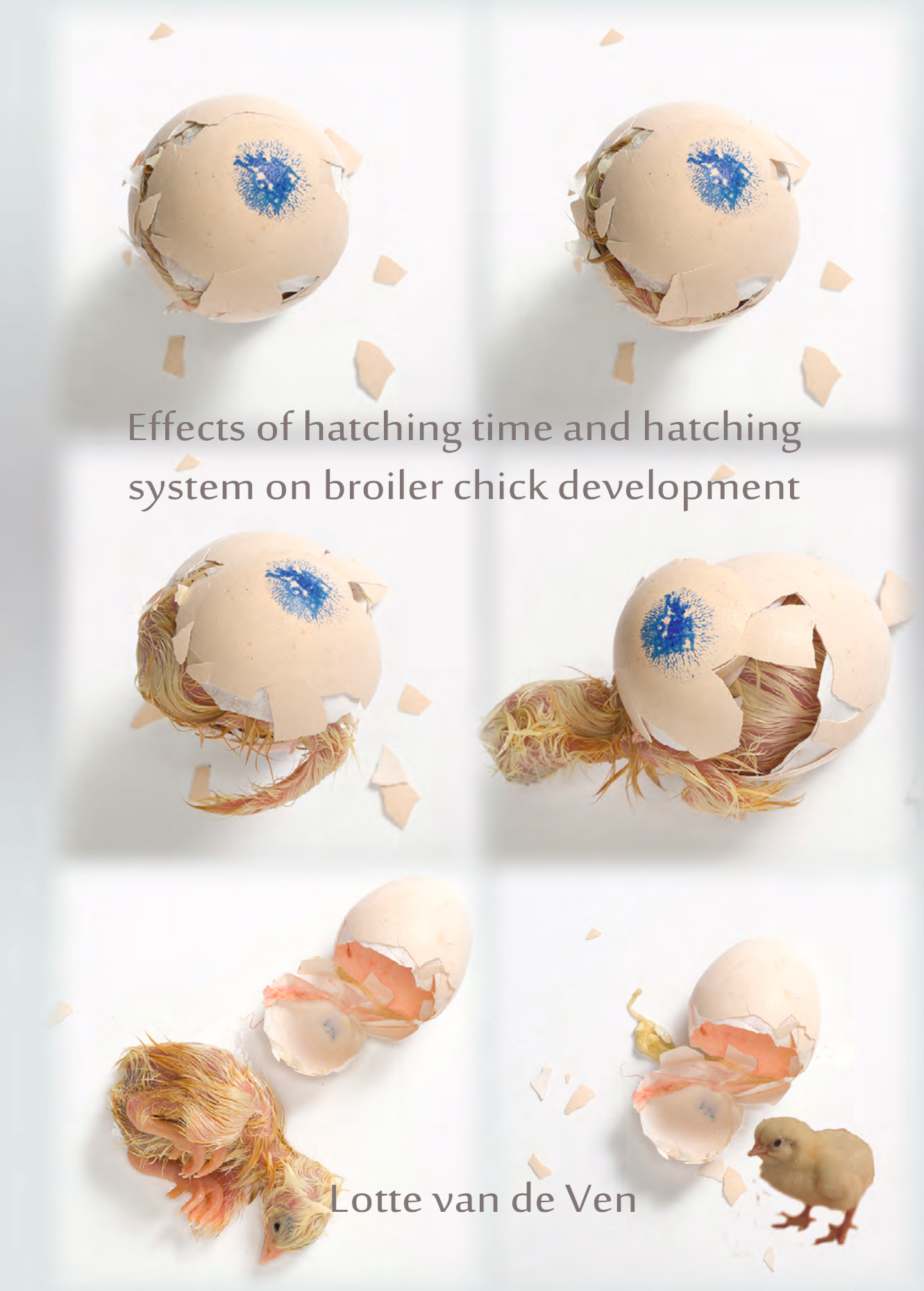

Effects of hatching time and hatching system on broiler chick development

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Effects of hatching time and hatching system on broiler chick development

