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Ingrid de Jong, Johan van Riel, Sander Lourens, Marc Bracke, Henry van den Brand

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I.C. de Jong[1], J. van Riel[1], A. Lourens[1], M.B.M. Bracke[1] H. van den Brand[2]

1 Wageningen University and Research, Wageningen Livestock Research

2 Wageningen University and Research, Adaptation Physiology Group

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P.O. Box 338, 6700 AH Wageningen, The Netherlands, T +31 (0)317 48 39 53,
E info.livestockresearch@wur.nl, www.wur.nl/livestock-research. Wageningen Livestock Research is part of Wageningen University & Research.

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Summary

The aim of the current study was to perform a systematic literature review and meta-analysis to determine effects of post-hatch food and water deprivation on chicken development, performance, health and welfare. Two types of meta-analysis were performed on peer-reviewed scientific publications: a quantitative meta-analysis (MA), and a qualitative analysis (QA). In the MA, effects of post-hatch food and water deprivation at various points in time were quantified, for measures related to production performance (body weight, food intake, food conversion ratio), mortality and relative yolk sac weight. With respect to other measures, insufficient data were available to perform a quantitative meta-analysis. In the QA, the number of significant positive, significant negative and non-significant effects were counted for all measures of which five records or more were present in the data set for one or more age/treatment combinations. This resulted in a QA for production measures and mortality; relative yolk sac, heart, liver and pancreas weight; plasma T3, T4 and glucose concentrations; relative duodenum, jejunum and ileum weight; duodenum, jejunum and ileum length; and villus height and crypt depth in duodenum, jejunum and ileum. Despite a number of studies on immunological measures and disease susceptibility in relation to post-hatch food and water deprivation, insufficient records for these measures were available for MA or QA.

Results of the MA showed that post-hatch food and water deprivation resulted in significant lower body weights compared to early fed chickens throughout the growing period up to 6 weeks of age, also after 24h (i.e. 12-36h) of food and water deprivation post-hatch. In addition, body weights and food intake were more reduced with increased food and water deprivation durations post-hatch (24, 48, 72, >84h post-hatch). Food conversion rate was significantly increased up to 21 and 42 days of age with >84h post-hatch food and water deprivation compared to immediate feeding. Total mortality at day 42 was significantly higher with 48h post-hatch food and water deprivation compared to immediate feeding. First week mortality was higher in >84h post-hatch food and water deprived chickens compared to early fed chickens.

The QA for production measures showed similar results as the MA. The QA of mortality showed that individual studies found no significant effects of post-hatch food and water deprivation on total mortality, in contrast to the MA for total mortality, which combined the results of multiple studies.

The MA showed no effect of post-hatch water provision, i.e. the consequences of food deprivation cannot be reduced by providing the food deprived chickens with water.

The MA and QA for relative yolk sac weight showed inconclusive results of post-hatch food and water deprivation, but we found an effect of providing water. I.e. for 72h (60-84h) food and water deprivation relative yolk sac weight at Day 3 was significantly lower, and no effects were found for 24h and 48h food and water deprivation. However, when water was provided post-hatch to chickens that were deprived of food for 72h post-hatch, relative yolk sac weights were significantly higher. The QA for plasma T3, T4 and glucose concentrations indicated mainly short-term decreases in T3 and glucose in food and water deprived chickens compared to early fed chickens.

Relative weights of liver, pancreas and heart were lower after post-hatch food and water deprivation, but only in the first week of life and not thereafter, as indicated by the QA. A reduced development of gut segments (duodenum, jejunum and ileum) was found in the first week of life, measured by decreased length, decreased relative weight, decreased villus height and crypt depth in these gut segments. However, these effects were not found in all studies, and negative or positive effects on these measures were sometimes observed after the first week of life when chickens were subjected to post-hatch food and water deprivation. This indicates short-term, but no long-term effects of post-hatch food and water deprivation on small intestine length and weight, villus height and crypt depth.

A wide variety in immune measures and disease challenges has been applied with hardly any overlap or repetition. It was therefore impossible to perform a QA or MA on these variables, so we cannot provide conclusions on the effect of food and water deprivation on health or disease susceptibility.

Studies indicate that chickens start to peck and ingest food a few hours after hatch. It is unclear whether early food- and water deprivation actually leads to enhanced feeding motivation and/or a change in behavioural development.

Various factors may influence the response of chickens to post-hatch food and water deprivation. These were divided in biotic factors (e.g., type (layer vs broiler), strain, parent stock age) and abiotic factors (e.g. incubation conditions, early housing conditions, diet composition) and discussed. These may explain the large variation in outcomes of the various studies with respect to the effects of post-hatch food and water deprivation. The extent to which these factors affect the response to post-hatch food deprivation is actually unknown.

Based on a thorough analysis of the existing literature, it is concluded that 48h (36-60h) post-hatch food and water deprivation leads to lower body weights and higher total mortality in chickens up to 6 weeks of age. From the existing scientific literature, it is unclear whether the delayed development of post-hatch food deprived chickens compared to early fed chickens has any consequences on welfare (including health, production and development) on the long-term, e.g., whether it affects the susceptibility for diseases for example. There might be compensation for altered growth or physiological development during the life span of the chicken, since post-hatch food deprivation may also cause a delay in development, instead of a more long-lasting or permanent effect.

Samenvatting

Het doel van dit rapport was om een systematisch literatuuronderzoek en een meta-analyse uit te voeren om effecten van voer- en waterdeprivatie van kuikens na het uitkomen te bepalen op de ontwikkeling, de productie, de gezondheid en het welzijn van kuikens. Er zijn twee soorten meta-analyse uitgevoerd op basis van de beschikbare wetenschappelijke literatuur: een kwantitatieve meta analyse (MA) en een kwalitatieve analyse (QA). In de MA zijn de effecten van de verschillende duur van voer- en water deprivatie na het uitkomen gekwantificeerd. Dit is uitgevoerd voor parameters gerelateerd aan productie (lichaamsgewicht, voeropname, voederconversie), mortaliteit en relatief dooierzakgewicht. Van andere parameters waren er onvoldoende gegevens beschikbaar om een MA uit te voeren. In de QA is het aantal significant positieve, significant negatieve of niet-significante effecten geteld voor alle parameters waarvan meer dan 5 records in de dataset aanwezig waren voor één of meerdere combinaties van behandeling en leeftijd. Dit resulteerde in een QA voor productievariabelen en mortaliteit; voor relatief dooierzakgewicht, relatief hart-, lever- en pancreasgewicht; voor plasma T3, T4 en glucose concentraties; voor duodenum, jejunum en ileum lengte en gewicht; duodenum, ileum en jejunum villus lengte en crypt diepte. Ondanks dat immunologische parameters zijn gemeten in een aantal publicaties waren er onvoldoende data beschikbaar om een MA of QA uit te kunnen voeren voor de immunologische parameters.

Resultaten van de MA toonden aan dat de lichaamsgewichten van voer- en water gedepriveerde kuikens in vergelijking met kuikens die na uitkomen meteen voer en water verstrekt kregen significant lager waren tot en met zes weken leeftijd, ook bij 24h (12-36h) voer- en water deprivatie na uitkomen. Met een toenemende duur van voer- en waterdeprivatie na uitkomen (24, 48, 72, >84 uur na uitkomen) werd een grotere verlaging van het lichaamsgewicht en de voeropname tot zes weken leeftijd gevonden. De voerconversie gemeten op dag 21 en 42 was significant hoger voor >84 uur voer- en water gedepriveerde kuikens in vergelijking met niet-gedepriveerde kuikens. De totale mortaliteit van dag 0-42 was significant hoger wanneer kuikens 48 uur (36-60 uur) geen water en voer kregen na het uitkomen in vergelijking met kuikens zonder voer- en waterdeprivatie na uitkomen. De mortaliteit in de eerste week na uitkomen was significant hoger bij >84 uur voer- en water deprivatie vergeleken met niet-gedepriveerde kuikens.

De resultaten van de QA voor de productievariabelen waren vergelijkbaar met die van de MA. De QA van de totale mortaliteit liet zien dat in individuele studies geen effect werd gevonden van voer- en waterdeprivatie na uitkomen op de totale mortaliteit van dag 0 tot 42, in tegenstelling tot de resultaten van de MA voor totale mortaliteit waarin de resultaten van verschillende studies werden gecombineerd.

De MA toonde aan dat er geen effect was van waterverstrekking na uitkomen, dus, de consequenties van voerdeprivatie na uitkomen waren niet anders wanneer kuikens na het uitkomen water verstrekt kregen.

De MA en QA voor de effecten van voer- en waterdeprivatie op relatief dooierzakgewicht lieten geen consistente resultaten zien. Bij 72h (60-84h) voer- en waterdeprivatie was het relatief dooierzakgewicht op 3 dagen leeftijd significant lager dan bij niet-gedepriveerde kuikens, en er werden geen effecten van 24 en 48 uur voer- en waterdeprivatie op relatief dooierzakgewicht gevonden. Echter, wanneer water werd verstrekt na uitkomen aan kuikens die voer gedepriveerd werden gedurende 72 uur na uitkomen, waren de relatieve dooierzakgewichten in de 72 uur gedepriveerde kuikens significant hoger dan in de niet-voer gedepriveerde kuikens. De QA voor de effecten van voer- en waterdeprivatie op plamsa T3, T4 en glucose concentraties liet zien dat dat er met name korte-termijn dalingen waren in plasma T3 en glucose concentratie in voer- en water gedepriveerde kuikens ten opzichte van niet-gedepriveerde kuikens.

De QA liet zien dat relatieve gewichten van de lever, pancreas en het hart lager waren na voer- en waterdeprivatie in vergelijking met gewichten van deze organen in niet-gedepriveerde kuikens. Ook werd een effect gevonden van voer- en waterdeprivatie op de ontwikkeling van darmsegmenten in de eerste levensweek. De lengte, het relatieve gewicht, de villus hoogte en crypt diepte van de duodenum, het jejunum en het ileum waren vaak minder bij voer- en water gedepriveerde kuikens dan bij niet-gedepriveerde kuikens. Deze effecten werden echter niet in alle studies aangetoond. Enkele studies lieten positieve- of negatieve effecten zien van voer- en waterdeprivatie op]indicatoren voor darmmorfologie na de eerste levensweek. Dit geeft aan dat er met name korte-termijn effecten zijn van voer- en waterdeprivatie op de lengte en het relatieve gewicht van de darm en op de villus hoogte en crypt diepte.

Een grote variatie in gebruikte challenges en toegepaste immuunparameters na voer- en waterdeprivatie werd gevonden in de wetenschappelijke literatuur, met nauwelijks overlap of herhaling tussen studies. Daarom was het niet mogelijk om een QA of MA uit te voeren op deze indicatoren, dus we kunnen geen conclusies trekken over het effect van voer- en waterdeprivatie na uitkomen gezondheid en ziektegevoeligheid van kuikens.

Onderzoek heeft laten zien dat kuikens na een paar uur na het uitkomen beginnen te pikken naar voer. Het is niet duidelijk of voer- en waterdeprivatie na uitkomen leidt tot een verhoogde motivatie om voer op te nemen en /of veranderingen in de ontwikkeling van het gedrag.

Verschillende factoren kunnen de respons van kuikens op voer- en waterdeprivatie beïnvloeden. Deze werden onderverdeeld in biotische (bijvoorbeeld type (leghen versus vleeskuiken), ras, leeftijd ouderdieren) en abiotische factoren (bijvoorbeeld incubatie condities, huisvestingsomstandigheden, voersamenstelling) en besproken in het rapport. Deze factoren kunnen mogelijk de grote variatie in uitkomsten tussen studies naar de effecten van voer- en waterdeprivatie verklaren. De mate waarin deze factoren invloed hebben op de effecten van voer- en waterdeprivatie is echter onbekend.

Gebaseerd op een grondige analyse van de wetenschappelijke literatuur wordt geconcludeerd dat 48 uur (36-60 uur) voer- en waterdeprivatie na uitkomen van kuikens leidt tot een verminderd lichaamsgewicht en een hogere totale mortaliteit van 0 tot 42 dagen leeftijd in vergelijking met kuikens die niet werden gedepriveerd. Het is niet bekend of de vertraagde ontwikkeling van kuikens die na uitkomen gedepriveerd worden van voer en water, in vergelijking met niet-gedepriveerde kuikens, het welzijn (inclusief gezondheid, productie en ontwikkeling) beïnvloedt op de lange termijn (zoals de gevoeligheid voor ziekten). Uit deze literatuuranalyse is niet helder geworden of de lagere groei na voer- en waterdeprivatie het gevolg is van een vertraagde start van de voeropname of dat het komt door een pemanente minder goede ontwikkeling van de kuikens.

1 Introduction

As a follow up of a legal procedure that started in 2014, in 2016 the NGO 'Wakker Dier' took legal action against the Minister of Economic affairs ('Staatssecretaris van Economische Zaken') at the 'College van Beroep voor het bedrijfsleven (CBb)', requesting that the Minister enforces current welfare legislation, involving the provision of food and water to chickens in hatcheries. The current legislation concerns the 'Wet dieren' [1] and 'Besluit houders van dieren' [2]. Legislative details will be further explained in paragraph 1.2 and in Appendix 1.

'Wakker Dier' [3, 4] argues that hatcheries should provide food and water to newly-hatched broiler and laying hen chickens. In a commercial hatchery, broiler and laying hen chickens and turkey poults (from here onwards we will refer to these as chickens) usually hatch over a period of 24-48 hours (the 'hatch window') [5-8]. Newly-hatched chickens remain in the incubator until almost all chickens have hatched, after which all chickens are collected. This is usually performed at 21.5 days (broilers and laying hens) after the onset of incubation. After collection, also called pulling, the chickens undergo hatchery treatments, such as selection of second-grade chickens, vaccination, sex determination and sorting. Thereafter, the chickens are transported to the farm, which may involve a further period of food and water withdrawal. This all means that the duration of the whole process, i.e. the total time between hatching and first food and water intake at placement on the farm, is variable and dependent on the aforementioned factors such as hatch window, hatchery treatments and transport duration [6-10]. The time until first food and water intake ('holding period') can be 50 hours or more and may take up to 72 hours if long transportation is involved [7, 8].

According to the Minister of Agriculture, and based on a report by Lourens and Leenstra [6], chickens are able to survive on the yolk sac reserves during 72 hours after hatching. 'Wakker Dier' recognises that yolk sac reserves are important for survival of chickens, but also thinks that these yolk sac reserves are insufficient to overcome the first 72 h after hatching. They also indicate that food and water deprivation after hatching has long-term negative consequences for the welfare of chickens, and that the animals' behavioural and physiological need for food and water are not met in the current hatchery practice [3]. Therefore, in 2014, 'Wakker Dier' requested, via a lawsuit, the State Secretary of Economic Affairs to force hatcheries to provide food and water immediately after hatching and preceding the transport of day-old chickens to the farm. This request of 'Wakker Dier' was considered not to be legally based, after which 'Wakker Dier' started a legal action via the CBb.

Following this legal procedure, on June 29, 2016 the CBb dissolved the refusal of the State Secretary of Economic Affairs to force hatcheries to provide food and water immediately after hatching [11]. On June 29, 2016 the CBb decided that the State Secretary of Economic Affairs should ask Wageningen University and Research to provide comments to the response of 'Wakker Dier' [4] to the report of Lourens and Leenstra [6], and to provide insight in the effects of food and water deprivation after hatching on the development, performance, health and welfare of chickens based on findings from scientific studies. The detailed questions of CBb to Wageningen University and Research are listed in paragraph 1.2.

1.1 Legislation in relation to the current study

The NGO 'Wakker Dier' requests that the Secretary of State enforces current welfare legislation involving the provision of food and water to chickens in hatcheries. According to 'Wakker Dier' hatcheries break the existing legislation, i.e. 'Wet Dieren [1] and 'Besluit Houders van Dieren'[2], if they do not provide chickens with food and water post-hatch. The full text of all legislation related to the current report can be found in Appendix 1 (in Dutch). Here, we provide a short summary as far as this is relevant to the question whether post-hatch food and water deprivation affects the welfare of chickens.

Article 1.3. of ‘Wet Dieren’ indicates, in summary, that animals have an intrinsic value and should be provided with essential care. This includes that animals are free from (a) thirst, hunger or inappropriate feeding, (b) physical and physiological discomfort, (c) pain, injuries and disease, (4) fear and chronic stress and (e) restrictions in their natural behaviour (see Appendix 1). However, according to CBb, article 1.3. of ‘Wet Dieren’ is, as such, not enforcing. Therefore, in addition, additional articles of ‘Wet Dieren’ and ‘Besluit Houders van Dieren’ should be applied; i.e. Article 2.2. sub 8 of ‘Wet Dieren’ and Article 2.4. sub 6 of ‘Besluit Houders van Dieren’.

Article 2.2. sub 8 of ‘Wet Dieren’ states that it is forbidden to withdraw animals from essential care. Article 1.7. e and Article 2.4. sub 6 of ‘Besluit Houders van Dieren’ state that the person that keeps an animal should provide the animal with an appropriate amount of food according to the stage of development and age of the animal, and that the animal should receive food with intervals according to its physiological need. Article 1.7 f of ‘Besluit houders van Dieren’ states that ‘the person that keeps an animal, should provide a sufficient amount of water of sufficient quality to the animal or fulfil the need for water in another way’. These articles replace previous legislation. For details see Appendix 1.

In addition, the report of Lourens and Leenstra [6] refers to the Council Regulation (EC) No 1/2005 of December 22, 2004 on the protection of animals during transport, Appendix 1, Chapter 5 [12]:
‘2. Other species
2.1. For poultry, domestic birds and domestic rabbits, suitable food and water shall be available in adequate quantities, save in the case of a journey lasting less than:
(a) 12 hours disregarding loading and unloading time; or
(b) 24 hours for chickens of all species, provided that it is completed within 72 hours after hatching.’

However, this regulation refers to the provisioning of food and water during the duration of transport of 0-3-day-old chickens and, hence it is not relevant in relation to the main research question in the current report, which concerns (primarily) the question whether food and water should also be provided prior to transport.

1.2 Aim and approach of the study

The aim of the current study is to provide an overview of the state-of-the-art of the scientific literature with respect to the effect of food and water deprivation on the development, performance, health and welfare of chickens. The definition of animal welfare and health as applied in the current report will be explained in paragraph 2.1.

This report shows the results of a systematic analysis of scientific literature conducted on this subject, either in a quantitative meta-analysis (MA) or in a qualitative analysis (QA). The results of the analyses will be discussed in Chapter 4. In the discussion section, the detailed questions as posed by CBb to Wageningen University and Research will be taken into account:

- To provide comments to the response of Wakker Dier [4] on the report of Lourens en Leenstra [6];
- To include the scientific publications, that were referred to in the publication of Wakker Dier [3, 4], in the above requested comments;
- Refer to the current legislation in relation to the request of Wakker Dier (done above and in Appendix 1);
- To provide clarification to the response of Wakker Dier [4] that the content of the report [6] and the context (current legislation) it refers to are not correct (the basic assumptions in [6] referred to legislation stating that food and water should be provided within 72 h after hatching. However, the period of 72 h concerns transport of day-old chickens according to the EU transport Directive and does not relate to providing food and water after hatching; see also Appendix 1 related legislation);
- To indicate how long chickens, immediately after hatching, can survive on the yolk sac reserves (only), without depriving them from essential care, because they do not receive food and water after hatching. In addition, it is indicated that the current report should not only concern the

survival of the chickens, but also whether their welfare and health requirements are matched if they are deprived from food and water after hatching.

2 Materials and methods

2.1 Animal welfare definition

One of the questions to be answered in the current report is whether the welfare requirements of chickens are met if they are deprived from food and water immediately after hatching. We here define animal welfare as follows. Animal welfare has been defined as the quality of life as perceived by the animals themselves [13]. Feelings and perceptions, however, cannot be measured. Hence, animal-welfare scientists measure various aspects related to biological functioning and the animal's ability to cope [14]. In order to assess overall welfare, the various welfare needs have to be assessed. Each welfare need may be regarded as a cognitive-emotional control mechanism, which has evolved in evolution (and domestication) to deal with a variable environment [15]. Each need has an emotional dimension (affect; making the need relevant for welfare), a behavioural dimension (e.g. including behavioural elements associated with explorative and consumatory aspects of behaviour), a physiological dimension and a patho-physiological (health) dimension.

The need for food and the need for water are important welfare needs, because they are necessary for survival. Their emotional dimensions are commonly referred to as hunger and thirst. Behavioural elements include foraging (searching for food, e.g. scratching) and consumatory behaviours (e.g. pecking). The physiological dimension of food and water intake have to do with feeding behaviour (e.g. muscular activity involved in pecking; stress related to food deprivation) and metabolism (passage of food through the GI system, nutrient uptake, etc.). Patho-physiological measures concern situations when normal homeostatic or allostatic control mechanism are overtaxed, potentially leading to pathology/illness. These patho-physiological measures related to health include immunological measures (cellular and humoral) and disease susceptibility and morbidity and mortality measures.

Production performance measures (growth, food conversion) are also part of the normal physiology of the animal. Performance in relation to the capacity of the animal (i.e. production performance rather than welfare performance) is particularly relevant to assess the animal's welfare (and physiological) need for food (and water), because efficient metabolism is primarily beneficial for biological functioning (as well as for zootechnical/economical functioning).

2.2 Literature study

Between October 5-28, 2016 literature searches were done using Web of Science. The following key words were used: (broiler* OR laying hen) AND (early food* OR early fasting OR post hatch food*) AND >2000 publication year. In addition, the literature cited in the reports by Lourens and Leenstra [6] and 'Wakker Dier' [3] was retrieved and collected in Endnote. The Endnote files from the Web of Science search, the two reports and two existing Endnote files on this subject from the authors of this report were merged. Finally, relevant cross-references of reviewed papers were added to the database.

A step-wise approach was used to assign the papers to categories; the meta-analysis (MA) category was used for scientific papers that likely contained data on the effect of post-hatch food and water deprivation on production, health and welfare measures and could be used for the meta-analysis (both the qualitative and quantitative meta-analysis, see 2.3.). The review report (RR) category was used for scientific papers that were considered relevant for the discussion/review section of the report.

Many measures have been reported in scientific papers to determine effects of post-hatch food and water deprivation. When screening/classifying the scientific papers, we discriminated between production measures, i.e. body weight or body weight gain, yolk-free body mass, food intake, food conversion, mortality and other measures, such as (other) health measures (morbidity; immunological parameters such as antibody levels), behaviour, physiology (e.g. hormones; organ weights: heart, liver, gut, yolk), crude protein and crude fat (of breast fillet), muscle growth, gut development (e.g.

villus height, crypt depth and length, microbiota). Only peer reviewed scientific publications describing scientific experiments were included in the category MA; non-peer reviewed publications (e.g., conference abstracts, unpublished thesis chapters), but considered relevant for the study were included in category RR. Studies on post-hatch food deprivation in which access to food and water was confounded with another factor (e.g. housing system or food supplement) were not included in MA. Incidentally, a paper was not included, because it was not possible to extract sufficiently reliable quantitative scores from the paper (e.g. when numerical data could not be retrieved from a graph).

When reviewing the scientific papers for MA, it became clear that the age of the chickens had not been described into detail in all studies. We therefore classified the scientific papers as follows:
Category 1: Experiments in which the biological age (see also definitions in [8]) of the chickens was known. In these studies chickens were collected periodically (e.g. every 2 to 6 hours) from the incubator. If the publication specified that the down was still being wet, this indicated that the chickens were collected less than 3 hours post-hatch [16];
Category 2: This category included all experiments where the chicken age was specified after pulling/collection from the hatcher, including ‘day-old chickens’, which is the usually applied description used for chickens after pulling. This refers to the chronological age of the chickens [8]. In these studies the biological age was unknown;
Category 3: For these experiments, it was not clear at what age the chickens were being fed (as nothing was specified about age, only e.g. that ‘newly hatched chickens’ were used, which can be after hatching or upon arrival at the farm).

