el informe del Comité de Brambel (1965) y muy conocido en todo el mundo. Estas 5 libertades son las siguientes (FAWC, 1992):

1. Ausencia de sed, hambre y malnutrición.
2. Ausencia de incomodidad física y térmica.
3. Ausencia de dolor, heridas y enfermedades.
4. Ausencia de miedo y distrés.
5. Posibilidad de expresar los patrones de comportamiento normales de la especie.

Las 5 libertades se han utilizado frecuentemente como base de la legislación sobre bienestar animal. Sin embargo, las 5 libertades presentan unas limitaciones que hicieron necesaria su adaptación a los 5 principios de Mellor (Mellor y Reid, 1994). Una de estas limitaciones es que el concepto de las 5 libertades se creó haciendo énfasis en la ausencia de experiencias negativas, sin tener en cuenta la presencia de experiencias positivas. Otra limitación importante es que las 5 libertades están descritas de manera que no son completamente independientes entre sí, sino que existe una superposición entre ellas. Por esto, Mellor (2016) propuso el principio de los 5 dominios argumentando que, si la base de la preocupación por el bienestar de los animales es que éstos son seres sintientes (es decir, capaces de sufrir y de experimentar emociones), el aspecto más relevante del bienestar son precisamente las emociones. Por tanto, este nuevo enfoque se basa en que el bienestar de un animal debe medirse en función del balance entre las emociones positivas y negativas.

Según el principio de los 5 dominios de Mellor, las emociones surgen de los cuatro dominios funcionales (nutrición, ambiente, salud y comportamiento) y pueden ser negativas o positivas (Figura 1.1).

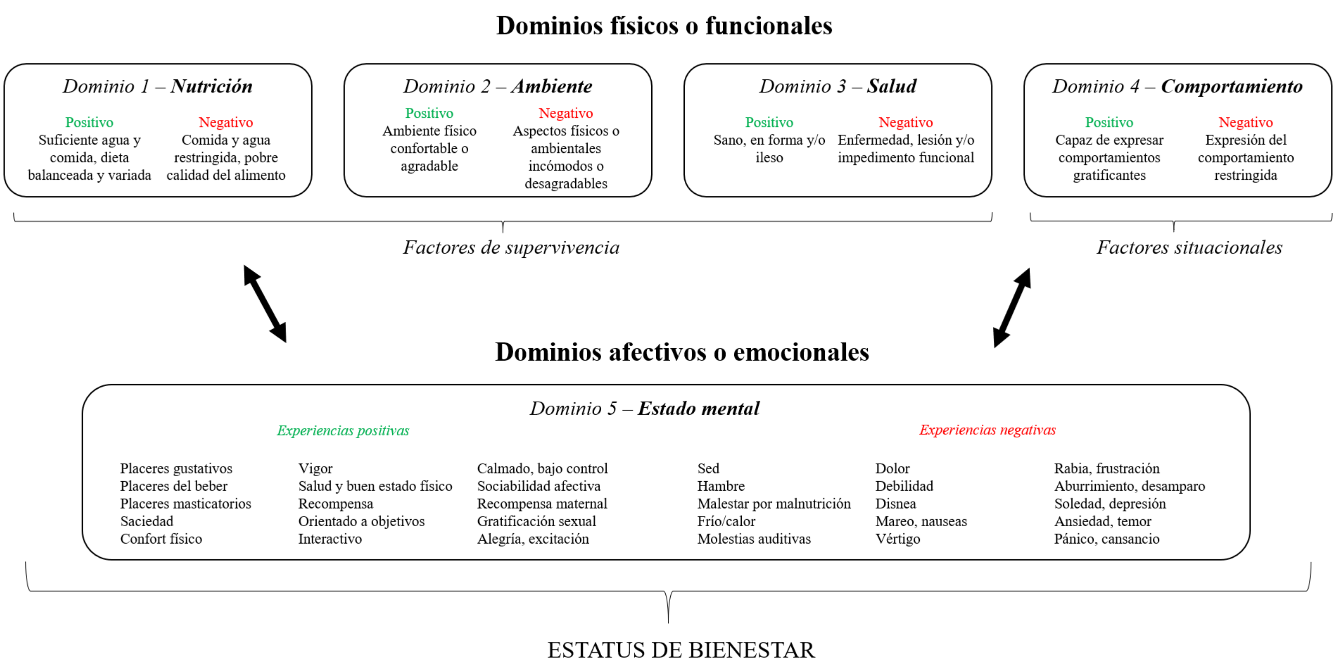

Figura 1.1. Esquema del modelo de los 5 dominios, versión adaptada (Mellor, 2016). Resume los factores de supervivencia y situacionales y su asociación con los dominios físicos o funcionales, y proporciona ejemplos de experiencias positivas y negativas asociadas al dominio mental. La experiencia afectiva general en el ámbito mental equivale al estado de bienestar de los animales.

Bienestar animal contextualizado en la producción

Durante los últimos 10 – 20 años se han desarrollado diversos protocolos de evaluación del bienestar en animales de granja. Los protocolos más sofisticados y utilizados más comúnmente son los elaborados por el proyecto 'Welfare Quality®' (EFSA, 2012). Estos protocolos incluyen 4 principios, que coinciden con los 4 dominios funcionales de Mellor: buena alimentación, buen alojamiento, buena salud y comportamiento apropiado. Cada uno de estos principios incluye diferentes criterios, y dentro de los criterios se describen los diferentes indicadores utilizados para evaluar los principios.

Para evaluar el bienestar animal debe tenerse en cuenta, en primer lugar, que el bienestar es una variable latente, es decir, que no puede medirse de forma directa. En realidad, la mayoría de las variables complejas que resultan de la combinación de diferentes elementos son variables latentes. Esto no significa, ni mucho menos, que no puedan medirse, sino que deben medirse utilizando indicadores, es decir, parámetros que sí que pueden medirse de forma directa y que aportan información sobre el bienestar de los animales. Estos indicadores se clasifican en dos categorías: indicadores basados en el ambiente e indicadores basados en el animal. Muchos de los indicadores basados en el ambiente tienen la ventaja de ser más fáciles de medir que los indicadores basados en el animal. Sin embargo, una determinada característica ambiental puede tener un efecto variable y a menudo impredecible sobre el bienestar de los animales, ya que su efecto dependerá del temperamento de cada individuo y de su interacción con otras variables ambientales. Por esta razón, los indicadores basados en los animales, que proporcionan información directa sobre su bienestar, son generalmente preferibles (Manteca y col. 2016).

Los protocolos de evaluación del bienestar 'Welfare Quality®' presentan una descripción amplia, científicamente sólida y basada en resultados (Blokhuis y col. 2010), cuyos fundamentos conceptuales reflejan las opiniones de las distintas partes interesadas pertenecientes a diversos ámbitos, incluyendo el científico, el científico-social y el público general (Miele y col. 2011). Los protocolos describen un sistema de tres niveles de puntuación, en el que las medidas recogidas en la granja evaluada se incluyen en 12 criterios agrupados en los 4 principios que hemos mencionado antes (Tabla 1.1), obteniendo de esta manera una puntuación para cada uno de ellos. Por último, las puntuaciones obtenidas para cada principio se combinan en una clasificación general de bienestar para cada explotación (No clasificada, Aceptable, Mejorada o Excelente).

Tabla 1.1. Principios de bienestar y criterios identificados en el protocolo Welfare Quality®.

Principios	Criterio de bienestar	Indicadores (ejemplos)
Buena alimentación	1. Ausencia de hambre prolongada	Este criterio se mide en matadero
	2. Ausencia de sed prolongada	Revisión de abastecimiento de agua
Buen alojamiento	3. Confort durante el descanso	Limpieza plumaje, calidad yacija, test del polvo
	4. Confort térmico	Jadeo, animales acurrucados
	5. Facilidad de movimiento	Densidad en granja
Buena salud	6. Ausencia de lesiones	Cojeras; dermatitis de contacto en la pechuga y el corvejón; pododermatitis
	7. Ausencia de enfermedad	Mortalidad en granja, descartes en granja
	8. Ausencia de dolor inducido por procedimientos de manejo	En esta situación este criterio no se aplica
Comportamiento apropiado	9. Expresión de comportamientos sociales	No hay medidas desarrolladas para evaluar este criterio
	10. Expresión de otros comportamientos	Acceso al exterior.
	11. Buena relación humano-animal	Test de evitación de distancia
	12. Estado emocional positivo	Evaluación cualitativa del comportamiento (QBA)

Los protocolos Welfare Quality se utilizan con fines legislativos, para la certificación voluntaria, para la gestión de las granjas o para uso en investigación (Botreau y col. 2009)

Bienestar animal en la producción avícola

En general, la producción avícola se cuenta entre las formas más intensivas de producción animal y muchas de las condiciones y procedimientos a los que se ven expuestos los pollos y gallinas a lo largo de su vida comprometen su bienestar.

La mortalidad es uno de los indicadores más utilizados y fáciles de medir y se acepta que los sistemas de producción o transporte que resultan en mortalidades elevadas no son adecuados desde el punto de vista del bienestar de los animales. Los daños físicos, como las heridas producidas por el canibalismo, las fracturas óseas y las enfermedades, pueden evaluarse fácilmente mediante sistemas de puntuación y diagnósticos veterinarios establecidos, y está generalmente aceptado que estas condiciones perjudican el bienestar de los animales (Bessei, 2018).

Sin embargo, los criterios fisiológicos y etológicos son más difíciles de medir y su relación con el bienestar no resulta siempre fácil de determinar. Así, por ejemplo, el estrés como respuesta fisiológica a los estímulos ambientales se menciona con frecuencia en el contexto del bienestar (Rushen, 1991). La liberación de corticosterona y epinefrina, y el cambio en la proporción de linfocitos/heterófilos (Maxwell, 1993) se utilizan generalmente como indicadores de estrés. La validez de estos criterios como indicadores de un bienestar deficiente ha quedado demostrada en condiciones ambientales extremas y en situaciones experimentales. No obstante, su aplicación en condiciones comerciales (en producción) es difícil y no siempre puede relacionarse claramente con el estado de bienestar (Moe y col. 2010).

Los cambios de comportamiento también se utilizan para evaluar el bienestar de los animales (Figura 1.2.). Los comportamientos anormales y/o lesivos para otros individuos (tales como las estereotipias, la agresividad, el picaje y el canibalismo, por ejemplo) indican una falta de bienestar, es decir, son indicadores negativos de bienestar. Por otra parte, se asume que un estado emocional positivo resulta en la expresión de conductas de confort, que indicarían por lo tanto un bienestar satisfactorio. En la práctica, sin embargo, estos indicadores positivos de bienestar se utilizan menos que los indicadores negativos comentados anteriormente (Bessei, 2018).

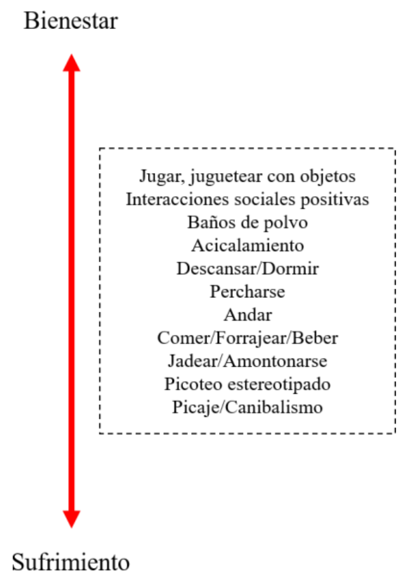

Figura 1.2. Adaptación de Bessei, 2018. Clasificación sugerida para los distintos comportamientos en avicultura en una escala de bienestar que va del sufrimiento total al bienestar total.

1.3. Relación entre el bienestar animal y los pollitos de engorde

Importancia del pollito y su supervivencia

Los pollos y el resto de las aves de producción son especies precociales, es decir, capaces de desplazarse y alimentase de forma autónoma desde el momento del nacimiento (Widowski y Torrey, 2017). Sin embargo, durante las primeras semanas de vida los pollitos requieren una atención y cuidados específicos. Además, si consideramos el periodo de vida completo de estos animales en términos productivos, la primera semana de vida en los sistemas de producción actuales representa entre el 16 y el 20% del total de su vida productiva. Por tanto, se trata de una etapa nada desdeñable en la cual los pollitos se encuentran en una fase de transición en la que estarán expuestos a multitud de novedades y desafíos. A todo esto, se añade que, en un periodo muy breve de tiempo, los pollitos pasarán de unas condiciones muy concretas en la planta de incubación a otras muy distintas y menos controladas en la granja (Yassin y col. 2009).

Durante esta etapa de transición, muchos aspectos de la fisiología del pollito no están plenamente desarrollados. Uno de estos aspectos es la termorregulación. En efecto, al nacer los pollitos tiene un sistema de termorregulación inmaduro que no será completamente eficiente hasta los 7-10 días de vida (Tzschentke y Nichelmann, 1999). Por tanto, el control post nacimiento de la temperatura ambiental será un punto crítico en el manejo de los animales (Shinder y col. 2007), con el objetivo de evitar estrés térmico. De hecho, en los estándares de bienestar de la Royal Society for the Prevention of Cruelty to Animals para los pollos de carne se recomienda que los pollos estén constantemente monitorizados con el propósito de prevenir estrés por calor y estrés por frío. El estrés térmico puede afectar de manera negativa tanto el bienestar como el rendimiento de las aves (Akşit y col. 2010; Baracho y col. 2010), provocando patologías e incluso la muerte (Deaton y col. 1996; Blahová y col. 2007). La mortalidad durante la primera semana de vida en los pollos de engorde es un importante criterio de producción ampliamente utilizado por el sector avícola. Además, es uno de los indicadores de bienestar animal contemplado en la Directiva Europea 2007/43/EC que tiene como objetivo mejorar el bienestar de los pollos de engorde.

Durante la primera semana de vida de los pollitos existen tres fases: la incubación, el transporte y la primera semana de adaptación en la granja de engorde (Figura 1.3). En cada una de estas fases, los pollitos estarán expuestos a múltiples factores externos de manejo y ambientales, que podrán disminuir su bienestar. Sin embargo, es importante recordar que existen diversos factores internos como la genética (estirpe), el sexo de los pollitos y la edad de las reproductoras que indirectamente juegan un papel importante en dicho bienestar (López y col. 2011; Bergoug y col. 2013).

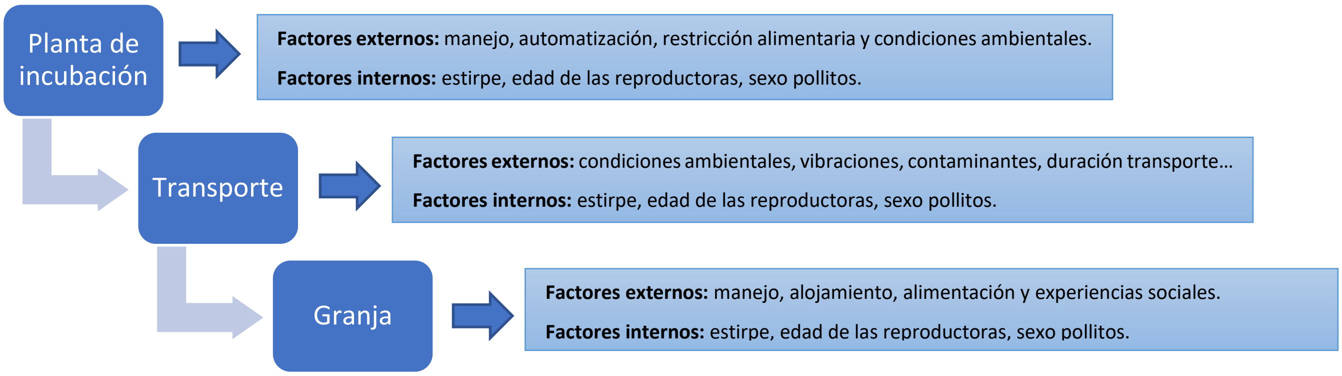

Figura 1.3. Esquema de las fases críticas y factores que pueden influir en el bienestar durante la primera semana de vida de los pollos de engorde.

Efecto del manejo y de los factores ambientales de las plantas de incubación en el bienestar de los pollos de engorde

Las plantas de incubación comerciales tienen la mayor concentración de aves de todas las unidades de producción avícola. A diferencia de las granjas de engorde, existe un número reducido de plantas de incubación y, a pesar de que los pollos pasan un período relativamente corto en ellas, los procedimientos y prácticas de manejo llevados a cabo en la planta de incubación pueden tener un importante impacto (positivo o negativo) en el bienestar de las aves a corto y largo plazo (Schwean-Lardner, 2017). Por ejemplo, los estudios de Heier y col. (2002) y Yassin y col. (2009) detectaron que la tasa de mortalidad durante la primera semana de vida se veía afectada por la planta de incubación de la que provenían los pollos.

El periodo de incubación puede resultar muy estresante ya que los pollitos están expuestos a cambios de temperatura y humedad, así como a ruidos, movimientos, varias prácticas de manejo, etc. Las incubadoras de pollo de engorde actuales suelen estar muy automatizadas y el manejo de los pollitos comienza con equipos que desapilan las cestas de nacimiento y las colocan en el separador de huevos, que es un equipo encargado de separar los pollitos vivos de los huevos no eclosionados y los restos de cáscara. Posteriormente, los pollitos son transportados mediante cintas automáticas a lo largo de toda la planta de nacimiento para ser sexados, triados y vacunados. Finalmente, los pollitos llegan a una contadora y a un apilador de cajas de expedición. Todo este proceso puede generar tanto lesiones como estrés físico y psicológico en los pollitos (Knowles y col. 2004).

A todas estas prácticas de manejo se une la restricción de alimentos y agua a la que son sometidos los pollitos recién nacidos. En efecto, tradicionalmente los pollitos no reciben ni comida ni agua hasta su llegada a la granja (pasadas 24 – 72h), de modo que el pollito utiliza el saco vitelino como fuente de nutrición (Nielsen y col. 2011). Sin embargo, recientemente se han desarrollado sistemas que evitan que los animales neonatos pasen tantas horas sin acceso a agua y comida. Por ejemplo, se han creado equipos dispensadores de suplementos alimenticios que permiten a los pollitos obtener una pequeña fuente de nutrientes y agua antes de pasar a las cajas de expedición (Figura 1.4). Otro sistema que también se utiliza actualmente consiste en que los pollitos recién nacidos caen a una cesta inferior donde tienen acceso directo a la comida y al agua (Figura 1.5).

Figura 1.4. Cevagel® producto para suplementar con nutrientes. Este producto permite a los pollitos obtener durante las primeras horas de vida en la incubadora una fuente de nutrientes e hidratación.
Fuente: Cortesía Ceva Salud Animal.

Figura 1.5. Equipo HatchCare®, que permite a los pollitos tener acceso a agua y pienso inmediatamente después de nacer. Los pollitos nacen en una bandeja superior, y después caen a una bandeja inferior, donde el pienso y el agua están disponibles para los pollitos.
Fuente: Cortesía de Hatchtech®.