Lotte J.F. van de Ven

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ABSTRACT

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Chicks hatch over a time window of 24-36 hours and are only removed from the hatcher when the majority of the chicks has hatched. Especially for the early hatching chicks this leads to delays in the first feed and water access and consequently negative effects on chick development. In an alternative hatching system, named Patio, the hatching and brooding phase are combined, thereby enabling direct posthatch feed and water access. Environmental conditions in Patio differ from those in hatchers, which may further influence chick quality, physiology, and growth. Chicks hatching at different moments may respond differently to these different conditions in both hatching systems. In this thesis, the first aim was to determine effects of hatching in the Patio system on hatchability, chick quality, and growth. The second aim was to determine the physiological status of chicks of different hatching moments, in the hatcher and the Patio system, at hatch, and at chick collection (21.5 d of incubation). Effects of hatching time and moment of first feed and water access on posthatch growth were also included.
Hatchability of fertile eggs was 1.03% higher in the Patio system compared to the hatcher, which was probably due to different climate conditions during the hatching phase. At hatching, chick physiology was not clearly affected by hatching system, but effects of moment of hatching in the hatch window were clear: longer incubation times led to increased organ weights and decreased yolk weights, suggesting a higher level of maturation in late hatching chicks. At the moment of chick collection, Patio chicks, having immediate feed and water access, showed larger body and organ weights, higher hepatic glycogen reserves, higher plasma glucose and T_3 levels, and lower corticosterone levels compared to hatcher chicks which were fasted between hatching and chick collection. Using a chick qualitative score based on physical traits and the incidence of second grade chicks, chick quality was lower in Patio than in hatcher chicks. However the quality scores used were not predictive for posthatch performance. Patio chicks showed improved posthatch growth compared to hatcher chicks, which was not related to different climate conditions during hatching, but to earlier feed and water access. Apart from higher growth from d0-7 in early and midterm vs late hatching chicks, effects of hatching time on growth were not clear from this thesis.
In conclusion, despite considerable differences in climate and other environmental factors, effects of hatching system on physiology of broiler chickens at hatch and growth performance up to slaughter age are limited. Perinatal chick physiology is affected by the moment of hatching in the hatch window, and by posthatch conditions in the hatching system, especially early feed and water access.

Key words: hatching time, hatching system, chick physiology, broiler growth, chick quality

VOORWOORD

Bij het afronden van mijn studie in november 2003 was ik vrij zeker van mijn beslissing: mijn toekomst lag niet in het onderzoek. Hoewel ik bij Pas Reform, een bedrijf wat broedmachines produceert, met veel plezier en motivatie aan mijn afstudeeropdracht had gewerkt, had ik toch geconcludeerd dat het stil zitten achter de microscoop en computer het niet voor mij was. Heel enthousiast begon ik in januari 2004 aan mijn eerste echte serieuze baan bij Pas Reform. Omdat ik niet precies wist wat ik wilde, mocht ik een soort van traineeship volgen. Zodoende heb ik gewerkt op de ontwikkelingsafdeling, bij marketing, was ik verantwoordelijk voor aftersales service en installaties, en heb ik uiteindelijk zelfs even aan verkoop mogen proeven. Geweldig mooi en super leerzaam. Na een dikke 4 jaar bij Pas Reform te hebben gewerkt, concludeerde ik toch dat ik wat diepgang miste in mijn werk. En volgde het telefoontje van Bas Kemp: of ik interesse had in een promotieonderzoek? In opdracht van Vencomatic? Onderzoek met praktische toepassing?

Eigenlijk klonk het zo gek nog niet en hoe meer ik erover na dacht, hoe enthousiaster ik werd. Henry van den Brand en Victor van Wagenberg werden mijn dagelijkse begeleiders. Bas Kemp mijn promotor, en Peter Groot Koerkamp mijn copromotor. In januari 2008 ben ik gestart met mijn onderzoek naar de ontwikkeling van de kuikens in de eerste levensdagen. En nu, na 4,5 jaar, is het zover en rond ik het onderzoek af. Met heel veel dank aan een lange lijst mensen.