2.3 Meta-analysis

Two types of meta-analysis were performed: a quantitative meta-analysis (**MA**), and a qualitative analysis (**QA**). To that end, data retrieved from scientific papers in the category MA were collected in an Excel sheet, including a description of the treatments applied and factors included in the study. Data of studies on broiler chickens, laying hen chickens and turkey poults were included. Age of first feeding or water provision was indicated (category 1 to 3). Additionally, breed, sex, parent stock age, hatchery type (experimental vs. conventional), type of first food, or type of water were added if specified in the paper. Finally, it was indicated whether effects of post-hatch food and water deprivation were significant positive, significant negative or insignificant. In the results section ‘positive’ and ‘negative’ effects refer to numerical statistically significant increases and decreases, respectively (hence do not directly refer to what is positive or negative for the chickens and/or their behavioural or physiological needs). Each record in the spreadsheet represented the result for a specific combination of treatment within an experiment. In case the experiment had a non-factorial design this implied that treatment average values for the specified measure (e.g. body weight, mortality, etc.) were collected in the spreadsheet. In case of a factorial design, data were collected in the spreadsheet only if effects were reported on interaction level. Thus, one paper could report more than one experiment, and also more than one treatment within an experiment. This could generate multiple records per scientific paper, dependent on the number of treatment/experiment combinations.

2.3.1 Quantitative meta-analysis (MA)

In the MA, effects of post-hatch food and water deprivation at various points in time were quantified on a continuous scale, for measures related to production performance (body weight, food intake, food conversion ratio), mortality, relative yolk sac, liver, pancreas and heart weight and for duodenum, jejunum, and ileum villus height and crypt depth. In the analysis, it turned out that insufficient records were available for a reliable MA of relative liver, pancreas and heart weight, and duodenum, jejunum and ileum villus height and crypt depth, thus these measures were excluded from the final results of the MA.

Two types of experiments could be discriminated in the MA with respect to water and food provision: either food and water provision were linked, so these were provided at the same time (**FW**), or water only was provided immediately after hatching regardless of the time of first feeding (**WO**). Thus all studies concerned early nutrition as primary treatment, and in these studies all chickens had access to water in WO experiments, but not in FW experiments (where only chickens that received food also received water). No experiments on water-deprivation only were included in the MA, because the number of such experiments was insufficient for meta-analysis. An analysis for possible interaction between FW and WO was included in the MA.

A wide variation in the duration of food and water deprivation after hatching has been reported in the scientific literature. To overcome this variation in the analysis, the following classes were made for the duration of food and/or water deprivation: 0-12h, 12-36h, 36-60h, 60-84h, >84h, these were labelled as **0, 24, 48, 72 and >84,** respectively and are as such presented in the results and discussion sections. For these classes a 24-hour interval was considered most appropriate, such that the focal time points 24, 48 and 72 hours of food deprivation were the middle points of a class.

A statistical analysis was performed on the effects of various durations of food deprivation at day 7, day 21 and day 42 for body weight, cumulative food intake, food conversion ratio and mortality. Because studies may include day 6, but not day 7, for example, day 7 and day 21 results include a variation of one day (6-8 and 20-22 days of age), and day 42 results include a variation of 2 days (40-44 days of age). The ages for analysis were chosen, because most of the studies used these time points to assess the (long-term) effects of food deprivation. For relative yolk sac weight the analysis was performed for day 1-6 of age. For the production measures, three analysis were performed: for all categories of papers, for category 1+2 only (biological and chronological age of the chickens known) and for category 1 only (biological age of chickens known). For relative yolk sac weight only an analysis of all categories of papers was performed, because there were insufficient records to separately analyse category 1/category 1+2 papers. The statistical analysis was a REML procedure in Genstat [17] done on food and water deprivation categories.

The final model used for estimation of main effects of duration of food deprivation was:

$$\underline{Y}_{ijkl} = \alpha_l + \underline{\varepsilon}_i + \underline{\varepsilon}_{ij} + \underline{\varepsilon}_{ijk} + \underline{\varepsilon}_{ijkl}$$ model equation 1.1

Where:

$\underline{Y}_{ijklm}$: LOG-transformed value treatment average from literature table, from paper i, experiment j (within paper, factorial treatments j (within experiment) and level of duration of food deprivation l (within experiment).

α_i : effect of level i of duration of food deprivation ; i=0h, 24 h, 48h, 72h, >84h

$\underline{\varepsilon}_i, \underline{\varepsilon}_{ij}$: Random effects of paper *i* , resp. experiment *j (within i).*

$\underline{\varepsilon}_{ijk}$: Random effects of factorial treatment k (in case of factorial design) within experiment j of paper i.

$\underline{\varepsilon}_{ijkl}$: Random residual variance (variance on level of record in the data file structure); unexplained variance of individual records with level of duration of food deprivation l within experiment j of paper i.

A pre-model was used to check the interaction effect of duration of food deprivation with the factor "Combination Food-Water deprivation Yes/No".

$$\underline{Y}_{ijklm} = equation\_1.1 + \beta_m + (\alpha\beta)_{lm}$$ model equation 1.2

Where:

$\beta_m, \alpha\beta_{lm}$: resp. effect of Combination of food-water-deprivation and interaction effect between duration of food deprivation and factor 'Combination Food-Water deprivation Yes/no'.

2.3.2 Qualitative analysis (QA)

In the QA, the number of significant positive, significant negative or insignificant effects were counted for all measures of which five records or more were present for one or more ages. In case less than five records were found for a measure, the results of the experiment will be described in the discussion section only. In the QA we focused on the contrasts between 0-24h food and water deprivation, 0-48h food and water deprivation and 0-72h food and water deprivation. Contrasts between 24-48h, 24-72h and 48-72h were not taken into account. Similar classes were used as in the MA to overcome the wide variation in durations of food and water deprivation in literature, i.e. 0-12h, 12-36h, 36-60h, 60-84h, these were labelled as **0, 24, 48 and 72h**. QA included all three categories of papers as defined above and results for long-term effects of food or food and water deprivation are presented for day 7 (±1), day 14 (±1), day 21(±1), day 28 (±1), day 35 (±1) and day 42 (±2). For relative weights of heart, liver, pancreas, duodenum, jejunum and ileum, relative lengths of duodenum, jejunum, ileum, villi height and crypt depth in duodenum, jejunum and ileum and plasma glucose concentrations, results are shown for day 1-6 of age and 1-6 weeks of age. For relative yolk sac weight and plasma T3 and T4 concentrations results are only shown for day 1-6 of age. No further statistical analysis was done in the QA, only counts of records are presented in the results. The same Excel spreadsheet was used for the QA as for the MA.

Two tables were generated, first a table where a discrimination was made between FW (food and water provided at the same time) and WO (water available immediately after hatching regardless of the time of feeding) studies, and second a table where no discrimination was made between FW and WO studies. As a separation of FW and WO studies did in general not lead to different results for all measures, therefore only the results of QA of all records (including both FW and WO studies) are shown.

3 Results

3.1 Literature screening

The literature searches resulted in a total of 84 papers including experiments with post-hatch food and water deprivation for meta-analysis. After screening, 75 papers were included in the MA and QA according to the criteria as described in paragraph 2.2.

MA for body weight included the following studies: [18-60]; MA for food intake included the following studies: [19, 27, 28, 42-44, 46, 49, 50, 54-58, 61]; MA for food conversion ratio included the following studies: [18, 19, 26, 27, 42, 44-46, 49, 53-63]; and MA for mortality included the following studies: [21, 23, 45, 46, 53-57, 60, 63, 64]; MA for relative yolk sac weight included the following studies: [40, 43, 57, 65].

All 75 studies were screened for inclusion in the QA; in case less than five records were available for a single measure, this measure was not included in the QA as described in paragraph 2.3.2. This resulted in QA based on experiments reported in the following studies: [5, 10, 18-88].

Appendix 2 shows the list of the various measures included in the scientific papers in each MA category, and indicates whether the measures were included in MA, QA and/or in the discussion section of this report. The discussion section includes measures for which insufficient records were available to perform QA or MA and the measure was relevant for a particular aspect of the discussion. Appendix 2 also specifies which measures were excluded from the report (in case the measure does not have any clear relationship with chicken welfare, e.g. carcass quality measures, or only a single study reported results for the specific measure).

3.2 Meta-analysis

Three measures included in the meta-analysis (MA) were related to production: body weight, food intake and food conversion ratio. Mortality is an indicator of health but often included when production is measured, and therefore here added to the category of production indicators (see Table 1). As indicated in the methods section, two types of experiments could be discriminated. Either food and water deprivation were linked (FW), so these were provided at the same time, or water was provided immediately after hatching regardless of the time of first feeding (WO). First, for each measure the interaction between WO and FW was analysed. No significant interaction was found for any of the production measures included in the MA. Thus, for these measures effects of food deprivation seemed to be independent of the provision of water post-hatch.

Table 1 shows the results of the MA, including all categories of scientific papers (category 1, 2 and 3). Results for only category 1 (biological age of the chickens known) and category 1+2 papers (biological and chronological age known) are shown in Appendix 3. In general, MA of all categories of papers showed similar results compared to MA of category 1/category 1+2 papers only. Significant negative effects of duration of post-hatch food and water deprivation were found for body weight, food intake and mortality (except for mortality at day 21). Significantly increased food conversion ratios were found if post-hatch food and water deprivation was more than 84h. Papers including a very long duration of food and water deprivation (> 84h) were all classified as papers in category 3. This very long food and water deprivation (> 84h) showed a pronounced reduction of body weight at day 7 (nearly half of the weight of non-deprived chickens), a negative effect on food conversion ratio at day 21 and 42 and caused a pronounced increase in first week mortality (Table 2). However, it should be noticed that only a small number of scientific papers included such long durations of food and water deprivation as the number of database records for food and water deprivations of more than 84h was relatively low (N=22 records).

The majority of the studies included body weight at day 7 as measure of the effect of food and water deprivation (Table 2). In general, body weight decreased with increasing duration of food and water deprivation (0, 24, 48, 72 and >84hrs post-hatch), and this effect was observed up to 42 days of age, although relative differences between the early fed and food deprived chickens decreased with increasing age. This indicates that there was some, but no full compensation in body weight gain in food and water deprived chickens up to 42 days of age. Cumulative food intake was reduced with increasing duration of post-hatch food deprivation, but relative differences between early fed and food deprived chickens were smaller at day 42 as compared to day 21 and day 7, also indicating that chickens may partially compensate during the growth period. However, at day 42 cumulative food intake was still lower following 48h and 72h food deprivation post-hatch, indicating that full compensation was not observed at the end of the rearing period for broiler chickens.

Food conversion ratio was not affected by post-hatch food and water deprivation, except when food and water deprivation was longer than 84h at day 21 and 42 of age (Table 2).

First week mortality was higher for >84h post-hatch food and water deprivation, whereas at day 42 a significantly higher total mortality was found for 48h post-hatch food and water deprivation compared to non-deprived chickens; 24h food and water deprivation did not affect total mortality at all ages (Table 1). Only few records (N=6) were available for total mortality at day 21 and no significant effect of post-hatch food and water deprivation was found for total mortality at day 21.

Table 1 *Results of the meta-analysis for production measures, showing effects of the various durations of food and water deprivation after hatching (0, 24, 48, 72 and >84hrs) on body weight, food conversion ratio, cumulative food intake and total mortality at day 7, 21 and 42 of age. Average values for the various measures/indicators are expressed as relative values/percentages compared to 0h (no food and water deprivation), which is set at 100 percent. An asterisk in a cell indicates that no analysis results were available due to a lack of sufficient data for a certain measure at a specific time point. N shows the number of records analysed per measure/age.*

	Relative value after food and water deprivation for[1]						
Measure/ Age 1st food	**0h** (0-12h)	**24h** (12-36h)	**48h** (36-60h)	**72h** (60-84h)	**>84h**	**P value[2]**	**N**
Body weight day 7	100^{a}	92.8^{b}	83.0^{c}	73.1^{d}	51.6^{e}	<0.001	204
Body weight day 21	100^{a}	95.0^{b}	89.3^{c}	79.5^{d}	*	<0.001	82
Body weight day 42	100^{a}	97.4^{b}	94.5^{c}	91.7^{c}	*	<0.001	50
Food conversion day 0-7	100	99.3	103.5	*	*	NS	37
Food conversion ratio day 0-21	100^{b}	99.6^{b}	98.7^{b}	106.1^{ab}	110.4^{a}	0.013	57
Food conversion ratio day 0-42	100^{b}	99.9^{b}	100.1^{b}	103.8^{b}	110.3^{a}	<0.001	47
Cumulative food intake day 0-7	100^{a}	92.1^{a}	67.4^{b}	63.5^{b}	*	<0.001	37
Cumulative food intake day 0-21	100^{a}	95.4^{a}	87.3^{b}	78.4^{b}	*	<0.001	39
Cumulative food intake day 0-42	100^{a}	98.0^{a}	95.1^{b}	89.2^{b}	*	<0.001	33
Mortality day 0-7	100^{bc}	81.4^{c}	143^{bc}	226^{b}	827^{a}	<0.001	39
Mortality day 0-21	100	102.3	200	*	*	NS	6
Mortality day 0-42	100^{b}	100.3^{b}	156^{a}	*	*	0.003	29

[1] Values within a row lacking a common superscript differ significantly (P<0.05).

[2] P-value for effect of post-hatch food and water deprivation

In addition to the production measure, a MA was performed for yolk sac weight (relative to body weight) (Table 2), for all categories of scientific studies (category 1+2+3). A significant interaction effect was found between early food and water deprivation (FW) and water availability at 0h (WO) for relative yolk sac weight at three days of age (P<0.001). Thus, for relative yolk sac weight at day 3, effects of food deprivation seemed to be dependent on water provision immediately after hatching. In case food and water were provided at the same time, following a deprivation period (of both food and water) for either 24 or 48h post hatch, relative yolk sac weights were not significantly different from early fed chickens. However, following a 72h deprivation period relative yolk sac weight was significantly lower (to 85%) compared to 0h deprivation. By contrast, relative yolk sac weight was higher (to 219%) following 72 hrs of food deprivation when these chickens had been provided with water as of 0h post-hatch (Table 2).

Table 2 *Results of the meta-analysis for yolk sac weight (relative to body weight) at 3 days of age, showing the effect of the various durations of food and water deprivation after hatching, for WO (water provided immediately after hatching) and FW (water and food provided at the same time). Average values for the relative yolk sac weight are expressed as relative values compared to 0h (no food and water deprivation), which is set at 100%. An asterisk in a cell indicates that no analysis results were available due to insufficient data for a certain measure at a specific time point. N=19 records were used in the analysis.*

	Relative value after post-hatch food and water deprivation for[1]				
Measure/ Age 1st food	**0h** (0-12h)	**24h** (12-36h)	**48h** (36-60h)	**72h** (60-84h)	**>84h**
Relative yolk sac weight (FW)	100[a]	118.0[a]	121.8[a]	84.6[b]	*
Relative yolk sac weight (WO)	100[a]	*	*	219[b]	*

[1] Values within a row lacking a common superscript differ significantly (P<0.05).

3.3 Qualitative analysis

3.3.1 Production measures and mortality

Figure 1 shows the results of the QA for body weight. For body weight we only found significantly negative effects (i.e. reductions in body weight) or non-significant effects of post-hatch food and water deprivation. In all records, deprivation of food and water for 72h significantly reduced body weight between 2-5 weeks of age compared to 0h, but the number of records including deprivation up to 72h compared to 0h food and water deprivation was relatively low (N≤9). For 24 and 48h post-hatch food and water deprivation compared to 0h deprivation the majority of the records showed a significantly reduced body weight up to 4 weeks of age, whereas at 6 weeks of age for 24h deprivation no record showed a significant effect and for 48h only a few records indicated a significantly reduced body weight. The number of records measuring body weight decreased with increasing age; most records are found for body weight in week 1 and 2 of age.

The QA for food intake also showed only records for a significantly reduced food intake or non-significant effects of post-hatch food and water deprivation on food intake (Figure 2). For 72h post-hatch food and water deprivation compared to 0h deprivation, all records reported a reduced food intake up to week 6 of age, but the number of records was low for all ages (N=2). For 24h post-hatch food and water deprivation compared to 0h food and water deprivation, most studies reported a reduced food intake in the first week, but at week 2, 3 and 6 most records indicated no significant effect. Only a few records were found for week 4 and 5 (N≤2). For 48h post-hatch food and water deprivation compared to 0h food and water deprivation, most records indicated a negative effect on cumulative food intake in week 1 and 2, and at week 6 the majority of records indicated that there was no effect. Furthermore, there were only a few records for week 4 and 5 (N≤2).

Most records reported no effect of 24, 48h or 72h post-hatch food and water deprivation compared to 0h post-hatch food and water deprivation on the food conversion ratio; for 72h food and water deprivation as compared to 0h there were nearly no records (Figure 3). For 24h and 48h post-hatch food and water deprivation compared to 0h food and water deprivation few studies reported a significantly increased food conversion rate at week 1 of age, but not thereafter.

There were no records showing a significant effect of 24, 48 or 72h post-hatch food and water deprivation compared to 0h food and water deprivation on mortality in week 1-6 (Figure 4). Also here, only few records were available for 72h post-hatch food and water deprivation, and for week 4 and 5 of age (N≤2).

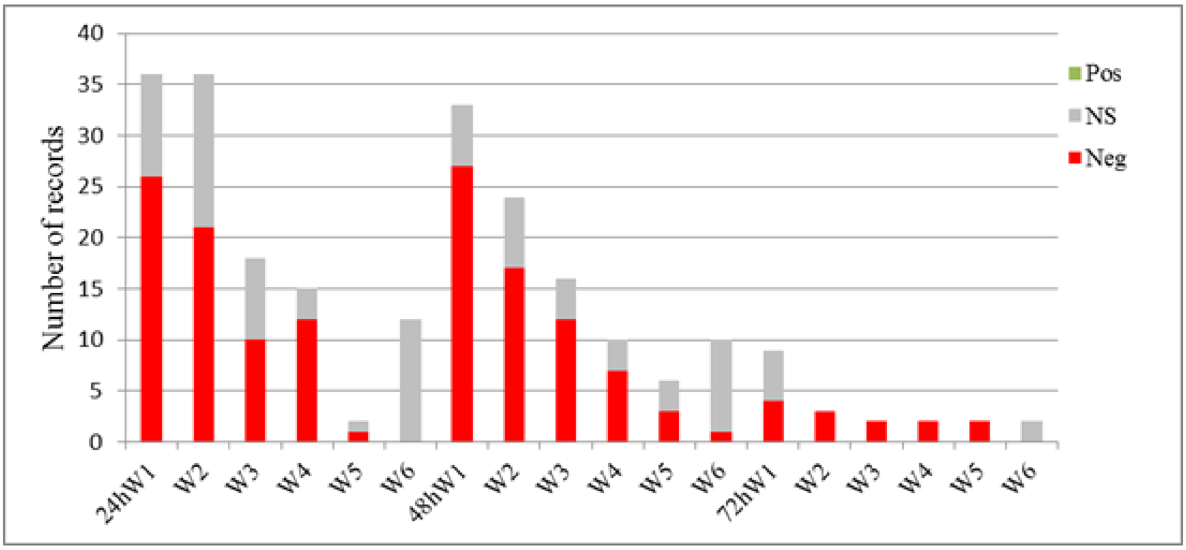


***Figure 1** Results of QA of records including the effect of post-hatch food and water deprivation on **body weight**. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand as measured at 1-6 weeks of age (W1-W6 on the horizontal axis).*

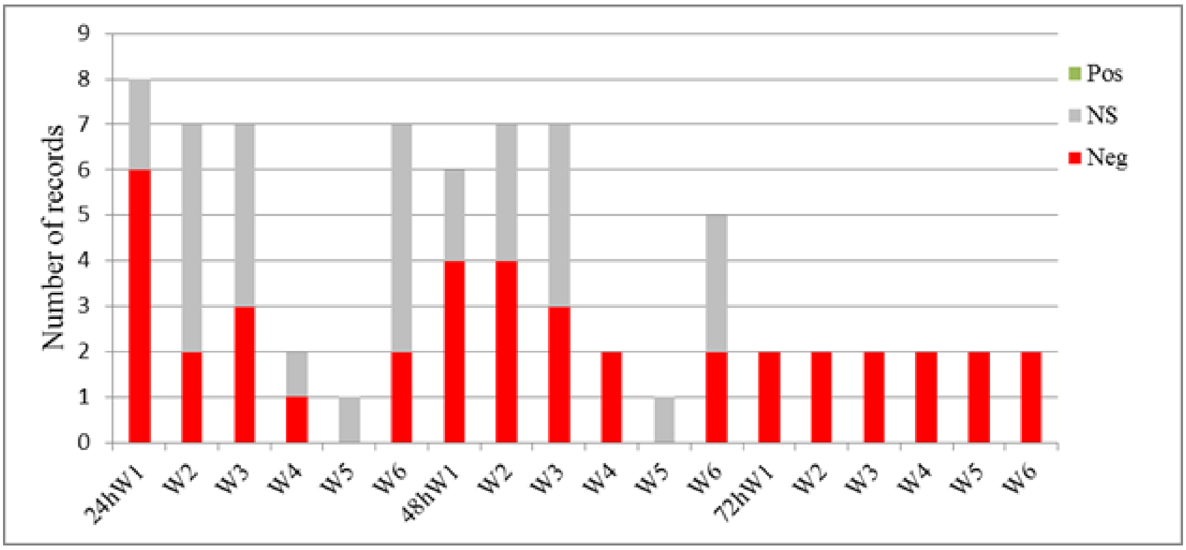


***Figure 2** Results of QA of records including the effect of post-hatch food and water deprivation on **cumulative food intake**. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand as measured at 1-6 weeks of age (W1-W6 on the horizontal axis).*

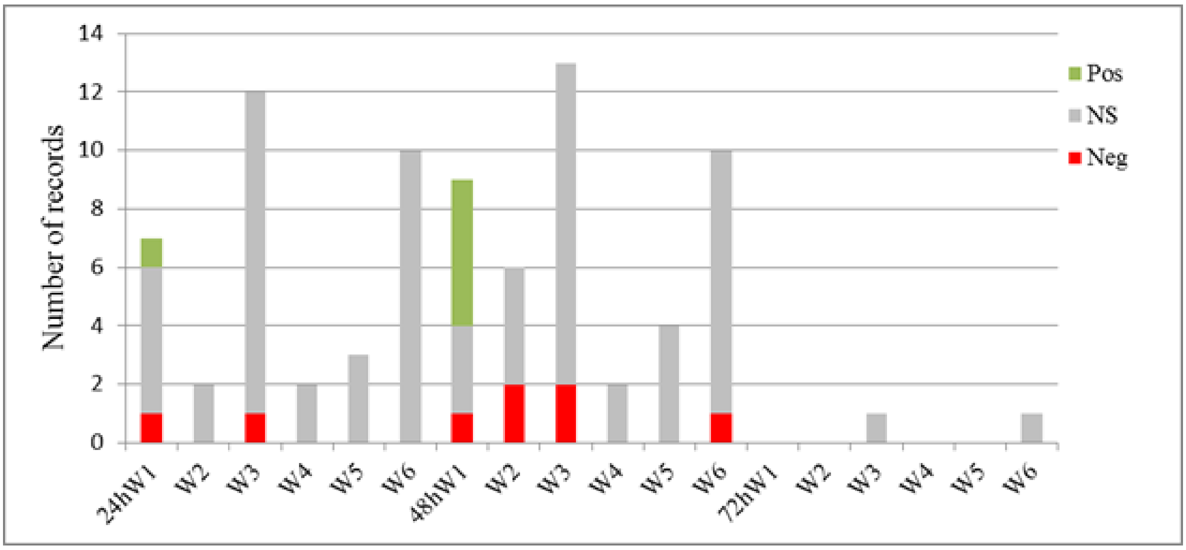


Figure 3 *Results of QA of records including the effect of post-hatch food and water deprivation on* ***food conversion rate****. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand as measured at 1-6 weeks of age (W1-W6 on the horizontal axis).*

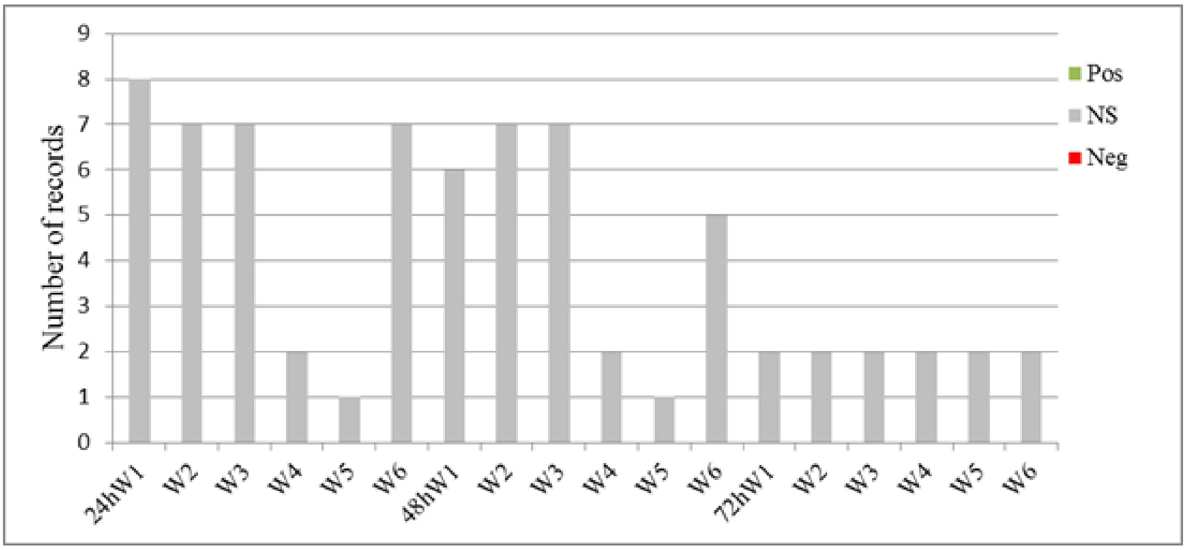


Figure 4 *Results of QA of records including the effect of post-hatch food and water deprivation on* ***mortality****. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand as measured at 1-6 weeks of age (W1-W6 on the horizontal axis).*

3.3.2 Relative organ weights

Figure 5 shows the results of the QA of relative yolk sac weight at day 1-6 of age. Either no effect or a significant positive effect of 24, 48 or 72h post-hatch food and water deprivation compared to 0h food and water deprivation was found for relative yolk sac weight in the first week of life, for both WO and FW treatments (Figure 5). In general, few records were available for the analysed contrasts (N≤5).

