Differences in intestinal health and performance between broilers hatched on the farm or at a hatchery.

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Contents

Abstract

In the modern poultry industry, most of the broilers are hatched in a hatchery ("traditional hatching"). Chicks generally do not have access to feed or water before the majority of the chicks is hatched and have been transported to the farm. This delayed feeding has been shown to have an impact on the development of the gut and immune system.

Coccidiosis is a very prevalent intestinal disease, caused by several species of the protozoan parasite *Eimeria*. This disease causes considerable economic losses in the broiler poultry industry. As coccidiosis is a gut infection and delayed feeding negatively influences gut development and immunity, it was hypothesized that on farm hatching may have beneficial effects on the response to infection with *Eimeria* spp.

In traditionally hatched (referred to as R) and Home Hatched (X) Ross 308 broiler chicks the response to an *Eimeria* infection and performance was compared in a field study and challenge experiment. In the field study, body weights of 100 R and 100 X chicks were determined twice per week and oocyst excretion was quantified in faecal samples on 8 occasions between day 11 and day 40. Furthermore, uniformity of the flock was assessed and clinical signs, mortality and intestinal lesions scores were determined. For the challenge experiment, 33 R and 33 X chicks were obtained from the field study flock at 2 days of age. At day 8 of age, 20 R and 20 X chicks were selected and challenged with 5400 sporulated *Eimeria acervulina* oocysts. Dividing R and X chicks in 2 replicate groups, resulted in groups R1, R2 and X1 and X2 with 10 chicks each. The excretion of oocysts was measured daily from day 12 to 22 and at days 25, 27, 29, 32, 36 and 40 of age. Furthermore, feed consumption was measured and the chicks were weighed two times a week.

In the field study, the mean body weight was not significantly different between groups, except at day 0 and 39 of age when respectively X and R chicks had a higher body weight. The qPCR of the GD Deventer is used to analyse the oocyst excretion in the field study. It is shown that the OPG of *E. acervulina* is lower than *E. tenella*, over the whole period. Besides that, it is notable that the Home Hatched broilers had a lower excretion than the traditional hatched chicks at each sampling time. The slaughterhouse data had shown that the X chicks had slightly more foot lesions and condemned chicks. In this data, it was also shown that the mean body weight of the R chicks was 69 gram higher than that of the X chicks.

Due to low feed consumption and low body weights in all groups, and especially in the R1 group, circumstances in the challenge experiment do not allow for drawing conclusions on differences in body weight or feed consumption between X and R chicks.
The oocyst excretion of the X chicks was significant higher compared to R chicks from day 12 to 17 of age, in the second period of 4 days the R chicks had a significant higher OPG (day 18-22 of age). The mean $\log_{10} AUC$ was equal for both groups at the end.

During clinical examination in the challenge experiment as well as in the field study, it was noticed that the R chicks were more aggressive and restless than the X chicks.

Differences in performance and health between traditionally hatched and home hatched chicks in this study were small and not clearly in favour of either system. However, positive experiences of farmers and scientific literature suggest that the potential benefits of farm hatching warrants further practical use and evaluation.

1. Introduction

In the modern poultry industry, most of the broilers are hatched in a hatchery (further referred to as "traditional hatching"). In this system, the chicks generally do not have access to feed or water before the majority of the chicks is hatched and have been transported from the hatchery to the farm.

Chicks hatch over a time period of 24 to 48 hours (Willemsen, Debonne et al. 2010). Hatching broiler chickens can be categorized as early, midterm and late hatchers. Because of differences in hatching time, chicks differ from each other in biological age from several hours up to 2 days but they are simultaneously taken out of the incubator. The biological age is explained in figure 1. The treatments at the hatchery and transport to the farm are the same for chicks of all biological ages. At the farm, they finally will all have access to feed and water *ad libitum.* This means that early chicks can often be between 24-72 hours old before they are delivered on the farm, and consequently have been deprived of feed and water for 1 to 3 days (Willemsen, Debonne et al. 2010, Box 2014).

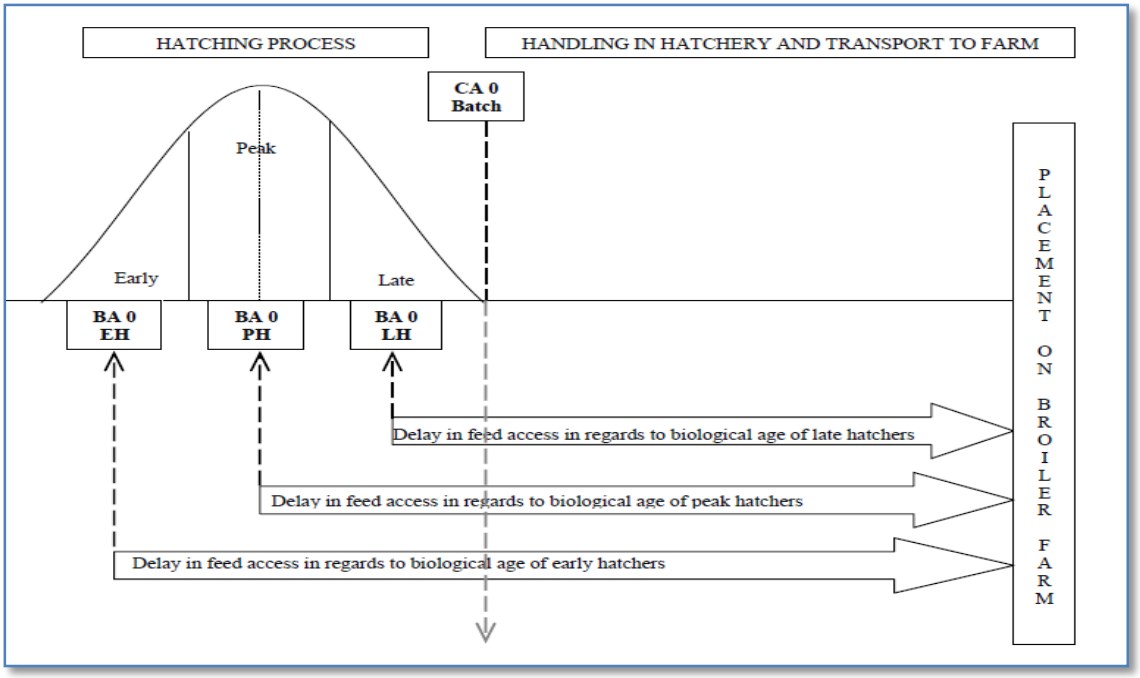

Figure 1: Biological age (BA in figure) of broiler chicks. The delay in feed access is longer for early hatchers than for midterm or late hatchers. The chronological age (CA in figure) is the age from the moment that all the chicks are hatched (Willemsen, Debonne et al. 2010).

Literature on feed deprivation after hatch clearly demonstrates the detrimental effects of any delay in feed access on performance of the chicks with respect to growth, immune system activation, digestive enzyme stimulation and organ development (Willemsen, Debonne et al. 2010). In addition to the health effects of a good developed digestive system, there are also economic effects. In case of a good developed digestive system, the chick will have a better production efficiency (Lamot, Linde van de, I. B. et al. 2014). In a study by the Animal Health Service, GD Deventer (Fabri, de Bruijn 2013), the impact of delayed feeding on morphological development of the intestinal tract was evaluated. Villus-crypt ratio (V:C ratio), villus surface area, villus length and goblet cell size was

determined for chicks with delayed and early feeding from day 0 to day 7 post-hatch. Early feeding significantly increased the villus surface area and villus length, whereas goblet cell size was not significantly different for animals with early or delayed feeding. This study shows that early feeding can accelerate morphological development of the intestinal tract.

Broilers are very susceptible for infections in the first few days of their life. The development of the gut is very important to handle these infections and can result in a reduced need to use antibiotics. Furthermore, a well-developed intestinal tract is important in reducing chance of entry of pathogens from the intestines (Fabri, de Bruijn 2013). When the broilers have immediately access to feed and water, the residual yolk sac can be used for thermoregulation and the development of organs and the immune system. In case of delayed feeding (traditional system of hatching), the chicks use the residual yolk sac to survive instead of for the development of the immune system. Because of that, it is thought that there is a difference in the response to infection between broilers after hatching in a traditional system (hatchery) or in an on farm hatching system. Gut associated lymphoid tissue (GALT) is a component of the mucosal immune system, which has evolved to provide protection against pathogens encountered by the gut. In a study of Shira, where GALT activity was determined by antibody production, it was shown that GALT activity in the hindgut and the gut-associated cloacal bursa was delayed in the first two weeks in case of 24-72 hours of withholding of feed. (Shira, Sklan et al. 2005)This suggest that delayed access to feed after hatching impairs the development of the intestinal immune system.

In the modern poultry, the use of antibiotics is under scrutiny, as it is associated with the increased risk for public health because of bacterial resistance. Clearly, a system that promotes intestinal and immune development in broiler chicks can have substantial beneficial effects on broiler health, welfare, production efficiency and public health. Because of the detrimental effects of delayed feeding at the start of the broiler chicks' life, solutions for this problem are sought by researchers and the broiler industry. A very recent example of a system developed to tackle this problem is the development of a hatcher in which feed and water are supplied (Hatchcare[1], Hatchtech BV, Veenendaal). Data on performance of chickens from these hatchers in scientific literature and actual application of this system in hatcheries is however still scarce. Other systems that have been developed have focussed on hatching chickens on the farm. In general, hatching on a farm instead of at a hatchery is called ''farm hatching''. There are many variations of this principle, for example the "Patio system" and ''X-treck'', both developed by Vencomatic and ''Home Hatching'', the system that is used in this study. The Home Hatching system is developed by two brothers from Schaijk, The Netherlands, in association with Pe-Da[2]. The Patio system, was developed by Vencomatic in the Netherlands and has been tested from 2006 to 2008, to evaluate effects on hatchability and early performance of broilers (van de Ven, L J F, van-Wagenberg et al. 2009). In all these systems, Patio, X-treck and Home Hatching, chicks have direct access to feed and water *ad libitum* after hatching, as they hatch in the poultry stable. Furthermore, these birds are not exposed to stressors that are associated with handling at the hatchery and transport to the farm.

[1] http://www.hatchtech.nl/incubationtechnology/hatchcare.php
[2] www.Pe-da.nl

Effects of moment of hatch and different hatching systems have been evaluated and published in scientific literature. In a study of Lamot, it is shown that early hatched chicks had a higher body weight until day 18 compared to the midterm and late hatchers. Relative breast meat yield at day 18, expressed as percentage of carcass weight, was higher for early (30,4%) than midterm (28,5%) and late hatchers (27,8%) (Lamot, Linde van de, I. B. et al. 2014). Effects on physical parameters of moment of hatching for the traditional (hatcher) and Patio hatching system were assessed by (van de Ven, L J F, van-Wagenberg et al. 2013). The weights of heart, lungs, stomach and intestines increased with hatching time, concurrent with a decrease in residual yolk weight, regardless of hatching system, and indicating that late hatchers are more matured. Weights of the heart, liver, stomach, and intestines were lower in hatcher than in patio chicks. Between hatch and E21.5, residual yolk weight decreased, whereas organ weights increased in both fasted hatcher and fed patio chicks, but at a higher rate in the latter. Chick physiology at chick pulling time was shown to vary both with time after hatching and post hatch conditions, especially feed access (van de Ven, L J F, van-Wagenberg et al. 2013). It was concluded that early feed and water access enabled early hatching chicks to compensate for their apparent disadvantage in development at hatching, whereas chicks subjected to fasting show metabolic adaptations to preserve nutrients.
A study of Wageningen University (Van Harn, Lourens et al. 2014) showed that higher body weights were only found on day 0 for farm hatched chickens compared to traditionally hatched chickens. On day 10, the weight differences were not significant anymore. No significant differences were found in growth rate, feed conversion, water and feed intake between the farm hatched and traditionally hatched chicks. There was a significant difference in the quality of the litter, which was much higher in the farm hatched group (Van Harn, Lourens et al. 2014).