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Hoewel ik nu, na 4,5 jaar onderzoek, opnieuw concludeer dat ik geen onderzoeker in hart en nieren ben, en me toch weer met andere zaken ga bezig houden, heb ik geen seconde spijt gehad van mijn beslissing een promotieonderzoek te gaan doen. De afgelopen jaren waren een investering in kennis van de kuikens, van onderzoek doen, en in nieuwe contacten met veel mensen. Een mooie basis voor de nieuwe uitdaging waar ik nu aan begin, in het zoeken van de juiste combinatie tussen onderzoek, ontwikkeling, en het vermarkten van nieuwe pluimveeconcepten voor Vencomatic. Ik heb er super veel zin in!

Lotte Staadegaard – van de Ven

CONTENTS

chapter 1

General introduction

Poultry meat consumption is expected to increase by 60% over the next 20 years and will be the most important meat category worldwide by 2030. In 2010, over 53 billion meat type chickens or broilers, being the most important supplier of poultry meat, were reared globally (Food and Agriculture Organisation, 2010). An important factor for the success in broiler production is the quality of day old chicks, and with increasing chicken meat consumption, the demand for day old chicks, presently produced by the hatchery, also grows. Although high quality of day old chicks was considered crucial to good broiler performance up to slaughter age (Tona et al., 2003, 2004; Decuypere and Bruggeman, 2007), it seems that chick quality and performance in terms of liveability and growth potential may not be optimal in current incubation systems and hatchery management procedures. This is due to two important factors. Firstly, chicks hatch over a time window of approximately 24-36 hours and are removed from the hatchers only when the majority of the chicks has hatched (Decuypere et al., 2001). Especially for early hatching chicks, this practice leads to delay in the moment of placement in the farm, and first access to feed and water, which is associated with increased early mortality (Misra and Fanguy, 1978; Kingston, 1979; Fanguy et al., 1980), and suboptimal growth posthatch (Fanguy et al., 1980; Gonzales et al., 2003; Kornasio et al., 2011). Secondly, conditions in the hatcher may not be optimal for modern broiler breeds. In the last phase of incubation, broiler hatching eggs and newly hatched chicks produce considerable amounts of heat (Tona et al., 2004). Field observations have shown that in large capacity setters and hatchers, inadequate heat removal from broiler eggs leads to hyperthermia, depressing hatchability, chick quality and posthatch performance up to marketing age (Lourens, 2001; Hulet, 2007; Leksrisompong et al., 2007, 2009).

Based on these two factors, it can be questioned whether the hatchlings' requirements are met or can be met in current hatchery practice. An alternative to traditional hatchery practice and related impacts on chick quality and posthatch performance, is to combine the hatching and brooding phase in one system. In the years 2002-2006, a multi-tiered hatching and brooding system for broiler chicks was developed and tested, named Patio (Vencomatic, the Netherlands). After 18 d of incubation in a conventional hatchery, apparently fertile eggs are transported to the Patio for the last 3 days of incubation. After hatching, chicks remain in this system during (part of) their growing period. Thus, for these chicks hatchery treatments such as counting, packaging and transportation are omitted and chicks have immediate posthatch access to feed and water. Also, the selection of non-saleable chicks (second grade chicks), which are removed by hatchery personnel after chick collection in hatchery practice, is omitted in the Patio system as a standard procedure after hatch. Furthermore, climate conditions in the perinatal phase in the Patio differ from those provided in hatcher cabinets, which may influence the quality and posthatch development of the broiler chicks. These different conditions may have consequences for chicks hatching at different moments in the hatch window.

Hatching time

Hatching time is known to be influenced by parental age, storage time and storage conditions, and incubation temperature (Tona et al., 2003; Careghi et al., 2005; Decuypere and Bruggeman, 2007). Although part of these factors can be controlled in hatchery practice, temperature gradients are present even in the most modern incubators, which influence embryo developmental rate (French, 1997; Van Brecht et al., 2003) resulting in differences in hatching time. Furthermore, within a batch of eggs from the same origin, additional intrinsic factors influence hatching time such as order of an egg in a clutch, time of lay during the day, eggshell conductance, and egg weight (Meijer and Siemers, 1993; Christensen, 2001; Decuypere et al., 2001), which can hardly be controlled in current hatchery practice. Consequently, it seems that in commercial hatcheries, a spread of at least 24 h in hatching time is inevitable.