No obstante, los estudios llevados a cabo hasta el momento no han mostrado diferencias en la concentración plasmática de corticosteroides entre pollitos con y sin acceso a comida y agua. Esto sugeriría que la falta de agua y comida inmediatamente después del nacimiento no es estresante, pero ciertamente son necesarios más estudios para determinar si los pollitos recién nacidos que no tienen acceso al agua y a la comida sufren hambre o sed (Gonzales y col. 2003; Tong y col. 2015).

Finalmente, otro aspecto acerca del que hay poca información pero que podría tener un importante impacto sobre el bienestar de los pollitos son las condiciones ambientales en las incubadoras. Algunos estudios sugieren que tanto las temperaturas muy altas como las muy bajas durante la cría pueden disminuir la productividad y aumentar la mortalidad de los pollos de engorde (Careghi y col. 2005; Bergoug y col. 2013; Piestun y col. 2017). Sin embargo, existen en general pocos estudios que permitan determinar de forma clara el efecto de las variables ambientales en la incubadora sobre el bienestar y rendimiento productivo de los pollos, especialmente en condiciones comerciales.

Efecto del transporte del pollito de un día de vida en el bienestar de los pollos de engorde

Las plantas de incubación son frecuentemente responsables del transporte de los pollitos a las granjas de engorde. Aunque hay pocos estudios acerca del bienestar de los pollitos durante el transporte, existen muchas evidencias que demuestran que el transporte es una fase crítica para el bienestar de los animales (Mitchell 2009) debido a la gran variedad de factores potencialmente estresantes que actúan sobre estos. Entre dichos factores destacan los cambios de temperatura. las aceleraciones, las vibraciones, el ruido, y la falta de espacio, entre otros (Mitchell and Kettlewell 1993; Mitchell 2009). Se ha demostrado que el transporte de los pollitos desde la planta de incubación hasta la granja tiene un efecto importante en el posterior desempeño de los pollos (Mitchell 2009; Jacobs y col. 2016; Valros y col. 2008).

Los vehículos utilizados para el transporte de los pollitos suelen ser caros y deberían disponer de sistemas de climatización del ambiente, así como de sensores de temperatura, humedad relativa y gases para el control ambiental en el interior del remolque. Sin embargo, a pesar de los esfuerzos para mejorar este proceso, los pollitos siguen transportándose durante largos períodos de tiempo bajo condiciones ambientales subóptimas (Bergoug y col. 2013; Jacobs y col. 2016). Por ejemplo, Nazareno y col. (2015, 2016) detectó una considerable variabilidad de la temperatura y la humedad relativa dentro de la zona de carga de los camiones y ambos parámetros se encontraban a menudo fuera de los rangos recomendados. Dichos rangos varían según los autores, Meijerhof (1997) y Weeks y Nicol (2000) sugirieron un rango de temperatura ambiental de 24 – 26ºC, mientras que Marques (1994) recomendó temperaturas entre 22 – 31ºC y una humedad relativa del 50%. Nazareno y col. (2016) detectó que la temperatura media dentro de la zona de carga era de 28ºC y la humedad relativa de 40,5%.

Además de las variables ambientales, existen otros factores involucrados en el transporte que parecen tener múltiples efectos sobre los pollitos. Uno de estos factores es la distancia hasta la granja o duración del trayecto. Según Bergoug y col. (2013), los transportes tanto de 4 como de 10 horas reducen el peso de los pollitos en comparación con aquéllos que no son transportados. Valros y col. (2008) intentaron examinar una serie de indicadores de bienestar en transportes largos (14 horas) versus cortos (4 horas). Sus resultados no fueron consistentes, pero permitieron describir cambios en el comportamiento de las pollitas una vez en la granja que dependían de la duración del transporte. Concretamente, las pollitas que habían sido transportadas durante períodos largos de tiempo utilizaron antes las perchas y tardaron más en comer que las pollitas que habían sido transportadas durante períodos cortos, lo que puede sugerir que las primeras estaban más estresadas o teína más miedo que las segundas.

En resumen, muchos aspectos del transporte han sido poco estudiados, incluyendo cuáles son los parámetros ambientales adecuados para el transporte de pollitos de un día de vida y el efecto de dichos parámetros sobre el bienestar de los pollitos.

Efecto de las condiciones de alojamiento y otros factores durante la primera semana de vida sobre en la adaptabilidad y el bienestar de los pollos de engorde

El periodo de cría es crítico para el comportamiento y el desarrollo fisiológico de las aves. Después de someterse a múltiples procedimientos en la incubadora, los pollitos son transportados e instalados en un ambiente nuevo con otros individuos de su edad, y deben aprender a comer, beber y desarrollar un comportamiento social adecuado en grupos de cientos o miles de animales. A menudo se presta poca atención a este período de la vida de las aves, aunque los factores relacionados con el manejo, el alojamiento, la alimentación y las experiencias sociales pueden tener consecuencias significativas en el comportamiento adulto de las aves, la fisiología del estrés y el desarrollo neuromuscular, e incluso pueden tener efectos en la progenie (Widowski y Torrey, 2017).

En las prácticas ganaderas actuales, los pollitos se separan de sus madres y deben aprender a comer y beber junto a congéneres de su misma edad. Como resultado de ello, los pollitos no pueden aprender las pautas normales de comportamiento de alimentación y pueden sufrir inanición y deshidratación durante la primera semana de vida, disminuyendo las probabilidades de supervivencia durante este periodo. Por esta razón, en las últimas décadas se ha llevado a cabo una importante labor para mejorar las especificaciones de los nutrientes a fin de contribuir al desarrollo temprano de los animales (Noy y Uni, 2010; Sklan, 2001). Sin embargo, sigue existiendo el desafío de conseguir que las aves coman (particularmente durante los dos primeros días de vida).

Además de este desafío, otro aspecto importante está relacionado con el comportamiento social durante este período de transición. En condiciones naturales o seminaturales, los pollos viven en grupos sociales definidos. Durante la cría, los pollos jóvenes se agrupan con sus madres y otras gallinas adultas, y utilizan estímulos auditivos y visuales para aprender el comportamiento social de sus madres y congéneres (Wood-Gush, 1955). No obstante, las condiciones actuales de alojamiento difieren significativamente de las condiciones naturales o seminaturales, ya que los pollos de engorde se suelen criar en grupos de un solo sexo con cientos o miles de individuos de la misma edad.

Existe muy poca información sobre el efecto de las experiencias sociales tempranas en aves y su posterior capacidad de adaptación. Sabemos, por ejemplo, que los pollos prefieren estar más cerca de las aves con las que se han criado (Lindberg y Nicol, 1996). Algunos estudios (Heier y col. 2002; Chou y col. 2004) han concluido que un mayor tamaño de lote durante la primera semana de vida podría ser beneficioso, reduciendo la mortalidad. Según Heier y col. (2002), esto podría estar relacionado con una menor perdida de calor, disminuyendo así el estrés por frío, y con una mayor probabilidad de encontrar fácilmente las fuentes de alimento y bebida.

En resumen, existe poca información acerca de cómo los distintos factores relacionados con el manejo, el alojamiento, la alimentación y las experiencias sociales durante la primera semana de vida afectan a la supervivencia y bienestar de los pollos de engorde.

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Capítulo 2
Objetivos

"Our greatest weakness lies in giving up. The most certain way to succeed is always to try just one more time."

Thomas A. Edison

La presente tesis doctoral trata de dar respuesta a un problema del sector avícola pocas veces estudiado de forma global y normalmente abordado desde un enfoque meramente productivo. La mortalidad durante la primera semana de vida de los pollos de engorde es un problema que ha existido siempre. Sin embargo, en los últimos años ha aumentado el interés por este problema debido a su importancia y su relación con el bienestar animal. En este contexto, la Facultad de Veterinaria de la Universidad Autónoma de Barcelona, la empresa Ceva Salud Animal y el grupo Sada plantearon en el año 2016 abordar el tema conjuntamente para mejorar el bienestar de las aves y disminuir la tasa de mortalidad acumulada durante la primera semana de vida.

Todos los estudios realizados en el transcurso de la presente tesis doctoral se han llevado a cabo bajo condiciones comerciales de producción y se han utilizado estirpes de animales que son representativas de las condiciones de producción españolas. Con objeto de proponer medidas que pudieran ser aplicadas en la práctica.

La revisión bibliográfica realizada en el capítulo anterior ha puesto de manifiesto el carácter multifactorial de la mortalidad acumulada durante la primera semana de vida. No obstante, la mayoría de los estudios realizados hasta la fecha han considerado únicamente el posible efecto de las condiciones en la granja, obviando la posible influencia de las condiciones de transporte y de la fase de incubación.

En este contexto, el objetivo general de la presente tesis doctoral ha sido estudiar los principales factores que contribuyen a aumentar la mortalidad de los pollitos de engorde durante la primera semana de vida, en cada una de las tres fases críticas de este periodo (planta de incubación, transporte y condiciones en granja), así como proponer algunas medidas de carácter práctico que permitan reducir dicha mortalidad y mejorar el bienestar de los pollitos.

Para poder dar respuesta a este objetivo general, se formularon 5 objetivos específicos:

1. Identificar los factores en la planta de incubación que aumentan el riesgo de estrés térmico en los pollitos de un día de vida (Capítulo 3).
2. Determinar si existe una relación entre el estrés térmico de los pollitos de un día de vida y la mortalidad acumulada a siete días de vida (Capítulo 3).
3. Estudiar el efecto del transporte de los pollitos de un día de vida sobre la mortalidad y la pérdida de peso (Capítulo 4)
4. Evaluar las condiciones ambientales del transporte que han tenido un efecto sobre la mortalidad y la pérdida de peso de los pollitos durante el transporte (Capítulo 4).
5. Identificar mediante una metodología basada en el análisis de riesgo, los factores internos (propios de los individuos) y externos (relacionados con el manejo y el ambiente) asociados con la mortalidad acumulada durante la primera semana de vida de los pollos de engorde, y cuantificar el riesgo atribuible a cada factor (Capítulo 5).

Para la consecución de los cinco objetivos anteriores, se llevaron a cabo dos pruebas experimentales en condiciones comerciales y un estudio observacional que serán objeto de un análisis en profundidad en los siguientes capítulos.

Capítulo 3

Thermal stress in day-old chicks: risk factors and effects on mortality

"A comfort zone is a beautiful place, but nothing ever grows there."

Unknow

This chapter is based on following manuscript submitted to *Animal Welfare* and currently under review:

Thermal stress in day-old chicks: risk factors and effects on mortality

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3.1. Abstract

The aim of this study was to identify risk factors for thermal stress in the hatchery under commercial conditions, and to determine their relationship with chick mortality during the first week of life. Risk factors were classified as pre-hatch and post-hatch factors. Cloacal temperatures of day-old chicks were sampled at three points in the hatchery: immediately after hatching, before their placement in the expedition room and in the expedition room. Chicks' cloacal temperature was significantly affected by breeder age, chick gender, incubation process, waiting time until the start of sampling, sampling time and whether the ventilation system was on or off at sampling. It was observed that, as time passed since hatching, pre-hatch factors had less influence on cloacal temperature, whereas post-hatch factors became more significant. Furthermore, cloacal temperatures below the optimum range could increase first-week mortality by up to three times. These results suggest that it is important to consider post-hatch factors and to avoid cold stress in commercial conditions, as these may decrease chick welfare and increase first week mortality.

3.2. Introduction

Thermal stress has a major effect on welfare, performance and mortality in broiler chickens (Nawab et al 2018). The effects of thermal stress are even more important in day-old chicks, as their thermoregulatory system is not completely mature until they are 10 days old (Tzschentke & Nichelmann, 1999). One indicator of thermal stress in day-old chicks is cloacal temperature (Abreu et al 2017; Nascimento et al 2012), which should fall between 39.7 and 40.3ºC (Malheiros et al 2000). Therefore, the control of the post-hatch environment temperature is vital in the management of neonatal chicks (Shinder et al 2007). For example, the welfare standards for meat chicken of the Royal Society for the Prevention of Cruelty to Animals (RSPCA), recommend that chicks are continuously monitored to prevent heat (HS) and cold stress (CS). Similarly, the standards of the World Organization for Animal Health (OIE) recommend avoiding extreme heat, humidity and cold (Stevenson et al 2014).

Several studies have described the negative effects of thermal stress on welfare and performance of day-old chicks (Baarendse et al 2006; Akşit et al 2010; Baracho et al 2010). For example, Zhang et al (2011) found that CS negatively influenced the intestinal system in broiler chickens. Also, CS increased the likelihood of cold ascites and mortality, reduced feed conversion efficiency, increased energy requirements (Deaton et al 1996; Blahova et al 2007); and decreased weight gain at 21 days (Deaton et al 1996). Similarly, HS reduces both performance and welfare of chicks (Baziz et al 1996; Yahav et al 1996; Hadad et al 2014). Hulet et al (2007) found that chicks incubated at high temperatures (shell temperature 39.7ºC) had significantly lower body weight and poorer feed conversion at 21 days of age compared with chicks incubated at middle temperatures (shell temperature 38.6ºC). Furthermore, several studies have demonstrated that embryos frequently become overheated during the incubation process, even when the incubator is operating correctly (Meijerhof & Van Beek 1993; Hulet 2007) and as a result chicks suffer HS and are more likely to suffer dehydration (Bergoug et al 2013; Piestun et al 2017) and weight loss (Careghi et al 2005).

The factors that can contribute to thermal discomfort in chicks can be divided into pre-hatching and post-hatching factors. The most important pre-hatching factors are gender, breed, breeder age and egg storage (Tona et al 2005; López et al 2011; Bergoug et al 2013). The most important post-hatching factors are the hatcher environment, the hatch window - i.e.. time interval between the hatching of the first and last chick - (Meijerhof 2009), environmental temperatures, air flow, stocking density, feed restriction and the vaccination process (Nääs et al 2014).

Most of the studies carried out have been implemented under experimental conditions. Therefore, there is not much information available under commercial conditions that verify how the environment and the mentioned factors during the first hours of life could influence the thermal stress of the chicks and its consequences. For this reason, this study was carried out with two objectives: (1) to identify the factors that increase the risk of thermal stress in day-old chicks between hatching and expedition and (2) to ascertain if there is a relationship between thermal stress in day-old chicks and mortality at seven days of age.

3.3. Material and methods

This study consisted of two trials. Trial one was divided into two phases and trial two had one phase. The project was carried out in a poultry commercial hatchery located in Zaragoza, Spain. In both trials, chicks (*Gallus gallus domesticus*) of Ross and Cobb breed and of both genders were used. The same digital thermometer was used for all the sampling (Indiglo® Timex) and was inserted 2 cm into the colon to obtain the cloacal temperature measurements.

Trial 1 - Evaluating cloacal temperatures in different phases in the hatchery room

Trial one was carried out in May 2016. Flocks composed of eight trolleys and with waiting times in the expedition room of at least 2 hours were included in the trial. Each flock was assessed twice: immediately after hatching and in the expedition room. The information referring to the flocks was recorded as independent variables : flock ID, breed, breeder age (weeks), number of hatcher, trolley position inside the hatcher (1 or 5), hatcher basket position inside the trolley (upper, middle and bottom), departure time of the hatcher (00:00), sampling date (xx/xx/xxxx), sampling hour (00:00), sampling time (t0, t1, t2), waiting time until the start of sampling (in minutes) and expedition box position inside the trolley (upper, middle and bottom).

Phase 1 - Evaluating cloacal temperature of chicks immediately after hatching

Sampling was performed over six days. Immediately after the trolleys were removed from the hatchers, two hatchers and two flocks were selected on each sampling day. For each hatcher, two trolleys from the same flock and hatcher position were sampled: position one and five (Figure 3.1). The hatcher baskets to be sampled were taken from three different heights of the trolley: upper, middle and bottom (Figure 3.2), always discarding the two upper and the two lower baskets of the trolley. For each hatcher basket, the cloacal temperature of 12 randomly chosen chicks was measured. A total of 12 flocks, 24 trolleys and 856 chicks were assessed.

Phase 2 - Evaluating cloacal temperature of chicks in the expedition room

In the expedition room, a subsample from each of the 12 flocks previously assessed in the hatching room was randomly selected. Each sampling day, two flocks that included one trolley per flock were selected. The chick boxes to be sampled were selected following the same procedure as in Phase 1.

The cloacal temperatures were measured at three different sampling times: t_0 = moment of entry to the expedition room, t_1 = chicks in the expedition room after one hour, t_2 = chicks in the expedition room after two hours. In addition, the temperature (ºC) of the expedition room and whether the ventilation system was on or off at the moment of sampling was registered. For each expedition box, the cloacal temperature of 12 randomly chosen chicks was measured. Finally, a total of 12 flocks, 12 trolleys and 1296 chicks were assessed.

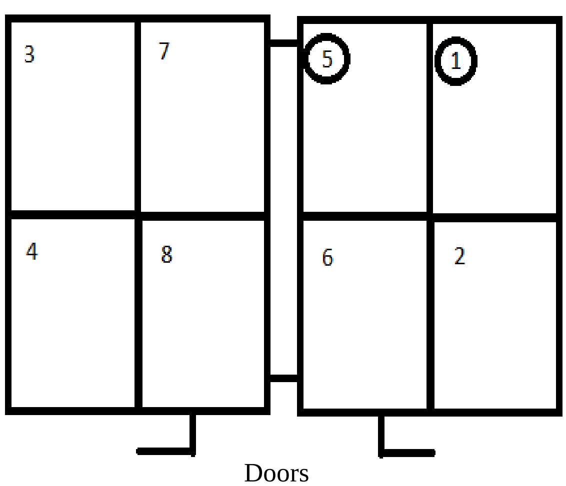

Figure 3.1. Outline of a hatcher specifying the different positions of the trolleys. The position of trolleys 1 and 5 is marked with a circle.

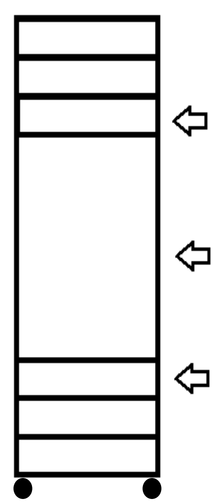

Figure 3.2. Drawing of the positions of the hatcher baskets. The baskets marked with an arrow are from which samples are taken. The 2 upper boxes and the 2 lower boxes are discarded in the sampling.

Trial 2 – Cloacal temperature in the hatchery and its relationship with mortality at seven days

Trial two was carried out between January and June 2017. Only chicks placed on farms coming exclusively from one flock were included in the trial. From each flock the information referring to the chicks was recorded and considered as independent variables: flock ID, breed, breeder age (weeks), egg storage (days), number of hatcher, departure time of hatcher (00:00), sampling date (xx/xx/xxxx), sampling hour (00:00), waiting time until the start of sampling (in minutes) and gender.