In this study we aimed to determine whether on farm hatching has significant benefits on broiler health and production efficiency compared to traditional hatching. The study consisted of a field trial on an actual broiler farm and an experiment under controlled circumstances at the Faculty of Veterinary Medicine. For the field trial, a broiler house was divided in two parts, with home hatched chickens on one side, and regular, traditionally hatched, chickens which were transported to the farm, on the other side. All chicks originated from the same parent flock and were exposed to the same feed, water and climate conditions. To assess the production efficiency of the chicks, technical data of the home hatched and traditionally hatched chicks of this flock were evaluated, such as the body weights, feed conversion and mortality of the chicks.

In addition to a good production performance, good (intestinal) health and reducing the use of anticoccidial or antibiotic drugs are essential for sustainable broiler production that meets current demands of consumers and retailers. Therefore, effects of farm hatching on broiler health are very relevant to assess as well. A very prevalent disease in broilers, affecting intestinal health, is coccidiosis. Coccidiosis is caused by several species of the protozoan parasite *Eimeria*. This disease causes considerable economic losses in the broiler poultry industry. These costs consists of poor performance of the broiler chicks, costs of treatments and control. Despite of many measures (hygienic measures, in-feed anticoccidials etc.) it isn't possible to prevent infection with *Eimeria* spp. in broiler flocks (McDougald 2013).

As coccidiosis is a gut infection and delayed feeding influences gut development and immunity, it is likely that effects of on farm hatching on intestinal health can be assessed by studying how flocks

respond to infection with *Eimeria* spp. Furthermore, the degree of infection is measurable by counting the oocysts in faeces, which can be assessed with more simple and less expensive techniques compared to measuring virus or bacterial load. Therefore we studied the response of the broilers to a gut infection with *Eimeria* spp. both in the field study and in a challenge experiment. In the field study the chicks were naturally exposed to the *Eimeria* spp. that were present in the poultry house. They were also exposed to many other pathogens and circumstances. Therefore, a challenge experiment was done as well, because of more controlled conditions. Chickens were challenged with *Eimeria acervulina* because this is the most prevalent species, affecting practically all broiler flocks, and the clinical signs of this species of *Eimeria* are mild. It mainly affects body weight and feed conversion. Furthermore, its oocysts can be determined in colonic faeces, whereas *E. tenella* is only shed with the caecal faeces which complicates faecal sampling. In the challenge experiment the research facility and materials were completely disinfected before arrival of the chicks and only a few people had access to the research facility and no vaccines or treatments were given. Under these controlled circumstances, effects can be attributed largely to the challenge infection with *E. acervulina*, which facilitates evaluating differences between the hatching systems.
This report describes the results and conclusions of both the field study and challenge experiment. Although this work can be regarded as a pilot study, it will provide a modest contribution to evaluation of systems aimed to improve broiler health and productivity.

2. Materials and Methods

This study consists of a field study and a challenge experiment. In the field study, the technical data of the flock was analysed, the chicks were weighed twice per week and individual and pooled faecal samples were taken 8 times to assess the level of infection with *Eimeria* spp. throughout the flock cycle. To determine the differences in the response to an infection with *Eimeria acervulina* under controlled circumstances, a challenge experiment was carried out. The birds for the challenge study were collected from the flock used for the field study. All the broiler chicks in this study originated of the same parent flock (Ross 308, 47 weeks of age).

The field study is done at a broiler farm in Schaijk, in the province "Noord-Brabant" in the Netherlands. This farm consists of 6 poultry houses. The study was carried out in poultry house 4, which had a surface of 914m^2. The flock in poultry house 4 consisted of 20.000 broiler chicks. The two groups were separated by a plastic netting, situated in the middle of the poultry house (photo 1). The poultry house was split in two parts, which had exactly the same surface (457m^2). The density of the chicks in this broiler house was 35,9kg per m^2 at the end of the production cycle. On the right side of the house, 10.000 broilers were hatched with the "home hatching system" (referred to as group B2 or X chicks). These chicks hatched at the 10th of September 2014. At the 11th of September, 10.000 one-day-old broiler chicks from the hatchery were placed at the left side (referred to as group B1 or "regular (R) chicks"). The arrival day from the one-day-old chicks (11th of September 2014) is referred to as day 0 of the study (which corresponds with day 0 of age, the chronological age, as explained in figure 1 of the introduction). The challenge experiment therefore started at day 2 (of age). Throughout this report, day of the experiment corresponds with day of age for both the field and challenge experiment.

Photo 1: Poultry house where the field study was carried out. The separation of home hatched and regular chicks with plastic netting is indicated by the green arrows.

2.1 Challenge experiment

2.1.1 Chickens and management

On day 2 of age, 66 broilers were randomly selected at the farm and transported to the research facility at the department of Farm Animal Health (Faculty of Veterinary Medicine, Utrecht University, Utrecht, The Netherlands). At the research facility, the chickens were initially housed in two cardboard floor pens (1180x780x780mm, widthxdepthxheight), 33 chickens of the R group (hatchery, regular) and 33 of the X group (home hatched chickens). These cardboard floor pens are shown on photo 2. The broiler chicks were all given an individual number, by using neck tags (see photo 2). The cardboard floor pens were equipped with a thermometer, a heat lamp, one feed box and four water bottles (during the first two weeks, there was also a simple bell drinker in each pen). The floor of the pen was covered with approximately 1 kg wood shavings per m^2. After day 8, 20 chicks of the X and

20 chicks of the R groups were selected (see below) and housed in four floor pens (0,91x0,91x0,70cm, depthxwidthxheight), with 10 chicks per group (at a bird density of 12 chicks per $m^{2,}$ which corresponded with 24,5kg per m^2 at the end of the experiment at day 43). Group composition is given in appendix 1. The chicks were fed with a broiler ration without coccidiostatic or antibiotic drugs from Research Diet Services (Wijk bij Duurstede, The Netherlands) and water, which was available *ad libitum*.

Room temperature on chicken level was gradually decreased from 33°C at day 2 to approximately 23°C at day 43. A lighting scheme of 19-20 hours of light per day was given, except for the first days. The lights were kept on for 24 hours during days 2 and 3, followed by a one hour dark period for days 4 to 6. From day 7 onwards there were two dark periods each day (from 20:00/23:00 or 24:00 and for one hour before 7:30). The complete light scheme is given in appendix 2. Until day 37 there was no refreshment of the litter. At day 37, the pens were provided with some fresh wood shavings, because of the presence of mild foot lesions caused by wet litter.

The chicks were observed every day for signs of illness or welfare impairment and were housed, handled and treated following approval by the Animals Experiments Committee of Utrecht University (Utrecht, The Netherlands), in accordance with the Dutch law on experimental animals. All chickens were killed at day 43 by electrocution followed by debleeding.

Photo 2: 33 X broiler chicks, housed in a big cardboard box, with a density of 12 chicks / m^2. Note that each chick had a neck tag with an individual number.

2.1.2 Body weight and feed consumption

The chicks were weighed at day of arrival (day 2 of age) and then approximately twice a week at days 5, 8, 12, 15, 19, 22, 26, 29, 33, 36, 40 and 43. To measure total feed consumption for each cage all feed that was placed in the feeders was weighed, which occurred on days 8, 16, 21, 25, 29, 32 and 36. The weight of remaining feed in the feeders, which was replaced due to contamination with faeces, was also determined.

2.1.3 Selection of broiler chickens

On day 8 of the experiment, 40 chicks were selected from the total of 66 chicks to participate in the challenge experiment. The aim of this selection was to obtain four groups that were comparable in terms of mean weight and uniformity. Birds that deviated most (with lower or higher values) from the mean weight and for which abnormalities in behaviour or health were noted before, were not selected. With random number generators, remaining birds were randomly assigned to one of the two R or X groups. Some birds that were assigned to a certain group were changed for another chick

when, during inoculation, abnormalities were observed. For example, a selected chick that showed respiratory problems was replaced by another chick with a similar body weight. The selected and inoculated chicks were placed into 4 floor pens of 10 chicks.

During the whole procedure, the chicks of the R and X group, were housed separately, and any direct or indirect contact was avoided (see § 2.1.5.). The separate housing in 4 different cages is shown in photo 3.

Photo 3(left): The four wooden cages the chicks are housed in, from day 8 onwards until the end of

Photo 4(right): A transport box with a plastic inlay for individual dropping collection.

2.1.4 Inoculation

After the selection, the selected chicks were orally inoculated with a dose of 5400 sporulated oocysts of *Eimeria acervulina* (strain Weybridge W119, supplied by the Animal Health Service, GD, Deventer, The Netherlands). Before inoculation, the non-selected chicks of the X and R group were separated from the selected chicks, by putting them in a cardboard box for each group separately. Then, the selected chicks of the X group were placed in 2 separate cardboard boxes, according to their assignment to groups X1 and X2, as described in § 2.1.3, and the same was done for the R group. After all birds had been placed in the temporary cardboard boxes, according to the assigned group, the chicks were inoculated with 0,5 mL of the inoculum (10.800 oocysts/mL). One researcher took the chicks out of the cardboard box and the other stretched the neck and inoculated the bird directly into the crop with a syringe without a needle. After inoculation, the bird was directly placed in their new cage (see photo 3). The inoculation took 10-15 minutes per group. In appendix 3, the exact time schedule of the inoculation procedure is shown. After the inoculation, the non-selected chicks were killed by cervical dislocation.

2.1.5 Detection and quantification of oocyst excretion

To determine infection status of the chicks before experimental infection with *Eimeria acervulina*, faecal samples were collected from X and R chicks at day 8. The samples were collected from the litter from the two cages. Also, the chicks were placed in temporary cardboard boxes for 1 ½ hours after which the faeces in these boxes was collected and examined with both the McMaster and sedimentation-flotation technique (see below).

Oocyst excretion of individual birds was determined throughout the experiment to evaluate the response to the experimental infection with *E. acervulina*. For this, single individual faecal droppings were collected daily from day 12 until 22 of age. After day 22 of age the droppings were collected on

day 25, 27, 29, 32, 36 and 40 of age. Near the end of the dark period in the morning, each chick was placed in a numbered transport box using a head light. When all birds were in the transport boxes, the lights were turned on again and the birds remained in the boxes for 1-2 hours. This procedure has proven to be an effective method to obtain faecal samples (Velkers, Bouma et al. 2010). The boxes are shown on photo 4. Every chick had his own box, with a plastic inlay. The faecal droppings of each individual chicken were collected in 50 mL plastic tubes that where marked with the chick number and weighed before sampling. The tubes were weighed again after sample collection to determine weight of the faecal dropping and stored at 5°C in the lab until further processing within the same day.