Several studies demonstrated differences in liveability and posthatch growth in chicks of different hatching times in eggs from the same hatch (Williams et al., 1951; Kingston, 1979; Hager and Beane, 1983; Careghi et al., 2005). Higher mortalities at 10 d of age were observed in chicks that hatched late (52.9%) in the hatching window, compared with chicks hatched early (3.2%) or the peak of hatch (1.2%; Kingston, 1979). Chicks hatching early in the hatch window seem to have higher potential for posthatch growth compared to later hatching chicks (Williams et al., 1951; Hager and Beane, 1983). Furthermore, broiler chicks hatching late within the hatch window of one flock, respond differently to delayed feed access compared to chicks hatched early (Careghi et al., 2005). Blood T_3 levels at day 1 and 2 posthatch were lower in late hatching chicks compared with earlier hatching chicks that were deprived of feed for 48 hours posthatch, suggesting a lower metabolic rate, but this effect was not observed in chicks with immediate access to feed. It appears that there are intrinsic factors influencing posthatch performance that relate to age at hatching, which are aggravated by feed deprivation (Careghi et al., 2005; Decuypere and Bruggeman, 2007). These factors are unknown, but may be related to a difference in rate of yolk absorption and/or to differences in thermoregulatory, intestinal, immunological and muscular development. Due to the variation in hatching time and the fixed moment of chick collection from the hatcher, early chicks are subjected to feed and water deprivation and posthatch climate conditions in the hatching system for a longer period than late hatching chicks, which may lead to different chick quality (partly due to dehydration) and posthatch performance. This factor has so far largely been ignored in previous studies and in hatchery practice.

First feed and water access

In hatchery practice, fixed management schedules often leave little room for flexibility and thus the moment of chick collection has usually been set at 21.5 days. Consequently, a slight delay in the moment of hatch or a more pronounced variation of it may decrease hatchability, as opening the hatchers too early means that eggs with viable chicks inside are wasted. On the other hand, postponing the moment of chick collection will lead to a higher percentage of chicks dehydrating (Bamelis et al., 2005). Further management practices at the hatchery, such as chick counting, vaccination, packaging and transportation procedures increase the time until placement in the

broiler house and thus first feed and water intake, for part of the flock by up to 50 h or more (Sklan et al., 2000). Next to impaired posthatch growth, delayed access to feed reduces development of the gastrointestinal tract (Noy and Sklan, 1999b; Bigot et al., 2003), immune functioning (Dibner et al., 1998; Bar-Shira et al., 2005), and capacity to withstand cold exposure (Van den Brand et al., 2010).

After the chick emerges from the egg, the adaptation to its new environment involves several dramatic physiological changes such as switching from the chicks' exclusive dependence on the embryonic lipid rich yolk sac to the utilization of carbohydrate rich exogenous feed (Noy and Sklan, 1999a). In order to accommodate this change in nutrient intake, rapid intestinal development occurs during the immediate posthatch period, with notable increases in villus size and volume, and crypt depth in the small intestines (Geyra et al., 2001; Sklan, 2001). This early development of the gastrointestinal tract is characterized by a large increase in intestinal weight relative to body mass in the first days after hatching (Noy and Sklan, 1999b; Bar-Shira et al., 2005). In several studies it was shown that early access to feed and water stimulated the absorption of residual yolk in the abdominal cavity and the development of the digestive tract (Noy and Sklan, 1998, 1999a, 1999b). It appears that a key stimulator to the development of the digestive tract in chicken is the physical exposure to feed in the intestines, and feed deprivation impairs intestinal development.