Phase 1 – Evaluating cloacal temperature of chicks before their placement in the expedition room

Sampling was carried out over six months. The boxes to be sampled were randomly chosen and the total number of chicks sampled per box was 20. A total of 5117 chicks distributed in 10 flocks and 14 farms were evaluated. The measurement of cloacal temperature was made at the end of the conveyor belt (after gender determination and vaccination) and before their placement in the expedition trolleys. After taking the cloacal temperature of the chicks, the sampled animals were divided into three boxes and marked with three different colors (red, black and blue) depending on the cloacal temperature range they presented. Each colour was assigned to a temperature range. The individuals marked with black were those that presented a temperature within the optimal range (39.7 - 40.3ºC). Those marked in red and blue, respectively, were individuals with temperatures above (> 40.3ºC) and below (< 39.7ºC) the range considered optimal. The dyes used for sampling were of food origin, in powder form, in 3 g jars and typically used to make decorative edible paint (Rainbow Dust®). The area selected to mark the individuals was the head, since it is one of the regions in which the natal down is maintained for a longer time and is the last areas in which the process of moulting begins (Leeson & Walsh 2004).

Finally, daily mortality was recorded on farm during the first seven days of life, according to the colour of the chicks and their gender (total deaths marked in blue, total deaths marked in red, total deaths marked in black and total deaths not marked).

Statistical analysis

Statistical analysis was carried out using the statistical software SAS v9.4, (SAS Institute Inc., Cary, NC, USA) for Windows. The significance level was set at 0.05. The study unit was the flock, and cloacal temperatures of each flock were considered repeated measures. Cloacal temperature data and their residuals were normally distributed. The categorization of the cloacal temperature was performed in the following blocks: < 39.7ºC, 39.7 - 40.3ºC (optimal range) and > 40.3ºC. The explanatory variable breeder age was categorized too; the blocks were created according to the Cobb guide (2008): ≤ 33 weeks (young breeders), 34 - 50 weeks (prime breeders) and ≥ 51 weeks (old breeders). A bivariate linear regression analysis was carried out to examine the relationship of the explanatory variables collected and the response variables. The correlations study was carried out using the Spearman correlation coefficient. Also, the study of interactions was made between hatcher basket position and trolley position. A mixed model was used because the data contained fixed effects, random effects and repeated measures inside the same statistical unit. The p-values were adjusted by multiplicity of contrasts with the Tukey correction. In the case of mortality data, the data was considered to have a binomial distribution (total of death chicks/total of marked death chicks), and a proportions study was made (OR).

3.4. Results

Trial 1 - Evaluating cloacal temperatures in different phases in the hatchery room

Phase 1 - Evaluating cloacal temperature of chicks immediately after hatching

The mean cloacal temperature of the 864 chicks sampled was 40.31ºC (std = 0.76), which falls within the optimal temperature range (39.7 to 40.3ºC). Categorized breeder age and hatcher basket position were statistically related to cloacal temperature in broiler chickens (P = 0.0249 and P = 0.0015, respectively). The differences in the cloacal temperature between hatcher basket position inside the trolley were significant for upper and bottom position (t = - 3.62; P = 0.0019), and there were also significant differences between the middle and bottom position (t = - 2.90; P = 0.0147). There were no statistically significant differences between the trolleys for the same hatcher basket position (P = 0.1341; P = 0.8871; P = 0.9579). The mean cloacal temperature was higher in the lower hatcher basket inside the trolley, and the lowest mean cloacal temperature was detected in the upper hatcher basket. In the case of breeder age, there were significant differences between chicks from young breeders (≤ 33 weeks) and chicks from prime breeders (34 - 50 weeks), (t = 3.43; P = 0.0216). Chicks from old breeders did not show significant differences when cloacal temperatures were compared with chicks from young and prime breeders (t = 1.15; P = 0.5148 and t = 1.44; P =0.3665; respectively). Chicks from prime breeders were those that showed a higher mean cloacal temperature (40.84ºC vs 40.07ºC and 40.33ºC).

Phase 2 - Evaluating cloacal temperature of chicks in the expedition room

The mean cloacal temperature of the 1296 chicks sampled was 39.73ºC (std = 0.88), which falls within the optimal temperature range (39.7 to 40.3ºC). Sampling time (h) and ventilation system being on or off during sampling were statistically related to cloacal temperature in broiler chickens (P < 0.0001 and P = 0.0008, respectively). There were differences between the three sample times: t_0 vs t_1 (t = - 7.97; P < 0.0001); t_0 vs t_2 (t = - 11.76; P < 0.0001) and t_1 vs t_2 (t = - 3.97; P= 0.0004). Cloacal temperatures increased significantly over time. In addition, differences were detected between cloacal temperatures depending on whether the ventilation system was on or off during sampling (t = 3.47; P = 0.0008). When the ventilation system was on, the cloacal temperature of the chicks was higher (39.85ºC), whereas when the ventilation system was off, the cloacal temperature of the chicks was lower (39.65ºC). However, the cloacal temperature and ambient temperature of the expedition room were not correlated (r = - 0.27; P = 0.6683).

Trial 2 – Cloacal temperature in the hatchery and its relationship with mortality at seven days

Phase 1 – Evaluating cloacal temperature of chicks before their placement in the expedition room

The mean cloacal temperature of the 5116 chicks sampled was 40.77ºC (std = 0.52), and it was above the optimal temperature range (39.7 to 40.3ºC). Categorized breeder age, chick gender and waiting time were statistically related to cloacal temperature in broiler chickens (P < 0.0001, P < 0.0001 and P = 0.0001, respectively). Waiting time has a significant effect (t = 3.86, P = 0.0001). However, the correlation between the variables was weak (r = 0.03075). As for gender, there were statistically significant differences between male and female chicks (t = - 7.48; P < 0.0001). The results show that the mean cloacal temperature of the females was significantly higher than that of males (40.76ºC vs 40.63ºC). Regarding breeder age, there were statistically significant differences between chicks from prime breeders (34 - 50 weeks) and chicks from young breeders (≤ 33 weeks), (t = 18.59; P < 0.0001). Chicks from prime breeders were those that showed a higher mean cloacal temperature (40.88ºC vs 40.50ºC).

Mortality

Chick mortality at seven days was statistically related only with the categorized cloacal temperature in broiler chickens (P = 0.0132). Table 3.1 shows the estimated proportions and confidence interval for chick mortality at seven days for all the parameters considered in the analysis. The differences of mortality proportions between the groups above and below the optimal temperature range (> 40.3ºC and < 39.7ºC, respectively) were statistically significant (t = 3.15; P = 0.0096). As shown in Table 3.1, chicks that had a cloacal temperature below the optimal temperature range (< 39.7ºC) in the hatchery were three times more likely to die than chicks that had a cloacal temperature above the optimal temperature range (> 40.3ºC).

Table 3.1. Estimated proportion and confidence intervals for first week mortality for the significant parameters: breed, gender, breeder age and cloacal temperature (10 flocks, 5,116 chicks; Spain 2017).

	Parameter		Estimated proportion	CI $_{95\%}$	
Trial 2	Breed	Ross	2.34%	1.37%	3.95%
		Cobb	1.47%	0.58%	3.66%
	Gender	Male	2.01%	1.07%	3.72%
		Female	1.71%	0.79%	3.64%
	Categorized breeder age (weeks)	≤ 33	1.80%	0.75%	4.27%
		34 - 50	1.91%	1.07%	3.38%
	Categorized cloacal temperature (ºC)	< 39.7	4.19%	1.69%	10.03%
		39.7 - 40.3	1.32%	0.62%	2.75%
		> 40.3	1.14%	0.65%	2.01%

3.5. Discussion

Risks factors can induce thermal stress

There were pre-hatch and post-hatch factors that influenced the cloacal temperature. In the case of cloacal temperature measured immediately after hatching, two significant pre-hatch factors were found: breeder age and hatcher basket position inside the trolley. Regarding cloacal temperature before the placement of the chicks in the expedition room, two significant pre-hatch factors were found: breeder age and chick gender, and one post-hatch factor, named waiting time previous to the sampling. Finally, in the case of cloacal temperature in the expedition room, two significant post-hatch factors were found: sampling time (t0, t1, t2) and whether the ventilation system was on or off during sampling.

As the results show, a few hours after hatching, the pre-hatch factors become less important for cloacal temperature, whereas the post-hatch factors became more significant.

Pre-hatch factors

Breeder age

Chicks from prime breeders showed a higher mean cloacal temperature compared with old and young breeders. These differences detected between chicks from different breeder ages could be related with thermoregulatory capacity, hatchability and chick weight. In fact, the results coincide with the findings of Weytjens et al (1999), who found differences in the cloacal temperature and T_3 (triiodothyronine) values, between chicks from old breeders (65 weeks) and chicks from young breeders (25 weeks). As T_3) is a hormone involved in the thermoregulatory process, these authors discussed the possibility of a functional difference in thermoregulation between chicks originating from breeders of different ages. Also, Yalçin et al (2008) detected a significant interaction of breeder age with incubation and rearing temperatures. At control rearing temperatures (from d 21; 22 ± 1ºC and 50%), cloacal temperatures were similar among breeder ages regardless of incubation temperature. Whereas at high rearing temperatures (from d 21; 32 ± 1ºC and 70%), the greatest and lowest cloacal temperatures were for chicks from old breeders incubated at control (37.8ºC) and high temperatures (38.5ºC), respectively. Which also supports the idea of a possible functional difference in thermoregulation. There are several studies that confirm the influence of breeder age on hatchability. According to Rosa et al 2002, Almeida et al 2006 and Araújo et al 2016, the highest hatchability was for prime breeders (34 - 50 weeks) and the lowest was for older breeders (≥ 51 weeks). This is due to the decline in eggshell quality caused by increasing breeder age, resulting in higher embryo mortality. In the case of chick weight, and in line with the findings of Tona et al (2004) and Willemsen et al (2008), chicks from old breeders were heavier than chicks from young breeders. Supporting these results, Jacobs et al (2016) proposed a positive relationship between breeder age and the body weight of day-old chicks. Considering these two factors, body weight and hatchability, could help explain the fact that chicks from prime breeders in trial 1 had a higher average cloacal temperature. The explanation could be related to the fact that the highest hatchability occurs when breeders are in prime age. Therefore, the desity of chicks on the tray is lower when breeders are old, which may lead to lower cloacal temperature.

The difference in the thermoregulatory ability of broiler chicks considering only breeder age could imply the need for changes in the management of chicks in the hatchery. Chicks from young breeders are more resistant to heat whereas chicks from old breeders are more resistant to cold (Weytjens et al 1999), so post-hatch chicks could be selected according to the age of the breeder flock depending on the geographical area or kind of farm.

Incubation process

According to our results, chicks presented a mean difference of 0.3ºC in cloacal temperature comparing the bottom and upper hatcher basket inside the trolley. These cloacal temperature differences could be explained by the metabolic heat production. According to Meijerhof (2009), during the last days of incubation, the production of metabolic heat results in the the embryo temperature rising significantly above air temperature. Because of evaporation and differences in heat transfer conditions, large differences may be detected between eggs in terms of embryo temperature at different spots in the machine. Temperature variations of up to 1.5ºC are quite common and may explain why the chicks showed cloacal temperature variations at different heights of the trolley at the moment of hatching.

Gender

Female chicks showed higher average cloacal temperatures than males. ATo the best of our knowledge, there are no other studies on this issue However, there are studies that reflect differences between male and female chicks for other parameters. For example, Burke & Sharp (1989) conducted a study in which they found weight differences between sexes in chick embryos. The weight of the male embryos was significantly higher than the females at 11, 13 and 18 days of incubation. Rose et al (1996) mentioned that the amount of bone tissue in the tibia-tarsus and tibia, and the presence of bony deformities for the intertarsal joint were lower in females than males. They highlighted that the cortical growth of the bones was very different between males and females. And finally, another study pointed to the effect of sex on meat characteristics with males reaching a higher body weight, carcass weight and breast weight than females (López et al 2011).

Post-hatch factors

Before the chicks were placed in the expedition room, waiting time prior to sampling had an influence on the cloacal temperature of the chicks. For every additional minute, the cloacal temperature increased slightly. Once the chicks were in the expedition room, as time passed, the cloacal temperatures of the chicks were significantly higher. When the ventilation system was on, the cloacal temperature of the chicks was lower. Conversely, when the ventilation system was off, the cloacal temperature of the chicks was higher. Hai et al 1996 observed the influence of the environment temperature on the chicks' cloacal temperature. They exposed the chicks to different ambient temperature and humidity and found significant effects on cloacal temperature ($P < 0.01$). Also, Nääs et al 2014 studied heat loss in pullets in commercial conditions. They observed that pullets lost more heat in the vaccination room, because it was a room with lower temperature (25.5 ± 0.2ºC) than the hatching room (28.9 ± 0.2ºC). The detection of post-hatch factors that affected the cloacal temperature of the chicks revealed that it might be necessary to consider more environmental control points in the different areas of the hatchery and in different phases of the process. In addition, more studies are needed under commercial conditions in order to detect the pre- and post-hatch factors in the hatchery and to avoid thermal stress and ensuing y economic losses. Moreover, the influence of broiler chick sex and breeder age on the cloacal temperature should be considered as additional factors in thermoregulatory experiments. Finally, as Nääs et al 2014 suggested, more experiments on heat loss would be interesting, to have a precise estimation of this parameter, and to implement strategies that may help reduce these losses.

Mortality

Our results showed that cloacal temperature measures in the hatchery had an influence on mortality. Chicks that had a cloacal temperature below the optimal temperature range (< 39.7ºC) in the hatchery were three times more likely to die than chicks that had a cloacal temperature above the optimal temperature range (> 40.3ºC). Once again, this may be due to the absence of a mature thermoregulatory system, as the ability of the chicks to adapt their body temperature during post-hatching period is inefficient at least during the first 10 days of their life (Tzschentke & Nichelmann, 1999). It has been suggested that a variable body temperature during early life can be a predisposing factor for the development of ascites syndrome (Maxwell & Robertson, 1998) and might lead to mortality. However, when studying the effects of moderate housing temperatures in early life, Baarendse et al (2006) did not detect any significant effect of early-age ambient temperature on the prevalence of ascites and mortality. In this case, no studies have been found showing the effect of cloacal temperature in the hatchery on mortality at seven days of life. These results support the possibility of acclimatizing the chicks to hot and cold temperatures, and indeed there are several studies that explain the advantages of exposing chicks to high or low temperatures during incubation or at an early age. In fact, incubating chicks at higher or lower temperatures in the last week of embryonic development will determine their preference for higher or lower ambient temperatures during the first 10 days of life (Tzschentke & Nichelmann, 1999). Yalçin et al (2008) studied the effects of exposing the chicks to high temperatures during incubation in order to acclimatise them. After exposing them to high environmental temperatures during brooding, the authors observed differences between chicks that were acclimatised and chicks that were not. They concluded that exposing the embryos to high temperatures improves the thermotolerance of chicks to cyclical exposure to high environmental temperatures. This practice could help decrease thermal stress in broiler chicks and consequently reduce the impact on performance indexes and mortality.

3.6. Animal welfare implications

In conclusion, these data indicate that it is important to consider ambient conditions as post-hatch factors, as they affect the cloacal temperature of chicks, and consequently decrease their welfare. Although it has not been possible to directly relate the post-hatch factors with chick mortality at seven days of life, the cloacal temperature of chicks in the hatchery has been found to be a risk factor that can increase chick mortality by up to three times, when these cloacal temperatures are below the optimum temperature range (< 39.7 ºC). Therefore, cold stress could be a higher risk than heat stress for mortality at first week of life.

Moreover, as the findings suggest, the sex of the chick and breeder age could be used as management tools, together with the optimal control of the environmental conditions in the hatchery, with the aim of reducing possible thermal stress during the first hours of life, and the associated economic impact. However, regarding chick mortality at seven days of life, more studies are needed in order to consider the large number of factors that can have an influence in the first seven days of a chick's life.

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Capítulo 4

Effect of transport on chick weight loss and mortality.

"Live as if you were to die tomorrow. Learn as if you were to live forever."

M. Gandhi

This chapter is based on the following manuscript submitted to *Poultry Science* and currently under review

Effect of transport on chick weight loss and mortality

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4.1. Abstract

The present study had two objectives: the first was to analyse the possible impact of transport environmental conditions on weight loss and mortality during transport, and first-week mortality; whereas the second was to monitor those environmental conditions during transport with an effect on day-old chicks. Probe equipment was installed in a truck of a poultry company from Spain, including a total of 66 journeys made in commercial conditions between May and November 2017. Animal-based measures collected included body weight (before and after transport), mortality during transport, mortality during the first week of life, which were contrasted against a series of environmental variables including air temperature, relative humidity and carbon dioxide (CO2) atmospheric concentration for every journey, number of day-old chicks (%) per journey, transport duration (hours), zones inside the loading area (zone 1 – near to the cabin, zone 2 – in the central point and zone 3 – close to the back doors), height (1- top, 2- medium and 3- bottom), month (May to November), number of stops, type of stop during journey (farm stops and driver stops), time to start the journey, as well as other intrinsic factors of chicks (gender, breed (Ross and Cobb), breeder age (weeks) and egg storage days. Chick weight loss was positively associated with journey duration and relative humidity. No effect of environmental variables was found on mortality during transport. However, chick mortality during the first week of life was related with the number of day-old chicks (%) loaded per journey and chick gender. In conclusion, due to the environmental heterogeneity during transport and the effect of the environment on chick weight during transport and mortality at first week of life, there is an urgent need to refine the air-conditioning and ventilation systems of day-old chicks transport towards a greater environmental homogeneity.

Key words: transport; broiler; day-old chick; performance; animal welfare

4.2. Introduction

The transport of live animals is considered a great source of stress (Mitchell, 2009), due to the exposition to a variety of potential stressors involved, such as ambient temperature, acceleration, vibration, noise, space restrictions and air pollutants (Mitchell and Kettlewell, 1993; Mitchell, 2009). In chicks, despite efforts to improve the transportation process, they are still transported for extended periods under suboptimal environment conditions (Bergoug et al., 2013; Jacobs et al., 2016). There are still many knowledge gaps, such as the adequate level of carbon dioxide (CO_2) during transport, or the interaction between environmental variables (temperature, relative humidity or CO_2).

In addition to these knowledge gaps, chicks are neonatal animals with an immature physiology (Yassin et al., 2009), and they are exposed to many challenges during transportation. From hatching to arriving at the farm, they can be deprived from feed or water for more than 72 h. Hatching can range few hours within the same flock (up to 48 hours), which may result in some chicks being deprived of feed and water for longer than others within the same flock (Jacobs et al., 2016). Feed and water deprivation have been proven to negatively affect performance (Decuypere et al., 2001). Despite chicks are believed to sustain themselves without feed or water for 72 h after hatching by using reserves in their yolk sac (EFSA, 2011), modern genetic lines with high growth and metabolic rates, may deplete their energy reserves more quickly (EFSA, 2011). In addition, the transportation process could exacerbate the depletion of reserves and dehydration, through excessive thermoregulatory demands and stress, thus possibly affecting chicks' body weight and mortality rates (Bergoug et al., 2013; Jacobs et al., 2016, 2017). In fact, chick mortality during first week of life can reflect the stress of transportation process (Bayliss and Hinton, 1990; Yassin et al., 2009).