Protocol of McMaster:

The tubes with the droppings are filled with 20 mL of a sodium chloride solution (specific gravity 1.1). After homogenisation, 2 mL of this suspension is transferred into a centrifuge tube filled with 8 mL of a saturated sodium chloride solution (specific gravity 1.2). This suspension was used to fill two McMaster counting chambers (2x 0,15 mL). Further 10 fold dilutions were made if more than 300 oocysts were present per chamber. The number of oocysts per gram of faeces (OPG) was calculated: 333,3 ab/ sample weight (a = total number of oocysts; b = the dilution).(Velkers 2011)

The number of oocysts per gram of faeces (OPG) was quantified using the McMaster method as described in the green box. The OPG is calculated using Excel, after entering data of the counts and dropping weight. The formula is also shown in the green box on the right (Velkers, Blake et al. 2010).

If no oocysts were found for two or more days, a sedimentation-flotation (SF) technique was used (Long, Joyner et al. 1976) to determine whether the oocyst excretion had stopped completely. This test is more sensitive to low oocyst numbers. For the SF method, one gram of the dropping was used, which was taken from the faecal sample prior to further processing for the McMaster technique.

2.1.6 Hygienic measures

To avoid cross contamination between groups, researchers and animal caretakers handled birds with gloves and changed gloves between every group, both during weighing as during the whole procedure of collecting faecal samples.

The chicks were weighed in separate cardboard transport boxes for each group. In the first week, two boxes, one of the R group and one of the X group, were used. After day 8, every group of chicks had their own box (R1, R2, X1, X2). For the collection of individual faecal samples, each bird had its own transport box with plastic inlay. This plastic inlay (shown on photo 4) was rinsed after the faecal sample was collected. The individual box was cleaned with cotton wool and alcohol 70% when faecal contamination was present on parts of the box outside of the plastic inlay.

2.1.7 Post mortem examination

On day 43, all the chicks of the challenge experiment were killed by electrocution, followed by debleeding. Post mortem examination was done in order to determine the sex of each bird, determine weight of the spleen (outside of the scope of this study) and determine whether signs of disease or other abnormalities were present. Also, feathers were collected from the birds to determine level of corticosterone as an indicator of stress (outside of the scope of this study). The

sex of the broiler was evaluated to determine the number of males and females per group, as this can have a great influence on the body weights.

2.2 Field study

In addition to the challenge experiment, a field study was done. The broiler house was divided in two groups, 10.000 hatchery broiler chicks, transported to the farm at the 11th of September and 10.000 home hatching broiler chicks (hatched in this poultry house from the 10th of September onwards).

2.2.1 Chickens and management

The chicks were fed a prestart, start, growth (1), growth (2) and finisher broiler ration from Coppens Diervoeding (Helmond, the Netherlands). Start feed contained Nicarbazine and Narazin and the growth 1 and 2 feed contained Monensin as coccidiostats. The chicks were weighed twice a week, from day 1 of age until day 40. A pooled sample of faecal droppings was collected from the litter for each of the two groups for McMaster counting of *Eimeria* oocysts. In addition, on day 27, 34 and 39, individual faecal dropping samples were collected from 20 of the weighed chicks of each group (20 R and 20 X). An overview of all the faecal dropping collections of the Utrecht University is shown in table 1 (UU). In paragraph 2.2.3, this is further explained. The technical data of the flock, such as mortality, was written on the flock record sheet. Data about the weight of the chicks in the two groups, were also available from the slaughterhouse at the end of the period. In addition to this, the Animal Health Service, GD Deventer examined faecal dropping samples for quantification of *Eimeria* spp. using PCR and carried out post mortem examination on 5 birds of each group to determine coccidiosis lesion scores and determine weight of spleen and bursa. The moments of sample collection of the Animal Health Centre are also shown in table 1 (GD).

Table 1: Sampling schedule

Date	Day	Institution	Samples taken
15-09-2014	4	GD	2x5 chicks spleen/bursa examination, Villus/Crypte
18-09-2014	7	GD	2x5 chicks spleen/bursa examination, Villus/Crypte
22-09-2014	11	UU	Pooled faecal samples
24-09-2014	13	GD	2x5 chicks spleen/bursa examination and 2x5 chicks lesion scores
29-09-2014	15	UU	Pooled faecal samples
01-10-2014	20	GD	2x5 chicks spleen/bursa examination, 2x5 chicks lesion scores, qPCR faecal samples
01-10-2014	20	UU	Pooled faecal samples
06-10-2014	25	UU	Pooled faecal samples
08-10-2014	27	GD	2x5 chicks spleen/bursa examination, 2x5 chicks lesion scores, qPCR faecal samples
08-10-2014	27	UU	Pooled and individual faecal samples
13-10-2014	32	UU	Pooled faecal samples
15-10-2014	34	GD	2x5 chicks spleen/bursa examination, 2x5 chicks lesion scores, qPCR faecal samples
15-10-2014	34	UU	Pooled and individual faecal samples
20-10-2014	39	GD	qPCR faecal samples
20-10-2014	39	UU	Pooled and individual faecal samples
22-10-2014	41	GD	2x5 chicks spleen/bursa examination, 2x5 chicks lesion scores

2.2.2 Body weight evaluation

Of the total population of 10.000 regular chicks and 10.000 home hatched chicks, 100 chicks of both groups were randomly selected and weighed in this study. The birds were not marked, so a new selection of 100 birds was made during each weighing moment. During the first weighing moments, the chicks were transported in a box (10 chicks at a time) to the entrance of the poultry house (place were the weighing scale was set up). After day 25 this was not practical, because of the weight of the chicks. From day 25 onwards, a larger weighing scale was brought into the poultry house to weigh the birds in the house. The method of the first weeks is shown in photo 5 and 6.

Photo 5 (left): Broiler chick in the scale on day 1. This laboratory scale was used until day 11, after which a scale was used that could measure weights exceeding 500 g

Photo 6 (right): Chicks in the transport box.

The chicks were randomly selected from the whole section (B1 or B2 section) of the poultry house. During the selection and weighing the lights were turned off. Random selection was easier in a dark broiler house. A head light was used to avoid trampling of chicks in the house during selection of the birds. After day 25, a head light was also used during weighing of the chicks in the broiler house.

2.2.3 Detection and quantification of oocyst excretion

Pooled faecal samples were collected two times a week in both of the groups. In each group colonic faecal samples and caecal samples were collected and pooled separately. The colonic faecal samples were collected in a small plastic bag, the caecal samples were collected in a small plastic box. The colonic samples were taken with a glove, the caecal samples with a wooden tongue depressor. Each time, about 40 drops of colonic faeces were collected. We aimed at collecting a minimum number of caecal samples of 20 per group, but it appeared to be difficult to detect and collect this number of caecal samples from the litter. Therefore all detectable caecal samples were collected, but the actual number differed largely between sampling days. The method of sampling is shown in appendix 5. Four grams of these pooled samples was processed like the individual samples of the challenge experiment.

Individual colonic faecal samples were collected on day 27, 34 and 39 in both of the groups. This was done for the first 20 chicks that were weighed in both of the groups. We aimed at collecting 18 samples, but we put two extra chicks in a box to compensate for chicks that did not produce a faecal dropping. The chicks were placed in an individual, numbered, cardboard box. Than the other 80 chicks were weighed. After that, the faecal droppings of the 20 chicks were collected in 50 mL tubes that were marked with the chick number (1-20) and weighed before sampling. When less than 18 droppings were produced, a random fresh faecal dropping was collected from the litter and these tubes were marked as “stable samples”.

2.2.4 Clinical inspection of the broilers

Every time the chicks were weighed and faecal dropping samples were collected, a clinical inspection of the chicks was carried out. During this clinical inspection, attention was given to the behaviour, signs of illness and cleanness of the broilers. In addition, the technical data of the flock, such as broiler house temperature and mortality, was obtained.

2.2.5 Data analyses (challenge and field study)

The total feed conversion for the challenge study was calculated by adding standard consumption of Ross 308 broilers between day 0 and 8 to the total measured feed consumption and dividing this number with total weight gain between day 8 and 43. The statistical analyses were all done by using SPSS 22 (IBM SPSS Statistics for Windows, Armonk, NY: IBM Corp.). At first, some descriptive statistics were carried out for both the data of the field and the challenge study. These analyses included an evaluation of means and standard deviation of body weights and means of oocyst excretion parameters per animal, per group and per hatching system, expressed in tables and graphs.

A dataset was produced in Excel (Microsoft 2013) with all weights and oocyst quantification data and reference to the corresponding per animal, group (X1, X2, R1, R2) and hatching system (Regular and Home Hatching). The groups were coded as R1/R2/X1/X2 and numerically, with respectively 1/2/3/4. Differences between means of weight between the 4 groups, were analysed using an Analysis of Variance (ANOVA) test, followed by post hoc analyses using a Bonferroni correction for multiple comparisons. When hatching systems were compared an independent students T-test was performed. Differences in means were regarded significant when *P* value was below 0,05.

Oocyst counts were $\log_{10}$ transformed to normalize the data ($\log_{10}$ (OPG + 1)). The mean of the $\log_{10}$transformed OPG was calculated for R and X chicks per group and per hatching system, to obtain mean oocyst excretion patterns. As a measure for the total number of excreted oocysts, the *AUC* (area under the curve) of oocyst output was calculated per bird from the daily OPG results and was $\log_{10}$ transformed (referred to as $\log_{10}$ *AUC*). The mean $\log_{10}$ *AUC* for each group and hatching system were compared with ANOVA and independent students T-test respectively, similar to the body weight analyses described before. In addition the $\log_{10}$ *AUC* was calculated for three periods (day 12-17, 18-22, 25-40) of the experiment to facilitate comparisons between R and X birds (Velkers, Bouma et al. 2010). The periods were identified by evaluation of the mean oocyst excretion pattern graph (figure 8), which showed three distinctive peaks of oocyst excretion during the experiment. The periods were defined as period 1, 2 and 3 and contained oocyst output between day 12 and day 17 (period 1), day 18 to 22 (period 2) and day 25 to 40 (period 3). For these three periods the $\log_{10}$ *AUC* was compared for the two hatching systems with an independent students T- test.

3. Results

The results of this study will be discussed for the challenge and field study separately.

3.1 Challenge experiment

3.1.1 Body weights

The mean body weights and standard deviations measured throughout the experimental period of individual chicks are given in table 3. Also, graphs of individual body weights were made for each group but only those of the R1 and X1 group are shown in figure 2 and 3 as an example.