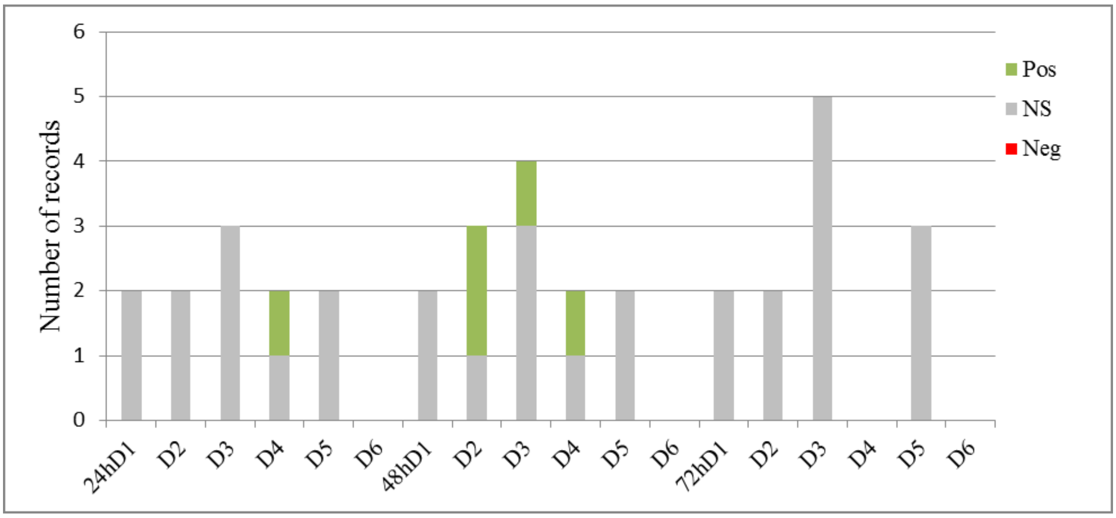
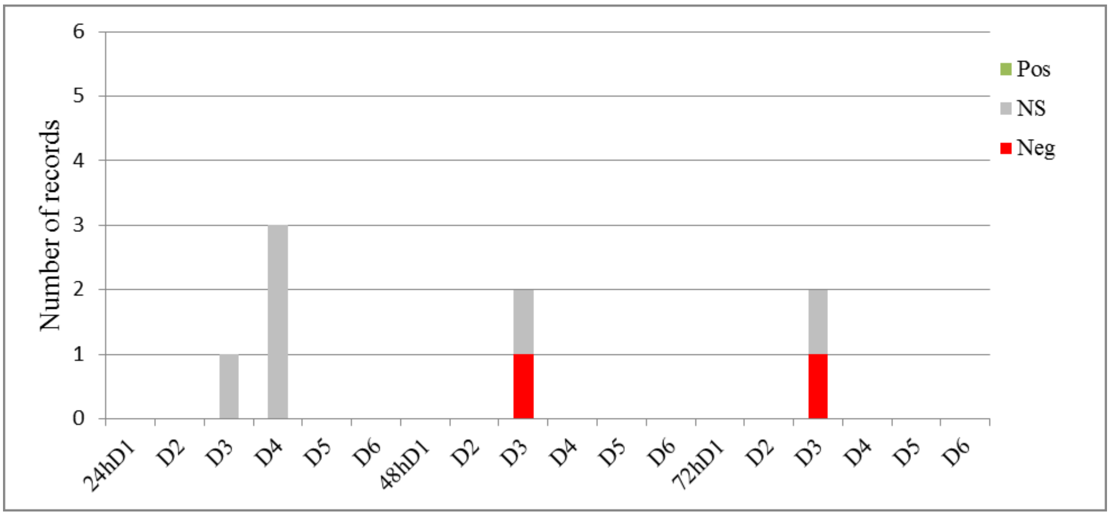


Figure 5 *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative yolk sac weight****. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand as measured at 1-6 days of age (D1-D6 on the horizontal axis).*

Results for relative liver weight are shown in Figure 6 a and b. There were only records showing a significant negative effect or no effect of 24, 48h or 72h food deprivation compared to 0h on relative liver weight in the first week of life (Figure 6a), with only two records showing a negative effect at day 3 of age for 48h an d72h post-hatch food and water deprivation compared to 0h. Records for long-term effects are shown in Figure 6b, indicating that the majority of the records did not find long-term effects of food and water deprivation on relative liver weight.



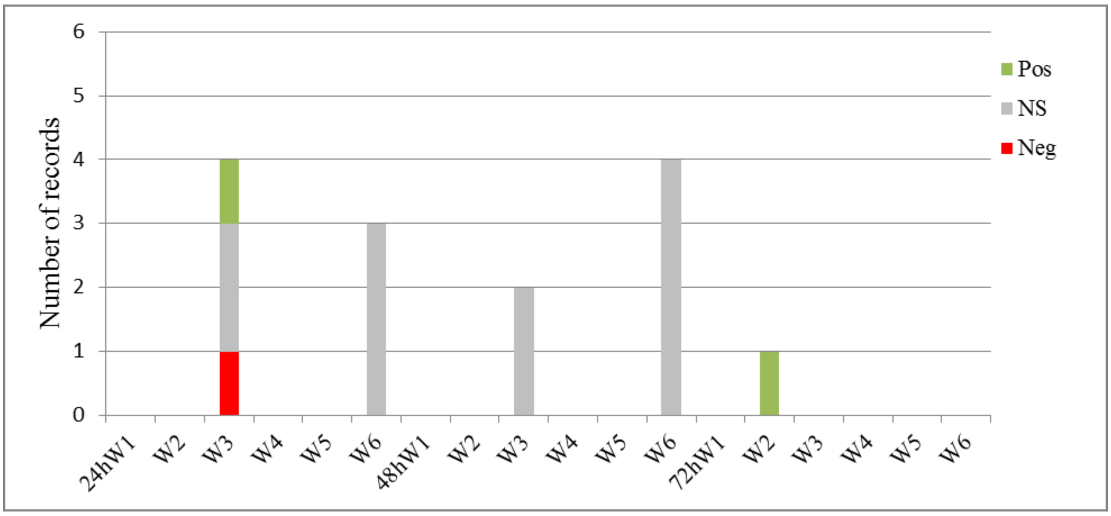
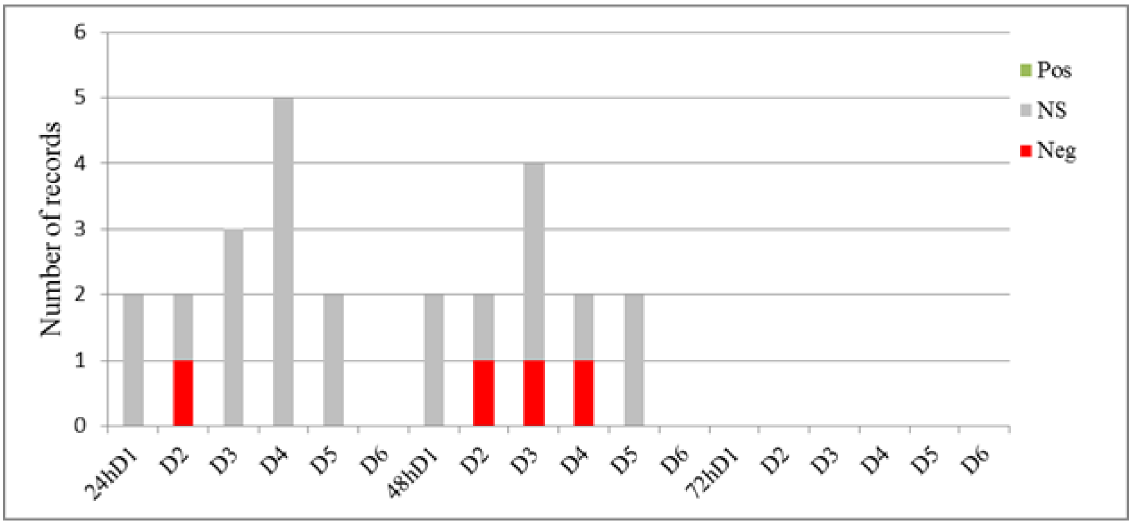


Figure 6 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative liver weight*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Figure 7 a and b shows results for relative pancreas weight in the first week of life and up to 6 weeks of age, respectively. Few records showed a significant negative effect of 24h and 48h post-hatch food and water deprivation compared to 0h deprivation in the first week of life, but the majority of the records did not show significant effects of food or food and water deprivation on pancreas weight in the first week (Figure 7b). Results up to 6 weeks of age had a similar pattern, with a few studies showing a significant positive or negative effect of 48h or 72h food or food and water deprivation as compared to 0h, but the majority of records showing no significant effect (Figure 7b).



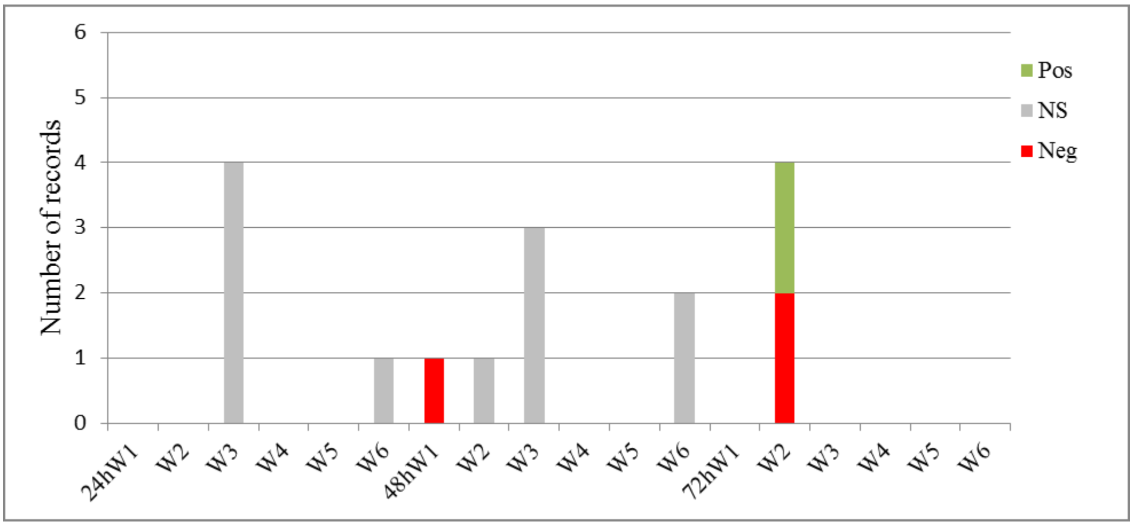


Figure 7 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative pancreas weight*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Results of relative heart weights are shown in Figure 8. Only three records were found for 24h food and water deprivation compared to 0h deprivation for relative heart weight at day 4 (Figure 8a). The majority of the records did not show any effect of 24h, 48h or 72h post-hatch food and water deprivation compared to 0h on relative heart weight. (Figure 8b).

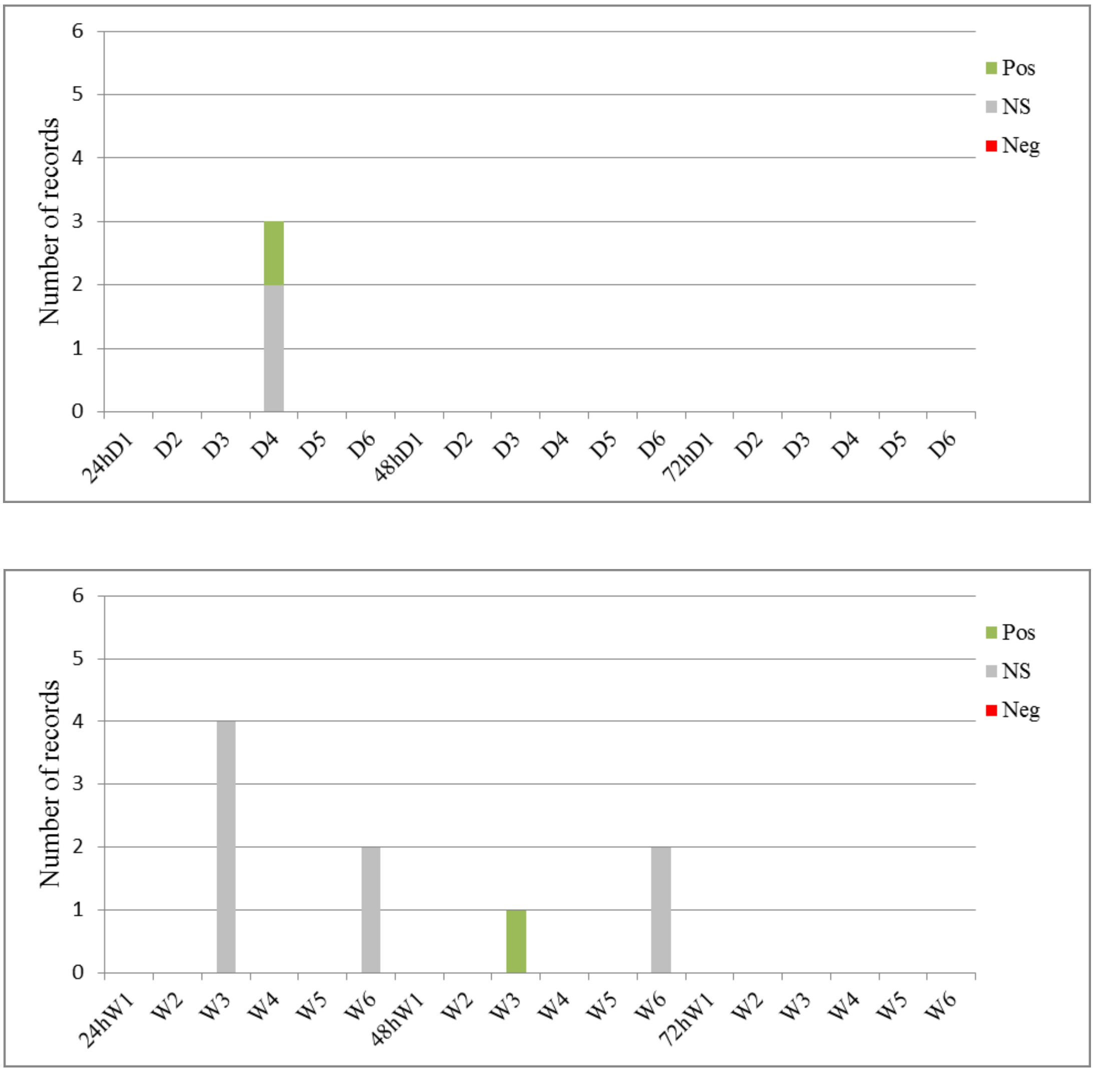


Figure 8 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative heart weight*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

3.3.3 Gut development

QA results for post-hatch food and water deprivation on duodenum, jejunum and ileum lengths and relative duodenum, jejunum and ileum weights are shown in Figures 9-14, for both effects on day 1-6 of age and on 1-6 weeks of age.

Figure 9 shows the results for duodenal length at 1-6 days of age and 1-6 weeks of age. For 24h and 48h food deprivation compared to 0h some records showed significant negative effects at day 3 and 5 of age. No significant effects were reported for 72h food and water deprivation compared to 0h (Figure 9a). There were no records showing significant effects of 24h and 48 post-hatch food and water deprivation compared to 0h between 1-6 weeks of age (Figure 9b). Figure 10 and 11 show the results for jejunal and ileal length, which are more or less similar to the results for duodenal length.

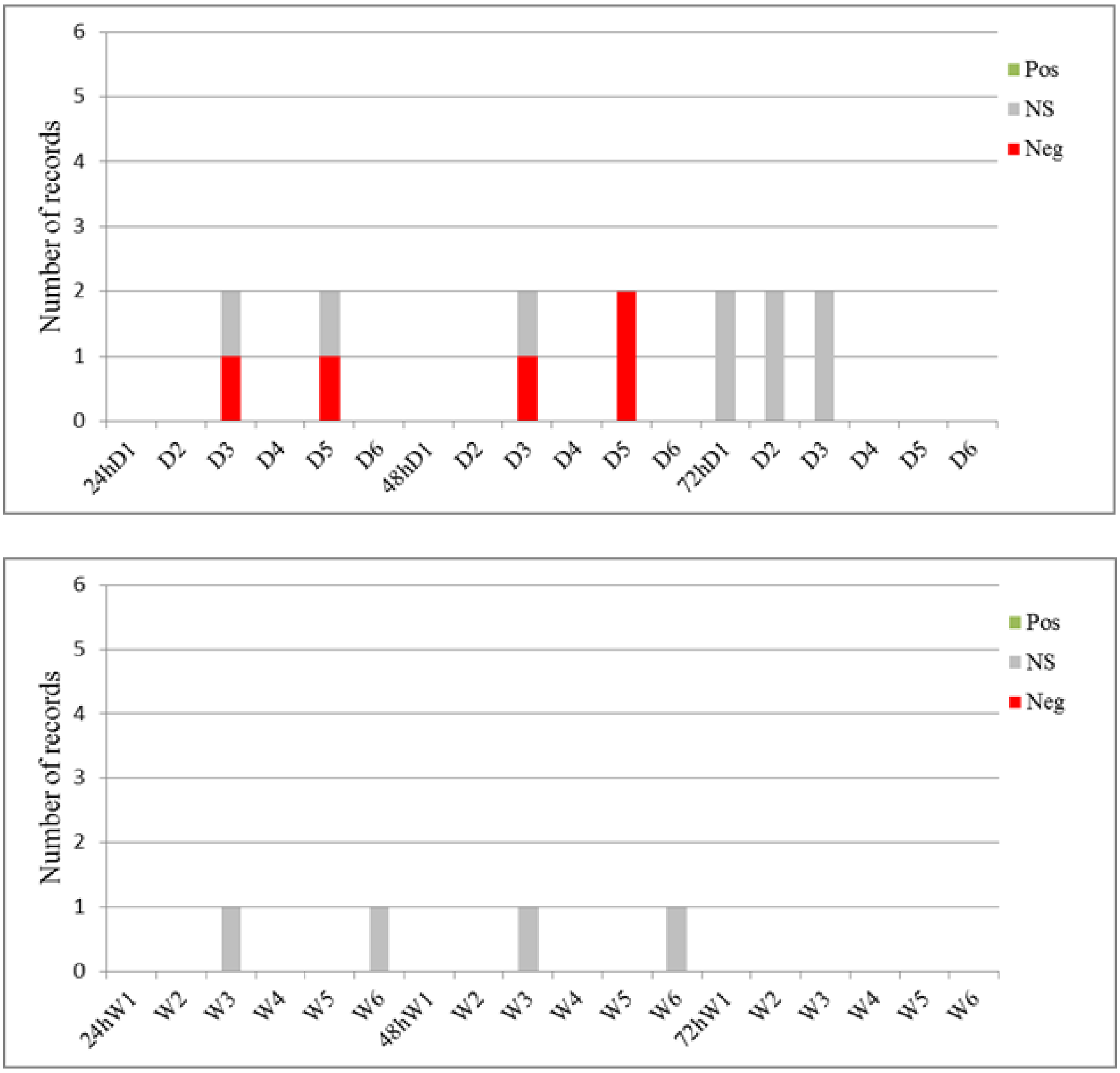
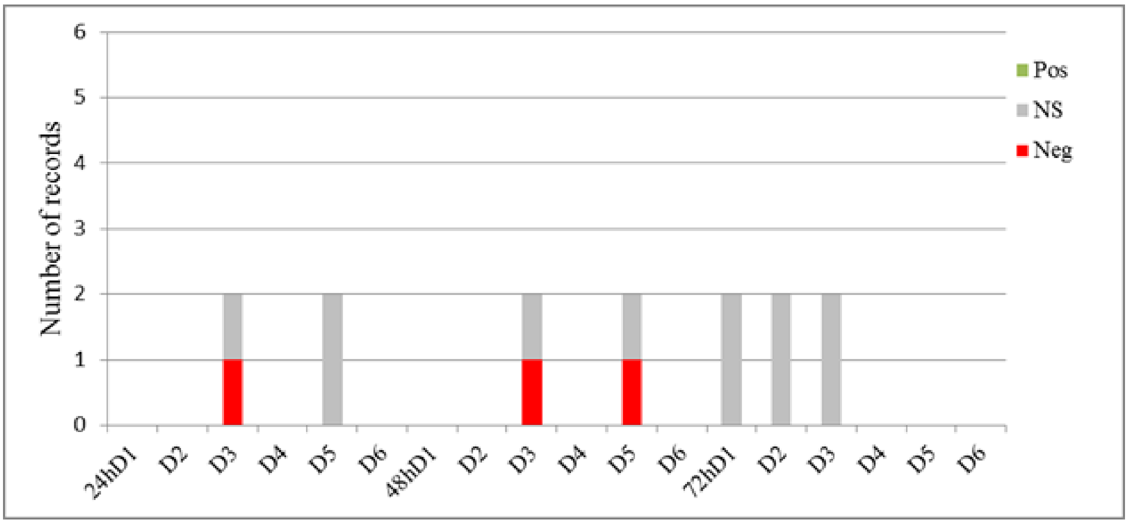


Figure 9 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***duodenal length*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*