Given the impact of transport, the general aim of this study was to assess the conditions during transport that may affect broiler chick's welfare and performance in Spain. To respond to this objective, two phases were carried out: (1) to analyse the possible impact of transport environmental conditions on weight loss and mortality during transport and first-week mortality, and (2) to monitor those environmental conditions during transport with an effect on day-old chicks.

4.3. Materials and methods

This study was conducted in a broiler hatchery of a poultry company from Spain. The experiment ran from May to November 2017, during which time 66 journeys were monitored. The study was divided into two Phases, Phase I was carried out with10 journeys that were used to investigate the association between the environmental conditions inside the container and weight loss during transport, mortality during transport and first-week mortality. In Phase II, the following 56 journeys were used to monitor the environmental profile inside the container. Data on the environmental conditions inside the truck was monitored using a digital probe equipment (model TRUCK RHT, Sinergia G6®) in one truck. The equipment consisted on one data logger, 27 probes for temperature – Tª (ºC) and relative humidity – RH (%) and one CO2 probe (ppm). Sensors recorded in 45 seconds intervals.

Truck

The trailer used to transport chicks was 13 x 3 x 2.5 meters long, wide and high respectively. It was equipped with thermal isolation of expanded polyurethane and had an internal and external structure coated with aluminium. The trailer had two back doors with non-hermetic enclosure. The inner door was a sailcloth sliding curtain and the outer door

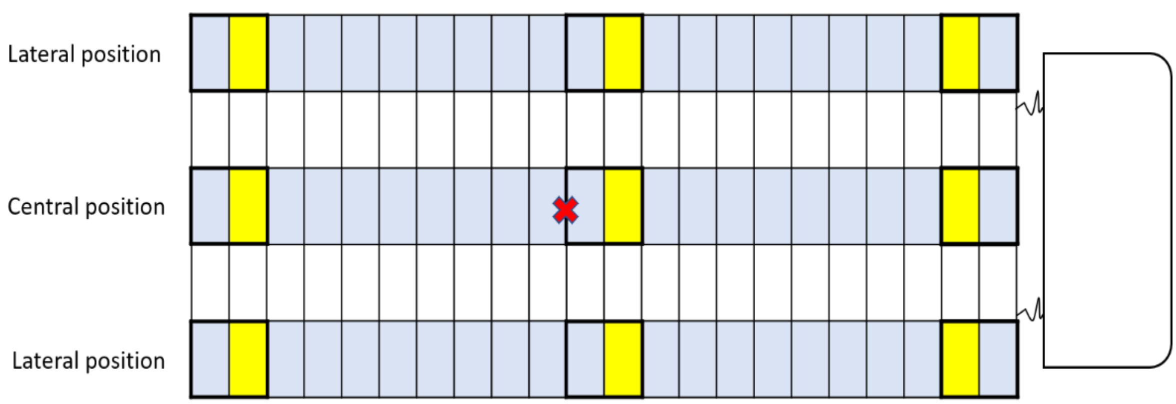

Figure 4.1. Air view, highlighting the center and lateral rows. The coloured squares in yellow were the boxes with temperature and relative humidity probes. The red X indicates the position of the CO_2 probe.

was a lifting platform. The trailer load was distributed in 5 rows of trolleys along the truck with a 10 cm space between them (Figure 4.1).

The load capacity was 750 boxes with 100 chicks per box approximately, which makes an average number of 150,000 chicks per journey. Inside the container, two probes were part of the truck's equipment and were located on the roof to record the environmental temperature. The temperature of the trailer was automatically controlled at 27°C ±2 using fans for ventilation. The air inlets were distributed along the sides of the container roof, and the air exits (ventilation) were located in the central area of the roof in the form of a grid with filter.

Day-old chick boxes

Chicks were transported in fully perforated plastic boxes (60 x 40 x 15 cm) putting on average 100 chicks per box, following regular procedures in commercial transports. Every day-old chick trolley carried 28 boxes distributed in two columns of 14 boxes (Figure 4.2).

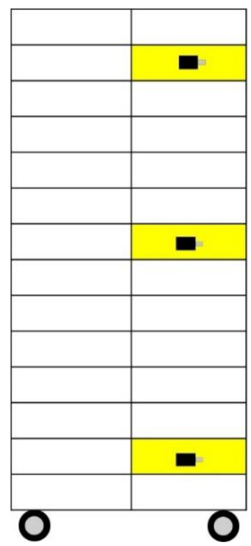

Figure 4. 2. Lateral view of day-old chick trolley. Distribution of the temperature and relative humidity probes inside the trolley. The coloured squares in yellow were the boxes with temperature and relative humidity probes.

Experimental design

Phase I. In the first 10 journeys the loss of weight and the mortality during transport and first-week mortality were monitored. For every journey, 27 boxes of day-old chicks were monitored using 27 Tª and RH probes. The distribution of the Tª and RH probes was different in every journey because the day-old chicks' boxes were randomly selected. The probes were installed in the day-old chick trolleys considering three height points (Figure 4.2) and the height of the probes remained constant in all the journeys.

The CO_2 probe was located at the central point of the container at the bottom height (Figure 4.1). The 27 boxes were randomly selected in the hatchery before every journey, and after loading the chicks, they were weighed (scale model NVL20000 Navigator XL, OHAUS®) and the number of chicks recorded. Upon arrival at the farm, the boxes were weighed once again and the number of dead chicks was recorded.

The company provided data on the following breeding variables for each flock: flock ID, breed (Ross or Cobb), egg storage (days), breeder age (weeks), chick gender and farm (house reference). The age of the breeders was categorized according to the Cobb guide (2008): ≤ 33 weeks (young breeders), 34 - 50 weeks (adult breeders) and ≥ 51 weeks (old breeders). In addition to temperature and relative humidity, the environmental variables considered were: time to start the journey, journey duration (h), number and type of stops during journey (farm stops and driver stops), number of chicks (%) per journey and month. Animal-based dependent variables recorded during the study were weight loss and number of dead chicks during transport, and dead chicks during the first week of life.

Phase II. In 56 journeys, the environmental variables found to affect chick's growth and/or survival during Phase I were monitored. Probes of T[a] and RH were distributed in three zones among the container: zone 1 – near to the cabin, zone 2 – in the central point and zone 3 – close to the back doors (Figure 4.3).

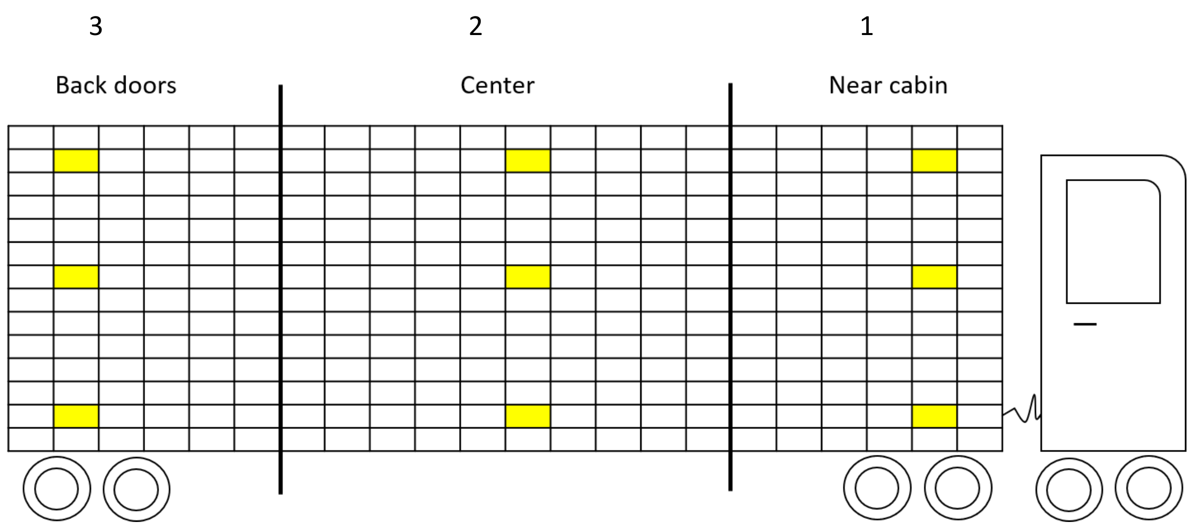

Figure 4.3. Lateral view of the container. Distribution of the temperature and relative humidity probes inside the container. The coloured squares were the boxes with temperature and relative humidity probes.

This probes distribution allowed the monitorization of potential differences between zones and positions (comparing the central row with the two sides of the container) in the container (Figure 4.1). This setup allowed to control 3 out of 5 trolley that were distributed horizontally at three different height points (top, medium and bottom), following the same distribution as in the first 10 journeys (Figure 4.2).

Statistical analysis

Statistical analysis was carried out using the statistical software SAS v9.4, (SAS Institute Inc., Cary, NC, USA) for Windows. The significance level was set at $P < 0.05$. The experimental unit was each journey. To respond the two objectives of the study, the statistical analysis was carried out in two parts, following the steps described below. Data on Tª, RH and CO_2 were normally distributed, based on the graphical evaluation of the residuals (histogram and QQ-plot). Correlations between continuous variables was assessed using Pearson correlation coefficient. A bivariate linear regression analysis was carried out between independent and dependent variables. Since the database included random factors, longitudinal data and repeated measures; a multivariate model was used for the two study phases. The independent variables considered in Phase I analysis were the Tª (ºC), RH (%), CO2 (ppm), number of day-old chicks (%) per journey, transport duration (h), height (1- top, 2- medium and 3- bottom), month (May to November), number of stops, type of stop during journey (farm stops and driver stops), time to start the journey; and the breeding variables were chick gender, breed (Ross and Cobb), breeder age (weeks) and egg storage (days). The dependent variables were weight loss (%) and mortality (%) during transport and at first week of life. The independent variables considered in the Phase II were the environmental variables including number of day-old chicks (%) per journey, transport duration (hours), zones inside the loading area (zone 1 – near to the cabin, zone 2 – in the central point and zone 3 – close to the back doors), position inside load area (0 – central row, 1 – lateral rows), height (1- top, 2- middle and 3- bottom), month (May to October), number and type of stops during journey and start time of the journey. The dependent variables were temperature (ºC) and RH (%). In addition, the following interactions were considered in the multivariate model of Phase II: height*position, height*zone, position*zone.

Each journey was considered a random factor and the variables were manually removed one by one from the model following a step-wise manner using a significance level of 0.05 as reference. The p-values of multiple comparisons were adjusted with the Tukey correction. Finally, the results will be presented using the abbreviation SD for standard deviation and ± for standard error.

4.4. Results

Phase I

Weight loss during transport

During transport, chicks lost an average body weight of 2.96% (SD = 1.22). The variables showing an effect over weight loss during transport were journey duration (P = 0.0006) and RH (P = 0.0188). The estimated effect of journey duration and RH level over weight loss was - 0.2407 (± 0.06889) and - 0.07745 (± 0.03269) respectively. The environmental T^a, CO_2, the number of day-old chicks per journey, height, month, number of stops, type of stop during journey, time to start the journey and the breeding variables did not show a significant effect on weight loss during transport.

Chick mortality during transport and at first week of life

Mean chick mortality during transport was 0.055% (SD = 0.043). which assimilates to 15 dead chicks out of 27,031 chicks monitored. The statistical analysis of this variable could not be carried out due to the low incidence of mortality during transport.

Chicks mean mortality during the first week of life was 2.02% (SD = 1.21). The number of day-old chicks (%) per journey and chick gender had effect on chick mortality during the first week of life (P = 0.0082 and P = 0.0087, respectively). The higher the number of chicks loaded in the truck the higher the mortality (estimate = 0.305 ± 0.114). As for chick gender, male chicks (estimate = 0.607 ± 0.052) had the highest mortality rate, followed by mixed chicks (estimate = 0.541 ± 0.099), and females (estimate = 0.498 ± 0.049) had the lowest mortality rate. The environment variables, journey duration, height, month, number of stops, type of stop during journey, time to start the journey and the other breeding variables did not have a significant effect on first week mortality.

Phase II

Microclimate profile

Container was at 29.38ºC (SD = 0.97) temperature and 47.97% (SD = 4.43) humidity. Table 4.1 shows the descriptive analysis of the average Tª and RH in the container, according to zone, position and height.

Table 4.1. Descriptive analysis of the average air temperature and relative humidity inside the container of the truck by zones (1- near cabin, 2- center container load and 3- back doors), position (central row, lateral rows) and height (1- upper, 2- middle and 3- bottom).

			N	Mean	Std Dev	Min.	Max.
Air temperature (ºC)	**Height**	Upper	60	28.80	0.971	25.88	31.04
		Middle	60	29.92	1.122	27.55	32.67
		Bottom	60	29.41	1.019	26.50	31.38
	Zones	Near cabin	60	30.19	1.186	25.43	33.11
		Center	60	28.80	1.434	25.62	31.00
		Near doors	60	29.01	1.458	25.37	31.97
	Positions	Lateral rows	60	29.07	0.948	26.66	31.24
		Central row	60	29.96	1.320	26.53	32.71
Relative humidity (%)	**Height**	Upper	60	48.82	4.643	40.91	59.08
		Middle	60	46.81	4.589	38.95	58.12
		Bottom	60	48.29	4.307	40.50	57.63
	Zones	Near cabin	60	47.24	4.621	38.27	60.63
		Center	60	48.75	5.596	39.73	61.37
		Near doors	60	49.06	5.645	38.61	63.85
	Positions	Lateral rows	60	48.38	4.307	40.10	57.47
		Central row	60	47.20	4.900	38.86	60.31

Relative humidity

The results showed that the HR was different among heights, positions and zones (all $P < 0.0001$). Related to height, upper height had a higher HR (estimate = 49.22% ± 0.628) compared with middle (estimate = 46.80% ± 0.627) and bottom (estimate = 47.95% ± 0.628) height. In the case of positions, the central row showed a lower HR (estimate = 47.41% ± 0.615), compared to lateral rows RH (estimate = 48.57% ± 0.615). Regarding zones, zone 1 showed a lower RH (estimate = 46.50% ± 0.638) compared to zone 2 (estimate = 48.56% ± 0.625; $P < 0.001$) and 3 (estimate = 48.90% ± 0.624; $P < 0.001$), whereas no significant differences were found between zone 2 vs. 3. The differences in RH between zones varied more than 2%, being zone 3 the most heterogeneous. The interaction height*position was significant ($P < 0.0001$), which means that the RH at certain height was different depending on the position (row), reaching differences of more than 5% within the same height among positions (Figure 4.4). These patterns could be associated with the association between air T^a and RH ($r = -0.55$, $P = 0.0001$), which means that when air temperature was high, RH was low and vice versa. The association between T^a and RH makes that variations in T^a among locations impacted the RH.

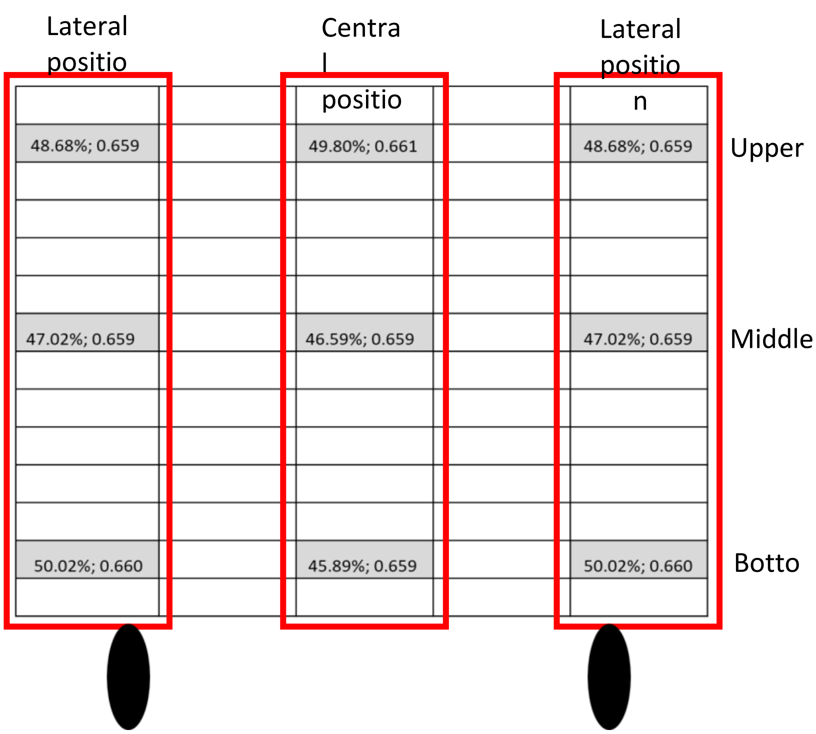

Figure 4.4 Estimate relative humidity values and standard error during day-old chicks transport. Interaction between height and position (back doors view).

No differences in RH levels were detected according to journey duration (h), type of truck stop during journey and number of day-old chicks per journey (%). However, differences were detected between months for the RH levels ($P = 0.0265$), being August and September the months with the highest RH levels (mean = 49.40 SD = 3.47, and 50.51 SD = 4.95; respectively) compared to May, June and July (mean = 46.16 SD = 3.49, 46.82 SD = 4.55, and 45.92 SD = 4.08; respectively).

Air temperature

The results showed that the temperature was different among heights and locations (all $P < 0.0001$). Related to height, there were differences between the upper vs. middle height and upper and bottom height (all $P<0.001$) middle height had the higher T^a (estimate = 29.97ºC ± 0.145) compared with upper (estimate = 28.66ºC ± 0.146) and bottom (estimate = 29.72ºC ± 0.146) height, the difference between mean temperatures varied on average 1.06ºC. With regard to positions, there were differences between central row and lateral rows, the central row was the one that showed a higher T^a (estimate = 29.84ºC ± 0.133), while the lateral rows showed lower air T^a (estimate = 29.06ºC ± 0.133). In the case of zones, there were different temperatures between zone 1 vs. 2 and 1 vs. 3 (all $P<0.001$). Zone 1 showed the highest mean temperature (estimate = 30.35ºC ± 0.154) compared with zone 2 (estimate = 28.95ºC ± 0.143) and 3 (estimate = 29.05ºC ± 0.143), the mean air temperature differences detected between zones varied more than 1ºC, being zone 3 the most heterogeneous. The interactions height*position and position*zone were statistically significant ($P < 0.0001$ and $P = 0.0170$; respectively). Figure 4.5 shows the temperatures estimate values according to the significant interactions. The interaction position*zone (Figure 4.5, A) showed that air temperature in a certain position (row) was different depending on the zone, this difference could vary by more than 1ºC. In addition, the differences detected in this interaction for upper and bottom height depending on position were significant ($P = 0.0005$ and $P < 0.0001$; respectively). However, the differences between positions at middle height were not significant ($P = 0.1348$). The height*position interaction (Figure 4.5, B) showed that the air temperature gradient by heights were more pronounced in the central position (row) than in the lateral positions (rows). Moreover, the differences detected in this interaction for zones 1, 2 and 3 depending on position were significant (P = 0.0263, P < 0.0001 and P = 0.0005; respectively).