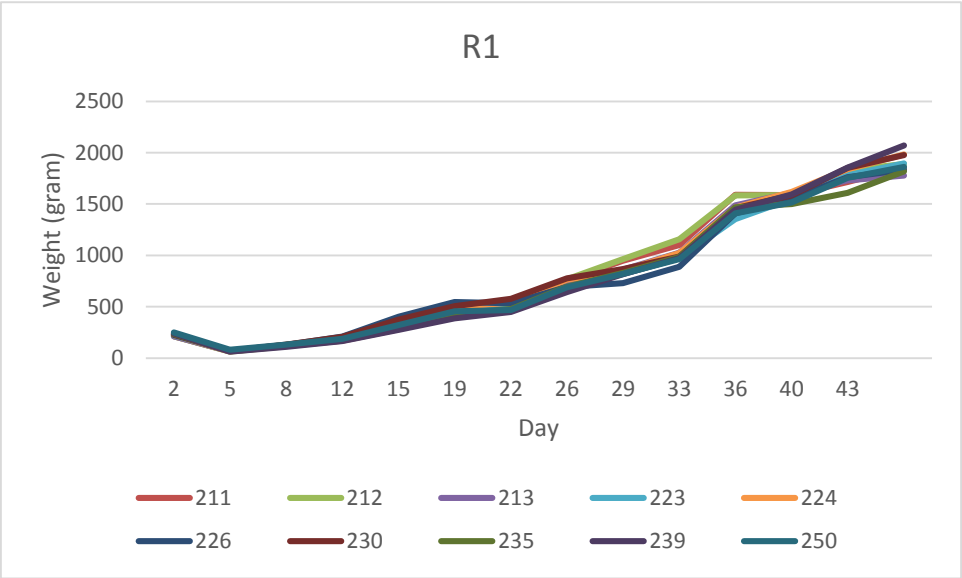

Figure 2: Weights of the R1 chicks per day.

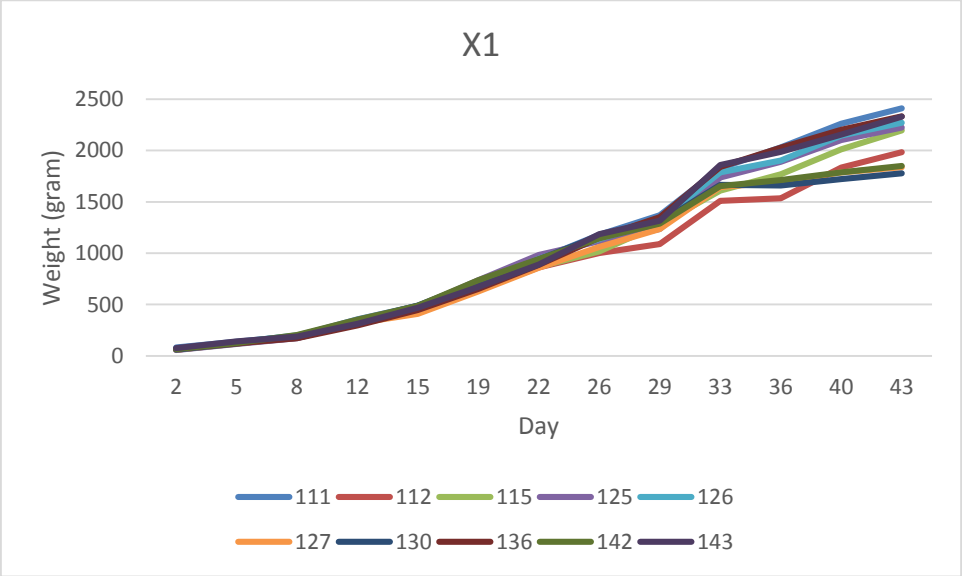

Figure 3: Weights of the X1 chicks per day.

From figures 2 and 3 follows that chicks of the X1 group have higher body weights than the R1 chicks (see also table 3), but the R1 chicks had a better uniformity, which also follows from the standard deviation given in table 3.

In figure 4 the mean body weights per group are visualized. Significant differences in mean weights between groups (comparing all 4 groups together) were found on day 19, 22, 26, 29, 33 and 36 and 40. Post-hoc tests, using Bonferroni corrections, showed that on day 19, 22, 26, 29, 33 and 36 the R1 group was significantly different from all the other groups due to a lower body weight (table 3). On day 22, the X1 group had a significantly higher body weight than the X2 group. On day 40, the R1 group was only significantly different from R2 and X1, due to a lower mean body weight of the R1 group.

In table 2, the number of male and female birds per group are shown. The sexes were determined during the post mortem examination.

Table 2: Number of male and female birds per group

Group	Male	Female
R1	5	5
R2	5	5
X1	10	0
X2	4	6

Group X1 is not comparable to the other groups, because there were only males in that group, whereas the other groups had about 50% males. It is known that male boilers are heavier than females, as shown in table 3 (Ross 308 performance objectives 2014). Because group X1 completely consists of male chicks, it may be expected that this group would have a higher mean weight. However, figure 4 and table 3 show that the mean weight of group X1, X2 and R2 is comparable. This was also shown with the ANOVA test, X1 was only significantly higher in body weight compared to X2 on day 22. It looks like the sexes don't have a big influence on the mean weights in these groups.

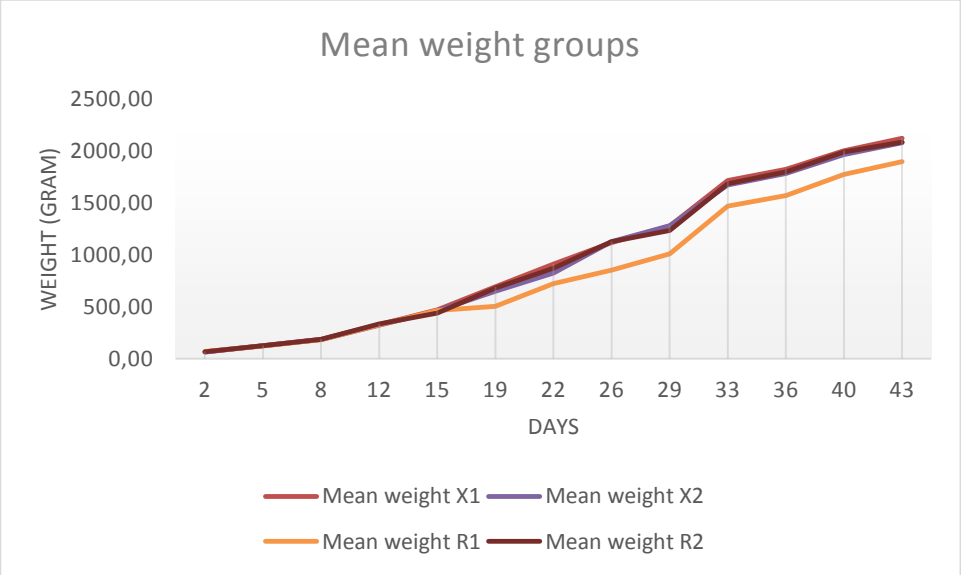

Figure 4: Mean weights of the four groups in the challenge experiment, per weighing moment.

Table 3: The mean weights and the standard deviation of the groups, per day.

Day	Mean R1	SD R1	Mean R2	SD R2	Mean X1	SD X1	Mean X2	SD X2	Ross 308 standards male (mean weight)	Ross 308 standards female (mean weight)
2	69	5,42	67	5,15	66	7,77	65	5,21	73	73
5	123	7,03	126	8,14	126	8,37	125	9,52	134	134
8	184	14,94	188	14,31	186	11,30	185	9,60	221	220
12	326	39,12	336	25,50	327	18,48	321	17,72	385	376
15	463	43,50	440	29,80	469	24,80	460	19,61	545	526
19	503	40,41	680	47,39	690	40,69	648	38,21	808	765
22	721	45,19	869	80,21	911	44,45	823	59,60	1040	969
26	851	67,38	1127	85,81	1116	67,01	1125	72,99	1388	1268
29	1008	75,08	1234	89,05	1277	79,27	1280	90,35	1673	1507
33	1467	73,97	1683	132,63	1713	115,15	1669	132,27	2075	1838
36	1569	39,23	1796	147,03	1820	171,43	1781	165,02	2388	2090
40	1772	76,51	1987	181,49	2000	200,43	1961	198,04	2811	2428
43	1895	89,28	2083	184,14	2121	235,69	2077	207,16	3129	2678

In table 3, the mean weights of the groups and the standard deviation are shown. The standard deviation is a measure of how widely values are dispersed around the average value (the mean).

From table 3 follows that, although the mean weight of group R1 was lowest, approximately from day 19 onwards, this group had the highest uniformity of all groups. X1 had the worst uniformity at the end of the research period. X2 had the best uniformity until day 19.

Table 3 also clearly shows that the body weight of all groups are much lower than weights recorded in the field study (see below) and Ross 308 performance standards, throughout the experiment. Feed consumption in the challenge experiment was also much lower than expected. Between day 8-43 the broilers had consumed on average 3,07 kg, whereas Ross 308 standard indicates a feed consumption of 4,75 kg. The total feed conversion for the challenge study for all groups together was 1.744, which is only slightly higher than Ross 308 standards indicate for 43 days of age (1.739), which suggests that the low body weight is most likely a result of the low feed intake.

In Figure 5, the weights of X1 and X2 are combined as X, R1 and R2 are combined as R. The figure is a comparison of the hatching systems. It is shown that the mean weight of the complete X group is higher than the R group. This is probably caused by the aberrant weight of the R1 chicks, which is shown in figure 4. Due to low feed consumption and low body weights in all groups, and especially in the R1 group, circumstances in this study do not allow for drawing conclusions on differences in body weight or feed consumption between hatching systems.

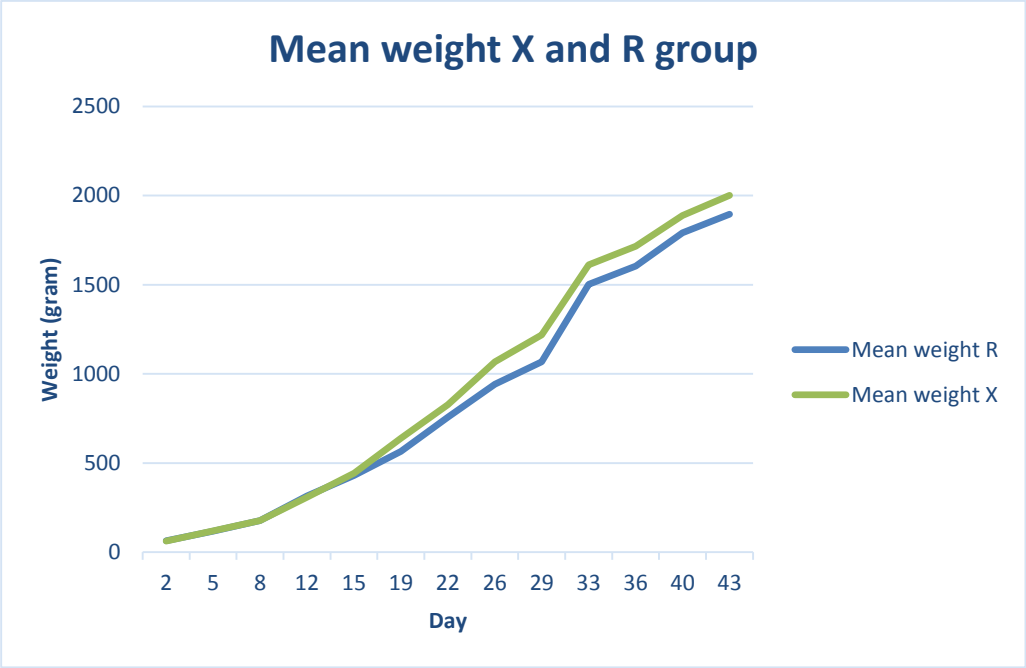

Figure 5: Mean weight of the X and R group

3.1.2 Excretion of oocysts

Excretion of oocysts was analysed in different ways. First the oocyst excretion per day was visualized to compare oocyst excretion patterns for the different groups throughout the experimental period. Also, the total oocyst output for the different groups was compared by comparing cumulative output using mean $\log_{10}$ *AUC* for the entire experiment and for different periods between groups and hatching systems.