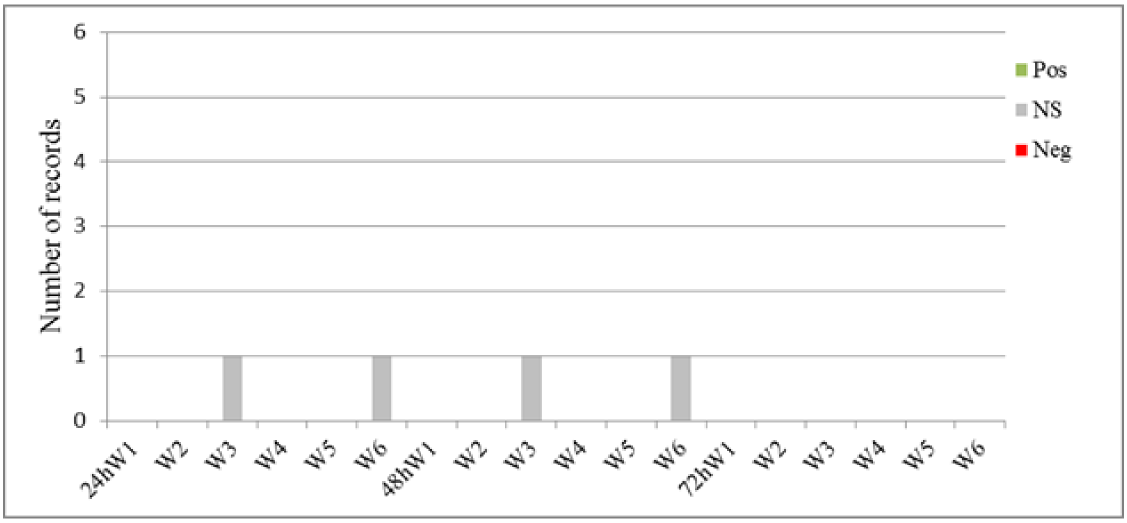


Figure 10 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***jejunal length*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

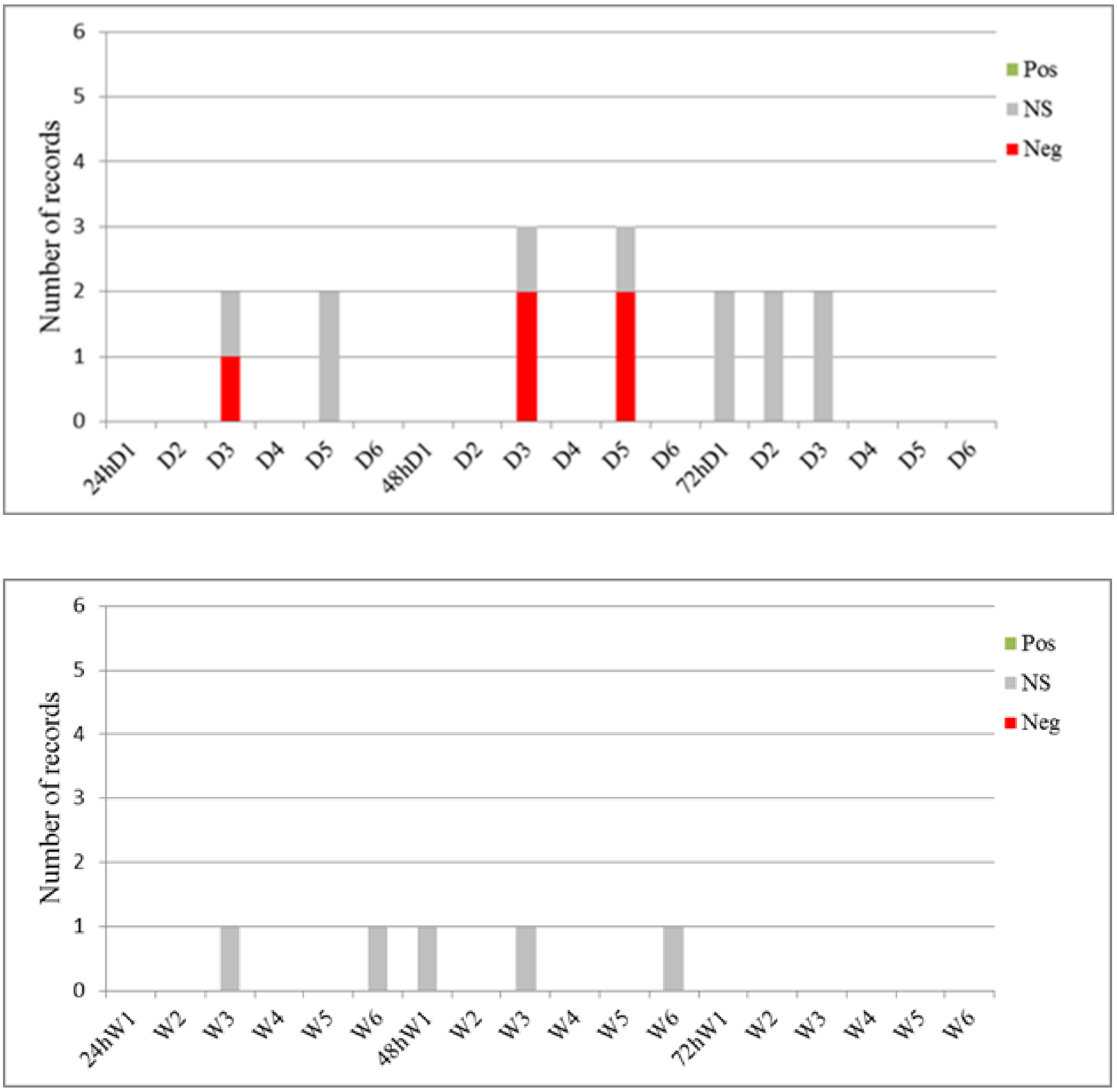


Figure 11 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on **ileal length** in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

With respect to relative duodenum weight between day 1-6 of age, the majority of records indicated no significant effects on duodenum weight in the first week of life of 24h, 48h and 72h food and water deprivation as compared to 0h (Figure 12a); results for 1-6 weeks of age were similar (Figure 12b). If a record indicated a significant effect, effects were mainly positive (Figure 12a and b).

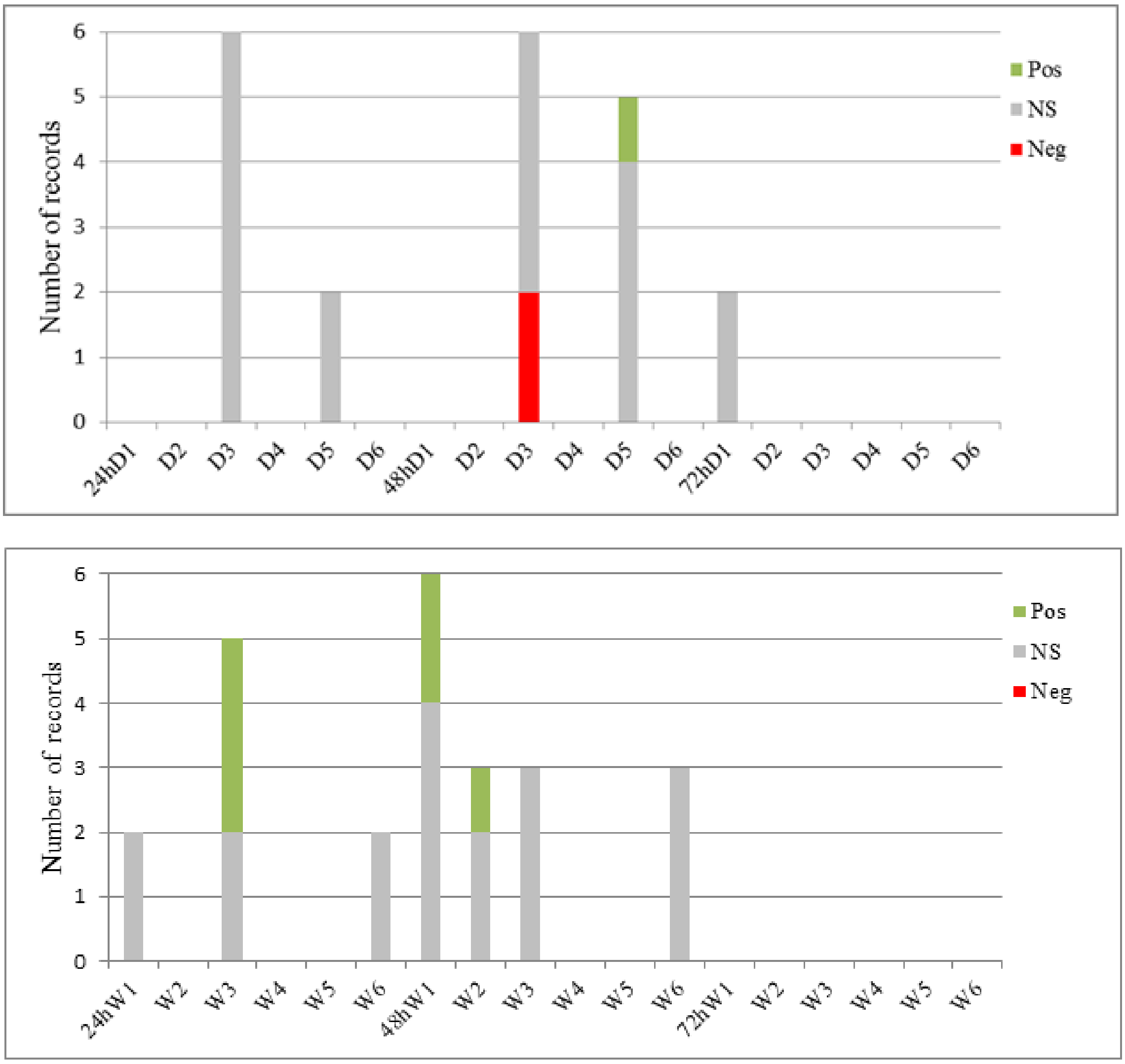


Figure 12 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative duodenal weight*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Relative jejunum weights were measured at day 1 and 5 of age. Again, one record showed a significant positive effect for 48h food deprivation compared to 0h, but here a couple of records indicated negative effects of 24h and 48h food and water deprivation at day 1 of age as compared to 0h. In addition two records indicated negative effect of 48h food or food and water deprivation compared to 0h deprivation for relative jejunum weight at day 5 (Figure 13a). Records for relative jejunum weights were found for week 1, 3 and 6 of age for 24h and 48h food or food and water deprivation compared to 0h. The majority of records indicated no effect of food or food and water deprivation, except four records showing a negative effect of 48h food deprivation compared to 0h on jejunum weight at 1 week of age (Figure 13b).

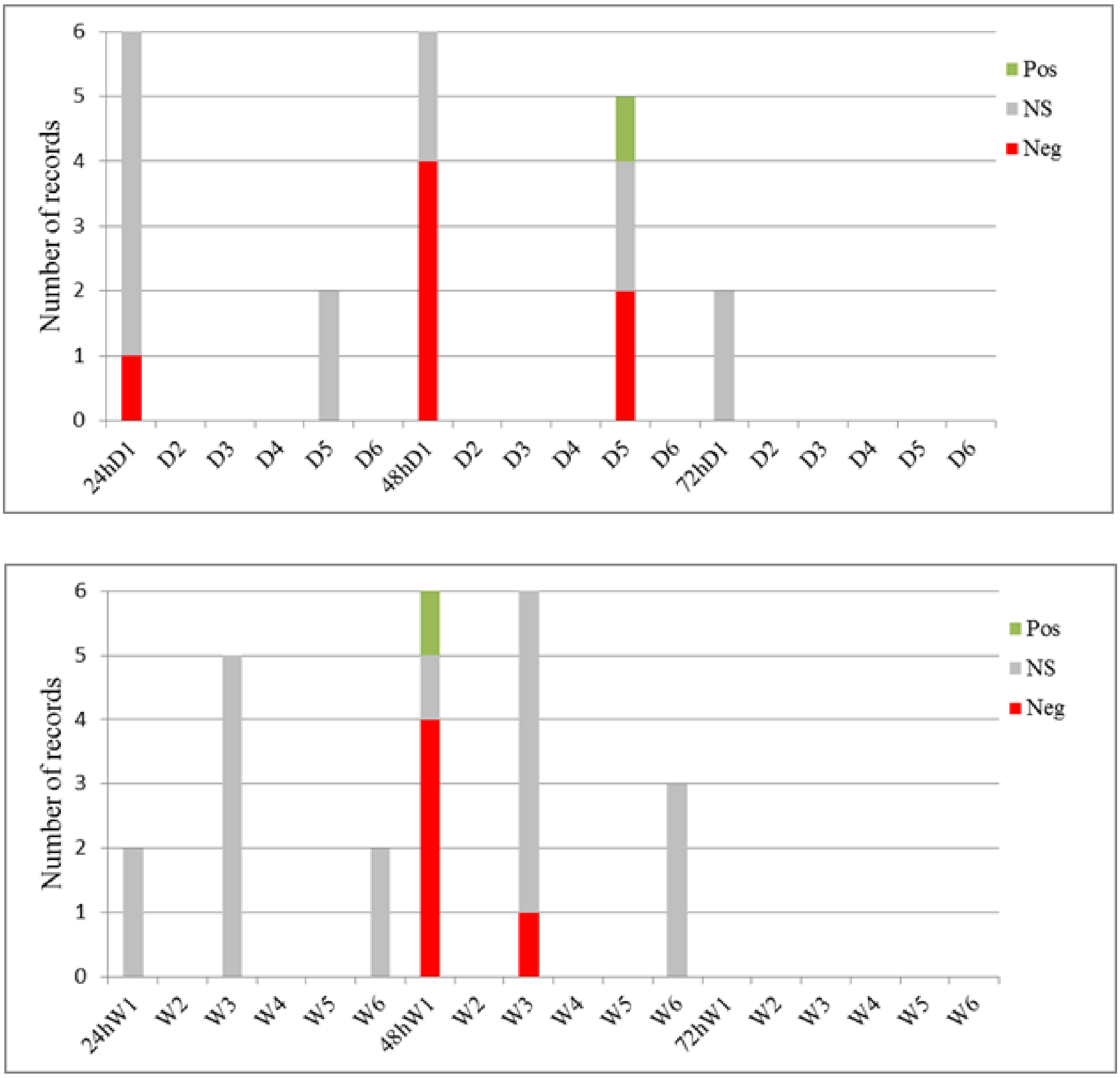
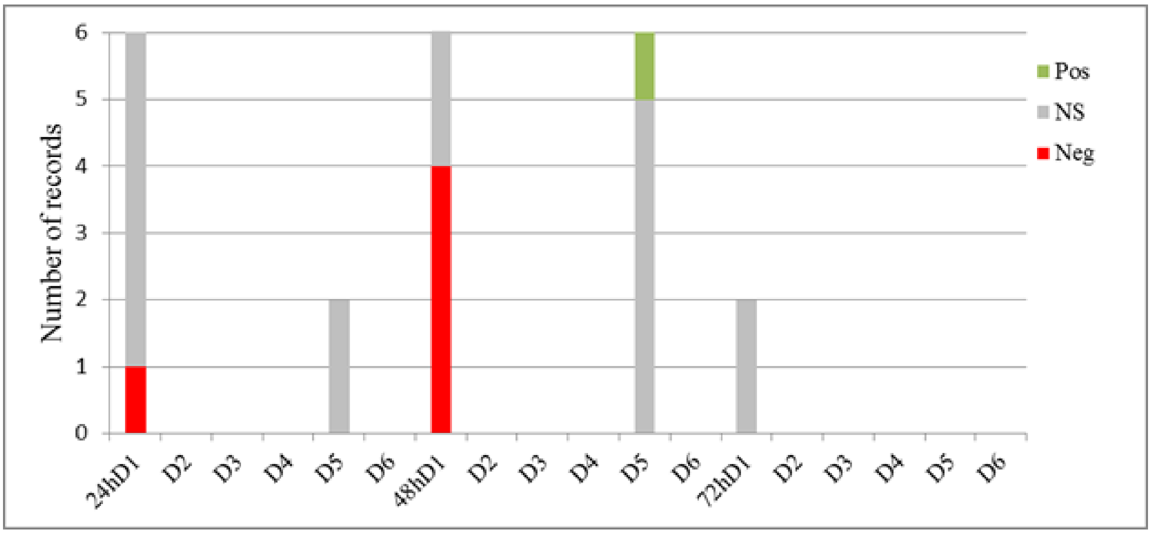


Figure 13 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative jejunal weight*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Relative ileum weights were measured at day 1 (24, 48 and 72h food and water deprivation compared to 0h) and day 5 (24h and 48h food and water deprivation compared to 0h). Significant negative effects of 24h and 48h food and water deprivation and 48h food deprivation compared to 0h were found in a records for day 1. Only one record indicated a positive effect at day 5 for 48h food deprivation compared to 0h (Figure 14a). The majority of records did not show significant effects of 24h and 48h food and water deprivation in ileal weight between 1-6 weeks of age (Figure 14b).



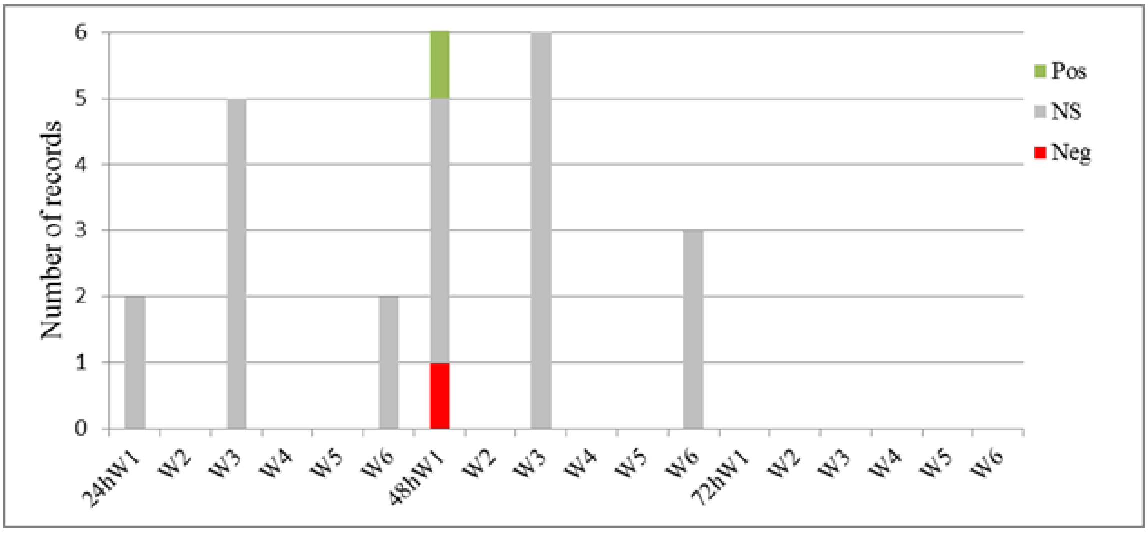


Figure 14 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on **relative ileal weight** in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Results for QA of records on the effect of post-hatch food and water or food deprivation for duodenum, jejunum and ileum villus heights are shown in Figures 15-17, for both effects on day 1-6 of age and on 1-6 weeks of age.

Duodenal, jejunal and ileal villus height records were found for day 2 (48h food and water deprivation) and 4 (24h and 48h food and water deprivation) of age, but the total number of records wais low (Figures 15a, 16a, 17a). For duodenal villus height, one record indicated a significant negative effect of 48h food and water deprivation on day 2, and two records a significant negative effect of 48h food and water deprivation on day 4, compared to 0h deprivation (Figure 15a). For jejunal villus height, two records indicated a negative effect of 48h food and water deprivation on day 4 compared to 0h deprivation (Figure 16a). Only one record indicated a significant negative effect on ileal villus height at day 4 when 48h post-hatch food and water deprived compared to 0h (Figure 17a).

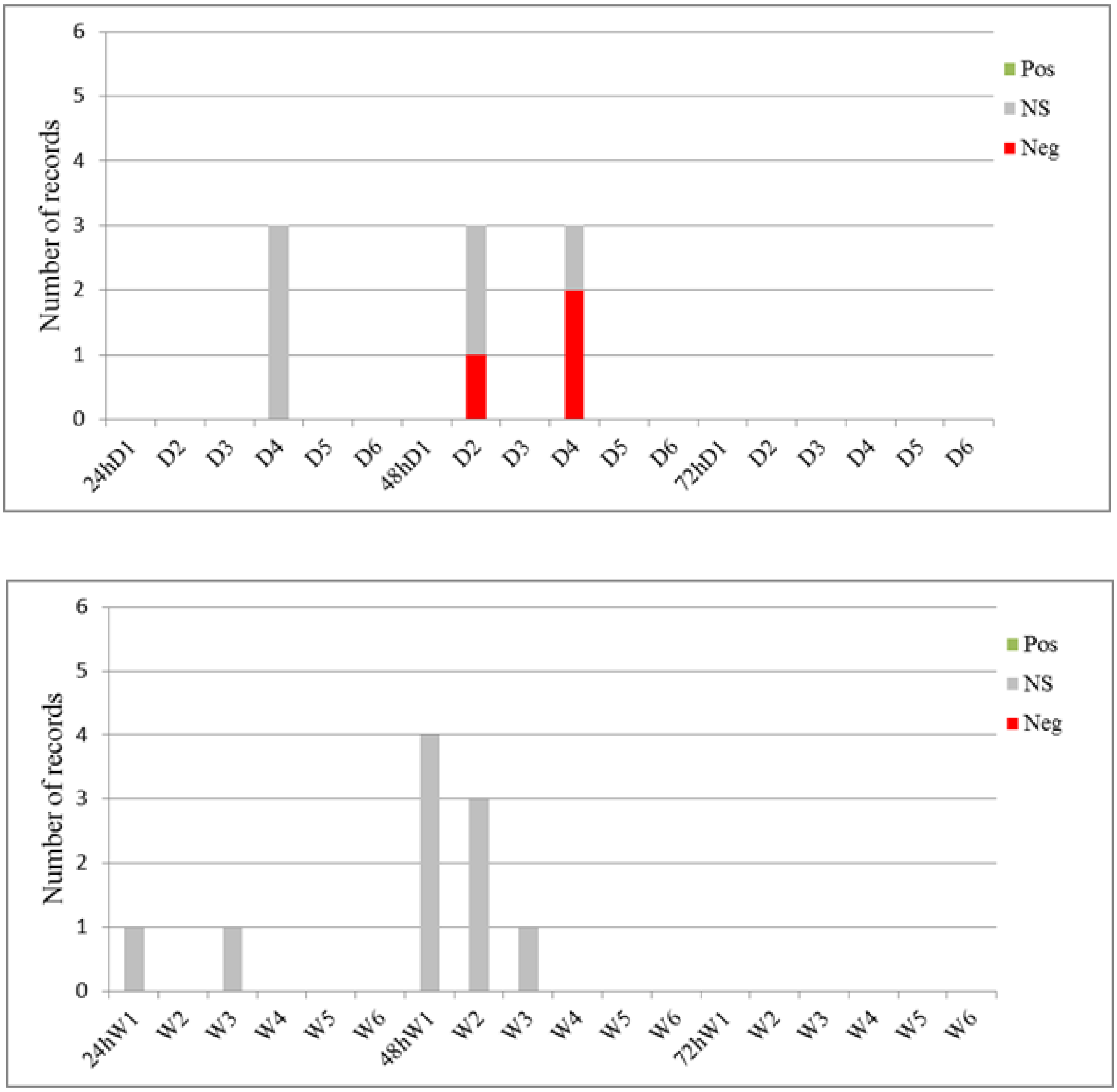
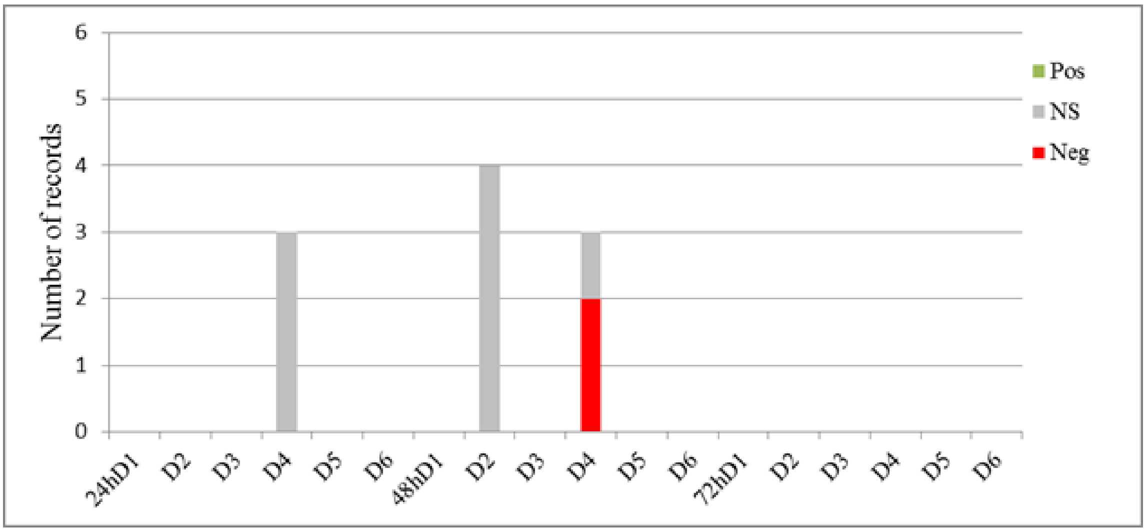