Finally, no differences in air temperature were detected depending on journey duration (h), type of stop, quantity of day-old chicks load per journey (%) and month.

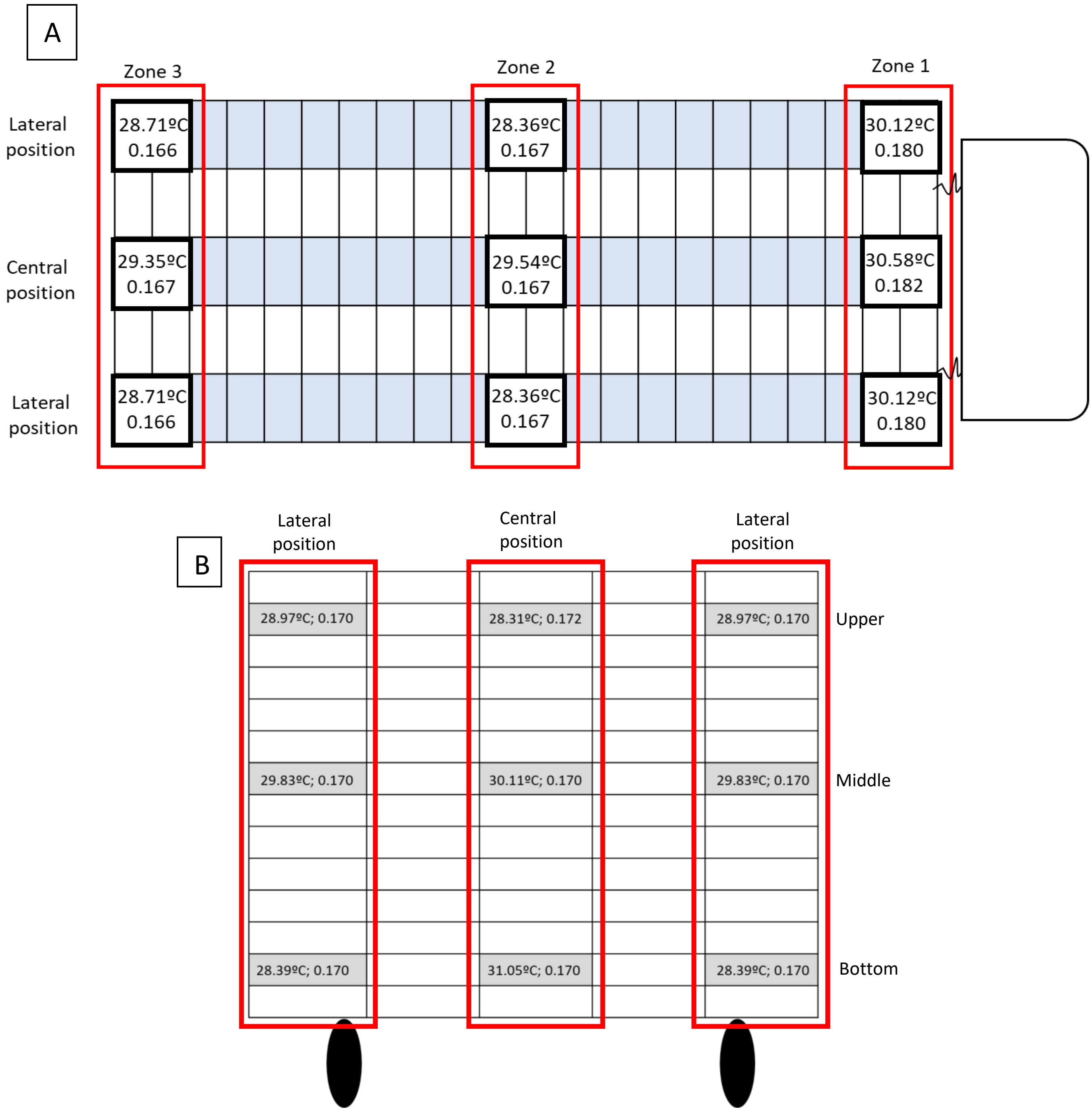

Figure 4.5 Estimate mean temperature values and standard error during day-old chicks transport. A - interaction between zone and position (air view). B – interaction between height and position (back doors view).

4.5. Discussion

Phase I

Weight loss during transport.

According to our results, weight loss during transport is associated with journey duration and RH, and these results coincide partially with those from Jacobs et al., (2016) and Bergoug et al., (2013b). The latter explained that a negative correlation between weight loss and journey duration was maintained over time, lasting up to d 21 after birth. Jacobs et al., (2016) suggested that any weight reduction after transport is probably due to delayed feed intake, as in their study the negative effect of transport on the chicks' weight did not persist until slaughter age. The same result was confirmed by other authors (Batal and Parsons, 2002; Bergoug et al., 2013). In fact, Baião et al., (1998) suggested that the negative effect observed on chick weight and subsequent impact at early ages may be more related to a longer interval between hatching and housing than to transport itself. Likely due to this reason, in their study, the weight and feed efficiency at the end of the growth period did not change.

In the case of RH, this environmental variable impacts the capacity for heat exchange (sensible and latent) of broiler chicks (Lin et al., 2005; Schimidt et al., 2009). The loss of evaporative heat increases with temperature but decreases with humidity. When the RH is above 60%, heat dissipation is reduced, which harms the reduction of body temperature (Nazareno et al., 2016). In line also with our results, when chicks are exposed to a heat challenge with high RH, the efficacy of the thermoregulation system may be compromised, and chicks will need to use other pathways to lose heat, accelerating metabolism. When this occurs, part of the energy that should be used to gain weight will be dedicated to thermoregulatory process, reducing performance (Abreu et al., 2017). Lin et al., (2005) suggested that broiler chicks are sensitive to changes in humidity, even when temperatures are thermoneutral, and observed the influence of RH on cloacal and peripheral temperatures. They showed that when temperature and RH are high, the evaporative and non-evaporative heat loss decrease, and chicks activate alternative physiological pathways to redistribute heat within the body to adapt to the environment. This, may explain why the higher environmental humidity in high temperatures results in greater weight loss in chicks.

Chick mortality during transport and at first week of life

No effect of transportation was found on mortality during transport, which confirms the findings of other studies (Bergoug et al., 2013; Jacobs et al., 2016). Ritz et al., (2005) emphasised the difficulty in associating mortality during transport with thermal stress or any other transport variable, or even with any effect during all the growth period. However, we found that transportation was indeed associated with mortality during the first week of life, and more precisely with the number of day-old chicks transported per journey. Similarly, Chou et al., (2004) found an association between mortality during the first week of life and transportation distance. However, this association is still controversial as other authors did not find any relationship between the same variables (Bergoug et al., 2013; Jacobs et al., 2016). This difference could be due to the fact that Chou et al. (2004) focused on journey duration without considering other independent variables related to transport such as environment conditions during transport or the number of chicks transported per journey.

Regarding the number of day-old chicks loaded per journey, according to Nazareno et al., (2016), there are two environments inside the truck (the container environment and the environment inside transport boxes), with the highest averages for air T^a and RH inside the chick boxes. Considering the existence of these two environments that can thwart environmental stability, in addition to the quantity of chicks' load per journey, the greater number of chicks per journey the higher the divergence between those environments, likely increasing temperature inside the transport boxes. These could lead to thermal heat stress for the chicks, making it more difficult for them to dissipate heat (Ernst et al., 1984; Yalçin et al., 1997). It is well established that heat stress may facilitate dehydration and increase rate mortality in early life (Bergoug et al., 2013; Piestun et al., 2017).

In addition to transport variables, we also found that the first-week mortality differs according to chick gender. Our results are according to those from Leitner et al., (1988), who detected significant differences for mortality between males and females during the first eight weeks of life. They hypothesised that males are more susceptible to pathogens and therefore have a higher mortality. However, infectious diseases were not monitored in the present study, and therefore we cannot confirm the hypothesis of Leitner et al., (1988).

Phase II

Taking into account that there were transport related variables affecting the welfare and productivity of broilers, it was considered important to study the performance of environmental variables during transport, and the most likely reasons to explain environmental variations inside the trailer.

Microclimate profile

The findings obtained in the microclimate assessment (Table 4.1) indicate an environmental heterogeneity inside the container, and coincide with the studies conducted by Knezacek et al., (2010); Barbosa Filho et al., (2014) and Nazareno et al., (2015b). Marques (1994) and Quinn & Baker (1997) attributed this heterogeneity to low air circulation, caused by problems in the air-conditioning and ventilation system of trailers, as well as to the non-standardisation of load density in journeys.

Relative humidity and air temperature

As shown in Phase I, the RH had an effect on weight loss during transport. Humidity affects the animal depending on the environmental temperature. For this reason, the importance of the association between T^a and RH is discussed. It is important to clarify the temperature difference between the integrated probes and the trial probes. In the case of integrated probes there were only two probes installed at the truck's roof with roughly 25 cm between the probe and day-old chicks' trolleys whereas there were 27 trial probes located next to the chicks' boxes. The localisation as well as the number of the trial probes may have offered a more realistic overview of what is happening inside the truck than the integrated probes. The variability detected in RH and temperature inside the container was in line with other studies. However, there is a lot of variation between authors as regards the thermal and RH range inside the container. Meijerhof (1997) and Weeks & Nicol (2000) suggested a temperature range between 24 and 26ºC, without alluding to RH, whereas Marques (1994) recommended temperatures between 22 – 31ºC and a RH of 50%. Finally, Nazareno et al. (2016) found that the temperature and RH means were 28.5ºC (23.3 – 32.2ºC) and 40.5% (31.4 – 54.1%), respectively. This was the study that had the most similar results to our study.

There are several possible reasons for this environmental variability. First, as mentioned earlier, it could be the influence of air circulation added to an inefficient ventilation system, causing a wide range of temperature and RH (12ºC and 40%) (Nazareno et al., 2015a), with particularly conflictive hot spots. Quinn and Baker (1997) suggested that the distribution of dead chicks in the truck does not follow a random distribution but reflects the variation in ventilation and areas with critical environmental conditions. The second reason could be heat metabolic production (Yalçin et al., 2001), as it could exacerbate the existing thermal gradient between zones, positions and heights inside the container. Also, the combination of the two environmental variables (RH and T^a) could compromise transpiration, exacerbated by the imposed high number of birds in a transport container and in the day-old chick boxes (Mitchell and Kettlewell, 1998). The third reason is the existence of two environments, one inside the container and the other inside the chick box, the later having the highest averages for temperature and RH (Nazareno et al., 2016). Therefore, in order to achieve a comfortable environment for the transport of day-old chicks, the truck design and the air-conditioning system efficiency should facilitate the homogenisation of the environment throughout the trailer, avoiding significant fluctuations of humidity and temperature among the container.

In conclusion, under commercial conditions, transportation of day-old chicks increased weight loss during transport, with journey duration and RH having a negative impact on chicks' body weight. No effect of transportation was detected on mortality during transport. However, mortality during first week of life was affected by the number of chicks loaded per journey. Variations in RH and air temperature during transport may have a negative effect over welfare and productivity of day-old chicks. To address these, there is an urgent need to refine the air-conditioning and ventilation systems of day-old chicks transport to achieve a greater environmental homogeneity in trailer.

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Capítulo 5
Factors associated with cumulative first-week mortality in broiler chicks

"Son necesarios cuarenta músculos para arrugar una frente, pero sólo quince para sonreír."

S. Sivananda

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Factors associated with cumulative first-week mortality in broiler chicks.

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5.1. Abstract

First-week mortality is an important performance index as well as an important welfare indicator. The aim of the present study was to identify internal (individual-dependent) and external (management or environmental) factors that could influence the cumulative first-week mortality of broilers. To carry out this study, field data obtained from a hatchery company were used, in which 2,267 flocks of broiler chicks (from 2015 to 2018), were analyzed. A generalized linear mixed model was used to analyze the data. Farm ID and house by farm were incorporated as random effects. The Odds Ratio was estimated for each factor, determining the effect of each explanatory variable. First-week mortality was significantly related to breeder age ($p < 0.0001$), chick gender ($p < 0.0001$) and breed ($p < 0.0001$) as internal factors, and type of broiler house ($p = 0.0129$), presence or absence of drip cup ($p < 0.0001$), egg storage ($p < 0.0001$), study year ($p < 0.0001$) and season ($p < 0.0001$) as external factors. Therefore, these factors should be considered in the decision making of poultry breeding companies, in order to reduce possible welfare problems and increase productive performance.

5.2. Introduction

In current broiler production systems, the first week of the chicks' life represents between 16 to 20% of their productive life. These first days of life are a transition stage in which many things happen in a very short period. First, chicks change from a very conditioned and controlled life in the hatchery to a more independent life on the farm (Yassin et al. 2009). At this transition stage, many of the chicks' internal systems are immature. Hence, it is during this sensitive stage that the greatest changes will occur requiring an adaptation time (Vieira and Moran, 1999). Therefore, during this first week of life chicks are submitted to a high degree of stress as they try to adapt to all these challenges and maximize their chances of survival. All these factors that can negatively influence chick morpho-physiology during this stage will have an impact on welfare and, if animals fail to adapt, could lead to increased mortality in the first week.

Mortality during the first week of life in broilers is an important production criterion widely used in poultry production. It is also one of the animal welfare indicators covered by the 2007/43/EC European Directive (European Union. Council Directive 2007). In addition, first-week mortality is a common parameter used to assess chick quality. Chick quality can be defined as chicks that are optimally developed during incubation and show a high-performance potential and survivability (Tona et al. 2005). Expanding on this concept, Ulmer-Franco et al. (2010) described that chicks hatched with good quality are those that are active, have a body weigh between 40-44 g, their navel is healed upon removal from the hatcher, and their down is dry. Importantly, the quality of day-old chicks is directly related to the survival rate at the first week of life (Goodhope, 1991). This measure is useful to provide information about the breeder farm, the hatchery and the individuals. Chick viability and chick quality depend on internal (individual-specific) and external (management or environmental factors) factors. Breed and breeder age can affect chick quality and in consequence the performance and welfare of the chicks (Vieira and Moran, 1999; Decuypere et al. 2001; Peebles et al. 2004). There are also external factors that can influence first-week mortality such as season (Imaeda, 2000), flock size, density of chicks housed in the farm, type of ventilation and type of drinkers (Heier et al. 2002; Chou et al. 2004; Yassin et al. 2009). For instance, according to Chou et al. (2004), an increase of the size of the flock decreases the cumulative first-week mortality.

Previous studies that assessed risk factors of first week mortality, included only a limited number of them. However, the present longitudinal study enlarges this list including an extensive number of factors (up to 24) related to chick, environment and management. The aim of this study was to identify internal and external factors that could influence the cumulative first-week mortality of broilers.

5.3. Materials and Methods

Data collection

For the study, information related to first-week mortality was requested from a Spanish broiler company, located in Zaragoza (Spain). As well as information on the characteristics of farms, the company provided the following databases:

- A descriptive database of the different farms that belong to the company, in which characteristics are described for 253 broiler houses of 104 farms.
- A database including the first-week mortality of the chicks during the last four years: 2015, 2016, 2017 and 2018. This database contains 6,979 mortality records, including the flock, gender and broiler house (or half broiler house). A total of 2,267 flocks, 70,211,678-day-old chicks and 68,949,774 seven-day old chicks were studied.

Factors under study

To carry out the study, 24 variables were considered as risk factors for chick mortality. These variables were classified as internal and external factors and are described in table 5.1.

Internal factors

Three variables were considered factors specific to the individual: breeder age - intervals expressed in weeks and categorized according to the Cobb guide (2008) (25 – 33 weeks, 34 – 50 weeks and 51 – 68 weeks and unknown), chick gender (male, female and mixed) and breed (Cobb and Ross). These internal factors were chosen due to the relationship detected in other studies with the viability and quality of the chick (Yassin et al. 2009; Vieira et al. 1999; Peebles et al. 2004).

External factors

There were 21 variables classified as environmental or management factors. The factors under study are listed in Table 5.1. It should be noted that the variables 'farm entry density' (chicks/m2) and 'flock size' (considering the number of chicks received) were categorized according to 25th, 50th and 75th percentiles. These external factors were considered because there is evidence showing its importance on the viability of the chicks. Factors such as season (Imaeda, 2000), transport (Chou et al. 2004), stocking density, flock size, feeding and drinking system, ventilation and insulation at the broiler farm (Heier et al. 2002) were related to first week mortality. In addition, Yassin et al. (2009) detected that egg storage days was negatively correlated with first week mortality and there was a significant difference in first week mortality in the different study years.

Table 5.1. The distribution of independent variables classified as external (environmental or management) and internal (individual-specific) factors, and the total mean cumulative mortality during the first week of life (study of 2,267 flocks in 104 farms and 253 broiler houses, Spain, 2015-2018).