In figure 6, the total mean oocyst excretion per group is shown, per day. Visual inspection of the graph suggest that R1 group had a lower total excretion in comparison to R2 and X1, until day 19. R2 and X1 had a lower excretion than R1 and X2 after day 19.

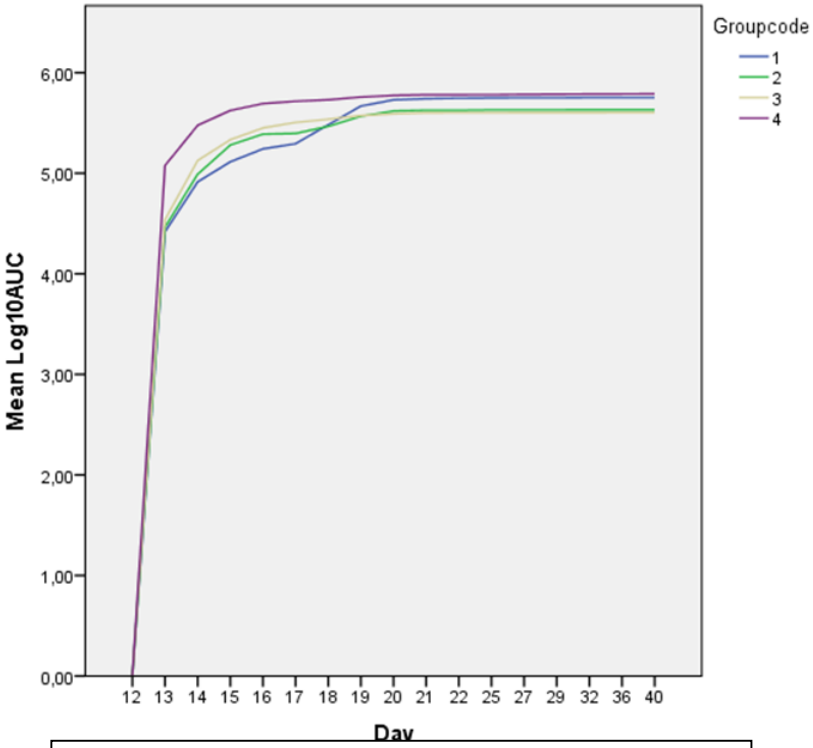

Figure 6: Mean $\log_{10}$ AUC per group, per day.
1=R1, 2=R2, 3=X1, 4=X2

In figure 7, the mean log_{10} *AUC* is shown per hatching system. The Regular broilers (R) had a lower excretion from day 13 until day 19 compared to the X chicks. The excretion of the R chicks rise from day 17 until day 19. Visual inspection of the graph suggest that from day 19 onwards, the mean log_{10}*AUC* is equal in both groups. An independent T-test was done on day 40. This test had also shown that there were no significant differences between the groups in total oocyst excretion.

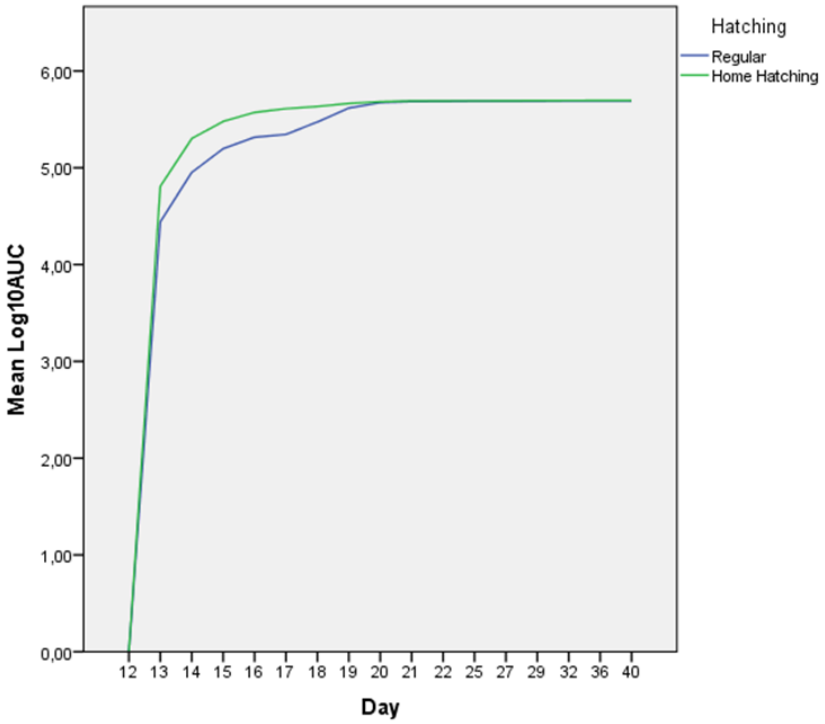

Figure 7: log10 cum AUC per hatching system.

In figure 8, the log_{10}OPG is shown in the whole period of the challenge experiment. It is shown that the Home Hatched broilers (X) had visually a higher log_{10}OPG on day 13 until 17 and 25-32. The Regular broilers (R) had a peak excretion on day 18, which was much higher than the peak of the Home Hatched (X) group. An independent T-test was done for each day to check if the visual differences in mean log_{10}OPG were significant. The T-Tests showed that the differences were only significant at days 16,18,20,22 and 27.

In figure 8, three different periods of oocyst excretion peaks can be identified in the graph. These three periods, were day 12-17 (period 1), 18-22 (period 2) and 25-40 (period 3). The mean log_{10} *AUC* of the two hatching systems were compared with the T-test for these periods. In the T-test, it is shown that the log_{10} *AUC* was only significantly different in the first two periods. In the first, the log_{10} *AUC* of the Home Hatched (X) chicks was higher, in the second period, the Regular chicks(R) had a higherlog_{10} *AUC*.

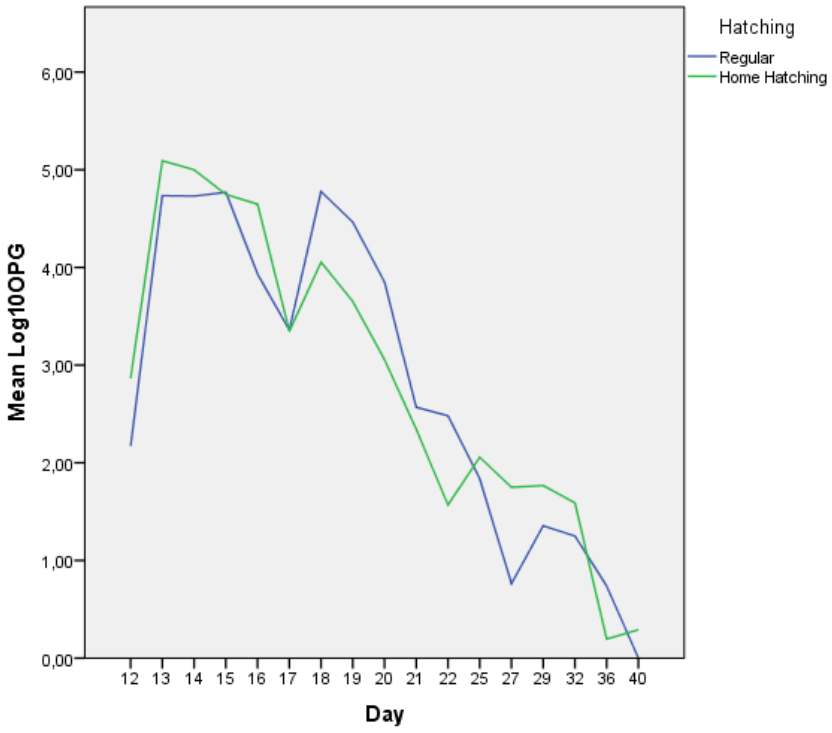

Figure 8: log10 OPG per hatching system.

To determine whether different groups show differences in the oocyst excretion patterns with regard to high oocyst excretion peaks, figure 9 was made. The graphs shows that there was a high peak of oocysts excretion on day 19 in all groups, and this peak was highest for the R1 group. Figure 6 also showed that there was an increase of cumulative numbers of oocysts from day 17 until 19 which was steeper in R1 than in other groups.

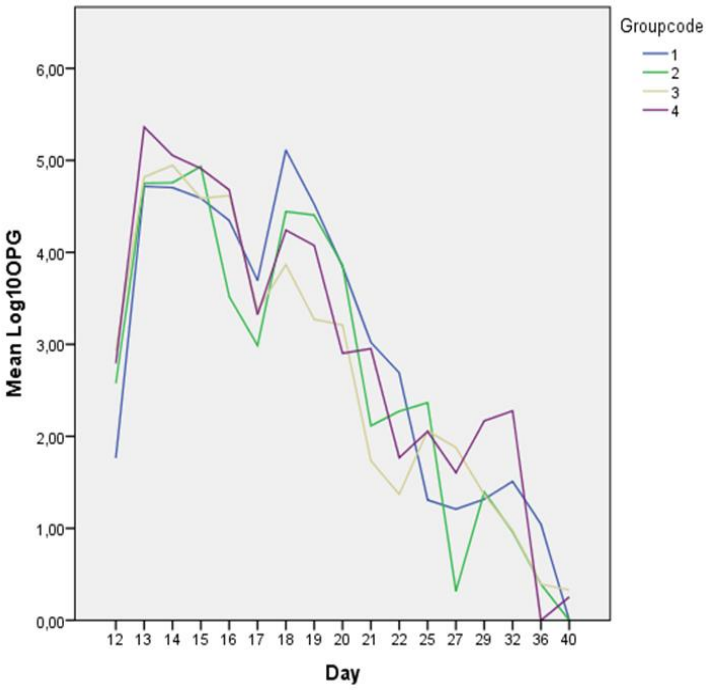

Figure 9: log10 OPG per group

3.1.3 Clinical examination

During the challenge experiment, a welfare and clinical inspection log was filled out daily. During the hour of darkness, when the chicks were in the transport boxes to produce a faecal dropping, the R1 chicks were very restless. When boxes were opened after the lights had been switched on to determine whether a dropping had been produced, the chicks of this group sometimes jumped out of the boxes. Also during caretaking of the animals and weighing R1 and R2 chicks seemed to show more fearful and more aggressive behaviour towards flock mates than X1 and X2 birds. It was also observed that the litter of the X2 group was very wet from day 28 onwards. The litter of the R1 cage was very dry compared to the other groups, because R1 was placed underneath a ventilation shaft and air flow seemed quite high. This was observed on day 32 after which the ventilation direction was altered and wet litter was also observed in this cage from day 36 onwards. On day 33 and 37, wood shavings were refreshed by adding additional wood shavings on top of the present wood shavings in all cages, as described in chapter 2.