Apart from the effects on the gastrointestinal tract, early feed and water deprivation decreases body weights of broiler chicks and poults up to 8% of the initial body weight during the first 24 h posthatch (Noy and Sklan, 1999a, 1999b; Geyra et al., 2001; Bigot et al., 2003; Gonzales et al., 2003; Careghi et al., 2005). Several studies demonstrated that the effects on body weight last until marketing age in broilers and turkeys (Noy and Sklan, 1999a, 1999b; Halevy et al., 2000; Gonzales et al., 2003). Furthermore, an increase in percentage of breast muscle at marketing weight was shown as a result of early feeding in birds when compared to broiler chicks or poults that were held in transport boxes for 34 or 48 h, respectively (Halevy et al., 2000; Noy and Sklan, 1999a). Feed deprivation during the immediate posthatch period delays the maturation of muscle fibers, which probably is the cause for less hypertrophy and muscle mass observed in starved birds (Halevy et al., 2000). Finally, suboptimal thermoregulatory capacity was associated with delayed feeding at d 2 and 3 posthatch (Van den Brand et al., 2010).

Combining the hatching and brooding phase as in the Patio system, enables immediate feed and water access posthatch, and may result in different early development and posthatch growth compared with chicks that are deprived from feed and water until placement in the farm. Moreover, in both hatching systems chicks of different hatching times may respond differently to the early feed and water access (Careghi et al., 2005).

Environmental conditions during hatching

Day old chick quality and posthatch performance are influenced by environmental conditions during incubation, and temperature has commonly been recognized as a critical factor. A number of papers were published on climate conditions during the first 18 days of incubation in setters (French, 1997; Lourens, 2001; Van Brecht et al., 2003; Elibol and Brake, 2008), but few data

are available on climate conditions during hatching in commercial hatchers. Due to increases in embryonic heat production, egg temperatures in the second half of incubation may be higher than set air temperature (Lourens et al., 2005). Egg temperatures of 39-41°C in commercial incubators were reported at incubation day 18 and 19 (Lourens, 2001; Leksrisompong et al., 2009). Apart from effects on chick quality and posthatch growth (Lourens, 2001; Hulet et al., 2007; Leksrisompong et al., 2007, 2009), temperatures higher than 38.8°C during late incubation reduced incubation time (Molenaar et al., 2010a,b, 2011), and weights of several organs, especially the heart (Wineland et al., 2000; Leksrisompong et al., 2007; Lourens et al., 2007; Molenaar et al., 2011), compared to the control temperature of 37.6-37.8°C. Effects of lower egg temperatures (35.5°C) during late incubation (E16-18.5), on broiler physiology were limited, but incubation time increased (Willemsen et al., 2010).

In previous studies on effects of incubation conditions, chicks were examined either at the moment of hatching (Givisiez et al., 2001; Molenaar et al., 2010a; Willemsen et al., 2010) or at the moment of chick collection from the hatcher, after about 21.5 d of incubation (Hulet et al., 2007; Leksrisompong et al., 2007; Lourens et al., 2007). Up to now, the variation in hatching time was not taken into account in these studies, and there is little information on the development of broilers during the early posthatch phase until chick collection from the hatcher.

Climate set points in the hatcher during the last 3 d of incubation are an air temperature of approximately 36.5°C and a relative humidity of approximately 50% (Hatchery managers, personal communication). These set points differ considerably from climate set points in the Patio, where the air temperature is set at 35.0°C, which was found to lead to the highest hatchability in the system, and relative humidity is set at 30%. In Patio, the CO_2 level reaches approximately 0.1 volume% during hatching and maximum air velocity is 0.2 m/s, while in the hatcher, CO_2 may reach 0.8-0.9 volume%, and air velocity is variable (Hatchery managers, personal communication). Another difference is the egg position during hatching, which is horizontal in baskets in the hatcher, and vertical in setter trays in the hatcher.

Due to different environmental conditions in the hatcher and the Patio system, chicks hatching at different moments within the hatch window are subjected to different conditions for a variable length of time, which may lead to different chick physiology at hatch and at the typical moment of chick collection (d 21.5 of incubation), and also to different growth posthatch.

In conclusion, it is hypothesized that chicks hatching at different moments within the hatch window respond differently to conditions in the late prenatal and early postnatal phase in the hatcher and the Patio system. In the majority of the studies on early development of broiler chicks, the moment of first feed and water access and influences of climatic factors were investigated for a batch of chicks considered to be homogeneous at removal from the hatcher without taking into account the actual moment of hatching.