Figure 15 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***duodenal villus height*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*



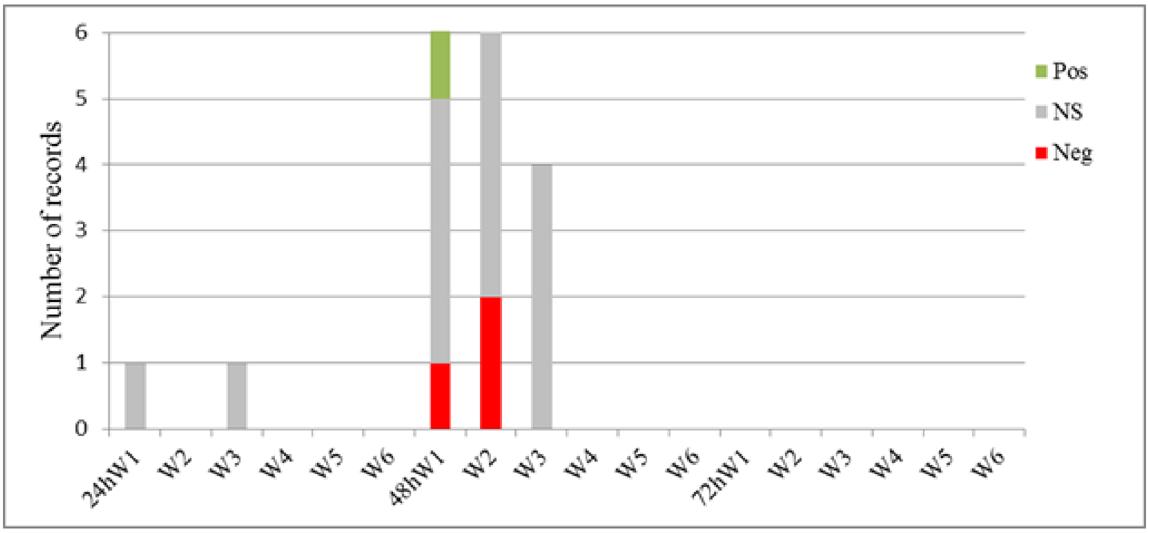
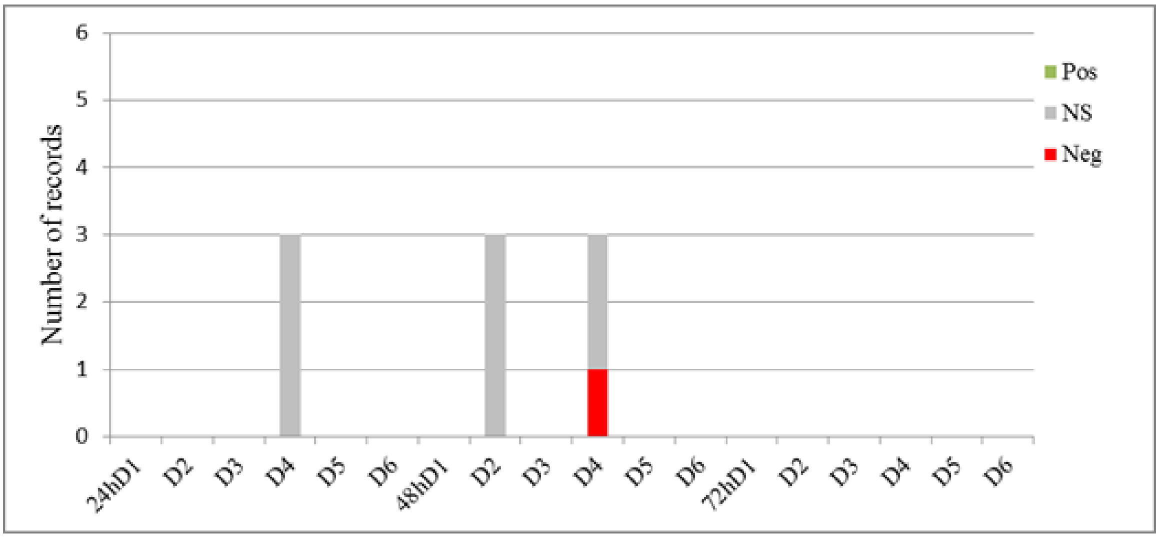


Figure 16 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***jejunal villus height*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*



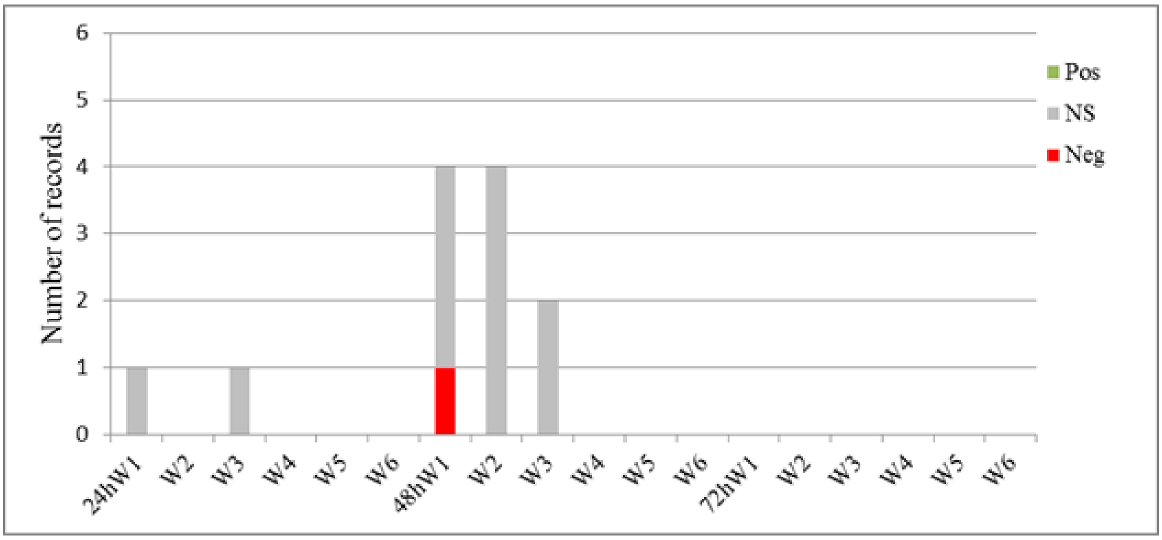


Figure 17 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on **ileal villus height** in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Duodenal, jejunal and ileal villus height records were found for week 1, 2 and 3 of age, but not for later ages and only for 24h and 48 food or food and water deprivation compared to 0 h. There were no records indicating a significant positive or negative effect of 24h or 48h food and water deprivation on duodenal villus height at week 1,2 and 3 of age (Figure 15a). For jejunal villus height, two records found a positive effect of 48h food and water deprivation at week 1 of age, one record found a significant negative effect of 48h food and water deprivation at one week of age and two records found a significant negative effect of 48h food and water deprivation at two weeks of age compared to 0h deprivation, but the majority of records did not show significant effects (Figure 16b). Additionally, for ileal villus height the majority of records did not indicate significant effects (Figure 16b).

Results for QA of records on the effect of post-hatch food and water deprivation for duodenum, jejunum and ileum crypt depth are shown in Figures 18-20, for both effects on day 1-6 of age and on 1-6 weeks of age.

Duodenal and ileal crypt depth records were found for day 1, 2, 4 and 5 of age (48h food and water deprivation). Jejunal crypt depth records were found for day 1, 2 and 4 of age (48h food and water deprivation). For duodenal crypt depth, two records indicated a significant positive effect at day 4 and one record a significant negative effect at day 2 of 48 food deprivation compared to 0h, but the majority of the records did not indicate significant effects (Figure 18a). For jejunal crypt depth, one record found a significant negative effect of 48h food and water deprivation at day 2, and two records found a negative effect of 48h food deprivation on day 4 (Figure 19a). For ileal crypt depth the majority of records did not indicate a significant effect of food and water deprivation (Figure 20a).

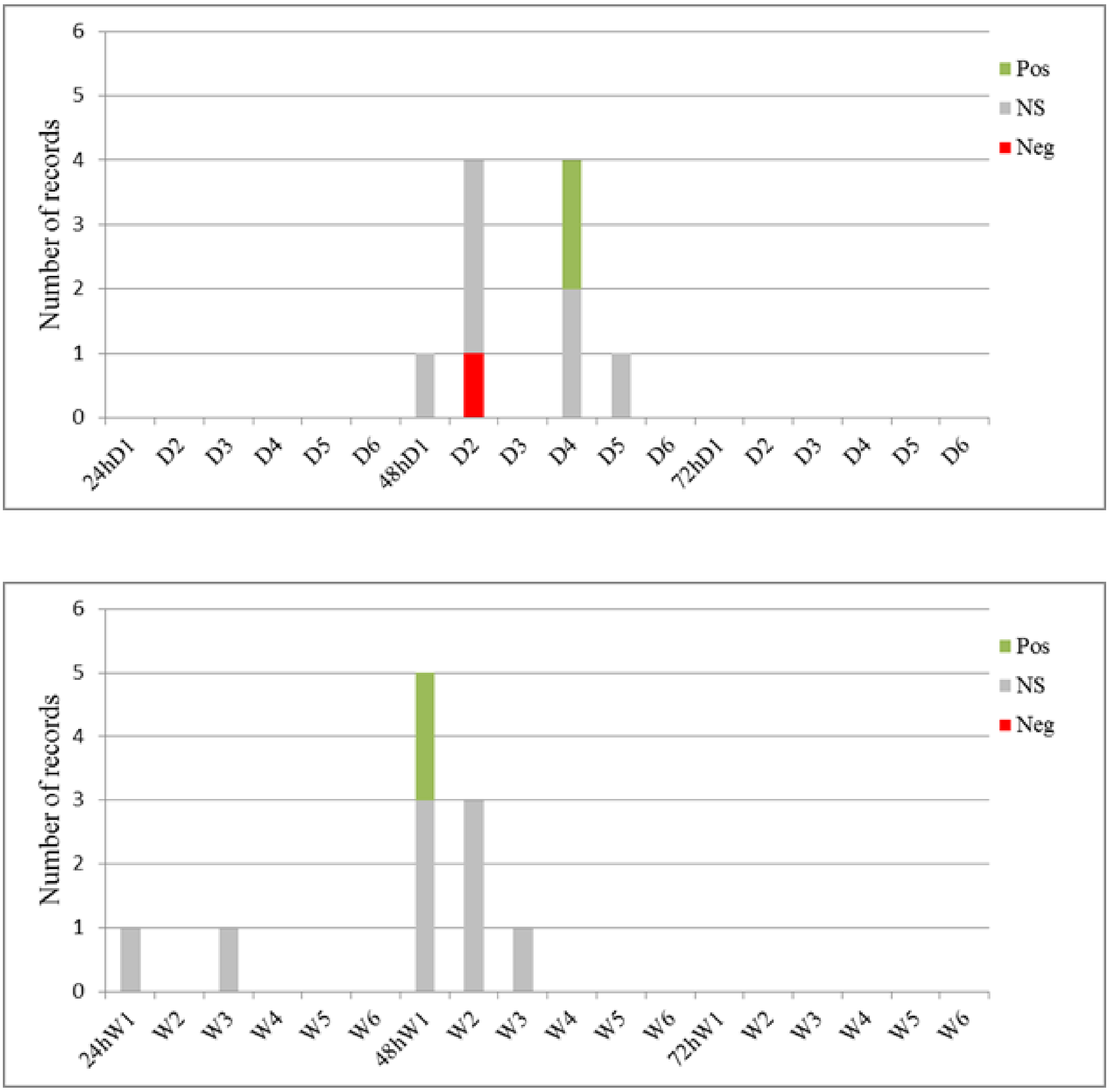


Figure 18 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative duodenal crypt depth*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

Duodenal, jejunal and ileal crypt depth records were found for week 1, 2 and 3 of age, but not for later ages and only for 24h and 48 food or food and water deprivation compared to 0 h. Two records indicated a significant positive effect of 48 food or food and water deprivation at 1 week of age compared to 0h deprivation on duodenal crypt depth (Figure 18b). For jejunal crypt depth, few records indicated significant negative effects for week 1-3 of age and 48 food and water deprivation, and one significant positive record was found for week 2, but the majority of the records did not indicate significant effects of 48h food or food and water deprivation compared to 0h on jejunal crypt depth at week 1-3 of age (Figure 19b). For ileal crypt depth, only a single record indicated a significant positive effect at 48 h food deprivation compared to 0h at week 1 of age and two records indicated significant negative effects at week 2 and 3 of age with 48h food and water deprivation (Figure 20b). Thus, the majority of records did not find significant effects of 48h food or food and water deprivation compared to 0h for duodenal, jejunal and ileal crypt depths between week 1-3 of age.

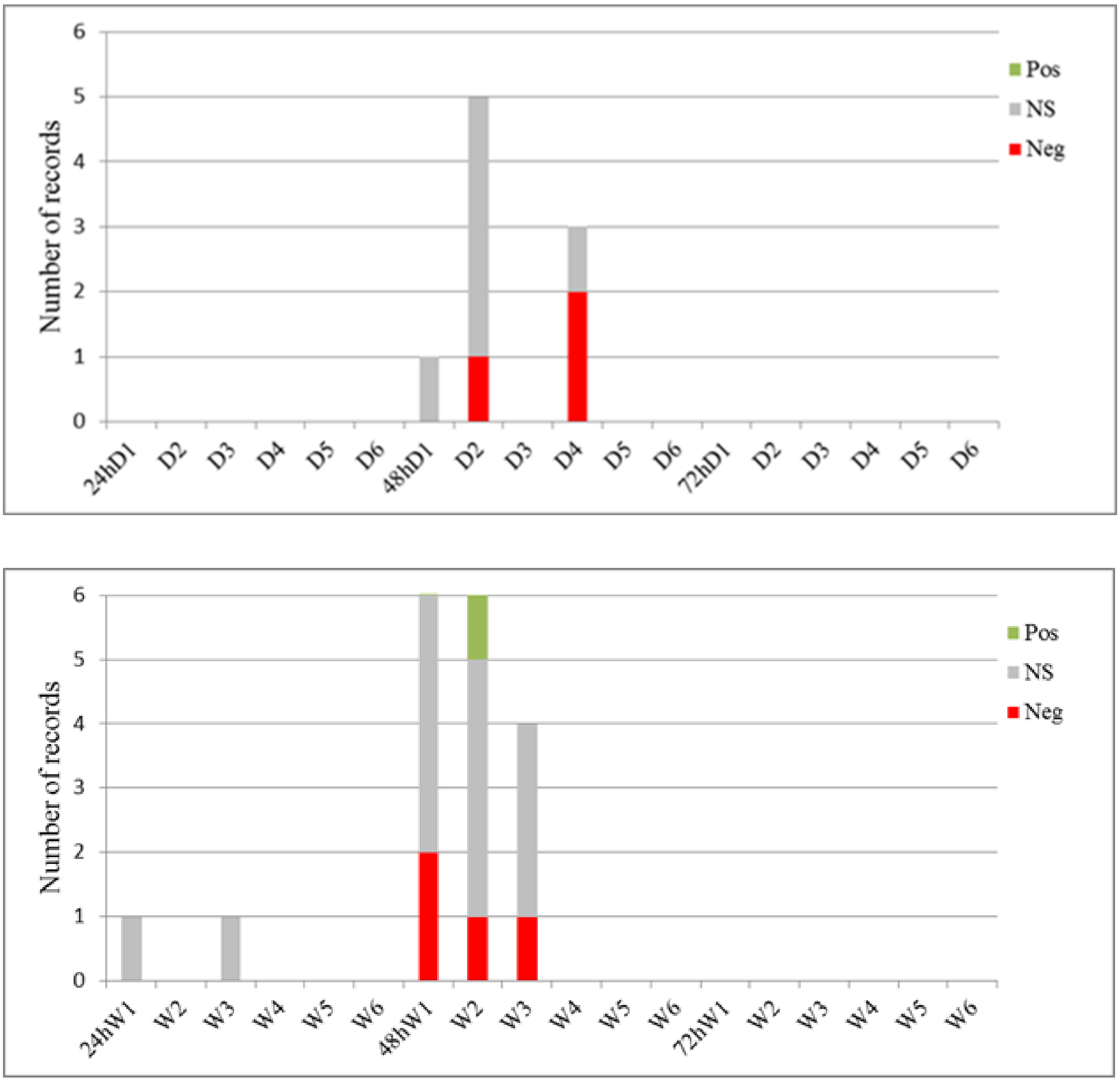


Figure 19 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative jejunal crypt depth*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

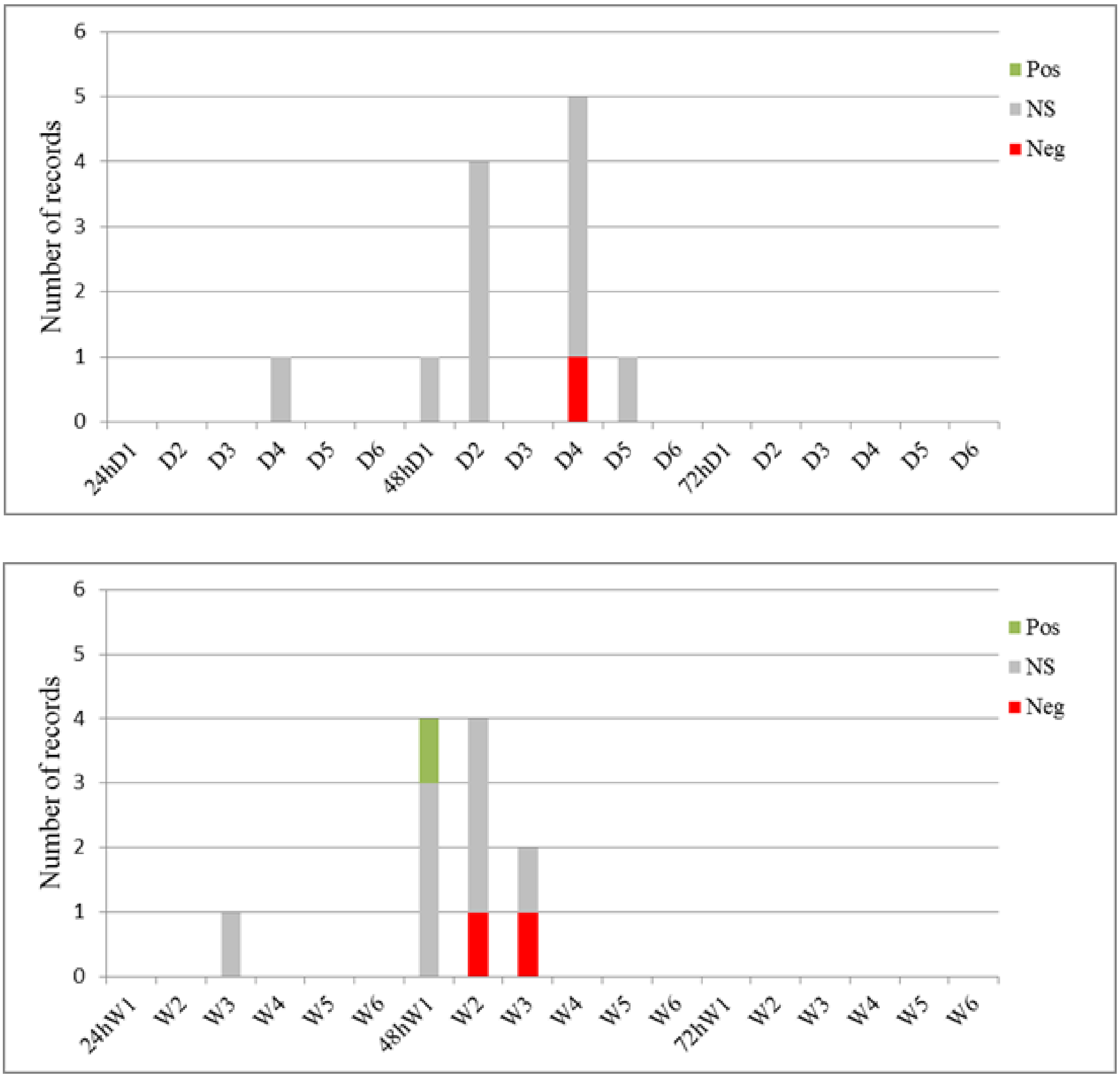


Figure 20 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***relative ileal crypt depth*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

3.3.4 Plasma T3, T4 and glucose concentration

Figures 21 and 22 show the number of records indicating an effect on plasma triiodothyronine (T3) hormone and thyroxine (T4) concentrations in the first week of life. Shortly after or during fasting, i.e. on day 1 and 2, records showed a significant negative effect on plasma T3 concentration for 24h and 48h food and water deprivation compared to 0h deprivation. Thereafter, no significant effects are observed (Figure 21). Records did not show effects of post-hatch food deprivation on plasma T4 concentrations (Figure 22).