More than other groups, R1 chicks were very aggressive when the researcher put them back in their floor pen after droppings collection. They were immediately drinking and eating, similar to chicks in the other groups, but the chicks of the R1 group were pecking aggressively to each other and their water bottles. The chicks had red spots on their beaks, caused by pecking very hard on the water bottles, this was noticed by caretakers on day 25. This little wounds were shown on photo 7. On day 31 caretakers of the animals noticed obstructions of some of the water bottles. Perhaps some of the bottles had not been working properly for a longer period, which may have affected feed intake and may have induced the aggressive pecking to the water bottles in R1. It could not be determined whether bottles in the R1 functioned worse than in other groups. This problem was resolved on day 31 by adding additional water bottles and extra water inspections during the day.

Figure 7: little red spot on the pecker of this R1 chick. It is caused by aggressive pecking on the water bottles.

3.2 Field study

3.2.1 Body weights

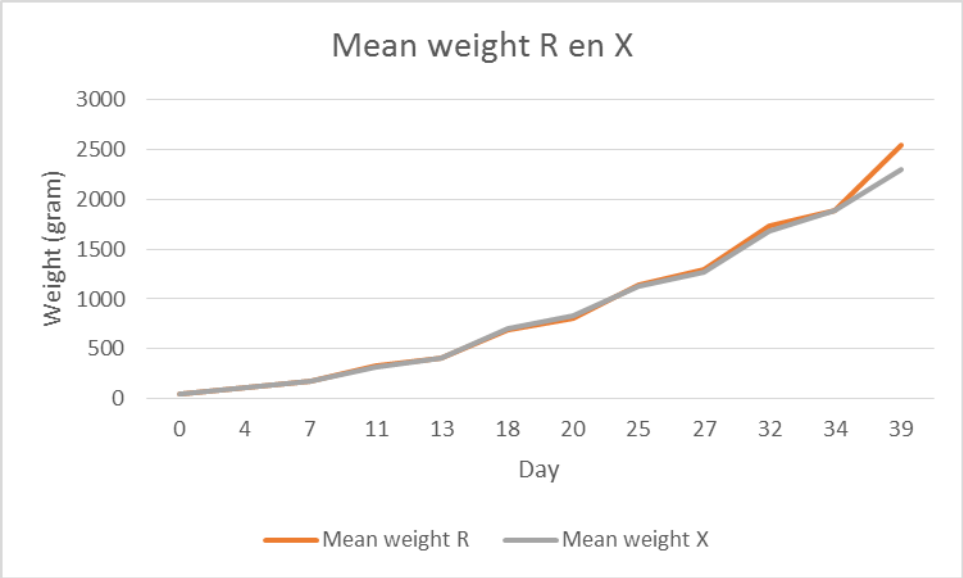

Figure 10: Mean weights of the R and X group, per day.

In figure 10, it is shown that the groups were very comparable over the whole period, excluding day 39. A T-test was done to check the significance of the differences per day. Figure 11 and 12 show that significant differences in body weights between the R and X group were only found on day 0 and 39. On day 0 the body weight of X chicks was slightly higher than of the R chicks, whereas on day 39 the R chicks weighed on average 246 grams more than X chicks.

Day = 0

Group Statistics[a]

	Groupcode	N	Mean	Std. Deviation	Std. Error Mean
Weight	1	75	45,468	3,0200	,3487
	2	75	47,283	3,3209	,3835

a. Day = 0

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Weight	Equal variances assumed	,349	,555	-3,501	148	,001	-1,8147	,5183	-2,8389	-,7904
	Equal variances not assumed			-3,501	146,685	,001	-1,8147	,5183	-2,8390	-,7903

a. Day = 0

Figure 11: Independent sample T-test of the weights in group R (1) and X (2) on day 0.

Day = 39

Group Statistics[a]

	Groupcode	N	Mean	Std. Deviation	Std. Error Mean
Weight	1	100	2553,820	372,9102	37,2910
	2	100	2308,140	365,6117	36,5612

a. Day = 39

Independent Samples Test[a]

		Levene's Test for Equality of Variances		t-test for Equality of Means						
									95% Confidence Interval of the Difference	
		F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	Lower	Upper
Weight	Equal variances assumed	,823	,365	4,704	198	,000	245,6800	52,2239	142,6935	348,6665
	Equal variances not assumed			4,704	197,923	,000	245,6800	52,2239	142,6932	348,6668

a. Day = 39

Figure 12: Independent sample T-test of the weights in group R (1) and X (2) on day 34.

3.2.2 Excretion of oocysts and lesion scores

As described in §2.2.1, the Animal Health Service, GD Deventer determined lesion scores weekly and carried out qPCR tests on faecal samples collected by the farmer to determine excretion of oocysts. Simultaneously, UU also collected and examined faecal samples (see table 1 in §2.2.1).

In figure 13, the $\log_{10}$ OPG is shown per group, per day. Only *E. acervulina* and *E. tenella* were present in the faecal samples according to the qPCR results. It is shown that the OPG of *E. acervulina* is lower than *E. tenella*, over the whole period. Besides that, it is notable that the Home Hatched (B2) broilers had a lower excretion than the traditional hatched (B1) chicks at each sampling time. The GD used collected samples of three days, the days on the X-axis were the last days of each sampling.

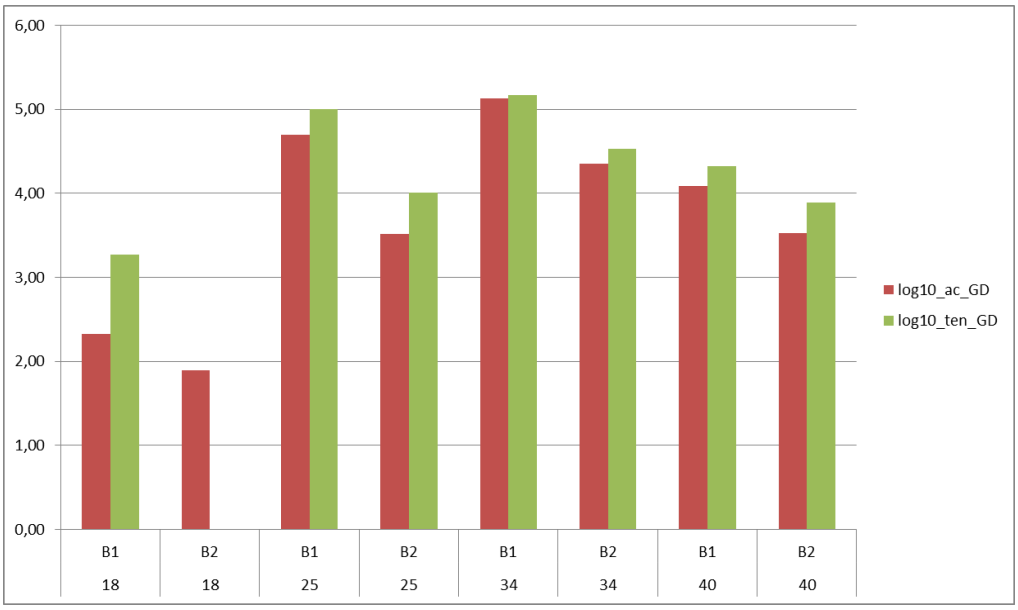

Figure 13: Log 10 OPG of E.acervulina and E.tenella in Regular (B1) and Home Hatched broiler chicks (B2)

In case of a high *E. tenella* excretion, a high mortality rate is expected. *E. tenella* excretion peaks at day 25 and 34. In figure 14, the mortality is shown. In this figure, little peaks of mortality were shown around day 25 and 34, but these were the same for both groups. The mortality peaks from day 0 until 12 cannot be attributed to *E. tenella.* Due to the prepatent period of 7 days and the time it takes before a flock becomes infected, excretion and clinical signs are not expected before day 14 of age.

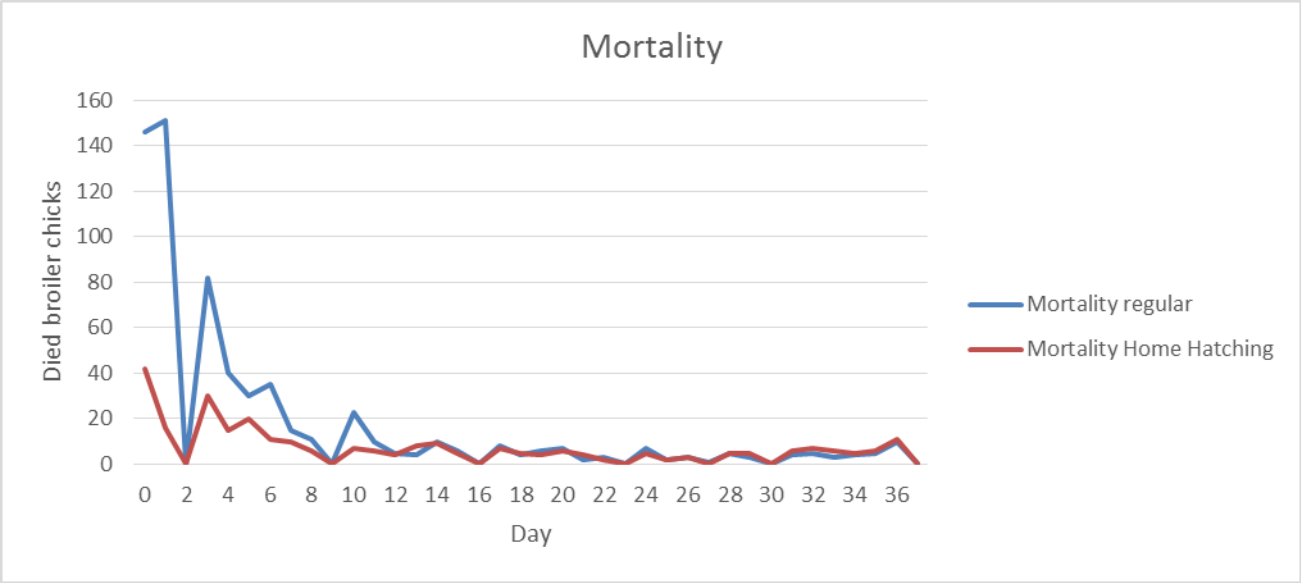

Figure 14: Mortality of broilers in the regular and Home Hatching group.

The Animal Health Centre measured the lesion scores during weekly post mortem examinations. The mean lesion score for *E. acervulina* and *E. tenella* was the highest on day 27 for both groups. The peak of the regular group was higher than that of the Home Hatched group. This peak can explain the increase of OPG on day 34, as generally lesions are caused by multiplication of the parasite, after which these are excreted.