Factor *	Category	N Farms	N houses	N flocks	Mean FWM
Farm ID	.	104	253	2,267	1.82
Broiler house by farm	.	.	.	.	.
Province	Barcelona	3	7	60	1.76
	Huesca	39	95	880	1.87
	Lleida	34	99	847	1.69
	Tarragona	6	14	150	1.73
	Teruel	1	1	14	1.79
	Zaragoza	19	37	316	2.05
Age of the broiler house	< 10 years	23	43	404	1.83
	10 – 20 years	27	56	555	1.70
	> 20 years	67	152	1,301	1.87
	Unknown	2	2	7	1.59
Heating system	Air generators	58	153	1,419	1.79
	Heat screens	34	73	612	1.91
	Cannon	7	14	99	1.81
	Other types	7	10	117	1.69
	Unknown	3	3	20	1.57
Litter	Straw	8	24	193	1.71
	Shavings	18	40	341	1.89
	Rice hulls	70	173	1,565	1.80
	Oat hulls	3	6	62	2.28
	Unknown	6	10	106	1.80
Farm entry density – chicks/m^2	< 17.5	26	65	611	1.95
	≤ 17.5 – < 30	23	54	442	1.74
	≤ 30 – < 36	32	69	668	1.71
	≥ 36	29	65	546	1.86

Study year	2015	.	.	591	1.93
	2016	.	.	612	1.75
	2017	.	.	610	1.80
	2018	.	.	454	1.79
Type of broiler farm	One floor	80	178	1,951	1.83
	Multiple floors	30	73	309	1.75
	Unknown	2	2	7	1.82
Type of insulation	Bridge work	75	179	1,544	1.81
	Sandwich panel	34	66	657	1.85
	Cosma	4	6	59	1.64
	Unknown	2	2	7	1.82
Feeder brand	Tigsa	15	28	257	1.74
	Chore-time	24	50	432	1.93
	Roxell	30	61	613	1.67
	Ska	24	47	429	1.89
	Other brands	22	47	393	1.93
	Unknown	10	20	143	1.73
Egg storage days	< 7	.	.	688	1.94
	7 - 10	.	.	1,368	1.76
	> 10	.	.	209	1.76
	Unknown	.	.	2	1.70
Lighting	LED	14	26	218	2.10
	Non-adjustable fluorescent	56	135	1,287	1.77
	Adjustable fluorescent	17	34	307	1.90
	Bulb	28	57	452	1.76
	Unknown	1	1	3	2.08
Season	Winter 22/12 – 20/03	.	.	523	1.93
	Spring 21/03 – 21/06	.	.	575	1.69
	Summer 22/06 – 22/09	.	.	600	1.81
	Autumn 23/09 – 21/12	.	.	569	1.86
Constructed area (m^2)	.	.	.	.	.
Drinker brand	Lubing	17	28	229	1.74
	Plasson	18	42	414	1.70
	Corti zootecnici	63	143	1294	1.89
	Other brands	16	38	323	1.74
	Unknown	2	2	7	1.82
Drinker flow rate	High	55	112	1,044	1.70
	Low	52	134	1,161	1.92
	Unknown	5	7	62	1.88
Presence of drip cup	Yes	59	130	1,152	1.66
	No	51	119	1,083	1.98
	Unknown	59	130	32	1.92
Ventilation	Natural	6	18	116	1.47
	Tunnel	27	51	446	1.78
	Transverse	57	125	1,044	1.83
	Transverse + Tunnel	24	55	630	1.92
	Natural + Tunnel	1	2	24	1.22
	Unknown	2	2	7	1.82
Fuel	Gas	74	163	1,490	1.93

	Diesel	12	31	229	1.62
	Biomass	14	41	366	1.66
	Other types	7	16	175	1.47
	Unknown	2	2	7	1.82
Flock size	< 6.700	.	.	1,133	1.86
	≤ 6.700 - < 9.300	.	.	521	1.80
	≤ 9.300 - < 13.000	.	.	309	1.83
	≥ 13.000	.	.	304	1.78
Breeder age	25 – 33 weeks	.	.	541	1.79
	34 – 50 weeks	.	.	1,292	1.76
	51 – 68 weeks	.	.	433	2.10
	Unknown	.	.	1	1.72
Chick gender	Mixed	.	.	149	1.91
	Male	.	.	962	1.89
	Female	.	.	1,156	1.74
Breed	1	.	.	1,717	1.85
	2	.	.	550	1.72

* External factors considered in this table were extracted from the following references: Yassin et al. 2009; Vieira et al. 1998b; Peebles et al. 2004; Heier et al. 2002; Chou et al. 2004; Thoghyani et al. 2010 and Rozemboim et al. 1999.

Statistical analysis

Statistical analysis was carried out using the statistical software SAS v9.4, (SAS Institute Inc., Cary, NC, USA) for Windows. The significance level was set at 0.05 and the experimental unit was the flock. According to the graphical evaluation of the residues (histogram and QQ-plot), the analyzed data was not normally distributed.

The cumulative first-week mortality (FWM) was calculated as a percentage. The total number of dead chicks plus discarded chicks during the first week of life was used as a numerator, and the number of chicks received in the farm as a denominator. Criteria for excluding chicks from the study were the same for all farms and chicks that showed clinical signs of severe disease, abnormalities or chicks with inequality in growth (little chicks) were excluded.

Secondly, a univariate descriptive analysis between mortality at first day of life and FWM was carried out. A bivariate linear regression analysis was performed to examine the relationship between the explanatory variables collected and the response variable FWM.

The correlations study was conducted using the Spearman correlation coefficient. In order to detect risk factors related to FWM, a generalized linear mixed model was used using a binomial distribution (number of chicks dead + discarded chicks at first week / number of total chicks received at the farm). 'Farm ID' and 'house' by farm were incorporated in the model as random effects. All significant explanatory variables detected in the bivariate analysis were included in a preliminary multivariable model. The variables were removed manually one by one from the model using a significance level of 0.05. For each factor, the objective was to estimate the probability of mortality (p) using Odds Ratio (OR) (p/(1-p)), determining the effect of each explanatory variable.

$$\ln\left(\frac{p}{1-p}\right) = \beta_0 + \sum_i \sum_j \beta_i * \left(\text{Variable}_i = \text{Category}_j\right)$$

each parameter was estimated (β) considering:

- Variable_i = each of the study factors (explanatory variables).
- Category_j = each category of the explanatory variable.

For each significant variable, the estimation of the proportion and confidence intervals was obtained, as well as the differences between each pair of categories of every significant variable (differences were measured in terms of OR). The confidence interval and confidence level were intended to estimate the error range. This proposes a range of plausible values for an unknown parameter (in this case mortality risk ≈ OR) and these intervals have an associated confidence level (0.05) where the true parameter is in the proposed range. We analysed the categories from each factor, comparing categories and obtaining the mortality risk which is equivalent to OR.

5.4. Results

The FWM of the studied flocks ranged from 0.13% (0.05th percentil) to 2.25% (0.95th percentil) with an average of 1.82% (± 0.99%). The generalized linear mixed model identified the following factors as significant ($P < 0.05$): breeder age, chick gender, breed, type of broiler house, presence of drip cup, egg storage, study year and season (Table 5.2.). External factors related to the environment (ventilation or type of insulation), feeding and bedding did not have a significant influence on FWM.

Table 5.2. The results from the generalized linear mixed model of the cumulative first-week mortality in the study of 2,267 flocks in 104 farms with 253 houses

Type III Tests of Fixed Effects				
Factor	**Num DF**	**Den DF**	**F Value**	**Pr > F**
Type of broiler house	1	242.6	6.28	0.0129
Drip cup	1	230	23.67	< 0.0001
Study year	3	6,380	424.32	< 0.0001
Season	3	6,380	449.73	< 0.0001
Egg storage days	2	6,380	491.92	< 0.0001
Breeder age (weeks)	2	6,380	3,210.88	< 0.0001
Chick gender	2	6,380	1,762.58	< 0.0001
Breed	1	6,380	1,350.97	< 0.0001

(Spain, 2015-2018).

To determine the effect of each explanatory variable, Table 5.3. shows the results obtained in the OR magnitude between each pair of categories. A one by one analysis for every factor was performed, in order to obtain the first-week mortality risk depending on the category.

Table 5.3. The results of the cumulative first-week mortality in the study of 2,267 flocks in 104 farms with 253 houses (Spain, 2015-2018) for the differences between each pair of categories of every significant variable (differences were measured in terms of OR).

Factor	Category a	Category b	P-value	OR[1] (CI [2])
Type of broiler house	One floor	Multiple floor	0.0129	1.09 (1.02 – 1.15)
Drip cup	No	Yes	<0.0001	1.17 (1.10 – 1.25)
Season	Winter	Autumn	0.0027	1.01 (1.00 – 1.02)
	Winter	Spring	<0.0001	1.10 (1.09 – 1.10)
	Winter	Summer	<0.0001	1.05 (1.04 – 1.05)
	Autumn	Spring	<0.0001	1.08 (1.08 – 1.09)
	Autumn	Summer	<0.0001	1.03 (1.03 – 1.04)
	Summer	Spring	<0.0001	1.05 (1.04 – 1.05)
Study year	2015	2016	<0.0001	1.09 (1.08 – 1.09)
	2015	2017	<0.0001	1.06 (1.06 – 1.07)
	2015	2018	<0.0001	1.10 (1.09 – 1.10)
	2017	2016	<0.0001	1.02 (1.01 – 1.03)
	2016	2018	0.0107	1.01 (1.00 – 1.02)
	2017	2018	<0.0001	1.03 (1.02 – 1.04)
Egg storage days	< 7	7 – 10	<0.0001	1.07 (1.06 – 1.07)
	7 – 10	> 10	<0.0001	1.03 (1.02 – 1.04)
	< 7	> 10	<0.0001	1.10 (1.09 – 1.11)
Breeder age (weeks)	34 – 50	25 – 33	<0.0001	1.04 (1.03 – 1.05)
	51 – 68	25 – 33	<0.0001	1.26 (1.25 – 1.26)
	51 – 68	34 – 50	<0.0001	1.22 (1.20 – 1.22)
Chick gender	Males	Females	<0.0001	1.11 (1.11 – 1.12)
	Mixed	Females	<0.0001	1.17 (1.17 – 1.18)
	Mixed	Males	<0.0001	1.05 (1.04 – 1.05)
Breed	Ross	Cobb	<0.0001	1.08 (1.07 – 1.08)

[1] OR – Odds Ratio

[2] CI – 95% Confidence Intervals

According to our results, chicks housed in farms with one floor had a higher FWM (1.83%) and had 9% more mortality risk than those chicks housed in multiple floors (1.75%). The FWM was higher in chicks from old breeders (51 – 68 weeks) (2.10%) compared with chicks from young (25 – 33 weeks) (1.79%) and prime breeders (34 – 50 weeks) (1.76%). In addition, chicks from old breeders had a 26% and 22% higher mortality risk during the first week of life than chicks from young and prime breeders, respectively. Irrespective of differences, mortality risk was low (4%). Regarding chick gender, the FWM was higher for mixed (1.91%) and male chick flocks (1.89%) compared with female chick flocks (1.74%). In fact, male and mixed chick flocks had a mortality risk 11% and 17% higher than females, respectively. In the case of breed, Ross chicks had a higher FWM (1.85%) than Cobb chicks (1.72%). Regarding mortality risk, chicks from Ross breed had a mortality risk 8% higher than chicks from Cobb breed. In the case of chicks housed on farms without presence of drip cup, the FWM was higher (1.98%), as it was the mortality risk, which was 17% higher than that of chicks housed on farms with presence of drip cups (1.66%). The FWM was higher in chicks from eggs stored less than 7 days (1.94%) compared with chicks from eggs stored from 7 to 10 days (1.76%) and eggs stored more than 10 days (1.76%). The mortality risk was higher in chicks from eggs stored less than 7 days. Chicks from eggs stored less than 7 days had 7% higher mortality risk compared to 7 to 10 days storage. Chicks from eggs stored for more than 10 days had a 10% lower mortality risk than 7 days storage. Regarding seasonality, autumn and winter had the highest FWM (1.86% and 1.93%, respectively) compared with spring and summer (1.69% and 1.81%, respectively). Equally, the mortality risk during the first week of life was higher in autumn and winter compared to spring and summer. In winter, the mortality risk was 10% higher than in spring, 5% higher than summer and 1% higher than autumn. In autumn, the mortality risk was 8% higher than spring and 3% higher than summer. In addition, chicks reared in summer had a 5% higher mortality risk than chicks reared in spring. Regarding the study year, 2015 had the highest FWM (1.96%) compared with 2016 (1.75%), 2017 (1.80%) and 2018 (1.79%). The highest mortality risk was found in 2015. In 2015 the mortality risk was 9% higher than in 2016, 6% higher than in 2017 and 10% higher than in 2018. In addition, the mortality risk increased by 2% and 3% in 2017 compared to 2016 and 2018; respectively. Also, the mortality risk in 2016 was higher (1%) than in 2018.

5.5. Discussion

On the farm, the weekly mortality rate changes over time; according to Heier et al. (2002) the average of FWM was 1.54% and 0.48% per week during the rest of the growth period. According to Chou et al. (2004), the average FWM was 1.32% and according to the results of Awobajo et al. (2007) the average FWM was 1.7%. Therefore, the results of the mean FWM obtained in Awobajo's study seem to be in line with the findings observed in the present study.

In the study conducted by Yassin et al. (2009) the data included information from years 2004, 2005 and 2006 from 2 hatcheries and 482 broiler farms, and they also included in their analysis data from 16,365 flocks. Heier et al. (2002) used data from 1996 to 1999 from 1,664 flocks in 132 farms, whereas Chou et al. (2004) used data from year 2000 in a study of 68 hatcheries and 4,796 flocks in 848 farms. As in our study, all the information obtained for the various studies mentioned was collected and volunteered registered by the companies involved.

All internal factors considered in the present study were significantly related to FWM. However, of the 21 external factors considered, only five were significantly related to FWM. As in the studies conducted by Heier el al. (2002) and Chou et al. (2004) the ventilation had no effect on FWM. This may be because the fresh air requirement during the first days of life is very low and there is no significant accumulation of ammonia. Therefore, the necessity for using active air supply in this period is questionable. In relation to feeding no significant effect on FWM was detected in our study in contrast to other studies, where it was found that feeding on paper affected FWM (Yassin et al. 2009; Heier et al. 2002). In contrast to our results Heier et al. (2002) detected that the insulation of the farm had an influence on FWM. In their study they analyzed the presence or absence of floor insulation and this may be the reason why our study did not find an influence, because we analyzed the different types of insulation. Finally, related to type of litter no influence on mortality was detected, and these results coincide with what was found in the Thoghyani et al. (2010) study. In their study, no influence was detected on mortality at 42 days depending on the type of litter, however differences were detected in feed intake, body weight and behavior during the rearing period.

Internal factors

Breeder age

Yassin et al. (2009) and Peebles et al. (2004) claimed that there is a close relationship between FWM and breeder age. According to our results, the older the breeders, the higher both the FWM rate and mortality risk during the first week of life. In contrast to our results, other studies suggest that the highest rate of FWM occurs in chicks from young breeders (25 – 33 weeks), followed by chicks from old breeders (51 – 68 weeks) (Wilson, 1991; Suarez et al. 1997). These differences might be because these studies only used two of the three breeder ages used in the present study. Wilson (1991) used chicks from young and old breeders, and Suarez et al. (1997) used chicks from young and prime breeders.

The explanation for the results obtained in the present study could be due to the correlation between breeder age with egg size and incubation time (Wilson, 1991; Suarez et al. 1997; Pedroso et al. 2005). Due to the interaction between breeder age and egg size, eggs from young breeders need more hours of incubation compared with eggs from prime and old breeders (Suarez et al. 1997). As a result, eggs from old breeders hatch earlier (Suarez et al. 1997), and therefore the risk of chick dehydration increases with breeder age. Dehydration of chicks at an early age may result in increased FWM (Tona et al. 2004; Shinder et al. 2007). Another possible hypothesis is that chicks from old breeders have low chick quality and viability and a worse healing navel, increasing the probability of suffering diverse pathologies and infections of the yolk sac, which might increase FWM (Tona et al. 2004; Yassin et al. 2009).

Therefore, in everyday hatchery practice, breeder age should be an essential factor for the management of the hatching window and the removal of the chicks from the hatcher. In order to improve the welfare of the chicks, breeder age should be considered a major factor in any decision related to management or production, since the negative effect of this factor persists during the performance of the chicks until the end of the growth period (Peebles et al. 2004; Willemsen et al. 2008).

Chick gender

Female chicks obtained a lower FWM and a higher probability that this rate would decrease, compared to male and mixed chicks. The differences observed between males and females is in line with the results obtained in the study by Leitner et al. (1988). From week two to week eight of life, they observed large differences in the mortality rate between males and females. These differences were found in the activity of the T and B lymphocytes, despite the participation of other regulatory cells could not be ruled out. They concluded that females developed effective immune responses before than males. This difference in the rate of development of immune response is what could make males more susceptible to pathogens, and therefore have a higher FWM.

However, Wu et al. (2012) obtained opposite results for the mortality rate during embryonic development. Wu et al. (2012) suggested that the possible causes for these differences in embryo mortality rate could be related to significant differences in embryonic development between males and females, leading to this bias in premature death. Another possible mechanism they suggested was that lethal sex-linked genes could account for the differences in early mortality observed between the sexes. The last cause they considered is related to the environment temperature to which embryos are exposed during incubation. Male embryonic mortality is higher at high temperatures, while female embryonic mortality is higher at low temperatures, with similar mortality in both sexes when incubation temperatures are intermediate.

Therefore, in order to reduce FWM, two separate strategies should be considered, one for the embryonic period and another for the postnatal period. During the embryonic period, an intermediate and homogeneous incubation temperature should be ensured to avoid possible embryonic thermal stress. During the postnatal period, adequate vaccination of all chicks should be ensured with special emphasis on male chicks. In this case, the potential cost of implementing the suggested measures would vary depending on the incubators, hatchers and vaccination system used in the hatchery. Nevertheless, due to the high risk of mortality according to the chick gender, the implementation of these measures would be cost-effective in the short term. Finally, in order to confirm these recommendations further experimental trials would be necessary.

Breed

Chicks from Ross breed had a higher FWM and a higher probability of increasing this rate compared to Cobb breed chicks. These findings agree with the results obtained in other studies in relation to differences between breeds (Awobajo et al. 2007; Yassin et al. 2009). However, the breeds used to carry out the comparison were different in all the studies. The International Agriculture Report (1994) explains that some breeds are prone to higher FWM due to genetic factors. In fact, Awobajo et al. 2007 observed that this difference in mortality rate depending on breed was maintained throughout the entire growing period. Some breed-related factors influencing chick viability and quality and consequently FWM are the difference in egg weight (Vieira and Moran, 1998b) and the daily metabolic activity of the embryo during incubation (Hamidu et al. 2007). As these breed-related factors influence chick quality, hatchery managers should take them into account when deciding which eggs to place together in each hatchery. Therefore, it would be important to consider the attributes of each breed before selecting the one that best suits the needs of each company, location and type of production.

External factors

Type of broiler house

According to the results, chicks housed on broiler farms with multiple floors had a lower FWM than chicks housed on broiler farms with one floor. Although farms with multiple floors tend to be older, and therefore more complicated to manage (Bell and Weaver, 2002), these types of facilities may be a good option because when broiler houses are preheated to receive chicks, temperature tends to rise, producing a comfortable litter temperature in the upper floors. In line with this hypothesis, Deaton et al. (1996) explain that chicks raised at low temperatures have a higher mortality, mainly due to ascites.

Considering these results, we hypothesize that FWM would be more related to the temperature of the house than to its space distribution. Therefore, at an early age, it should be ensured that the house reaches an adequate environmental and litter temperature, and that it is homogeneous throughout the housing facilities.

Drip cup

We found that chicks raised without drip cups had a higher FWM than chicks raised without, and a higher mortality risk during the first week of life. There are currently several water supply systems on farms. However, the most widely used in the Spanish poultry industry is the nipple system, with or without drip cup (drip cup is a part of nipple drinkers to avoid wet litter). The main reasons for its widespread use are that they require less labour to bell drinkers (Goan, 1994), the improvement in the microbiological quality of the water (Valias and Silva, 2001), and the increase in the control of litter moisture (Ipek et al. 2002).

However, this system alters the drinking behaviour of poultry, since it is very different from that which would naturally take place. In particular, 'spooning', in which the bird lowers its head, takes water in its beak and then raises its head again, is completely absent (Houldcroft et al. 2008). In addition, birds must learn a necessary action for drinking, which is to apply pressure with the beak on the nipple (Appleby et al. 2004). Despite the important management reasons for choosing this type of system, it does not seem to be the best option for chicks. In addition to this inconsistency with their natural behaviour (spooning), a study by Heier et al. (2002) comparing different water supply systems, showed that chicks raised with nipple drinkers had a higher FWM. Carpenter et al. (1990) compared performance indices in chicks raised with nipple drinkers or with nipple drinkers supported by supplementary drinkers (plate type). They detected that mortality decreased in the latter case.