Outline of the thesis

The first aim of this thesis is to evaluate the effects of conditions in the Patio system on hatchability, chick quality, liveability and posthatch growth, in comparison to the conventional hatcher. The second aim is to determine the physiological status of chicks at the moment of hatch and at chick collection, and the posthatch growth potential in relation to time of hatch in both hatching systems, including time of access to feed and water.

The second chapter describes the Patio system in detail, and summarizes the first results on hatchability, early growth, and liveability of broilers. In the third chapter, an experiment is described where the physiology of chicks right after hatch was investigated in relation to hatching time, in a conventional hatcher and the Patio system. Indicators of energy metabolism and stress physiology were measured. The fourth chapter describes an experiment with comparable set-up, however the physiological development in the early posthatch period, until the typical moment of chick collection from the hatcher was included. In chapter 5, the effects of hatching moment within the hatching window on posthatch growth was investigated, of chicks that were either fed or deprived from feed until after day 21.5 of incubation. In the 2 experiments described in chapter 6, the effects of egg position, namely horizontal, vertical with the air cell up, or vertical with the air cell down, on hatching parameters and chick quality were investigated. Chapter 7 describes an experiment, where the quality was investigated of day old chicks that hatched in a hatcher or in the Patio system. Chapter 8 is the general discussion, in which the data from the previous chapters are combined to discuss effects of different conditions during hatching and the early posthatch phase on hatchability, chick physiology, quality, and performance later on.

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chapter 2

Effects of a combined hatching and brooding system on hatchability, chick weight and mortality in broilers

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ABSTRACT

Chicks hatch over a time window of approximately 36-48 h and are removed from the hatchers only when the majority of the chicks has hatched. Consequently, chicks are exposed to prolonged posthatch holding periods and delays in feed and water access, leading to dehydration and impaired posthatch performance. It is questionable whether the hatchlings' physiological requirements can be met with current hatching systems. An alternative system that may better match the hatchlings' requirements, is a system that combines the hatching and brooding phase, so that feed and water can be provided immediately after hatch. Such system, named Patio, was developed in the Netherlands and tested from 2006-2008, to evaluate effects on hatchability and early performance of broilers. This paper describes the Patio system and the results from these tests.

A total of 21 broiler production trials (780,686 eggs) in the Patio system were evaluated at 3 locations, and compared to control hatches of eggs of the same parental flock in the hatchery. Hatchability in the Patio was on average 1.45%, 1.83% and 1.86% higher at location 1, 2 and 3, respectively. However, in the calculation of the hatchability in the Patio, possible second grade chicks were included, whereas these were excluded in the calculation of hatchability in the hatchery. Additionally, in the hatchery the hatching process was interrupted earlier than in the Patio, meaning that possible late hatching chicks remained in the flock in the Patio, but not in the hatchery. In 3 trials, the Patio chicks were 11.6-16.3% heavier at d 0, when the hatchery chicks were placed in the broiler house. Mean cumulative 7-d mortality was only assessed in the Patio and was 1.27%, 1.09% and 1.43% at location 1, 2 and 3, respectively. The Patio system appears to function as an alternative to current hatching and brooding systems. Further studies are required to determine to what extent the higher hatchability is due to second grade and to late hatching chicks.

(*Key words*: broiler, hatching system, hatchability, chick weight, delayed feeding)

INTRODUCTION

Hatching eggs are commonly incubated for 18 d in incubators, after which they are candled to verify the presence of an embryo inside the eggs. Following common practice, only the apparently fertile eggs are transferred to hatcher baskets and placed in hatcher cabinets for the last 3 d of incubation. Chicks hatch over a time window of approximately 36-48 h and are removed from the hatchers only when the majority of the chicks has hatched (Careghi et al., 2005). The variation in hatching time depends on factors such as age of the parent flock, egg handling, egg storage time, and incubation conditions (Decuypere et al., 2001). In addition, fixed management schedules at commercial hatcheries often leave little room for flexibility and thus the moment of chick collection has usually been set at 21.5 d. Consequently, a slight delay or a more pronounced variation in the moment of hatch may affect (and decrease) hatchability, as opening the hatchers too early means that eggs with viable chicks inside are wasted. On the other hand, postponing the moment of chick collection will lead to a higher percentage of chicks dehydrating and reduce chick quality (Bamelis et al., 2005; Tona et al., 2005).