In addition a comparison was made of the two methods, the qPCR on pooled faeces by GD and McMaster on pooled faeces by UU. It should be noted that the qPCR was done with a pooled sample of the preceding week. In figure 15 (and also figure 13), the day that is given on the X-axis is the day of the third and last sample of the preceding week. The OPG determined by Utrecht University (UU)

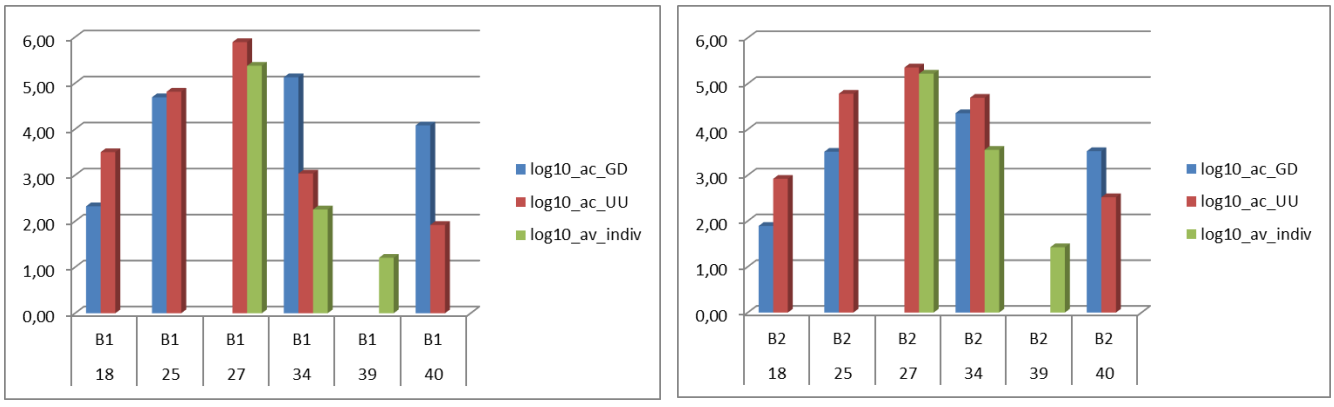

Figure 15: A comparison between the qPCR test and the McMaster.

was determined on the day that is given in the graphs. In addition, on three occasions (day 27, 34 and 39), individual droppings were tested with a McMaster by UU. This comparison is shown in figure 15. Agreement between individual dropping results and pooled McMaster results seem reasonable. Differences between qPCR test and UU pooled samples are fairly high, but can be explained by the fact that the qPCR represents oocyst output from the preceding week.

3.2.3 Clinical inspection and slaughterhouse data

Every time the chicks were weighed and faecal samples were taken, a clinical inspection of the chicks was carried out. During the first two weighing moments (day 4 and 7), the Home Hatched chicks seemed less shy than the traditional hatched chicks. The feathers of the chicks of both groups were clean. From day 27 the density of the chicks seemed to be higher in the Home Hatched group, but this was not confirmed by the farmer. The chicks were calm in the Home Hatched group, the traditional hatched chicks were more aggressive to the researcher upon handling. From day 34 onwards, the Home Hatched chicks appeared to be more fearfull then before: they ran away when the researcher wanted to weigh them or take the pooled faecal samples. The traditional hatched chicks were more aggressive and screeched when they were picked up, they also pecked at feet and hands of the researchers. The farmer has also indicated to see these differences between regular and home hatched chicks over several production rounds: generally regular chickens are more fearfull, more aggressive whereas home hatched chickens seem much calmer throughout the entire production period. To look if there is a difference in stress level between the two groups, feathers were collected from the birds of the field study (day 40) and challenge study (day 43) to determine the level of corticosterone as an indicator of stress according to procedures described by Carbajal (Carbajal, Tallo-Parra et al. 2014).This is outside of the scope of this study and therefore not described further in this report.

The chicks of both groups were slaughtered on the 22th of October (day 41 of age). At the slaughter house, the chicks were weighed, uniformity was determined and were scored for different diseases and quality of the carcass. The foot lesion scores are given in table 4. A higher lesions score indicates more severe lesions.

Table 4: Foot lesion scores of the traditional hatched (TH) and the Home Hatched (HH) chicks in the slaughter house examination.

Group/Lesion score	**0**	**1**	**2**
R	100%	0%	0%
X	88%	12%	0%

It is shown in table 4 both groups had low lesions scores suggesting that the litter quality was good in both groups. The traditional hatched chicks had a slightly better foot lesion score than the Home Hatched chicks.

In the slaughter house, it is also counted how many chicks were not good enough for human consumption. The number of condemned chickens was low and did not differ much between the traditional hatched and Home Hatched groups with respectively 78 and 99 condemned chicks. The mean weight of the traditional hatched chicks was 2633 grams and the mean weight of the Home Hatched chicks was 2564 grams. The traditional hatched chicks were heavier than the Home Hatched chicks, which is in agreement with what was found at day 39 in the field study of this report.

4 Discussion

When broilers are hatched at a hatchery, they generally do not have access to feed and water until arrival on the broiler farm. This may have negative effects on broiler health, production performance and welfare. The aims of this study were to determine whether on farm hatching, where broilers are hatched at the broiler farm and have immediate access to feed and water, has significant benefits with regard to production efficiency and the response to *Eimeria* infections. The study consists of two parts, a challenge experiment under controlled circumstances and a field study on a broiler farm, which are discussed separately below.

4.1 Challenge experiment

The challenge study was done to determine whether differences could be found in response to an *E. acervulina* infection under controlled circumstances. Birds were transported from the farm to the research facility at day 2, and at day 8 of age 5400 sporulated *E. acervulina* oocysts were given orally to artificially infect the birds. The response to the infection was determined by evaluating oocyst excretion in faecal samples of individual birds and assessing body weight development. The experiment was done with 2 replicate groups (X and R) of 10 birds each. Before inoculation, infection status was determined in the chicks. A very small number of oocyst resembling structures were found in the faecal samples, but the researchers were not completely convinced that these were actual oocysts. It cannot be ruled out that the chicks were infected in the stable before they were transferred to the research facility. Whether this was the case or not, if they were, the numbers were small and present in all groups, and most likely this did not play a significant role in the experiment.

All birds were successfully inoculated as they all started excreting oocysts from day 12 onwards, 4 days after inoculation. Considering a prepatent period of 96 hours according to literature (Velkers 2011) and inoculation time at day 8 between 13-14 hours and sampling at day 12 at 10, the prepatent period in this experiment was relatively short. After the first peak of excretion, between day 12-17 (with highest oocyst outputs on day 13-15) a second peak was present at days 18-19. This first peak was a result of the inoculation. The second peak was caused by ingestion of the sporulated oocysts from the litter from the first peak. The birds did not show clinical signs of disease due to the infection, although wet litter was observed from day 28 onwards but this was most likely caused by the local climate and water bottle leakage as discussed below.

As *E. acervulina* infections can have negative effects on body weight development, this was also an important parameter in this study. Unfortunately, due to low feed consumption and low body weights in all groups, and especially in the R1 group, circumstances in this study did not allow for drawing conclusions on differences in body weight or feed consumption between hatching systems.

In the introduction was explained that broilers that were hatched at the farm, should have a better start due to absence of a delay in feeding and a reduction of stressful events. The additional stress that hatchery broilers can have is caused by different things. For example the treatments at the hatchery, the transport to the farm and the delay of access to feed. In this challenge experiment, both the Home Hatched chicks and regular chicks were transported to the research facility at day 2 of age. During this transport, both groups may have had stress and also, were denied access to feed for 2 hours. The question is whether this has had a negative influence on the chicks. Perhaps the advantage the home hatched chicks had at the farm has been countervailed by transporting them to the research facility. During the research period, the handling method and treatments were exactly

the same for all the chicks so after arrival at the research facility circumstances were equal for the duration of the experiment.

The size of the experimental groups was large enough to detect significant differences in the excretion of oocysts, but most likely too small to analyse the weights and feed conversion. This was expected beforehand, and therefore weights were also recorded in the field study where 100 birds could be measured during each weighing moment. Furthermore, the body weight of the birds was much lower than expected based on the Ross 308 performance standards and also much lower than in the field study.

In the results, it is shown that the R1 group was most of the time, significantly different in body weight compared to the other groups. This was not due to a higher number of females in this group, compared to other groups. Most groups were comparable with regard to number of males and females, except for group X1 which consisted only of males. The body weight of this group however was not significantly different from the other groups in most cases, excluding sex as an explanation for differences in weight between groups. There were some local differences between housing and management of the groups that may explain some of the observed differences. For instance, there were some differences in the ventilation of the groups. R1 was placed underneath a ventilation shaft and air flow seemed quite high. The litter in this cage was very dry compared to the other groups. This was observed on day 32 after which the ventilation direction was altered and wet litter was also observed in this cage from day 36 onwards.

From day 31 onwards, there was also a problem with the water bottles, some of them were congested. As a reduced water intake can lead to reduced feed intake, the congested water bottles may have caused the low feed intake and low body weights. The total feed conversion for the challenge study for all groups together was 1.744, which is only slightly higher than Ross 308 standards indicate for 43 days of age (1.739), which suggests that the low body weight is most likely a result of the low feed intake.

In this report, the density in the challenge experiment was 12 chicks per m^2. This density was chosen to resemble the situation on the farm. However, at the end of the study at day 43, the broilers had a much lower mean body weight than expected (looking at the performance standard of Ross 308). This resulted in a density of 24,5 kg/m^2. If the chicks would have had the expected body weight, the density would have resembled that of the field study (35,9 kg/m^2).

The method of individual dropping collection was very accurate as it reflects oocyst excretion of each individual bird at the moment of sampling. The chicks all had an individual box and matching plastic inlay, to prevent cross contamination between birds and groups. This allowed for a thorough evaluation of oocyst excretion in time. Although the total oocysts output was not significantly different between groups, when certain periods were evaluated, some differences were found. It was shown that the X group had a higher excretion in the first period and the R group in the second period. The third period, none of the groups were significant higher. According to (Graat, Reilingh et al. 1996) performance is less impaired when the highest oocyst excretion occurs early in a production round, because then chicks have sufficient time to compensate the weight loss later in the production round. X birds started with a higher oocyst output, but R birds had a significantly higher excretion in the second period. This may suggest that X birds had a more beneficial course of infection. However, in the third period differences between X and R were not significant (with a

trend of higher excretion of X birds), so it is questionable whether these differences could have lead to significant differences in performance of the broilers.

The behaviour of the R chicks, mainly the R1 chicks, was very notable. They were more aggressive and restless than the chicks of X1 and X2. Whether significant differences in the level of corticosterone, as an indicator of stress, can be found in the feathers collected at the end of the study remains to be determined and falls outside of the scope of this study.

4.2 Field study

The field study was done to determine the differences in performance between traditional hatched and home hatched broilers. In the field study, body weights of 100 R and 100 X chicks were determined twice per week and oocyst excretion was quantified in faecal samples on 8 occasions between day 11 and day 40. In addition, on day 27, 34 and 39, individual faecal dropping samples were collected from 20 of the weighed chicks of each group (20 R and 20 X). Furthermore, uniformity of the flock was assessed and clinical signs, mortality and intestinal lesions scores were determined.

The field study was carried out on a broiler farm, in a broiler house with 20.000 chicks. The poultry house was split in two parts by using a plastic netting. The two parts of the house had exactly the same surface (457m^2). This results in a density of 35,9kg per m^2 at the end of the production cycle. On the right side of the house, 10.000 broilers were hatched with the “home hatching system”. These chicks hatched at the 10th of September 2014. At the 11th of September (day 0), 10.000 one-day-old broiler chicks from the hatchery were placed at the left side. The chicks stay in this broiler house until day 40, no transportation was needed in this period. In this broiler house, the chicks are exposed to different pathogens, such as different species of *Eimeria*. In the field study, there were much more chicks than in the challenge study, so a better funded conclusion can be made about performance.