All the previous literature indicates two possible reasons why chicks reared with nipple drinkers without drip cups had a higher probability of mortality. The first is that having this part of the drinker to collect water in a small plate gives chicks an easy and quick access to the water, so they do not need to learn how to drink in a new system. The second reason is that nipple drinkers without drip cup (lacking this water collection system) will be more susceptible to diseases associated to litter humidity. Therefore, the results of this study, suggest that the addition of the drip cup may improve the welfare of chicks by reducing the likelihood of dehydration and maintaining litter quality.

Egg storage days

Chicks from eggs stored for less than seven days had a higher FWM, and a higher mortality risk during the first week of life. These results contrast with the findings of Yassin et al. (2009) where the FWM increased slightly for each additional day of storage.

It should be noted that egg storage is a common practice in hatcheries in order to synchronise the different activities of the hatchery, such as egg reception, egg sanitization, preheating and schedule incubator and hatcher loading.

Egg storage for up to seven days has little or no effect on hatchability (Dymond et al. 2013). However, it is well documented that prolonged storage periods decrease hatchability and increases incubation times (Fasenko et al. 2001b; Fasenko, 2007), and decreases chick quality (Suarez et al. 1997; Tona et al. 2003). It has been suggested that this prolonged storage of the egg may induce embryonic stress, resulting in irreparable damage to the embryo, increasing embryonic mortality and decreasing performance (Fasenko, 2007; Hamidu et al. 2011). Because of this, various interventions have been developed to decrease the negative effects of prolonged egg storage. One of the most recently applied techniques is the use of short periods of incubation during egg storage (SPIDES). Several studies confirm that this technique provides a good restoration of hatchability and chick quality (French et al. 2011; Nicholson et al. 2011; Nicholson et al. 2012). Moreover, Dymond et al. (2013) reported that the performance during the first week of life in terms of chick body weight was greater using SPIDES.

The company in this study uses the SPIDES technique routinely, since it is usually applied to eggs stored for more than five days. According to our results, it is reasonable to expect that the FWM would be similar to that typically found with eggs stored for less than seven days. In fact, breeding companies suggest treating eggs when arriving in the hatchery in order to homogenize the temperature and embryonic phase. This could be the reason why the eggs stored for less than seven days obtained these results. Therefore, the results of this and other studies suggest that prolonged egg storage would not have a negative effect on performance, as long as the SPIDES technique is applied.

Study year

Mortality during the first week of life was significantly different between the different years of study. These results are consistent with those from Yassin et al. (2009) and Heier et al. (2002). However, a trend was observed in which FWM decreases over the years, in contrast to what was observed in the Heier et al. (2002). study. These authors did not expect this result because the two new breeds had been introduced in Norway two years before their study, and they hoped that the farmers were already familiar with the management of these genetically novel birds.

The trend of FWM annual decrease observed in the present study could be related to the implementation of more effective management and biosecurity measures on broiler farms. A major effort has been made in recent years to improve vaccination techniques in the hatcheries in order to improve and homogenize these practices, as well as to achieve an adequate immunization of the chicks. In addition, the main objective of the hatcheries is to obtain high quality chicks, and because of this reason the implementation of improvements in facilities and equipment may be another reason why FWM has tended to decrease. Therefore, it is expected that if both biosecurity and management improve, there would be a concomitant improvement in animal welfare reflected in performance indices such as lower mortality rates. However, the possible influence of genetics on these results was beyond the scope of this study and could not be ruled out.

Season

The highest FWM was detected in autumn and winter, whereas Yassin et al. (2009) observed higher FWM rates during the spring and lower rates during autumn. These differences could be related to the geographical location. The study of Yassin et al. (2009) was carried out in the Dutch poultry industry. The Dutch climate is temperate and very humid with narrower temperature ranges compared with the Spanish climate. The difference between summer and winter in Spain could be very extreme due to the influence of microenvironments.

The seasonality in mortality could be related to climate, since the lowest environmental temperatures are in winter. It should be considered that new-hatched chicks do not have a mature thermoregulation system, which do not become fully functional until day 7-10 of life (Tzschentke and Nichelmann, 1999). Therefore, if chicks are exposed to low ambient temperatures due to improper handling, the chances of increasing FWM are up to three times greater (Yerpes et al. Under Review). Another reason for this seasonality is that it could be related to market fluctuation. When demand is high, the hatcheries buy a lot of external eggs. These eggs, however, are mostly of variable quality and can therefore result in lower viability of chicks.

Given the influence of seasonality on the FWM, it would be important to ensure that possible seasonal fluctuations in the environment are controlled and minimized as far as possible. Production companies should review the environmental conditions of all their hatchery rooms, broiler farms and transport with the aim of reducing the possible impact on FWM.

5.6. Conclusions

Results indicate that breeder age, chick gender, breed, type of broiler house, presence or absence of drip cup, egg storage, study year and season were factors that influenced first-week mortality, which confirms and builds on the findings from previous studies Therefore, these factors should be key in the decision making of poultry breeding companies, in order to reduce possible welfare problems and increase productive performance.

Regarding the other factors detected, results suggest that they should also be considered. However, due to their variability, possibly caused by geographical location and human factors, more epidemiological studies are necessary.

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Capítulo 6
Discusión General

"Vieja madera para arder, viejo vino para beber, viejos amigos en quien confiar y viejos autores para leer."

F. Bacon

En la presente tesis se han investigado los factores de riesgo durante la primera semana de vida que tienen un efecto sobre el bienestar y la mortalidad de los pollos de engorde durante este periodo.

6.1. Mortalidad temprana: un indicador del bienestar de las aves

La mortalidad durante la primera semana de vida es un indicador medible que ofrece una visión objetiva del bienestar de las aves siempre y cuando se combine con otros indicadores (SCAHAW, 2000). Por lo tanto, se trata de un indicador útil que puede emplearse para demostrar tendencias dentro del mismo ciclo productivo, ofreciendo la posibilidad de tomar medidas y corregir posibles problemas in situ.

De acuerdo con Heier y col. 2002, la media de la mortalidad acumulada durante la primera semana fue de 1,54%, mientras que Chou y col. 2004 obtuvieron una mortalidad media de 1,32%. Awobajo y col. 2007 encontraron una mortalidad media durante la primera semana de vida de 1,7%. Por otra parte, nuestros resultados muestran una mortalidad media de 1,82%. Tal y como puede observarse, se trata de valores muy variables, lo que es debido a que la mortalidad es un indicador multifactorial. Por este motivo, en la presente tesis se estudiaron tanto factores de riesgo internos (propios de los individuos) como externos (propios del manejo y el ambiente). Estos factores fueron estudiados en cada uno de los puntos críticos considerados dentro de este período de cría (Capítulos 3, 4 y 5).

6.2. Importancia de los factores internos y su relación con la temperatura cloacal y la mortalidad durante la primera semana de vida

❖ ***Edad de las reproductoras: efecto sobre la temperatura cloacal***

Numerosos trabajos han estudiado el efecto de la edad de las reproductoras sobre distintos parámetros productivos tales como el peso del huevo, el porcentaje de nacimiento o el peso del pollito al nacimiento (Roque y Soares, 1994; Reis y col. 1997; Peebles y col., 2000). Tal y como hemos explicado en el Capítulo 3, la edad de las reproductoras tiene también un efecto sobre la temperatura cloacal de los pollitos, de modo que cuanto menor fue la edad de las reproductoras más baja fue la temperatura cloacal de los pollitos. Este hecho podría estar relacionado con la capacidad de termorregulación de los pollitos. En efecto, Weytjens y col. (1999) encontraron diferencias significativas en la temperatura cloacal y en los valores de T_3 (triiodotironina), de forma que los pollitos provenientes de madres viejas (65 semanas) tuvieron unos valores de T_3 al nacer más altos y una temperatura cloacal después de la exposición al frío también más alta que los pollitos provenientes de madres jóvenes (25 semanas). La T_3 es una hormona involucrada en el proceso de termorregulación (Decuypere y col., 1981), que actúa aumentando la tasa metabólica cuando la temperatura corporal de los pollitos disminuye.

Otra posible hipótesis que podría explicar las diferencias en la temperatura cloacal es el tamaño de los pollitos. Los pollitos provenientes de reproductoras jóvenes (25 – 33 semanas) tienen un tamaño menor en comparación con los pollitos provenientes de reproductoras viejas (51 – 68 semanas) (Tona y col. 2004). Dentro de una misma especie, cuanto más pequeño es el animal, más alta es su temperatura de confort. Esto es debido a la relación superficie/volumen: en efecto, un animal pierde calor en proporción a su superficie corporal, mientras que genera calor de manera proporcional a su volumen corporal. Como la relación superficie/volumen disminuye conforme aumenta el tamaño del animal, los animales pequeños son en general más susceptibles al frío que los animales grandes. Por tanto, los pollitos provenientes de reproductoras jóvenes tienen más probabilidades de sufrir estrés por frío.

Una propuesta para continuar con esta línea de investigación sería llevar a cabo un estudio en el que se exponga a 3 grupos de animales a distintos ambientes térmicos (temperatura baja, alta e intermedia) y se estudien las siguientes variables tamaño del pollito, edad de las reproductoras, temperatura cloacal y valores de T_3. De esta manera, a través de la monitorización de los individuos se podría estudiar de forma directa el efecto del tamaño de los pollitos y de la edad de las reproductoras sobre la temperatura cloacal y los valores de T_3 de los pollitos.

❖ *Edad de las reproductoras: efecto sobre la mortalidad*

Los resultados del Capítulo 5 indican que la edad de las reproductoras es un factor de riesgo que influye sobre la mortalidad de los pollitos, de forma que cuanto mayor era la edad de las reproductoras, mayor fue la mortalidad de los pollitos. Por el contrario, Suarez y col. (1997) y Yassin y col. (2009) obtuvieron resultados diferentes, según estos autores los pollitos con una mortalidad más elevada fueron aquellos provenientes de reproductoras jóvenes. Yassin y col. (2009) argumentan que las reproductoras jóvenes producen huevos más pequeños con una mayor proporción de albumen respecto a yema, y, por tanto, los pollitos provenientes de reproductoras jóvenes tienen un saco vitelino menor. La relación entre los nutrientes proporcionados por el saco vitelino y el desarrollo de los pollitos es directa, con lo que, los pollitos con un saco vitelino más pequeño tienen menos reservas de energía disponibles para su desarrollo. Este hecho esta frecuentemente asociado a una reducida viabilidad de los pollitos durante la primera semana de vida. No obstante, tal y como se argumenta en el Capítulo 5, es posible que este efecto se vea modificado por dos factores. El primero estaría relacionado con la metodología, ya que en los estudios realizados por Wilson (1991) y Suarez y col. (1997) no se utilizaron pollitos provenientes de reproductoras de las tres edades. El segundo factor puede relacionarse con la selección genética y la mejora de la nutrición. En efecto, los pollitos provenientes de madres jóvenes han compensado este menor tamaño de vitelo gracias a la selección genética y a la mejora en la nutrición. Sin embargo, los pollitos provenientes de reproductoras viejas continúan naciendo antes y tienen una peor calidad y cicatrización del ombligo, Esto explicaría porque, tal como se indica en el Capítulo 5 los pollitos pertenecientes a reproductoras viejas tuvieron una menor viabilidad.

❖ *Sexo de los pollitos: efecto sobre la temperatura cloacal y la mortalidad*

Nuestros resultados muestran que los pollitos macho tuvieron una temperatura cloacal inferior (Capítulo 3), así como una mortalidad más elevada durante la primera semana de vida (Capítulo 4 y 5) en comparación con las hembras. Estas diferencias no han podido ser contrastadas debido a la ausencia de estudios similares. Cabe pensar que si los pollitos macho muestran temperaturas cloacales inferiores también podrían tener una mayor tendencia a sufrir estrés térmico por frío. En efecto, cuando los pollitos tienen una temperatura cloacal por debajo del rango óptimo están más predispuestos a tener una mayor tasa de mortalidad durante la primera semana de vida (Capítulo 3). Por lo tanto, es de esperar que la tasa de mortalidad acumulada durante la primera semana de vida de los pollitos macho sea mayor al de las hembras. Sin embargo, no se pueden descartar otros efectos tales como la diferencia en el funcionamiento del sistema inmune. En efecto, Leitner y col. (1988) argumentan que estas diferencias en la viabilidad durante la primera semana de vida dependiendo del sexo podrían estar relacionadas con las diferencias halladas para la actividad de los linfocitos T y B entre machos y hembras. Ellos comentan que las hembras desarrollan una respuesta inmune efectiva antes que los machos, y, es esta diferencia en el desarrollo de la respuesta inmune, la que puede hacer que los machos sean más susceptibles a patógenos, traduciéndose en una mortalidad más alta durante la primera semana de vida.

En este caso, la propuesta de un próximo estudio en el que se considere el sexo como factor diferenciador para estudiar el mecanismo de termorregulación aportaría información novedosa acerca de cómo el dimorfismo sexual influye en la temperatura corporal de los pollitos. Por otra parte, la realización de más estudios epidemiológicos en los que se tenga en cuenta el sexo como uno de los principales factores de riesgo para la viabilidad de los pollitos aportaría más robustez a los hallazgos del presente estudio.

❖ *Estirpe de los pollitos: efecto sobre la mortalidad*

El factor interno "estirpe" tuvo una influencia sobre la mortalidad durante la primera semana de vida (Capítulo 5). Sin embargo, a diferencia de los otros factores internos, este factor únicamente se detectó en el último estudio (Capítulo 5). Otros autores encontraron también estas diferencias (Awobajo y col. 2007; Hamidu y col. 2007; Yassin y col. 2009); no obstante, las estirpes utilizadas fueron diferentes en todos los estudios. Los autores atribuyen estas diferencias a aspectos genéticos propios de cada estirpe como diferencias en el peso del huevo o en la actividad metabólica diaria del embrión durante la incubación. Es interesante mencionar, que Awobajo y col. (2007) observaron que estas diferencias para la tasa de mortalidad dependiendo de la estirpe se mantenían durante todo el ciclo productivo.

6.3. Importancia de los factores externos y su relación con la temperatura cloacal, la pérdida de peso y mortalidad durante la primera semana de vida

❖ *Factores externos en la planta de incubación: efecto sobre la temperatura cloacal*

Los factores externos que de acuerdo con el Capítulo 3 tienen una influencia sobre la temperatura cloacal de los pollitos son los siguientes: la posición de la cesta en el carro de las nacedoras, el tiempo que esperan los pollitos en la planta de incubación y el sistema de ventilación de la sala de expedición. Esto refleja que el sistema de termorregulación de los pollitos es inmaduro durante los primeros días de vida, y, por lo tanto, su temperatura corporal dependerá mucho de las condiciones ambientales. Otros autores obtuvieron resultados similares en los que quedaba patente la influencia del ambiente sobre la temperatura cloacal de los pollitos. Por ejemplo, Hai y col. (1996) expusieron pollitos a diferentes combinaciones de temperatura y humedad y detectaron efectos significativos sobre la temperatura cloacal. Nääs y col. 2014 detectaron que, dependiendo de la sala de la planta de incubación en la que se encontraran las pollitas, la pérdida de calor variaba según la temperatura ambiental que había en la sala. Este estrés térmico quedaría reflejado en la tasa de mortalidad durante la primera semana de vida, ya que los pollitos que en la planta de incubación tuvieron temperaturas por debajo del rango óptimo (< 39,7ºC) tuvieron también tasas de mortalidad más elevadas (Capítulo 3).

Sin embargo, al no realizar un seguimiento de los animales individualizado no se pudieron relacionar directamente los factores externos de la sala de incubación con la mortalidad durante la primera semana de vida. Por lo tanto, sería interesante realizar nuevos estudios similares a este, pero diferenciando a los pollitos por grupos según la posición de la cesta en el carro de las nacedoras, el tiempo que esperan los pollitos en la planta de incubación (1h, 2h, 3h o >3h), la temperatura media a la que están expuestos en la sala de expedición (<22ºC, 22-25ºC y >25ºC) y las veces que se acciona el sistema de ventilación de la sala de expedición.

❖ *Factores externos en el transporte: efecto sobre el peso*

En el Capítulo 4 de la presente tesis se detectó que la humedad relativa y la duración del transporte influyeron sobre la pérdida de peso de los pollitos. La humedad relativa es un factor externo que puede influir de manera directa sobre la temperatura de confort y sobre el intercambio de calor (sensible y latente) de los pollos de engorde (Lin y col. 2005; Schimidt y col. 2009). La pérdida de calor por evaporación aumenta con la temperatura, pero si la humedad relativa es alta, este intercambio disminuye. El efecto de estas condiciones sobre la termorregulación del pollito dependerá de la temperatura ambiental y de la edad del pollito (Lin y col. 2005; Shinder y col. 2007). Cuando la humedad relativa está por encima del 60%, la transmisión de calor desde el interior del cuerpo a la periferia se reduce. Por tanto, si la temperatura ambiental es elevada, el sistema de intercambio de calor puede verse comprometido y los pollitos necesitaran utilizar otras vías para perder calor a través de la aceleración del metabolismo. Parte de la energía será utilizada para el proceso de termorregulación y el peso del pollito puede verse reducido tal y como se observa en los resultados del Capítulo 4.

En cuanto a la duración del transporte, se detectó que cuanto más largo era el transporte, mayor era la pérdida de peso. Estos resultados coinciden con los obtenidos por Jacobs y col. (2016) y Bergoug y col. (2013). Sin embargo, estos autores sugirieron que cualquier reducción de peso después del transporte es probablemente debida al retraso en la ingestión de alimento. Tanto en sus estudios como en otros (Batal y Parsons, 2002; Bergoug y col. 2013), el efecto negativo del transporte sobre el peso de los pollitos no persistió hasta la edad de sacrificio.

Por último, para complementar los resultados obtenidos en nuestro estudio hubiera sido útil contemplar la posibilidad de añadir algún indicador fisiológico de bienestar. Por ejemplo, determinar la concentración plasmática de corticosterona antes y después del transporte nos habría permitido determinar el impacto del transporte sobre la respuesta fisiológica de estrés.

❖ *Factores externos durante el transporte: efecto sobre la mortalidad*

La cantidad de pollitos cargados por viaje tuvo una relación significativa con la mortalidad acumulada durante la primera semana de vida. Este hallazgo se podría relacionar con lo sugerido en el estudio de Nazareno y col (2016), en el que los autores explican que existen dos ambientes bien diferenciados, el ambiente en el interior de las cajas de pollitos y el ambiente en la zona de carga del camión. El ambiente del interior de la caja de pollitos tiene una temperatura y humedad relativa superiores a las del ambiente en la zona de carga. Si se considera la existencia de estos dos ambientes y el hecho de que se añaden más animales en un viaje, el gradiente creado entre estos dos ambientes puede tener una influencia sobre la temperatura corporal de los pollitos durante el transporte. En definitiva, si la temperatura ambiental y la cantidad de pollitos durante el transporte son elevados, esto llevaría a los pollitos a una posible situación de estrés térmico. Esto a su vez haría que los pollitos tuvieran más dificultades para disipar el calor en situaciones de altas temperaturas (Ernst y col. 1984; Yalçin y col. 1997). Finalmente, dicho estrés térmico podría causar deshidratación y aumentar la tasa de mortalidad durante los primeros días de vida (Bergoug y col. 2013; Piestun y col. 2017).