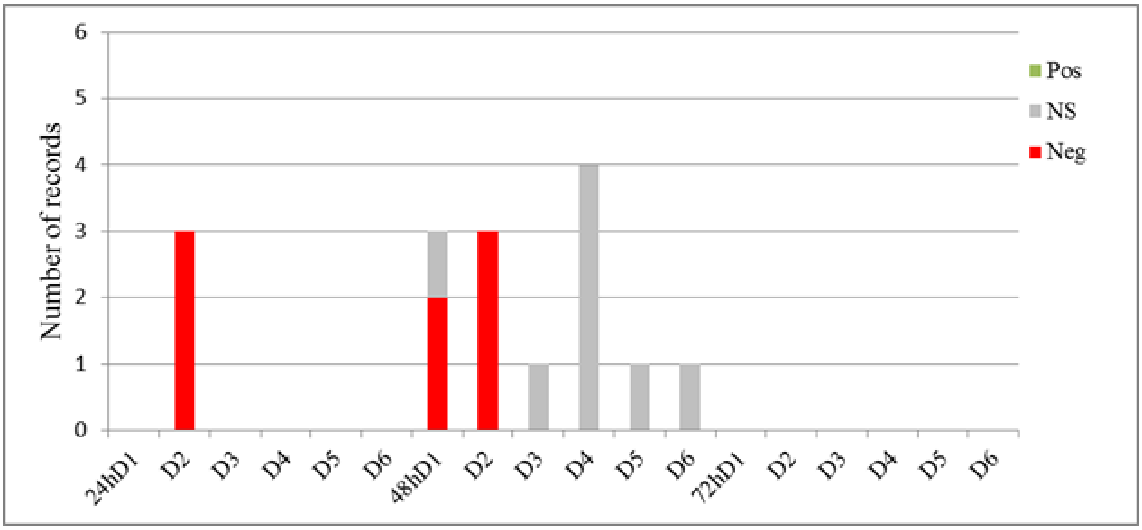


Figure 21. *Results of QA of records including the effect of post-hatch food and water deprivation on* ***plasma T3 concentration*** *in week 1 of age. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand for 1-6 days of age (D1-D6).*

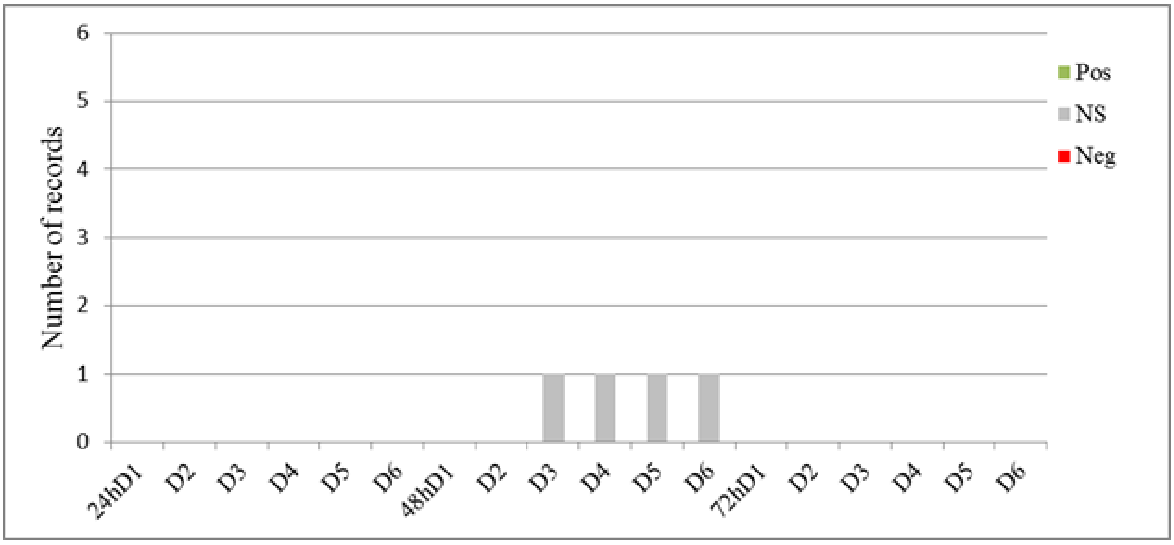


Figure 22. *Results of QA of records including the effect of post-hatch food and water deprivation on* ***plasma T4 concentration*** *in week 1 of age. The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days of age (D1-D6).*

Results for records on blood glucose concentration are shown in Figure 23. At 24h, food and water deprivation had a negative effect on blood glucose concentration on day 2 of age, but not at day 5 compared to 0h food and water deprivation (Figure 23a). A negative effect was also found for 24h food and water deprivation compared to 0h at week 1 and 2 of age (Figure 23b), but the number of records that was included in the QA was low (N=1); for 72h deprivation compared to 0h several records indicated a significant positive effect on blood glucose concentration in week 2 of age.

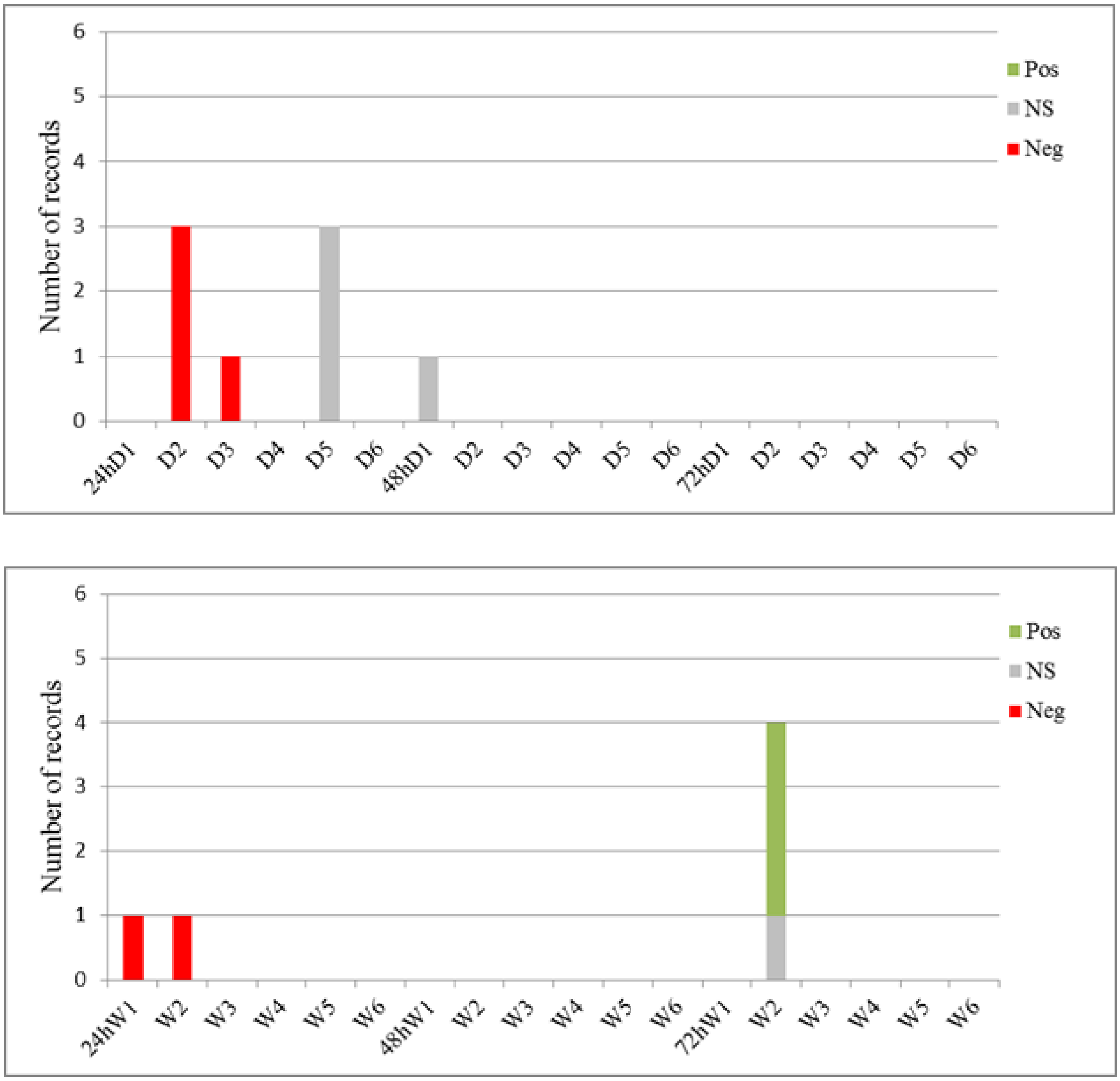


Figure 23 a (upper panel) and b (lower panel). *Results of QA of records including the effect of post-hatch food and water deprivation on* ***blood glucose concentrations*** *in week 1 of age (a) or between 1-6 weeks of age (b). The graph shows the number of records indicating a significant positive or negative effect, or indicating no significant effect, for the comparison between 0 hours of deprivation on the one hand and 24h, 48h and 72h post-hatch food and water deprivation (24h, 48h and 72h on the horizontal axis) on the other hand and for 1-6 days or 1-6 weeks of age (D1-D6 or W1-W6 on the horizontal axis).*

4 Discussion

In the current study, we systematically analysed the scientific literature with respect to effects of post-hatch food and water deprivation on measures of welfare (including performance, development and health) in chickens. A wide variation in measures on effects of post-hatch food deprivation has been found in literature. However, only a couple of measures have been sufficiently applied in studies to perform a meta-analysis. These are related to production (body weight, food intake and food conversion ratio), mortality and relative yolk sac weight. Despite a number of studies reporting immunological consequences of post-hatch food deprivation a meta-analysis of immunological measures was not possible, due to lack of use of common indicators; this topic will therefore be covered only in this section. For many other measures information was either reported incidentally or not in a standardised way, and/or, to our opinion, not sufficiently related to chicken welfare (such as slaughter yield characteristics). These are therefore not included in the current report (see also Appendix 2).

In the majority of studies, chickens were deprived from both food and water after hatching; few papers reported post-hatch feeding without water provision [5, 26] or subjected chickens to various duration of water deprivation [26, 40, 89], and some papers included a treatment group with immediate access to water after hatching [18, 20, 26, 31, 32, 40, 45, 59, 63, 65, 77]. This means that in the MA we could discriminate between treatments that received water immediately after hatching (WO) and treatments that received food and water at the same time (FW). This was not possible in the QA, except for production measures and mortality, due to insufficient records per measure for WO; results of the QA are therefore combined results of WO and FW treatment groups.

To determine whether post-hatch food deprivation affects chicken welfare, it is important not only to take into account whether there are short-term effects, but also whether there are long-term effects of food and water deprivation. There might be compensation for altered growth or physiological development during the life span of the chicken, since post-hatch food deprivation may also cause a delay in development, instead of a more long-lasting or permanent effect. We therefore included in the analysis and discussion section not only the short-term (days) but also the long-term effects (weeks), but it should be noticed that the number of studies including long-term effects is much smaller than the number of studies that have included relatively short-term effects of post-hatch food deprivation. In addition, it is often unclear whether the delayed development of the chicken has any consequences that affect welfare (including health, production and development) on the long-term; e.g., whether it affects the susceptibility for disease for example.

We performed a MA if sufficient records (N≥10-15 records) were available for a specific measure. MA uses a REML analysis to combine the results from multiple studies to increase power and to provide estimates of the size of effects [90]. This explains why results from the QA are not always in line with the MA; our QA only summarises findings from several studies, but does not combine these data for a more powerful/quantitative analysis. However, sufficient records need to be available to perform a MA. In the current study a MA of less than approximately 10-15 records yielded rather unreliable results, because e.g. several records from a single study could have a dominant effect on the results. In that case only a QA was performed. As with the MA, the reliability of a QA increases with increasing number of records. We applied a minimum of five records per measure to perform a QA, but some age/treatment combinations had fewer records. Results of QA with few records therefore need to be interpreted with care.

Due to a large variation in the duration of food and water deprivation reported in the various studies we defined classes of food and water deprivation times in our analyses (e.g. the 72h class covered the period from 60 to 84 hrs). This implies that if reported effects cannot be translated directly into a 'cut-off point'. In particular previous reports referred to 72h food and water deprivation as the time span over which a chick should be able to survive without food and water [6, 91]. In our analysis, however, the 72h deprivations class refers to the period between 60-84h (hence the cut-off is formally at 60 hours, but includes/may include studies up to 84 hours). In addition, studies differed with respect to the degree to which they reported the real age of the chickens, i.e. biological age, chronological age, and 'age unclear' (categories 1-3, respectively). These points need to be kept in mind when assessing the implications of this study on (existing or new) cut-off points.

4.1 Production measures and mortality

Delayed access to food and water after hatching causes weight loss during the holding (deprivation period) of the chickens, mainly due to dehydration of chickens and yolk and pectoral muscle utilisation, until the chickens receive food and water, after which body weight starts to increase [5, 9, 92, 93]. The results of the MA showed that post-hatch food and water deprivation has however long-term effects on production measures and mortality. With increasing duration of food deprivation body weight and food intake were negatively affected. Additionally, food conversion ratio and mortality in the first week were increased when food and water deprivation was longer than 84 h, whereas total mortality at day 42 was increased after 48 h food and water deprivation. Because the effects of post-hatch food and water deprivation on production measures and mortality may differ between studies that applied the treatments post-hatch and studies that applied the treatments post-pulling, we analysed category 1 and category 2 papers in addition to the analysis of all scientific papers (category 1+2+3) (Appendix 3). In Category 1 papers the biological age of the chickens is provided, i.e. in these papers the treatments were applied after hatching, whereas category 2 papers applied the treatments post-pulling. In theory, this may lead to different results, as in Category 2 papers early- and mid-hatching chickens are subjected to longer periods of food and water deprivation than late hatchers. This also concerns the control group (0h food and water deprivation post-pulling). However, separately analysing production measures for category 1 and 2 (where possible in case sufficient records available) did in general not lead to different results, despite that the effect of food and water deprivation was somewhat larger in Category 2 papers (e.g., a numerically lower body weight after post-hatch food and water deprivation for Cat 2 papers than for Cat 1 papers).

Results of the QA for production measures were in line with the results of the MA, except for total mortality, for which none of the individual studies included showed an effect of food and water deprivation. This may be explained by the fact that mortality is a more variable parameter (and can be recorded quantitatively only at group rather than individual level) which requires larger numbers (of studies) or an epidemiological approach to assess adverse consequences. Where records from single experiments did not report significant effects of post-hatch food and water deprivation on mortality, a meta-analysis on data of the combined studies showed that 48h post-hatch food and water deprivation or more had long-term effects on total mortality at day 42 of age. The results of the MA are statistically stronger compared to the results of single studies, as in the MA a greater number of observations are included and these derive from a wider range of subjects (broiler chickens, laying hens and poults) and experimental conditions.

The MA also showed that a very long duration of post-hatch food and water deprivation (>84h) had a pronounced negative effect on all production measures, including a large increase in total mortality compared to shorter durations of food and water deprivation. It should be noted that this long food and water deprivation has only been applied in category 3 studies, which means that the biological age of the chickens was unknown at the start of food deprivation. Therefore, the duration of food deprivation in these chickens may have been even longer than indicated; it is likely that early hatching chickens were included and possibly handling and transport took place before starting the experiments (e.g., [45]). In addition, the number of studies including such long post-hatch food and water deprivation duration is small. Nevertheless, the results of the analysis indicate that food and water deprivation of >84 h has major adverse effects on the performance of chickens.

Common hatchery procedures and transport usually may include a period of food and water deprivation of chickens for 50h or more before placement on the farm, and up to 72h if long transport is involved [7, 8]. This suggests that it is unlikely that in practice, at least in The Netherlands because of short transport duration, food and water deprivation for periods of more than 84h occur with very adverse negative effects on production measures. However, food and water deprivation of 48h is likely to occur more frequently in the commercial situation and is shown to have long-term consequences for growth and survival. The results of the MA indicate that up to day 42 there is no full compensation in body weight gain and food intake, whereas this could be suggested from the QA, because the QA in which the majority of the records indicated no significant effect of 24, 48 or 72h food and water deprivation compared to 0h on body weight at 6 weeks of age. Broiler chickens may be able to show compensatory growth when they are food deprived for a limited period during the first weeks of life [94], but this mechanism seems not to exist in neonatal chickens. It remains unclear whether the effects of post-hatch food and water deprivation with respect to growth and food intake are due to impaired development or only to delayed onset of growth. In case of a delayed onset of growth the actual growth starts with the moment of first feed intake and the same body weight will be reached at a later age but within a similar growth period as compared to early fed birds. We concluded that post-hatch food and water deprivation of 24h (body weight) or 48 h (food intake) have a long-term effect on performance and of 48h food and water deprivation has a long-term effect on mortality.

Few papers reported effects of early access to water (only, i.e. compared to deprivation of water) after hatching on production measures [26, 40, 89]. These studies indicated that broiler chickens and turkey poults provided with water immediately after hatching had a higher body weight up to a maximum of day 7 of age compared to water deprived and/or water and food deprived chickens or turkey poults, but for water deprivation alone (no food deprivation) no long lasting effects on body weight [26, 40] and mortality [40] were found. Thus, the transient responses to water intake immediately after hatching are suggested to represent enhanced hydration of the chickens or turkey poults with no long term effects on body weight and mortality [95]. This appears to be in contrast to the effects of food deprivation as reported in our MA. In this MA, we found that for none of the measures effects of food deprivation were dependent on the provision of water after immediately after hatching. However, only a limited number of studies focused on the effects of water and food deprivation separately.

4.2 Physiological measures

4.2.1 Yolk sac resorption and content

Towards the end of the incubation period, the part of the yolk which is not utilised during incubation will be absorbed into the abdominal cavity. The yolk provides immediate nutrition for maintenance and growth after hatching [51, 96, 97]. In the first 48h post-hatch, yolk contributes to small intestine maintenance and development. During this period, the chicken must make the transition from energy in the form of lipid from the yolk to utilisation of exogenous carbohydrate-rich food [73, 98]. It has been suggested that in the presence of food, the major route of yolk utilisation is via the yolk stalk into the small intestine. By contrast, in case of post-hatch food deprivation, yolk is mainly resorbed directly into the blood via the circulatory system. The presence of exogenous food in the gastro-intestinal tract stimulates release of yolk through the yolk stalk en enhances yolk secretion in the intestine [99]. Thus, early post-hatch feeding may stimulate yolk sac resorption in the intestine (e.g., [18, 54, 66, 95]), although studies also found that early post-hatch feeding did not stimulate yolk sac resorption (e.g., [20, 22, 23, 46, 56]). Based on these ambiguous results, effects of food and water deprivation on yolk sac resorption remain inconclusive. Higher resorption of the yolk sac is generally considered as positive for chicken development and has been suggested to stimulate the transport of immunoglobulins from the yolk to the chicken [66].

The inconclusive results of studies on the relation between post-hatch food deprivation and yolk sac resorption were confirmed by the results of the MA, showing that 24h and 48h post-hatch food and water deprivation did not have significant effects on relative yolk sac weight at day 3 of age. The reduction in relative yolk sac weight with 72h post hatch food deprivation compared to immediately post-hatch feeding and drinking may suggest that chickens subjected to long deprivation used the yolk sac for energy supply [45]. However, in case water was provided ad libitum post-hatch, but food was deprived unto 72h post-hatch, relative yolk sac weight increased, indicating reduced resorption of the yolk and overall body weight loss in the chickens. The difference between the FW and WO treatments was also described by Bierer and Eleazer [100], thus, early water provision by itself does not seem to stimulate yolk sac resorption. The results of the QA were in line with the results of the MA, with some records showing no effect of 24h, 48h or 72h food or food and water deprivation on relative yolk sac weight in the first week of age and others showing a significant positive effect (increased relative yolk sac weights), compared to immediate post-hatch feeding.

To our knowledge only one study examined effects of post-hatch food deprivation on yolk sac content in broiler chickens. 24h food only deprivation (water was available at libitum post-hatch) did not alter the lipid and protein contents in the yolk sack, but post-hatch food deprivation for 32, 40 and 48 h led to a retention of yolk lipid and a depletion of yolk protein [18].

4.2.2 Relative organ weights

Relative organ weights are usually assessed as indicators of chicken physiological development [87]. Several studies measured relative weights of organs directly involved in the ability of the chicken to digest and absorb food, such as the intestines (see next paragraph), liver, proventriculus, gizzard and pancreas (e.g., [18, 45, 65, 87]). It can be expected that in fed birds the digestive organs will increase more rapidly in size than in food-deprived birds. Thus, a delay in organ development is expected in post-hatch food deprived birds. Although a number of studies measured relative liver, heart and pancreas weight, these studies reported effects at different ages and hence insufficient records were obtained to perform a quantitative MA. Therefore, only a QA was done on organ weights.

With respect to relative liver weight, few records showed a significant negative effect of post-hatch food and water deprivation during the first week of age. Long-term effects (up to week 6) were, however, not found in the majority of studies. More or less the same results were found for relative pancreas and heart weight.

Relative gizzard and proventriculus weights were not included in the QA. These organ weights were not different in up to 48h food and water deprived chickens compared to early fed control chickens at day 7 of age [18]. El-Husseiny et al. [45] reported decreased proventriculus and gizzard weight up to day 7 of age when food deprived for 24-96h post-hatch compared to immediate feeding, but these values were not expressed as relative to body weight. Cengiz et al. [69] showed lower relative gizzard weight at 10 days of age for 36h post-hatch food and water deprivation compared to 0h food and water deprivation. At day 0, chickens provided with food and water immediately after hatching had higher relative stomach weights compared to chickens not provided with food [75]. Maiorka et al. [65] found that chickens that received ad libitum water post-hatch, but that were food deprived, had lower relative proventriculus and gizzard weights at 48h and 72h fasting compared to fed chickens. This was not observed in birds that fasted for 24h. Additionally, in turkey poults, food and water deprived for 56h post-hatch, gizzard weight was significantly reduced at day 7 compared to non-deprived poults [43].

We concluded that there does not seem to be a long-term effect of post-hatch food deprivation on relative weights of heart, gizzard, proventriculus, liver and pancreas. Although during the fasting period and the first week of life relative organ weight were lower in several studies, this seems to be a transient effect of post-hatch food and water deprivation due to a later onset of feed intake that is crucial for organ development.

4.2.3 Gut development

In the newly hatched chickens, the gastrointestinal tract is immature and in a process of substantial development and maturation, both physically (e.g. weight and length) and morphologically (villus height and area, maturation of enterocytes and goblet cells, organisation and establishment of the crypt region) [98, 101]. Intestinal development is also rapid with respect to enzymatic and absorptive activities [102]. It has been shown that the yolk contributes to small intestinal maintenance and development during the initial 48h post-hatch [73, 98, 101]. In addition, the intake of exogenous food is accompanied by rapid development of the gastro-intestinal tract and associated organs. Therefore, the timing (and form) of post-hatch feeding is critical for the development of the intestines [98] and may affect the digestibility and absorption of nutrients. During the same period, the gut-associated lymphoid tissue (GALT) is developing. It has therefore been suggested that post-hatch food deprivation may not only affect production performance, but also the immunological development [98], see also paragraph 4.3 (immunology).

Many studies included gut development to assess the effect of post-hatch food deprivation, the most frequent measures being intestinal length, weight, villus length and crypt depth (e.g., [20, 34, 43, 58, 65]). For duodenal, jejunal and ileal length, relative weight, villus length and crypt depth, it was possible to perform a QA. The QA showed that a number of records found that post-hatch food deprivation had a negative effect on duodenal, jejunal and ileal length, but no long-term effect of post-hatch food and water deprivation on length of gut segments was found. The QA of the weight of intestinal segments indicated that effects were mainly found for the jejunum in the first week of life, but long-term effects of food and water deprivation on intestinal segment weights appeared not to be present. For villus height, negative effects were mainly found for the duodenum (first week of age) and jejunum (week 1 and 2 of age); for crypt depth negative effects were mainly found for jejunum in the first weeks of life. However, for all measures of gut development, many records also indicated that there was no effect and some records indicated a positive effect.

It has been suggested that effects of post-hatch food deprivation effects can be more apparent in certain intestinal segments. E.g., Geyra et al. [34] and Uni et al. [39] observed that delayed development was present in duodenum and jejunum, but not in the ileum. The different intestinal segments have different functions. Consequently, effects of post-hatch food deprivation on the various segments might be different. The results of the QA also point into this direction, with most significant effects found for jejunum.

In addition various studies also reported additional measures, such as crypt size, villus/crypt ratio, crypt proliferation, villus area and the rate of enterocyte migration [34], mucosal enzyme activity [39], goblet cell development [84], and mucosal aspects [69]. Insufficient records were available to include these in the MA or QA.

The results of the QA indicate that the lack of access to post-hatch food may cause a depression or a delay in intestinal development, although studies showed ambiguous results. A delay in intestinal development can have long-lasting effects on performance by affecting digestion and absorption of nutrients [98]. This seems to be confirmed by the MA results on body weight gain. However, the QA also indicates that gut morphology effects (if present) are only observed during the first week(s) of life. This does not exclude that secondary effects (resulting from impaired digestion or absorption capabilities, e.g. impaired body weight gain) were found to be long-lasting.

4.2.4 Hormones and plasma glucose concentration

Plasma hormone concentrations were measured in a few studies. Only plasma corticosterone and thyroid hormones (triiodothyronine (T3) and thyroxine (T4)) were reported. Results for plasma corticosterone concentration in fasted chickens, just before post-hatch feeding started, or immediately after feeding, were inconsistent. Studies reported either a higher plasma corticosterone concentration in fasted chickens or no difference between fasted and fed birds [46, 75, 76, 103]. No long-term effects on plasma corticosterone concentration were found [46]. Plasma corticosterone concentrations are often measured as indicator of stress and thus potential welfare problems. However, corticosterone is also involved in regulation of the metabolism and may therefore respond to fasting (e.g. [87]), which makes it difficult to interpret the plasma corticosterone levels post-hatch.

Plasma T3 (triiodothyronine) levels were lower in post-hatch food deprived chickens during the period of food deprivation as compared to immediately fed birds, and T3 increased in response to feeding to similar levels as in the control early fed groups [5, 76, 104]. This was confirmed in our QA. Plasma T3 levels are indicative of the animal’s metabolic rate. Thus, lower T3 values indicate a lower metabolic rate in fasting birds [5]. QA did not show effects of post-hatch food deprivation on plasma T4 concentrations.