Before the start of the field study, protocols were made for the weighing method and the collection of the faecal samples. Although these protocols described exactly how to weigh the broiler chicks, it wasn’t possible to check if everyone randomly selected and weighed the chicks in the same way and according to these protocols. The fact that there were different researchers during this study, may have had an influence on the weight data. Especially from day 26 onwards, it was very difficult to select the chicks completely at random. The first weeks, the chicks were small and it was easy to pick them up and put them in the box. When they were heavier, you had to pick up the chicks one by one. Now, the chicks had more time to run away, and the researcher selected the chicks that he can catch. This is not completely random. At first it was assumed that the sudden significantly higher mean weight of the regular group on day 39 could be caused by the fact that another researchers had selected and weighed the birds. However, in the slaughterhouse data of day 41 it was shown that the traditional hatched chicks (R group) were heavier indeed, although the difference was 69 grams at slaughter whereas at day 39 (2 days before the slaughterhouse weight was determined) the difference was 246 grams. Therefore, an influence of the change of personnel on the weight of day 39 cannot be excluded completely. Because the mean weight of the chicks was almost equal for both groups until day 39, we cannot conclude that the R chicks had a better growth rate.

The mortality was much lower in the Home Hatched group compared to the traditional chicks. The differences was mainly apparent in the first twelve days. This difference could be a result of the

benefits that these chicks had from no stressful treatments and transport in the first 3 days of their lives. Besides that, the direct access to feed ensures that these chicks were stronger in the first days. One critical note has to be made, the Animal Health Centre, GD Deventer, had selected broiler chicks 7 times in both groups during the field study. It is plausible that this has had an influence on the mortality rate. The farmer had separated the clinical aberrant chicks, this pool of chicks was supplemented with random selected chicks. The clinical aberrant chicks weren't counted as died chicks, but as selected chicks. Normally these chicks would have died. Because of that the mortality rate may be lower than in case of random selection. If differences between groups would have been present with regard to prevalence of abnormal chicks, mortality rates may have been affected differently in both groups. This complicates drawing conclusions on differences in mortality rates between both systems.

The pooled faecal samples collected by Utrecht University were not very usable, because the samples of the colonic faeces were contaminated with caecal faeces due to inadequate sampling by the different researchers. These samples have to be collected separately as with a McMaster test the *Eimeria* species cannot be determined. Because colonic faeces mainly contains *Eimeria acervulina*, *E. maxima* (which can be recognized due to its large size) and *E. praecox* and *E. mitis* and caecal faeces mainly contains *E. tenella*, some indication can be given on prevalence of the different species. Therefore the results of the UU tests using McMaster counting was not the same as the qPCR. The individual samples were useful, but these were not taken at the same days as the samples of the qPCR, which made the comparisons very difficult. The Animal Health Service, GD Deventer carried out qPCR tests on faecal samples collected by the farmer to determine excretion of oocysts. This results of the qPCR test were more exact. In these qPCR results, it is shown that the OPG of *E. acervulina* is lower than *E. tenella*, over the whole period. Besides that, it is notable that the Home Hatched broilers had a lower excretion than the traditional hatched chicks every time.

During clinical inspection, it is noticed that the traditional hatched chicks were more aggressive and screeched when they were picked up, they also pecked at feet and hands of the researchers. At the beginning, the chicks of X group were calm. From day 34 onwards, they appeared to be more fearful then before: they ran away when the researcher wanted to weigh them or take the pooled faecal samples. The farmer has also indicated to see these differences between regular and home hatched chicks over several production rounds: generally regular chickens are more fearful, more aggressive whereas home hatched chickens seem much calmer throughout the entire production period.

The chicks of both groups were slaughtered on the 22th of October (day 41 of age). At the slaughter house, the chicks were weighed, uniformity was determined and were scored for different diseases and quality of the carcass. It is shown in the results, that both groups had low lesions scores suggesting that the litter quality was good in both groups. The traditional hatched chicks had a slightly better foot lesion score than the Home Hatched chicks. The researchers noticed that the litter on the X side of the broiler house was wet, more than on the R side of the house. In the slaughter house, it is also counted how many chicks were not good enough for human consumption. The number of condemned chickens was low and did not differ much between the traditional hatched and Home Hatched groups with respectively 78 and 99 condemned chicks.

This report is based on only one flock of broilers, from one broiler farm. The differences between the two groups can't be extrapolated to all broilers for these systems. To allow for a better funded

conclusion on body weights and feed conversion, this type of study may benefit from repetition in another research facility, for example Schothorst Feed Research (Lelystad), where facilities are available that resemble the field situations more (larger groups) but still provide controlled circumstances. Also field studies on more farms and in more flocks of broilers should be carried out. Also, the choice of pathogen to test health effects may also be altered in future studies. In this study *E. acervulina* was used. This is the most prevalent *Eimeria* species, it's affecting practically all broiler flocks, and the clinical signs of this species of *Eimeria* are mild. It mainly affects body weight and feed conversion. The mild character of *E. acervulina* could also have a disadvantage, because of the mild clinical signs the chance of finding differences in responses between groups is limited. This study could be repeated with *E. maxima* or *E. tenella*. In case of an *E. maxima* infection, the effects on feed conversion and growth are bigger than in case of *E. acervulina*. In case of *E. tenella*, mortality will increase. A combination between coccidiosis and *Clostridium perfringens* could also be made, in that case more aspects of the immune system have to be activated and maybe this will result in finding differences between the two systems.

4.3 Conclusion

In the introduction of this report, it was expected that there were difference between traditional hatched and Home Hatched chicks. This differences were expected on performance and welfare.

After the challenge experiment and the field study, it is difficult to make a clear conclusion which of these systems is better. The weights of the challenge experiments can't be used and the mean $\log_{10}AUC$ was equal for both groups. The difference was the period of the excretion peak, where the early peak of the X group seems to be better. In the field study, the X group had a lower oocyst excretion for both *E. acervulina* and *E. tenella* during the 6 weeks of study. The slaughterhouse data were not as positive as expected for the Home Hatched chicks. They had slightly more foot lesions and more carcasses were rejected. If we look to behaviour, the Home Hatched chicks (and X chicks in the challenge experiment) make a better impression. The traditional hatched chicks were more restless and had some little wounds (in the challenge experiment), which the Home Hatched chicks don't had. If you only look to performance, the traditional hatched chicks were better in this round, but the Home Hatched chicks may be better at welfare grounds. Besides the challenge and field study, the estimation of the farmer is, that the Home Hatched chicks had less stress and needed less antibiotics. The decrease of antibiotic use is very important to be measured in another study. In short, in this report, it is not shown that Home Hatching is better than traditional hatching. However, it isn't even shown to be worse. Overall, it is thought that it is definitely worth to further evaluate and study the Home Hatching system.

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Appendix 1 Group composition challenge experiment

The composition of the groups after the 19th of September.

X1	X2	R1	R2
Purple	Purple	Yellow	Yellow
111	113	211	222
112	118	212	225
115	121	213	227
125	122	223	228
126	124	224	229
127	129	226	233
130	132	230	243
136	133	235	245
142	135	239	248
143	141	250	249

Appendix 2 Light scheme challenge experiment

Day	Date	Day (age)	Target temp.	Target RV (in %)	Dark period 1	Darkperiod 2
			Chicken level	**40-80/60-70**		
Sa	13-9-2014	2	33	40-80	24 h light	
Su	14-9-2014	3	32	40-80	24 h light	
Mo	15-9-2014	4	31	40-80	05:30-06:30 uur	
Tu	16-9-2014	5	30	40-80	05:30-06:30 uur	
We	17-9-2014	6	29	40-80	05:30-06:30 uur	
Th	18-9-2014	7	28	40-80	20.00-23.00 uur	05:30-06:30 uur
Fr	19-9-2014	8	28	40-80	20.00-23.00 uur	05:30-06:30 uur
Sa	20-9-2014	9	28	40-80	20.00-23.00 uur	05:30-06:30 uur
Su	21-9-2014	10	27	40-80	20.00-23.00 uur	05:30-06:30 uur
Mo	22-9-2014	11	27	40-80	20.00-24.00 uur	06:30-07:30 uur
Tu	23-9-2014	12	26	40-80	20.00-24.00 uur	06:30-07:30 uur
We	24-9-2014	13	26	40-80	20.00-24.00 uur	06:30-07:30 uur
Th	25-9-2014	14	25	40-80	20.00-24.00 uur	06:30-07:30 uur
Fr	26-9-2014	15	25	40-80	20.00-24.00 uur	06:30-07:30 uur
Sa	27-9-2014	16	24	40-80	20.00-24.00 uur	06:30-07:30 uur
Su	28-9-2014	17	24	40-80	20.00-24.00 uur	06:30-07:30 uur
Mo	29-9-2014	18	23	40-80	20.00-24.00 uur	06:30-07:30 uur
Tu	30-9-2014	19	23	40-80	20.00-24.00 uur	06:30-07:30 uur
We	1-10-2014	20	23	40-80	20.00-24.00 uur	06:30-07:30 uur
Th	2-10-2014	21	22	40-80	20.00-24.00 uur	06:30-07:30 uur
Fr	3-10-2014	22	22	40-80	20.00-24.00 uur	06:30-07:30 uur
Sa	4-10-2014	23	22	40-80	20.00-24.00 uur	06:30-07:30 uur
Su	5-10-2014	24	22	40-80	20.00-24.00 uur	06:30-07:30 uur
Mo	6-10-2014	25	21	40-80	20.00-24.00 uur	06:30-07:30 uur
Tu	7-10-2014	26	21	40-80	20.00-24.00 uur	06:30-07:30 uur
We	8-10-2014	27	21	40-80	20.00-24.00 uur	06:30-07:30 uur
Th	9-10-2014	28	21	40-80	20.00-24.00 uur	06:30-07:30 uur
Fr	10-10-2014	29	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Sa	11-10-2014	30	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Su	12-10-2014	31	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Mo	13-10-2014	32	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Tu	14-10-2014	33	20	40-80	20.00-24.00 uur	06:30-07:30 uur
We	15-10-2014	34	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Th	16-10-2014	35	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Fr	17-10-2014	36	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Sa	18-10-2014	37	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Su	19-10-2014	38	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Mo	20-10-2014	39	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Tu	21-10-2014	40	20	40-80	20.00-24.00 uur	06:30-07:30 uur
We	22-10-2014	41	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Th	23-10-2014	42	20	40-80	20.00-24.00 uur	06:30-07:30 uur
Fr	24-10-2014	43	20	40-80	20.00-24.00 uur	06:30-07:30 uur

Appendix 3 Time schedule inoculation

The inoculation was done by two persons, in table 1 the time schedule of the inoculation is shown.

Table 1: Time schedule of inoculation

Time	Group	Person
13:15 – 13:30	X2	Francisca Velkers
13:30 – 13:40	X1	Francisca Velkers
13:40 – 13:52	R1	Fietje van Bochove
14:00 – 14:10	R2	Fietje van Bochove

Appendix 4 Sampling method field study

Sampling method, used by the Animal Health Centre (GD) and Utrecht University. The route was the same for caecal droppings and colonic droppings and was done twice, one time on the left side (regular chicks) and one time on the right side (Home Hatched chicks).