❖ ***Factores externos en la granja: efecto sobre la mortalidad***

Almacenamiento de los huevos

El almacenamiento de los huevos es una práctica común en las plantas de incubación para poder coordinar las actividades de la planta. La desinfección del huevo a su recepción o la coordinación de las cargas de huevos en incubadoras y nacedoras para programar nacimientos son algunas de estas actividades. De acuerdo con los resultados del Capítulo 5, el almacenamiento de los huevos influyó sobre la mortalidad de los pollitos durante la primera semana de vida. En este caso, a pesar de no estar relacionado directamente con la fase de granja, se trata de un factor externo muy interesante. Está demostrado que almacenamientos menores a siete días tiene poco o ningún efecto sobre la viabilidad de los embriones y el porcentaje de nacimiento (Dymond y col. 2013). Sin embargo, nuestros resultados indican que pollitos provenientes de huevos almacenados menos de siete días tuvieron una tasa de mortalidad superior a aquellos pollitos provenientes de huevos almacenados más tiempo. Está bien documentado que periodos de almacenamiento prolongados disminuyen la incubabilidad, el porcentaje de nacimiento (Fasenko y col., 2001b; Fasenko, 2007), y la calidad del pollito (Tona y col., 2003, 2004). Debido a esto, una de las técnicas recientemente más aplicadas son los periodos cortos de incubación durante el almacenamiento de los huevos ("*short periods of incubation during egg storage*" – SPIDES). Varios estudios confirman que esta técnica proporciona una buena restauración de la incubabilidad, del porcentaje de nacimiento y de la calidad del pollito (French y col., 2011; Nicholson y col., 2011; Nicholson y col., 2012). En nuestro caso, los huevos no eran tratados con la técnica de SPIDES hasta el día siete de almacenamiento. Debido a esto, cabría esperar que los resultados puedan ser similares con los obtenidos en huevos que sí que han sido tratados, demostrando la efectividad de esta técnica. De hecho, actualmente, las empresas de genética están recomendando el tratamiento de SPIDES, incluso, de los huevos recién llegados a la planta de incubación con el objetivo de homogeneizar tanto la temperatura de los huevos como la fase embrionaria.

Tipo de nave

Según nuestros resultados, el tipo de nave de engorde tuvo una influencia sobre la mortalidad durante la primera semana de vida. Concretamente, los pollitos alojados en granjas de una sola planta tuvieron una tasa de mortalidad más elevada que aquellos alojados en naves con múltiples plantas. A pesar de que las naves con múltiples plantas suelen ser más antiguas y consecuentemente más complicadas de manejar (*Bell y Weaver*, 2002), para los pollitos en edades tempranas este tipo de instalaciones pueden ser una buena opción. Cuando se realiza el precalentamiento de las naves para recibir a los pollitos, y teniendo en cuenta que el calor tiende a subir, las plantas superiores suelen acabar teniendo una temperatura de cama confortable. Tal y como hemos visto en capítulos anteriores de la presente tesis, la temperatura corporal de los pollitos y el ambiente al que se ven expuestos tienen un efecto muy marcado sobre su supervivencia.

Recuperador del bebedero

El tipo de sistema de abastecimiento de agua influyó sobre la mortalidad durante la primera semana de vida (Capítulo 5). Según nuestros resultados, los pollitos criados con bebederos tipo tetina sin recuperador tuvieron una mortalidad más elevada en comparación con aquellos que disponían de recuperador. Este resultado podría explicarse mediante dos hipótesis diferentes, una de ellas relacionada con la calidad de la cama y la otra con el comportamiento de los pollitos.

Los bebederos tipo tetina sin recuperador carecen de una pieza con forma de platillo que tiene como función recoger el agua que pueda caer de la tetina, evitando así problemas de cama húmeda. A largo plazo las camas húmedas pueden causar pododermatitis y a corto plazo pueden causar estrés por frío.

La segunda hipótesis es que, a pesar de que este sistema puede ofrecer múltiples beneficios para el sector productivo, altera el comportamiento de las aves de corral al beber agua. En particular, la acción llamada ‘cuchara’ en la que el ave baja la cabeza, toma agua en su pico y luego levanta la cabeza de nuevo, está completamente ausente (Houldcroft y col., 2008). Además, las aves tienen que aplicar presión con el pico en la tetina, acción que han de aprender para poder obtener el agua (Appleby y col., 2004). Aunque existen razones productivas importantes para escoger este tipo de sistemas, no parecen ser la mejor opción para los pollitos ya que suprimen una conducta natural y aumentan las probabilidades de obtener una tasa de mortalidad superior durante la primera semana de vida (Heier y col. 2002; Carpenter y col. 1990).

Para ampliar estos resultados sería interesante llevar a cabo un estudio adicional en condiciones comerciales en el que se estudiase la influencia del recuperador del bebedero. En este caso, dentro de una misma nave compartimentada, un grupo de pollitos se criaría con bebederos tipo tetina sin recuperador del bebedero y otro grupo de pollitos se criaría con recuperadores de bebedero. Para obtener unos resultados más robustos se deberían realizar, por ejemplo, 6x2 repeticiones (2 engordes en 6 naves). Con el fin de reducir la variabilidad, los pollitos del estudio deberían provenir de un mismo lote (misma estirpe y edad de las reproductoras) y deberían ser del mismo sexo. Por otra parte, además de considerar como variable de estudio la mortalidad durante la primera semana de vida, sería importante tener en cuenta la cantidad de agua ingerida por día y grupo, así como el consumo de alimento y el peso de los pollitos durante los siete días de estudio. Además, la adición de observaciones de comportamiento de los pollitos, como el número de veces que ingieren agua, enriquecería los resultados del estudio.

Año de estudio

La mortalidad durante la primera semana de vida fue diferente entre los distintos años de estudio. Estos resultados coincidirían con los obtenidos por Yassin y col. 2009 y Heier y col. 2002. Por otra parte, se observó una tendencia en que, a medida que pasaban los años, disminuía la tasa de mortalidad. Este hecho podría estar relacionado con la implementación de medidas de bioseguridad y manejo más efectivas. No obstante, no se puede descartar la influencia de la mejora genética de las estirpes de aves a lo largo de los años.

<u>Estacionalidad</u>

De acuerdo con los resultados del Capítulo 5, la estacionalidad tuvo una influencia sobre la mortalidad durante la primera semana de vida. La tasa de mortalidad más elevada se detectó durante el otoño y el invierno. La estacionalidad detectada en la mortalidad podría estar relacionada con el clima, debido a que las temperaturas más bajas se dan durante el invierno. Tal como hemos mencionado en párrafos anteriores, los pollitos carecen de un sistema de termorregulación maduro por lo que, si son expuestos a bajas temperaturas debido a un mal manejo, su tasa de mortalidad puede llegar a triplicarse (Yerpes y col. En revisión). Otra posible razón de esta estacionalidad podría estar relacionada con la fluctuación del mercado. Cuando la demanda es alta, las incubadoras compran muchos huevos a proveedores externos. Estos huevos, sin embargo, pueden tener una calidad muy variable y como resultado de ello los pollitos pueden tener una viabilidad menor.

Finalmente, cabe comentar que durante la realización de este (Capítulo 5) y otros estudios (Capítulo 3 y 4) no se contemplaron aspectos relacionados con la sanidad. Estos aspectos hubieran aportada información adicional, descartando la posibilidad de que la mortalidad durante la primera semana de vida pudiera deberse en parte a aspectos sanitarios y no a aspectos relacionados directamente con el manejo. Así pues, en el diseño de estudios futuros sería interesante contemplar la diferenciación de procesos o granjas con un estado sanitario óptimo o deficiente.

6.2. Estrategias de carácter práctico para mejorar el bienestar y disminuir la mortalidad durante la primera semana de vida

De acuerdo con los resultados obtenidos, a continuación, se abordan algunas estrategias prácticas encaminadas a mejorar el bienestar de las aves y como consecuencia disminuir su tasa de mortalidad durante la primera semana de vida:

1. Las diferencias en la capacidad de termorregulación de los pollitos considerando únicamente la edad de las reproductoras (Capítulo 3) puede implicar la necesidad de cambios en el manejo dentro de la planta de incubación. Así, considerando la variabilidad climática según el área geográfica o el tipo de granja, el hecho de seleccionar a los pollitos de acuerdo con la edad de las reproductoras podría ser útil para disminuir el estrés térmico, ya que los pollitos procedentes de madres jóvenes son más resistentes al calor, mientras que los procedentes de madres viejas son más resistentes al frío (Weytjens y col. 1999).

2. Los pollitos provenientes de reproductoras más viejas nacen antes. Por tanto, en la práctica diaria de las plantas de incubación debería tenerse en cuenta este factor para el manejo de la ventana de nacimiento y la retirada de los pollitos de la nacedora. Concretamente, para mejorar el bienestar de las aves solo se deberían cargar lotes de una misma edad en una misma nacedora. De esta manera se podría ajustar la ventana de nacimiento al máximo y la retirada de los pollitos se llevaría a cabo en un momento óptimo, evitando así el estrés térmico.

3. Los tratamientos de termotolerancia durante el desarrollo embrionario o incluso durante el periodo de cría se han descrito como estrategias para evitar el estrés térmico de las aves. Por ejemplo, Yalçin y col. (2008) concluyeron que la exposición de los embriones a altas temperaturas durante la incubación mejora la

termotolerancia de los pollitos. Esta práctica podría ser útil para la aumentar la adaptación de los pollos de engorde a distintos ambientes.

4. Las diferencias detectadas entre sexos en cuanto a la temperatura cloacal (Capítulo 3) y la mortalidad durante la primera semana de vida (Capítulo 4 y 5) sugieren dos estrategias distintas. La primera estrategia estaría dirigida al periodo embrionario, en el que debería asegurarse una temperatura intermedia y homogénea durante la incubación con el objetivo de evitar el estrés térmico embrionario y consecuentemente mejorar la viabilidad de los embriones. La segunda estrategia estaría dirigida al periodo postnatal; en este caso, debido a las diferencias del sistema inmune entre sexos, debería asegurarse una adecuada vacunación de los pollitos, haciendo especial énfasis en los machos.

5. Las diferencias detectadas en la mortalidad durante primera semana de vida según la estirpe (Capítulo 5), y la influencia de la misma sobre otros aspectos (Viera y Moran, 1998b; Hamidu y col. 2007) sugiere que este factor debe tenerse en cuenta en las decisiones de manejo de la planta de incubación. Concretamente, la carga de huevos idealmente solo debería contemplar un lote de la misma estirpe y edad por máquina. Por otra parte, sería útil considerar las características de cada estirpe antes de seleccionar una de ellas, con el objetivo de utilizar la que mejor se adapte a las necesidades y condiciones de cada empresa.

6. Deberían controlarse las variaciones de temperatura detectadas entre las cestas de nacimiento dependiendo de su posición en el carro de las nacedoras (Capítulo 3) con el objetivo de que estas variaciones sean mínimas.

7. La identificación de los factores externos que tienen una influencia sobre la temperatura cloacal de los pollitos en la planta de incubación (Capítulo 3) indica que es necesario considerar más puntos de control ambiental en las diferentes salas y procesos de la planta de incubación.

8. En relación con la pérdida de peso detectada durante el transporte y dependiente del tiempo (Capítulo 4), sería de especial interés tener en cuenta el aporte de una fuente de hidratación y de nutrientes.

9. En el Capítulo 4 se explicó que la pérdida de peso de los pollitos está relacionada con la humedad relativa durante el transporte (Capítulo 4). Por tanto, es posible que una mejora de los sistemas de climatización y ventilación de los camiones permitiera reducir esta pérdida de peso.

10. La heterogeneidad ambiental detectada en la zona de carga durante el transporte de los pollitos (Capítulo 4) indica que es necesario un mayor control de las condiciones ambientales durante el transporte. La adición de más sondas y la monitorización de los transportes podrían ser útiles.

11. El tipo de nave de engorde tiene un efecto sobre la mortalidad de los pollitos durante la primera semana de vida (Capítulo 5). Este hecho posiblemente está relacionado con la temperatura de la nave y su distribución en el espacio. Por lo tanto, es importante asegurar que las naves alcancen una temperatura ambiental y de cama adecuada y homogénea para mejorar el bienestar y la supervivencia de las aves.

12. Los resultados del Capítulo 5 sugieren que la adición de los recuperadores de bebederos puede ser una buena estrategia ya que reduciría el riesgo de deshidratación de los pollitos y consecuentemente la mortalidad durante la primera semana de vida, y aumentaría las probabilidades de mantener una calidad óptima de la cama.

13. El almacenamiento de los huevos puede influir en la calidad y viabilidad de los embriones. Sin embargo, como ha quedado reflejado en el Capítulo 5, el almacenamiento prolongado de los huevos no tiene por qué afectar de manera negativa, siempre y cuando se utilice la técnica de SPIDES. Por tanto, una

estrategia potencialmente útil seria implementar esta técnica incluso desde la llegada de los huevos a la planta de incubación.

14. La estacionalidad influye sobre la mortalidad durante la primera semana de vida (Capítulo 5). Por lo tanto, es importante controlar y minimizar en lo posible la variabilidad estacional asociada a la estacionalidad. Las empresas deberían realizar una revisión periódica de las condiciones ambientales de todas las salas de la planta de incubación, de las granjas y del vehículo de transporte.

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Por último, es importante recordar que los estudios realizados bajo condiciones comerciales resultan difíciles de ejecutar, entre otras razones por la dificultad que entraña controlar los factores que pueden interferir en los resultados, así como por la dificultad de disponer en todo momento de equipos o instalaciones adecuadas. Además, las plantas de incubación y las granjas tienen rutinas de manejo que pueden ser distintas y que no son fáciles de prever. A pesar de estas dificultades, esta tesis aporta datos que reflejan la importancia de realizar más estudios de campo en las condiciones reales que existen en las plantas de incubación y en las granjas. La recolección de datos de forma periódica y sistemática, junto con estudios de carácter más controlado, puede constituir una buena herramienta para alcanzar una mejora continuada en un sector altamente competitivo.

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Capítulo 7
Conclusiones

"Hermoso es lo que vemos. Más hermoso es lo que sabemos. Pero mucho más hermoso es lo que no conocemos"

N. Stensen

Objetivo específico 1: Identificar los factores en la planta de incubación que aumentan el riesgo de estrés térmico en los pollitos de un día de vida (Capítulo 3).

Conclusiones:

1. *Los factores que influyeron sobre la temperatura cloacal de los pollitos en la planta de incubación fueron los siguientes: edad de las reproductoras, sexo de los pollitos, posición de la cesta de nacimiento, tiempo de espera hasta el muestreo, tiempo de muestreo y ventilación en la sala de expedición.*

Objetivo específico 2: Determinar si existe una relación entre el estrés térmico de los pollitos de un día de vida y la mortalidad acumulada a siete días de vida (Capítulo 3).

Conclusiones:

2. *Una temperatura cloacal inferior a la recomendada (<39,7ºC) incrementó la mortalidad de los pollitos hasta tres veces.*

Objetivo específico 3: Estudiar el efecto del transporte de los pollitos de un día de vida sobre la mortalidad y la pérdida de peso (Capítulo 4).

Conclusiones:

3. *La humedad relativa y la duración del transporte tuvieron un efecto sobre el peso de los pollitos durante el transporte, de forma que una humedad alta y un transporte largo se asociaron a una mayor pérdida de peso.*

4. *Las condiciones climáticas, la duración del transporte, el número y tipo de paradas durante el viaje, la cantidad de animales cargados en cada viaje, el mes, la estirpe, el sexo de los pollitos, la edad de las reproductoras, el lote y los días de almacenamiento de los huevos no tuvieron ningún efecto sobre la mortalidad durante el transporte.*

5. *La cantidad de pollitos cargados en cada viaje y el sexo de los pollitos afectaron a la tasa de mortalidad durante la primera semana de vida, de forma que los pollitos macho y una alta carga de pollitos durante el transporte se asociaron a una mayor mortalidad durante la primera semana de vida.*

Objetivo específico 4: Evaluar las condiciones ambientales del transporte de los pollitos de un día de vida (Capítulo 4).

Conclusiones:

6. *Se detectó una acusada heterogeneidad ambiental en la zona de carga del camión que refleja la falta de control de los sistemas de climatización y ventilación durante el transporte.*

Objetivo específico 5: Identificar mediante una metodología basada en el análisis de riesgo los factores internos (propios de los individuos) y externos (de manejo y ambientales) asociados con la mortalidad acumulada durante la primera semana de vida de los pollos de engorde, y cuantificar el riesgo atribuible a cada factor (Capítulo 5).

Conclusiones:

7. *Existe una interrelación entre la mortalidad durante la primera semana de vida en la granja y los factores de manejo en las granjas de reproductores (edad de las reproductoras y estirpe), en las incubadoras (días de almacenamiento del huevo y manejo al nacimiento) y en la granja de engorde (tipo de nave, tipo de bebederos y estacionalidad).*

Capítulo 8
Curriculum del autor

Personal Information:

Surnames, Name: Yerpes Ron, Marta

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Location: Barcelona (Spain)

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Education:

2016-Present PhD Student in Animal Science (Thesis defense: February 2020)
Universitat Autònoma de Barcelona

2019 M.Sc. in Animal Welfare
Universitat Autònoma de Barcelona

2010-2016 Degree in Veterinary Medicine
Universitat Autònoma de Barcelona

Post-graduate courses:

2018 Training course for Researchers on Laboratory Animal management (FELASA)
Universitat Autònoma de Barcelona

2016 Statistical techniques in SAS®
Universitat Autònoma de Barcelona

Professional experience:

2016-Present Poultry technician in Ceva Salud Animal S.A.

- Collaboration with poultry team of Ceva Spain. Auditing vaccine preparations and equipment functioning. Carrying out embryonic development diagnosis, necropsies and other job-related functions.

2016-Present — Member of the Animal Nutrition and Welfare Service (SNiBA)

- Collaboration in research projects of the animal science field (experimental design, farm controls and samplings, laboratory and statistical analysis, reports writing and scientific divulgation).

Skills and competences:

Language

Spanish	●●●●●
Catalan	●●●●●
English	●●●●○

Software

Microsoft Office	●●●●●
SAS®	●●○○○

Scientific publications:

Yerpes, M., I. Hernández, and X. Manteca. 2019. Thermal stress in day-old chicks: risk factors and effects on mortality. Animal Welfare (*Under review*).

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Communications at scientific meetings:

Yerpes, M., M. Colvee, and X. Manteca. (2019). Factores de riesgo asociados a la mortalidad acumulada durante la primera semana de vida en pollitos de engorde. 56 Simposio AECA, Donostia/San Sebastián, Spain. Poster presentation

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