In addition to T3 and T4, plasma glucose has been measured as indicator of energy homeostasis, sometimes together with e.g. lactate [75, 76], protein or triglyceride levels [22]. QA could only be performed for plasma glucose levels. Results of the QA suggest a negative effect of 24h food and water deprivation on plasma glucose concentration during the first days and first two weeks of life compared to early feeding [22, 75, 76] and may indicate a physiological need for energy or is just a result of more glucogenic energy from feed. The positive effect of 72h deprivation on plasma glucose concentration at 2 weeks of age might indicate a physiological compensation to the early food deprivation.

4.3 Immunological measures

Several studies addressed effects of post-hatch food and water deprivation on (the development of) the immune system and the response to challenges [33, 38, 41, 47, 50, 68, 78, 81, 82, 105]. Since these studies reported a wide variety of immunological measures, however, we were unable to do a MA or QA on immunological measures , due to insufficient records for each distinct measure. The results of these studies will therefore be summarised and discussed below.

Early hatching chickens that remained 32h in the hatcher without food and water had less CD3+ cells in the spleen and ileum at opening of the hatcher than late-hatching chickens (0h and 16h without food and water). This may indicate a delayed development of cellular immunity in the 32h deprived chickens [78]. However, these authors did not investigate long-term consequences of food deprivation on immunological characteristics. Others showed that immediate post-hatch feeding had positive effects on the development and maturation of the gut associated lymphoid tissue (GALT). Concomitant with the rapid development of the intestines after hatching, a rapid development of the GALT occurs. Early feeding therefore seems to initiate microbial colonisation and consequently earlier immune maturation of the gut [106]. Dibner et al. [33] provided a nutritional supplement immediately post-hatch and subjected chickens to 72h post-hatch food and water deprivation (thereafter all chickens received a control diet). Immediate post-hatch feeding resulted in higher bursa weights, higher lymphocyte proliferation in the bursa, a more rapid appearance and a higher level of biliary IgA, and earlier and more rapid proliferation of germinal centres in the cecal tonsils measured until 21 days of age, suggesting enhanced humoral immunity. Bar-Shira et al. [68] showed that long-term effects of 72h post-hatch food deprivation were not found in the foregut (duodenum, jejunum and ileum), but they were found in the hindgut (cecum and colon). In the foregut, the development of lymphoid tissue was only temporarily impeded. However, in the hindgut and gut-related cloacal bursa, GALT activity was significantly delayed during the first two weeks of life. Systemic and intestinal antibody responses following rectal immunization to antigen were lower, colonization of the hindgut (cecum and colon) by T and B lymphocytes was delayed, as well as the expression of chIL-2 mRNA in hindgut T lymphocytes. It was also found that the increase of B and T population size in the cloacal bursa was delayed with time. Full recovery occurred from 2 weeks of age [68]. Simon et al. [38] found relatively limited effects of 72h food and water deprivation compared to no deprivation on ileal immune development (ileal cytokine and immunoglobulin mRNA expression), measured up to 42 and 140 days of age in broilers and laying hen chickens respectively, which was in line with the results found by Bar-Shira et al. [68]. In addition, inconsistent effects were found of 72h post-hatch food and water deprivation compared to no deprivation on natural antibody responses to keyhole limpet hemocyanin (KLH), measured up to 42 or 140 days of age for broiler chickens and laying hens, respectively [38]. Effects of post-hatch food deprivation on bursal weight (measured up to 42 days of age) were smaller in the study of Simon et al. [38] compared to earlier studies [33, 41, 68]. Studies on GALT development suggested that chickens with delayed access to food might be more susceptible to environmental pathogens than immediately fed chickens [33, 68], but whether this is indeed true is to our knowledge not investigated.

Some studies included the response of early fed chickens versus post-hatch food and water deprived chickens to vaccine or disease model challenges. Dibner et al. [33] showed that early feeding was associated with improved bird performance (in terms of body weight and food intake) following a coccidiosis vaccine challenge at 14 days of age compared to 72h post-hatch food and water deprivation. Simon et al. [50] showed that, in response to a non-infectious lung-challenge at 4 weeks of age, broiler chickens that received food and water immediately post-hatching had reduced clinical signs and better performance compared to broiler chickens subjected to 72h post-hatch food and water deprivation. Walstra et al. [105] showed that laying hen chickens had better intestinal immunity as measured by the response to an *Eimeria* infection at 53 days of age when kept under optimised incubation conditions and immediate post-hatch food and water provision as compared to sub-optimised incubation conditions and food delay (food and water provided to all chickens after pulling). In the same study, no treatment differences were found in response to a respiratory infection at 92 days of age. However, in this study early feeding was confounded with incubation conditions. Thus the protective effect to a coccidiosis infections cannot be attributed to early feeding per se. In another study, the response of laying hen chickens to *Eimeria* vaccination 6h post hatch/after hatching was delayed when the chickens were food deprived (up to 48h) compared to immediate post-hatch feeding. However, the overall response (oocyst shedding) was unaffected by the post-hatch feeding treatment [82]. Juul-Madsen et al. [47] found a tendency of a delay and a decrease in the amount of specific antibodies against a IBDV vaccine, administered at 10 days of age in broiler chickens subjected to 48h post-hatch food deprivation compared to broilers fed immediately or deprived for 24h. Nnadi et al. [81] observed a higher antibody response to Newcastle disease vaccination at 21 and 42 days of age in early fed chickens compared to 72h post-hatch food and water deprived chickens. In early fed chickens, a reduced mortality due to necrotic enteritis and enhanced T-cell proliferation 10 days post-infection were observed in response to a *Clostridium perfringens* challenge at two weeks of age, compared to 48h post-hatch food and water deprived broiler chickens [41].

From the various challenge studies, it has been suggested that post-hatch food deprivation seems to have adverse consequences for the immune response to infectious disease challenges later in life [41, 47, 50]. However, in only a few studies disease challenges were used. This means that effects of post-hatch food and water deprivation on disease resistance in later life are hardly investigated yet, and conclusions about effects of post-hatch food deprivation on disease resistance and health are largely based on suggestion and speculation.

To conclude, various studies indicate an effect of post-hatch food deprivation on the development of the immune system and the response to challenges, but it often involves single studies, effects may be temporary and results are sometimes inconsistent. It has been suggested that post-hatch food deprivation impairs health and disease resistance [3], but more evidence is needed to substantiate this conclusion. However it seems that immune development is affected by early feeding. It should be emphasised that the immune system is that complex that based on only one or a few parameters an increase or decrease cannot be related to an increase or decrease in disease susceptibility or health. Consequently, the actual meaning of the various measures in terms of robustness, disease resistance and performance of chickens merits further investigation. In addition, a possible relationship between post-hatch food deprivation and the need for antibiotic treatments in broiler production has not been confirmed, and the suggestion that antibiotic treatments could be reduced by providing early feeding [3] is to our knowledge only based on anecdotic information.

4.4 Timing of voluntary post-hatch food and water intake

Under natural conditions when chickens are raised with a mother hen, the first food normally eaten by a chicken will be offered to it by the mother hen [107]. In the absence of a mother hen, newly hatched chickens peck indiscriminately at non-food and food objects starting when they are a few hours old [108, 109], but by three days of age pecks are directed primarily at the food [109]. Until three days post-hatch, the pecking behaviour of newly hatched chickens seems to be independent of the nutritional state [110]. By pecking at edible and non-edible objects, chickens learn to discriminate between food and non-food objects [111]. The role of the mother hen to attract the chick to water is lacking. Newly hatched chickens show an innate response to peck at shining objects to learn where to find water. They recognise drinking behaviour of other chickens and are attracted by other chickens showing drinking behaviour [108, 112]. These studies were performed in non-commercial breeds or jungle fowl.

To our knowledge there is little information from peer-reviewed scientific publications on the timing of the first food and water intake of newly hatched commercial chickens in the hatcher or in systems where they hatch in the broiler house. Nielsen et al. [80, 113] observed broiler chickens feeding on day 1 post-hatch, but the moment of first food intake was not described. Pinchasov et al. [28] found that broilers with immediate access to food and water consumed 1.5 g of food during the first 24 h. Observations of broiler chickens in the Hatch-care system indicated that water intake was observed at 11.9h, 9.7h and 13.1h after hatching for early, middle and late hatching chickens and the authors indicated that body weight increased due to food intake started after water intake [114]. In a pilot experiment in small experimental hatching chambers, it was found that the first food intake of broiler chickens was between 1-2.5 h after hatching, dependent on moment of hatch after incubation and broiler breeder age. There was no information on the timing of the first water intake [16]. Observations around the peak of hatching in a system where broiler chickens hatched in the broiler house indicated that broilers were seen at the food at on average 2.5 h after hatching, with large variation among individual chickens (between 0:30 and more than 9 h after hatching). It was not checked whether chickens actually ingested food. Broilers were observed at the nipples after being observed at the food, at on average more than 5h after hatching [115]. However, in this system the distance from the hatching trays to food and water was larger compared to the studies of Van den Brand [16] and Van der Pol et al. [114].
Thus, although it is likely that the first food intake of newly hatched chickens is within the first 24h post-hatch and even within a couple of hours after hatching, the exact timing of first food and water intake of commercial breeds when water and food is available immediately post-hatch is unknown. Additionally, it is unknown which factors might affect the moment of first food and water intake. Studies indicate that food pecking starts a few hours after hatching, but these observations need to be confirmed in controlled studies.

In addition, it is unclear whether post-hatch food deprivation actually leads to stress and frustration, and thus reduced welfare, due to the unfulfilled behavioural and physiological need for food intake in chickens post-hatch. The only indication of stress due to food and water deprivation is from a study of Khosravinia et al. [116]. They measured behaviour during the final hour of the fasting period in groups of chickens subjected to food deprivation for 12, 24, 36 and 48 h. These chickens were held in boxes until placement in the pen. More chickens showed jumping and active wakefulness and less chickens showed sitting behaviour with increasing duration of food deprivation. This was interpreted as an increased motivation to search for food and water in chickens that were subjected to longer food deprivation [116] and might indicate that they experience stress or frustration. It is, however, difficult to state, based on a single study, that the physiological and behavioural needs of the post-hatch chicken are not met because of a delayed food intake post-hatch, as suggested by 'Wakker Dier' [3]; this area needs further investigation.

4.5 Effects of post-hatch food deprivation in relation to selection for efficient growth

In the report of 'Wakker Dier' [117] and the response of 'Wakker Dier' [4] to Lourens and Leenstra [6], it was suggested that due to the selection for efficient growth in the modern broiler chickens [118], broiler chickens have an increased need to eat and drink immediately post-hatch compared to breeds that do not have been selected, or have been selected to a lesser extent for efficient growth. In addition, it was suggested that the yolk sac reserves in modern broiler strains would be insufficient to overcome a period of post-hatch food deprivation [3].

The yolk stores in the newly hatched chick should represent energy and water supplies sufficient for three days survival without further provision of food and water (in the absence of excessive thermoregulatory demands). However, it has been suggested that because of the high metabolic rate in fast growing broiler strains yolk sac reserves are depleted more rapidly [91]. Indeed, fast growing broiler strains have a higher embryonic metabolic rate compared to slower growing strains [119], and it has also been shown that yolk sac resorption was faster in fast growing lines compared to slower growing lines or layer strains [23, 120, 121]. In addition, it has been suggested that food intake behaviour may develop more rapidly in broiler chickens as compared to jungle fowl [121]. Nielsen et al. [80] showed that post-hatch eating behaviour was delayed for about 4h in broilers from a slower growing strain compared to broilers from a fast growing strain. It is therefore likely that yolk sac reserves in modern broiler strains would be depleted earlier when fasting than in slower growing strains.

Few studies, however, included different genetic strains as a factor in their study on the effects of post-hatch food deprivation. Gonzales et al. [23] found that 36h post-hatch food and water deprivation compared to 8h deprivation had negative effects on body weight at day 7 for fast growing broiler chickens, but not for laying hen chickens or a slower growing broiler strain. However, Simon et al. [38] subjected broiler and laying hen chickens to 72h post-hatch food and water deprivation and early feeding, and found a decreased body weight gain up to 35 days of age in both breeds compared to early fed chickens from these breeds. Zhao et al. [32] showed that selection lines for high and low body weight gain differed in their response to fasting, indicated by blood glucose concentrations and relative pancreas weight. E.g., selection lines for low body weight showed lower relative pancreas weight when fed after 72h of food deprivation compared to selection lines for high body weight, which indicated a stunted development of the pancreas following 72h post-hatch food deprivation [32].

4.6 Potential causes for variation in effects of post-hatch food deprivation chickens

As demonstrated in both the MA and QA the variation between studies in the effects of post-hatch food and water deprivation on several measures is considerable. It may therefore be suggested that several other factors may interfere with the effect of early food and water deprivation on development and performance of chickens. In this paragraph, these factors are discussed briefly (Table 3). These factors can be classified into biotic and abiotic factors, i.e. in factors directly associated with the hatchling itself or external/environmental factors.

Table 3 *Overview of biotic and abiotic factors that may interfere with the effects of post-hatch food deprivation on chicken development and performance.*

Biotic factors	Abiotic factors
• Strain (e.g. layers vs broilers)	• Storage duration of eggs before incubation
• Breed (different genetic lines within layers or broilers)	• Incubation temperature
• Age of the parent stock	• Incubation relative humidity
• Egg size	• Incubation gas concentrations
• Hatching time	• Diet composition in early life • Transport (duration, quality)* • Vaccination protocol* • Housing temperature and ventilation* • Other environmental conditions (noise, vibrations, dust)* • Disturbances (e.g. inspections by people)* • Food (and perhaps water) quality, physical presentation, presence (accessibility; way of presentation)*
• Sex	

For some of the factors mentioned in the table above (marked *), , such as vaccination protocol, transport duration and quality [42], housing temperature [122], housing ventilation and maybe more, relatively little if anything is known. Therefore these factors will not be discussed further.

4.6.1 Biotic factors

- Type: layers and broilers are selected for different production purposes, which has led to physiological differences during incubation and post-hatching. For example, differences exist in egg composition [123], resulting in differences in chicken weight, residual yolk weight, and organ development (e.g. [123-126]). Whether these differences in embryonic development are associated with differences in chicken quality has not been established yet. Yet, available scientific knowledge indicates that broiler and layer chickens possibly differ in their response to early or delayed feeding (see also paragraph 4.5).

- Strain/cross: comparisons between layer lines on chicken quality have hardly been made, but comparisons between broiler lines on chicken quality have been investigated more often. Nangsuay et al. [127, 128] concluded that Ross 308 embryos had a lower nutrient efficiency, produce more heat during late incubation, and hatch later than Cobb500. In addition, Cobb500 broilers were more vulnerable to overheating during incubation (see abiotic factors). Also O'Dea et al. [129], Hamidu et al. [130] and Tona et al. [131] demonstrated differences between broiler breeds in egg composition, embryonic development and hatching time. This might affect chicken quality, and therefore it can be suggested that different breeds will respond differently to delayed or early feeding (see also paragraph 4.5).
- Age of the breeder: older breeders in general produce larger eggs, but even when eggs of the same size of young and old breeders are compared, it has been shown that egg composition differs between eggs from young and old breeders [132-134]. However, this does not automatically lead to differences in chicken weight at hatching, but differences in chicken composition will probably occur. Chickens obtained from older breeders demonstrated to have more protein and fat deposition in the body [132], which might make them less vulnerable for variable circumstances after hatching. This is for example demonstrated by Weijntjes et al. [135] who showed that chickens of older breeders are less sensitive to low ambient temperatures during the early post hatch period than chickens of younger breeders. Breeder age may influence effects of early food deprivation [49, 136], and because of that difference among studies in the effect of delayed or early nutrition might be related to differences in age of the breeder [44, 72].
- Egg size: The size of the egg, even within a given breeder age, largely determines the weight of the hatchling. Larger eggs produce more heat during incubation [137] and produce heavier hatchlings [132, 137], but also the protein and fat deposition in the body differs [132, 137]. This suggests that chickens of larger eggs are more mature at hatching and have other nutritional and environmental needs. Again, it is not clear whether egg size and consequently hatchling weight indeed influence effects of delayed or early nutrition.
- Hatching time: Physiological differences exist between hatchlings of the same hatch. It has been shown that early, midterm and late hatching chickens are physiologically different [29, 31, 58, 74, 86, 87]. This means that effects of delayed feeding may not only depend on the duration of post-hatch food deprivation, but also on the differences in hatching moment (early/midterm/late hatching) due to the differences in physiological status of the chickens. As has been demonstrated, early, midterm and late hatching chickens might differ in their response to delayed feeding [31, 86, 87]. It is thus important to consider this aspect when comparing studies that provided food immediately after hatching compared to studies that provided food immediately after pulling.
- Sex: Male and female chickens hardly differ in their embryonic development, although female chickens in general hatch somewhat earlier than male chickens [75]. Because in some studies on delayed or early feeding only one sex of chickens is used, it might be that the applied treatments are partially related to that specific sex. However, the aspect of difference in hatching time between females and males is hardly taken into consideration in experiments.

4.6.2 Abiotic factors

- Storage duration of eggs before incubation: Eggs are sometimes stored for up to 14 days before incubation is started. Prolonged storage has been shown to negatively affect chicken quality at hatching and later life performance of chickens in terms of growth [138-140]. It can be speculated that differences could existed between studies in storage duration and that there are differences in chicken quality at hatching. Furthermore, it can be speculated that chickens obtained from prolonged stored eggs, differing in residual yolk weight at hatching, will respond different to delayed or early nutrition than chickens obtained from eggs incubated shortly after oviposition. E.g., Careghi et al. [5] showed that long duration of egg storage depressed relative growth, not only in chickens with immediate access to food, but also in those subjected to post-hatch food deprivation, but this growth depression was significantly aggravated in eggs subjected to long storage time.

- Incubation temperature: A very important factor affecting chicken quality at hatching is the incubation temperature. E.g. Lourens et al. [141], Molenaar et al. [142], and Maatjens et al. [143, 144] demonstrated strong effects of incubation temperature on chicken quality at hatching. Furthermore, e.g. Hulet et al. [145], Leksrisompong et al. [146] and Molenaar et al. [142] showed that incubation temperature has long term effects on growth and development in later life. Particularly a high incubation temperature during the last week of incubation, that quite often occurs in practice [147] appears to affect chicken quality, particularly when the period between hatching and pulling is prolonged due to increased body weight loss as a result of evaporation. However, interaction between incubation temperature and delayed or early feeding has to our knowledge not been investigated yet.
- Relative humidity: Relative humidity in the incubator is affecting the weight loss of the eggs and consequently chicken quality. In general, it is assumed that egg weight loss between start of incubation and day 18 of incubation should be between 12 and 14% for optimal results. Van der Pol et al. [122] demonstrated that relative humidity is of less importance in case the incubation temperature remains optimal. However, particularly in relation to chicken quality, the period between hatching and pulling is of interest. In case relative humidity in the incubator is low, chickens loose more water via evaporation and consequently chicken quality might be affected. Possibly, chickens that are more dehydrated before pulling, will respond differently to post-hatch food deprivation than non-dehydrated chickens.
- Incubation gas concentrations: During incubation oxygen is used by the embryos to metabolise egg nutrients and to convert them into body tissues. Consequently, carbon dioxide is produced. Both gasses might affect chicken quality. Lourens et al. [148] and Molenaar et al. [149] demonstrated that lower oxygen concentrations than normal resulted in lower yolk free body weight and higher residual yolk weight at hatching. This phenomenon might also play a role when experiments are carried out at high altitude. Additionally, high carbon dioxide concentrations during late incubation affect hatching time (e.g. [150]), which might affect chicken quality at hatching. It can be speculated that both oxygen concentration and carbon dioxide concentration during incubation can interact with delayed or early post-hatch feeding, but this has not been demonstrated in scientific literature yet.
- Diet composition: A factor that does not affect chicken quality at hatching itself, but might explain differences between studies on post-hatch food deprivation is the diet composition [8]. Effects of diet composition in combination with delayed or early food provision has hardly been examined, but based on the limited studies done in this field [77, 151], it appears that diet composition indeed plays a role in the effects found of post-hatch food deprivation. In studies on post-hatch food deprivation the food composition is often not mentioned. However, it probably varies considerably between studies, which could explain differences in in the outcomes of studies on post-hatch food deprivation.

4.6.3 System comparisons

Systems have been developed that enable newly hatched chickens to eat and drink. Examples are the Hatch-care system, where broilers are provided food and water in the hatcher [114], and systems where eggs are transported to the farm at day 18 of incubation and broilers hatch in the house, where they have immediate access to food and water (e.g. Patio [7], One2Born [152] and X-treck [153]).

Few studies actually performed a system comparison, confounding the effects of post-hatch food and water deprivation with other factors, such as specific climate conditions (e.g. [10, 105]). These studies were excluded from the MA and QA in case the effects of food and water deprivation were not determined separately. For the Patio system, it has been suggested that hatchability, early growth and liveability were better compared to chickens subjected to conventional hatchery and transport procedures [7], but these results are from a single publication only. A study comparing the X-treck system (hatching in the broiler house) with the conventional hatching and transport procedures on commercial farms indicated improved litter quality and reduced footpad lesions for broiler chickens from the X-treck system compared to broilers subjected to standard hatchery procedures [154], but also these results are from a single study only.

4.7 Remarks and conclusions

The central aim of the current report was to determine whether chicken welfare is affected by post-hatch food and/or water deprivation. To assess welfare, animal scientists include various measures related to the biological function and the animal's ability to cope with stress [13]. With respect to effects of post-hatch food and water deprivation, available studies focused on certain aspects of animal welfare only: production measures (growth, food intake and food conversion rate), mortality, physiological development (organ development, some hormone concentrations, plasma glucose, gut development), immunological measures and resistance to disease challenges. Some dimensions of welfare, especially the behavioural dimension and the patho-physiological dimension (e.g., morbidity) in relation to post-hatch food deprivation have not been studied as extensively. In addition, few publications were found for the effects on behaviour and indices of stress or frustration during the fasting period. The current study integrates the results of available studies on post-hatch food and water deprivation, and provides the best possible overview of the state-of-the-art, given the actual state of knowledge in science. Yet, despite that not all welfare measures have been studied extensively, it gives indications to what extent chicken welfare is affected by post-hatch food and water deprivation.

With respect to the conclusions below, it should be noted that in our analysis we did the following to overcome differences between scientific publications in the duration of post-hatch food and water deprivation, and to overcome uncertainty in several papers with respect to the biological or chronological age. To overcome the variation in duration of post-hatch food and water deprivation the following classes were made: 0-12h, 12-36h, 36-60h, 60-84h, >84h, these were labelled as 0, 24, 48, 72 and >84. Thus, these labels should not be regarded as cut-off points referring to an exact time point but to a certain time window. This also means that the category '0h' refers to a control group which is fed within 0-12 h post-hatch if the biological age of the chickens is known. To overcome the (partially) unknown biological age of the chickens in various scientific papers, we labelled the papers into three categories according to the information provided in the papers. In Cat 1 papers the biological age (hatching moment) of the chicks was (more or less) known (in general plus/minus up to 6h accuracy). In Category 2 papers (chronological age, i.e. pulling moment known) and Cat 3 papers (age unknown; most probably post-pulling age but not clearly described in the papers) the control groups were most probably fed within 0-12 h post-pulling (rather than post-hatch). This implicates that a certain percentage of the chickens in the experiment (namely the early- and mid-hatchers) were subjected to (sometimes considerably) longer durations of food and water deprivation than indicated for the treatments. With respect to the question whether early hatchers need food and/or water, this implies that Cat 2 and Cat 3 studies were inaccurate in that in these experiments both deprived and control groups were composed of chicks with a range of biological ages.