Following chick collection from the hatcher, further hatchery procedures, such as sexing, vaccination, packaging and transportation increase the time until placement in the broiler house and thus first feed and water intake, for part of the flock by up to 50 h or more (Sklan et al., 2000; Careghi et al., 2005). If long transportation is involved, this period may be increased up to 72 h. Sub-optimal conditions during transport and a delay in the moment of placement and the first feed and water intake are associated with higher early mortality in chicks and poults (Kingston, 1979; Carver et al., 2002; Chou et al., 2004) and impaired performance throughout the grow out period (Halevy et al., 2000; Gonzales et al., 2003).

Although the first few days of a chicks' life are known to be crucial to later performance (Bruzual et al., 2000; Tona et al., 2005), it is questionable whether the hatchlings' physiological requirements can be met with current incubation systems and hatchery management procedures. An alternative system that can potentially overcome the negative effects of variation in hatching time and deprivation of feed and water, is a system that combines the hatching and brooding phase, in which feed and water can be provided immediately after hatch. In the period of 2002 to 2006, such a system was developed for broiler chicks. Thereafter, this system, named Patio[1], was tested at 3 locations in the Netherlands from 2006-2008, to evaluate consequences on hatchability and later performance of broilers. This paper describes the Patio system and the results of these trials.

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MATERIALS AND METHODS

At 3 locations, a total of 21 trials were evaluated in the Patio system (Table 1). At the first 2 locations (in total 18 trials), chicks were reared to an age of 7-14 d, after which they were transferred to a traditional broiler house. At both locations, bird density at the start of the trials varied between 55 and 90 birds per m^2. During the 3 trials carried out at the third location, the chicks remained in the system for the total grow out period. Bird density at the start of each trial was about 22 birds per m^2, which is similar to the Dutch average bird density in broiler houses (KWIN, 2007). The current paper describes the technical characteristics of the Patio system used at the third location. This system differs from the Patio system used at the first 2 locations mainly in terms of dimensions (Table 1), but also slightly in climate system. Data on hatchability of all 21 trials were compared to the results of control eggs that were simultaneously incubated until d 18 with eggs destined for Patio, and hatched in hatcher cabinets. Records of early mortality were collected of chicks that hatched in Patio at all 3 locations. At location 3, weights of chicks that hatched in the Patio and control chicks that hatched in the hatchery were recorded at d 0, which was the day of placement in the broiler house for the control chicks.

Table 1: Characteristics of the 3 locations where the Patio system was tested during 2006-2008.

Location	Period	Patio unit dimensions (m) (l x w x h)	Total living area of complete Patio system (m^2)	Bird age at removal from Patio (d)	Bird density (n / m^2)
1	May 2006 – Nov. 2006	32.2 x 1.43 x 0.40	553	7 - 14	55 – 90
2	Feb. 2007 – Aug. 2008	32.2 x 1.43 x 0.40	553	7 - 14	65 – 90
3	March 2008 – Aug. 2008	47.8 x 2.34 x 0.75	1.320	44 - 46	17 - 22

Patio System Description

The Patio system was built into a well insulated house (Figure 1) and was set up in 2 rows (**A**), each consisting of 6 identical levels (further referred to as Patio units) on top of each other. The rows were separated by a central corridor (**B**) and 2 corridors at each other side of the rows (**C**). The dimensions of one Patio unit were 47.80 m (length) x 2.34 m (width) x 0.75 m (height), mounting up to a living area for the chicks of 110 m^2 per unit. Based on a bird density of 22 birds/ m^2, each unit housed up to about 2,450 birds, resulting in a capacity of 29,400 birds for the total Patio system. The bottom of each level consisted of a synthetic moveable belt (further referred to as conveyor floor) on which the chicks were housed (Figure 2). At the start of each trial, the conveyor floor was covered with wood shavings (1 kg/m^2).