The thorough analysis of peer-reviewed publications in the MA and QA on the effects of post-hatch food and water deprivation on body weight, food intake, food conversion ratio, mortality and relative yolk sac weight resulted in the following conclusions:

- Post-hatch food and water deprivation of 24h[1], 48h, and 72h result in a significantly reduced body weight up to 42 days of age. It remains unclear whether the effects of post-hatch food and water deprivation on body weight are due to impaired development or only to delayed onset of growth;
- Body weights at 7, 21 and 42 days of age showed a linear reduction with increased food and water deprivation durations post hatch (24, 48, 72, >84h post-hatch) and similar results were found for food intake;
- Food conversion rate at 21 and 42 days of age was increased (i.e. less efficient utilisation of feed) from 48h food and water deprivation onwards;
- Although single studies did not find effects of post-hatch food and water deprivation on mortality, the MA showed that 48h post-hatch food and water deprivation leads to significantly higher total mortality at 6 weeks of age than 0 or 24 h of food and water deprivation;

[1] Note that to overcome the variation in duration of post-hatch food and water deprivation the following classes were made: 0-12h, 12-36h, 36-60h, 60-84h, >84h, these were labelled as 0, 24, 48, 72 and >84. Thus, cut-off points do not refer to an exact time point but to a certain time window.

- Very long (>84h) post-hatch food and water deprivation has adverse effects on almost all production measures and mortality;
- Results of post-hatch food or food and water deprivation on yolk-sac resorption were inconclusive;
- There was no effect of early water provision on body weight, food intake, food conversion and mortality (i.e. comparing chickens that were either only deprived of food or that were deprived of both food and water).
- The results of the MA showed that effects of post-hatch food and water deprivation on body weight, food conversion rate and food intake were in general similar for category 1 papers (biological age of the chickens known) and category 2 papers (treatments applied post-pulling, i.e. biological age of the chickens unknown), with sometimes somewhat larger effects found for Category 2 papers (see Appendix 3). For mortality, no discrimination could be made between category 1 and category 2 papers. This confirms that 48h (36-60h) food and water deprivation since hatching seems to be a cut-off point for the effects of food and water deprivation on production results, and indicates that for mortality this cut-off point also exists.

The additional QA showed that:

- There seems to be a temporary negative effect of post-hatch food and water deprivation on the development of liver and pancreas, as no long-term effects on relative organ weights were seen;
- Post-hatch food deprivation may delay the development of duodenum, jejunum and ileum, indicated by a decreased length, relative weight and reduced villus height and crypt depth. These effects are mainly observed in the first week of age and thus seem to be relatively short-lasting. Plasma T3 and glucose concentrations are negatively affected by post-hatch food and water deprivation during and shortly after fasting.

In addition, the following was concluded with respect to immunological studies:

- It is likely that post-hatch food and water deprivation changes the development of the immune system, but the actual benefit of early food and water provision for disease resistance is yet unknown.

With respect to the behavioural need to eat and drink post-hatch:

- Studies indicate that chickens start to peck at and ingest food within a few hours after hatch. However, whether delayed provision of food and water post-hatch actually leads to enhanced feeding motivation, behavioural deprivation, thwarting of the ethological need or a change in behavioural development later in life is yet unclear.

A range of biotic and abiotic factors may affect the response of chickens to early feeding or post-hatch food deprivation. These may explain the large variation in outcomes of the various studies with respect to the effects of post-hatch food deprivation. The extent to which these factors affect the response is largely unknown. To some extent this also concerns the impact of genetic selection for efficient growth on the response to post-hatch food and water deprivation.

4.7.1 Response to the points raised by 'Wakker Dier'

The current report takes into account the points of criticism raised by 'Wakker Dier' [3] in response to Lourens and Leenstra [6] and the questions posed by CBb, as listed in paragraph 1.2. The current paragraph provides a short answer or indicates where in this report the particular point has been discussed.

1. *The current report should include the scientific publications, that were referred to in the publication of Wakker Dier [3, 4].* In this report we included all published peer-reviewed studies we could find (including more recent publications) with respect to post-hatch food and water deprivation. 'Wakker Dier' refers multiple times to non-peer reviewed publications such as review reports [155-158] and publications in the popular press (e.g., [159-161]). These publications are summaries based on the experiments analysed in the current report. Furthermore, as a general rule, we included these types of publications only if we considered these added new information that was relevant to the discussion on the subject of the report.
2. *The current report should refer to the current legislation in relation to the request of Wakker Dier [3, 4].* The current and relevant legislation concerns 'Wet Dieren' and 'Besluit Houders van Dieren' and is summarised in paragraph 1.1. and copied in Appendix 1.

3. *The current report should provide clarification to the response of Wakker Dier [4] that the content of the report of Lourens and Leenstra [6] and the context (current legislation) it refers to are not correct.* This is answered in paragraph 1.1: the basic assumptions in [6] referred to legislation stating that food and water should be provided within 72 h after hatching. However, as 'Wakker Dier' indicates, the period of 72 h concerns transport of day-old chickens according to the EU transport Directive and does not relate to providing food and water after hatching; see also Appendix 1 related legislation.
4. *(a) The current report should indicate how long chickens, immediately after hatching, can survive on the yolk sac reserves (only), without depriving them from essential care because they do not receive food and water after hatching. In addition, it is indicated that the current report should not only concern the survival of the chickens, but also whether their welfare and health requirements are matched if they are deprived from food and water after hatching; and (b) Wakker Dier [117] indicates that according to Lourens and Leenstra [6] chicken welfare in the hatchery is not adversely affected by depriving them from food and water, and, that according to Lourens and Leenstra [6], there should be no effect of post-hatch food and water deprivation on mortality.* We refer to our conclusion section in paragraph 4.7 that summarises the results of the MA and QA and answers the question whether welfare and first week mortality are affected by post-hatch food and water deprivation. Our findings indicated that first week mortality significantly increased when chickens are food and water deprived for >84h post-hatch. Survival at 6 weeks of age is affected by a food and water deprivation period of 48h (36-60h) post-hatch.
5. *According to Wakker Dier [117] the ethological/behavioural need of chickens to eat and drink has not been taken into account in Lourens and Leenstra [6].* We included the behavioural needs of the chicken in paragraph 4.4. and refer also to the conclusion section, paragraph 4.7, final bullet point. Relatively little is known about the behavioural aspects of food deprivation, but we did find it likely that chickens will be motivated to consume food when given the opportunity.
6. *According to Wakker Dier [117] post-hatch food and water deprivation has a negative effect on growth, disease resistance and health.* Our findings indicate 'Wakker Dier' was right with respect to growth, but consequences of post-hatch food and water deprivation for disease resistance and health are unclear. The consequences of post-hatch food and water deprivation for growth may be explained by a delay in development rather than an impaired development. With respect to disease resistance and health, a wide variety in immune measures and disease challenges has been applied with hardly any overlap or repetition. We cannot provide conclusions on the effect of food and water deprivation on disease susceptibility. With respect to growth we refer to paragraph 4.1. of the discussion section and the MA/QA results. With respect to disease resistance we refer to paragraph 4.3. Disease resistance, health and growth are also addressed in the conclusions (paragraph 4.7).
7. *According to Wakker Dier [117], chickens should have both food and water post-hatch and optimised incubation conditions.* Both are addressed in the current report. How these interact or affect each other is yet unknown. Biotic and abiotic factors that may possibly influence/interact with the response of the chicken to post-hatch food and water provision are discussed in paragraph 4.6.

4.7.2 Recommendations for further research

As indicated in the discussion section, certain areas need further research to increase the understanding of the effects of post-hatch food and water deprivation on poultry welfare. To our opinion, most important areas for further research are:

- To study to what extent post-hatch food and water deprivation results in behavioural deprivation of the motivation to eat and drink and a change in behavioural development;
- To study the consequences of post-hatch food and water deprivation on chicken health and immunology. This includes the secondary effects of a delayed gut development and the consequences of a delayed or altered development of the immune system in response to post-hatch food and water deprivation on disease susceptibility in chickens;
- To study the influence/interaction of hatching and post-hatch conditions on the effects of post-hatch food and water deprivation, especially with respect to incubation conditions, climate

conditions in the broiler house and diet composition. Not only because these factors may aggravate or alleviate effects of post-hatch food and water deprivation, but also to define optimal procedures for incubation and housing of young chickens;

- To study the influence/interaction of type of bird or strain on the effects of post-hatch food and water deprivation. The majority of studies has been carried out in broiler chickens, whereas laying hens may show different responses to food and water deprivation. In addition differences between strains/crosses are likely to exist. Different hatching and early management procedures could be necessary for laying hens and broilers and different breeds, both from a production and a welfare point of view.

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Appendix 1 Related legislation

The text of current legislation in relation to the research question, and the legislation as mentioned in the request of 'Wakker Dier' are copied below (in Dutch), a short summary of the related legislation has been provided in the introduction section of the report (paragraph 1.2).

Artikel 1.3 Wet Dieren [1]
1 De intrinsieke waarde van het dier wordt erkend.
2 Onder erkenning van de intrinsieke waarde als bedoeld in het eerste lid wordt verstaan erkenning van de eigen waarde van dieren, zijnde wezens met gevoel. Bij het stellen van regels bij of krachtens deze wet, en het nemen van op die regels gebaseerde besluiten, wordt ten volle rekening gehouden met de gevolgen die deze regels of besluiten hebben voor deze intrinsieke waarde van het dier, onverminderd andere gerechtvaardigde belangen. Daarbij wordt er in elk geval in voorzien dat de inbreuk op de integriteit of het welzijn van dieren, verder dan redelijkerwijs noodzakelijk, wordt voorkomen en dat de zorg die de dieren redelijkerwijs behoeven is verzekerd.
3 Voor de toepassing van het tweede lid wordt tot de zorg die dieren redelijkerwijs behoeven in elk geval gerekend dat dieren zijn gevrijwaard van:
a. dorst, honger en onjuiste voeding;
b. fysiek en fysiologisch ongerief;
c. pijn, verwonding en ziektes;
d. angst en chronische stress;
e. beperking van hun natuurlijk gedrag;
voor zover zulks redelijkerwijs kan worden verlangd.

Some references to legislation were outdated, as in previous years new legislation came into force. Below the changes are indicated with reference to the relevant legislation and articles:

Artikel 37 Gezondheids- en welzijnswet voor dieren [162] ('Het is de houder van een dier verboden aan een dier de nodige zorg te onthouden'); is vervallen per 1-7-2014 en overgenomen in Artikel 2.2. lid 8 van de Wet Dieren : 'Het is houders van dieren verboden aan deze dieren de nodige verzorging te onthouden.'

Artikel 4, vierde en zesde lid van het Besluit welzijn productiedieren [163] ('4. Een dier krijgt een toereikende hoeveelheid gezond en voor de leeftijd en de soort geschikt voeder zodat het in goede gezondheid blijft en aan zijn voedingsbehoeften wordt voldaan' en '6. Een dier krijgt voeder met tussenpozen die bij zijn fysiologische behoeften passen'), zijn vervallen op 1-7-2014 en overgenomen in het Besluit houders van dieren [2] in Artikel 1.7.e respectievelijk Artikel 2.4. lid 6:
Artikel 1.7. e: 'Degene die een dier houdt, draagt er zorg voor dat een dier een voor dat dier toereikende hoeveelheid gezond en voor de soort en de leeftijd geschikt voer krijgt toegediend op een wijze die past bij het ontwikkelingsstadium van het dier'.
Artikel 2.4 lid 6: 'Een dier krijgt voedsel met ten minste de tussenpozen die bij zijn fysiologische behoeften passen.'
Artikel 5, achtste lid van het Besluit welzijn productiedieren ('Een dier heeft toegang tot een toereikende hoeveelheid schoon water of kan op een andere wijze aan zijn behoefte aan water voldoen'), genoemd in het handhavingsverzoek is overgenomen in het Besluit houders van dieren in artikel 1.7. f:
Artikel 1.7. f van het Besluit houders van dieren: 'Degene die een dier houdt, draagt er zorg voor dat een dier toegang heeft tot een toereikende hoeveelheid water van passende kwaliteit of op een andere wijze aan zijn behoefte aan water kan voldoen.'

Transportverordening, hieronder staat het voor dit rapport relevante artikel weergegeven:
Hoofdstuk V (Verordening 1/2005)/Transportverordening [12]

TUSSENPOZEN VOOR HET DRENKEN EN HET VOEDEREN,
ALSMEDE TRANSPORT- EN RUSTTIJDEN

2.
Andere soorten
2.1. Voor pluimvee en als landbouwhuisdier gehouden vogels en konijnen dient passend voeder en water in voldoende hoeveelheden voor handen te zijn, tenzij het transport korter duurt dan:
a) 12 uur; afgezien van de laad- en lostijden of
b) 24 uur voor kuikens van alle soorten, mits het transport binnen 72 uur na het uitkomen van de kuikens wordt voltooid.

Appendix 2 List of measures included in the scientific papers in the MA category

Table. *List of measures included in the reviewed scientific studies in the MA category.*

	Included in meta-analysis	Included in qualitative analysis	Results included in discussion section but not in MA/QA	Remark
Production measures				
Mortality	x	x		
Morbidity				Single study; excluded from the report [40]
Body weight (gain)	x	x		
Food intake	x	x		
Food conversion ratio	x	x		
Carcass weight, carcass%, carcass content: water%, protein%, fat%				No relationship with chicken welfare; excluded from the report.
Leg quarter %				No relationship with chicken welfare; excluded from the report.
Breast (muscle) weight, breast yield, breast muscle aspects: water%, protein%, fat%				No relationship with chicken welfare; excluded from the report.
Pectoral intra-muscular adipose tissue deposition aspects (gene expression, adipocyte cell aspects)				Single study; excluded from the report.
Abdominal fat%				No relationship with chicken welfare; excluded from the report.
Physiological indicators				
(Relative) yolk sac weight	x	x		
Yolk sac content/DM content: crude protein, fat			x	
(Relative) liver weight		x		
Genes involved in liver lipogenesis				Single study; excluded from the report
(Relative) heart weight		x		
(Relative) pancreas weight		x		
(relative) lung weight				Single study; excluded from the report
Pancreas tissue protein content				Single study; excluded from the report
Pancreas digestive enzyme activity: maltase, sucrose, alkaline phosphatase, chymotrypsin				Single study; excluded from the report
(Relative) proventriculus/gizzard weight			x	
Intestine length				Length of intestinal sections included in QA
(Relative) intestine weight				Weight of intestinal sections included in QA
(Relative) small intestine weight				Weight of intestinal sections included in QA
Intestintal contents: lactobacilli, enterobacteriacae, coliform bacteria, total aerobic bacteria				Single study; excluded from the report
(Relative) duodenum, jejunum, ileum weight and length		x		
(Relative) caeca weight and caeca length				Single study; excluded from the report
Gut muscle wall thickness				Single study; excluded from the report
Duodenum, jejunum, ileum crypt		X (crypt depth		Single study; excluded

	Included in meta-analysis	Included in qualitative analysis	Results included in discussion section but not in MA/QA	Remark
depth, villus height, surface area		and villus height)		from the report (surface area)
Number of cells per crypt or villus				Single study; excluded from the report
mucosal tissue protein content				Single study; excluded from the report
digestive enzyme activity: maltase, sucrose, alkaline phosphatase				Single study; excluded from the report
Goblet cell development/activity				Single study; excluded from the report
Bone strength, mineral density, mineral content			x	Single study, excluded from the report
Plasma/blood glucose, lactate, uric acid concentration		X (glucose)		Single study, excluded from the report (lactate, uric acid)
Plasma T3/T4		x		
Plasma corticosterone			x	
Plasma oestradiol and testosterone				Single study; excluded from the report
Glycogen hatching muscle				Single study; excluded from the report
Liver glycogen				Single study; excluded from the report
Ghrelin expression				Single study; excluded from the report
Immunological indicators				
Thymus, bursal, spleen, caecal tonsil weight weight			x	
Packed cell volume, leukocyte count, white blood cell count			x	
T-cell proliferation scores			x	
The relative expression of MHC I molecules on the cell surface of leukocytes; the relative expression of MHC II molecules on the surface of MHC II positive leukocytes; the percentage of MHC II positive leukocytes % MHC IIþ cells			x	
The relative expression of CD4 molecules on the surface of CD4 single positive leukocytes; the percentage of CD4 single positive Leukocytes % CD4þ cells; the relative expression of CD8 molecules on the surface of CD8 single positive leukocytes; The percentage of CD8 single positive leukocytes % CD8þ cells; The relative expression of CD4 molecules on the surface of CD4CD8αα double positive leukocytes; the relative expression of CD8 molecules on the surface of CD4CD8αα double positive leukocytes; the percentage of CD4CD8 double positive leukocytes			x	
The relative expression of BU-1 molecules on the surface of BU-1 positive leukocytes; the percentage of BU-1 positive leukocytes; % BU-1þ cells			x	
Duodenum, jejunum, ileum, colon CD3 gamma/delta expression (beta actin mRNA ratio)/ratio fed/food withheld			x	
Duodenum, jejunum, ileum, thymus, spleen CD3+ cells			x	
Colon ChIL2 mRNA expression ratio fed/food withheld			x	
Colonization of cloacal bursa by lymphocytes			x	
Colonization of cloacal bursa by			x	

	Included in meta-analysis	Included in qualitative analysis	Results included in discussion section but not in MA/QA	Remark
CD4+/CD8+lymphocytes				
Anti-Hemocyanin respons			x	
Anti-Hemocyanin positive cultures			x	
Anti-BSA respons (A450), Anti-BSA positive cultures			x	
Anti-KLH IgY titer, Anti-KLH IgM titer			x	
Ab titer to NCD vaccination			x	
#CFU/g digesta *Clostridium perfingens*, necrotic enteritis lesion scores			x	
Ab titer to IBDV			x	
Eimeria vaccine (oocyst) shedding			x	
Cecal tonsil germinal centers (N)			x	
Biliary immunoglobulin A (IgA) levels			x	
Ileum IgM, IgY, IgA mRNA expression			x	
Ileum IGA synthesizing cells			x	
Ileum Il12p40, IL-1beta, IFN-gamma, IL-10, TGF-beta mRNA expression, IL6			x	
The total amount of IgM in serum IgM, the total amount of IgG in serum IgG			x	
Serum protein, albumin, globulin			x	
Behavioural indicators				
Open-field behaviour			x	Single study, included in section on timing of first feeding
Feeding behaviour post-hatch			x	Single study, included in section on timing of first feeding
Other welfare indicators				
Pododermatitis				Single study; excluded from the report
Heterophil:lymphocyte ratio				Single study; excluded from the report

Appendix 3 Meta-analysis of production measures for all categories of scientific studies

Results of the meta-analysis for production measures, showing the effect of the various durations of food and water deprivation after hatching on body weight, food conversion ratio, food intake and mortality at day 7, 21 and 42 of age. Results are shown for all categories of papers (Cat 1+2+3) and for category 1+2, 2+3, category 1 and category 2 papers only (column Cat). Average values for the various indicators are expressed as relative values as compared to 0h (no food and water deprivation), which is set at 100. An asterisk in a cell indicates that no analysis results were available due to a lack of sufficient data for a certain measure at a specific time point. N shows the number of records analysed per measure/age.

	Relative value after food and water deprivation for[1]							
Measure/ Age 1st food	0h **(0-12h)**	24h **(12-36h)**	48h **(36-60h)**	72h **(60-84h)**	>84h	P value	N	Cat
Body weight day 7	100^{a}	92.8^{b}	83.0^{c}	73.1^{d}	51.6^{e}	<0.001	204	1+2+3
	100^{a}	92.4^{b}	80.8^{c}	75.2^{d}	*	<0.001	166	1+2
	100^{a}	94.2^{b}	81.2^{c}	74.1^{c}	*	<0.001	114	1
	100^{a}	87.9^{b}	78.7^{c}	75.2^{c}	*	<0.001	52	2
	100^{a}	90.3b	83.8^{c}	72.2^{d}	51.2^{e}	<0.001	90	2+3
Body weight day 21	100^{a}	95.0^{b}	89.3^{c}	79.5^{d}	*	<0.001	82	1+2+3
	100^{a}	94.3^{b}	86.7^{c}	79.8^{c}	*	<0.001	49	1+2
	100^{a}	96.8^{a}	86.8^{b}	79.8^{b}	*	<0.001	24	1
	100^{a}	92.9^{b}	86.0^{c}	*	*	<0.001	25	2
	100^{a}	94.4^{b}	89.9^{c}	*	*	<0.001	58	2+3
Body weight day 42	100^{a}	97.4^{b}	94.5^{c}	91.7^{c}	*	<0.001	50	1+2+3
	100^{a}	97.2^{b}	94.3^{c}	91.7^{c}	*	<0.001	35	1+2
	100^{a}	97.8^{b}	94.1^{c}	91.7^{c}	*	<0.001	24	1
	100^{a}	95.8^{b}	93.3^{c}	*	*	0.01	11	2
	100^{a}	97.2^{a}	94.6^{b}	*	*	0.002	26	2+3
Food conversion day 7[2]	100	99.3	103.5	*	*	NS	37	1+2+3
	100^{b}	102.1^{b}	114.0^{a}	*	*	0.064	21	1+2
	100	102.1	114	*	*	0.06	13	2
	100	96.2	102.8	*	*	NS	29	2+3
Food conversion day 21	100^{b}	99.6^{b}	98.7^{b}	106.1^{ab}	110.4^{a}	0.013	57	1+2+3
	100	99.3	98.6	*	*	NS	30	1+2
	100	99.8	100.7	*	*	NS	13	1
	100	98.6	97.9	*	*	NS	17	2
	100^{b}	99.7^{b}	98.5^{b}	106^{ab}	110.3^{a}	0.01	44	2+3
Food conversion day 42[2]	100^{b}	99.9^{b}	100.1^{b}	103.8^{b}	110.3^{a}	<0.001	47	1+2+3
	100	99.6	99.8	*	*	NS	20	1+2

Measure/ Age 1st food	Relative value after food and water deprivation for[1]							
	0h **(0-12h)**	24h **(12-36h)**	48h **(36-60h)**	72h **(60-84h)**	>84h	P value	N	Cat
	100	100.5	100.4	*	*	NS	15	2
	100^{b}	100.2^{b}	100.3^{b}	103.9^{b}	110.5^{a}	<0.001	42	2+3
Cumulative food intake day 7[2]	100^{a}	92.1^{a}	67.4^{b}	63.5^{b}	*	<0.001	37	1+2+3
	100^{a}	92.0^{b}	63.3^{c}	63.2^{c}	*	<0.001	21	1+2
	100^{a}	92.3^{b}	60.3^{c}	63.2^{c}	*	<0.001	15	1
	100^{a}	912.2^{b}	68.9^{b}	*	*	0.002	22	2+3
Cumulative food intake day 21[2]	100^{a}	95.4^{a}	87.3^{b}	78.4^{b}	*	<0.001	39	1+2+3
	100^{a}	94.2^{b}	85.3^{c}	78.5^{c}	*	<0.001	18	1+2
	100^{a}	96.1^{ab}	88.9^{bc}	78.6^{c}	*	0.008	11	1
	100^{a}	949^{b}	86.8^{b}	*	*	0.004	28	2+3
Cumulative food intake day 42[2]	100^{a}	98.0^{a}	95.1^{b}	89.2^{b}	*	<0.001	33	1+2+3
	100^{a}	96.0^{b}	93.2^{c}	89.2^{d}	*	<0.001	22	1+2
	100^{a}	94.5^{b}	91.6^{c}	*	*	0.007	24	2
	100^{a}	98.5^{a}	95.5^{b}	*	*	0.051	39	2+3
Mortality day 7[2]	100^{bc}	81.4^{c}	142.8^{bc}	225.9^{b}	826.7^{a}	<0.001	39	1+2+3
	100^{b}	100^{b}	123.1^{b}	296.6^{b}	1197^{a}	<0.001	26	1+2
Mortality day 21[2]	100	102.3	200	*	*	NS	6	1+2+3
Mortality day 42[2]	100^{b}	100.3^{b}	155.6^{a}	*	*	0.003	29	1+2+3
	100^{b}	101^{b}	165^{a}	*	*	0.004	24	2+3

[1] different letters within a row indicate a significant difference between the various durations of food and water deprivation.

[2] Missing records for Category 1 and/or 2 an/or 1+2 and/or 2+3 due to insufficient records for a reliable MA

To explore the potential of nature to improve the quality of life

Wageningen Livestock Research
P.O. Box 338
6700 AH Wageningen
The Netherlands
T +31 (0)317 48 39 53
E info.livestockresearch@wur.nl
www.wur.nl/livestock-research

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