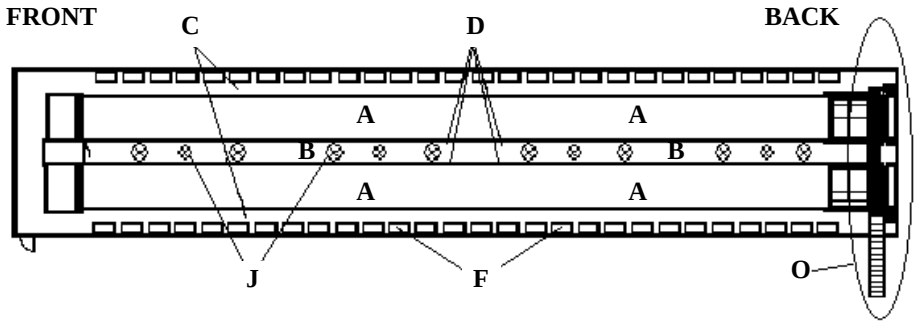

Figure 1. Schematic top view of the Patio system at location 3, consisting of 2 rows (A), with one central (B) and 2 outer corridors (C). Furthermore are indicated: position of the sensors used for control of the climate conditions (D), air inlet to outer corridors (F), exhaust fans (J) and the conveyor belt used for removal of the birds from the house (O).

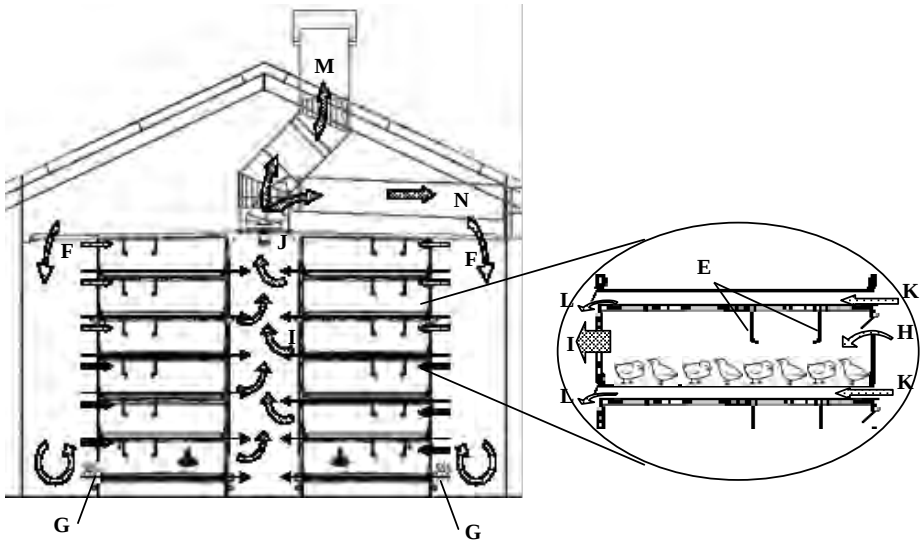

Figure 2. Schematic overview of the ventilation system in a Patio house at location 3: (left) front view of the entire house and (right) detail view of a Patio unit with (E) egg tray holder, (F) air inlet to outer corridors, (G) water heating system, (H) air inlet to the Patio unit, (I) air outlet Patio unit, (J) exhaust fan, (K) airflow between conveyor floor, (L) air outlet from space between conveyor floor, (M) air outlet to outside air, (N) ducted return air connection.

In the center of each Patio unit, at a height of 0.45 m above the conveyor floor, a rail system was installed to hold egg trays during hatching (**E** in Figure 2). Egg trays containing 18-day-incubated eggs were inserted at the front end of the system by means of an automatic elevator and a chord conveyor system. Eggs were positioned in the tray in a vertical position, with the air-chamber up. At the side facing the central corridor between the 2 system rows, low capacity drinking nipples[2] were provided (Type 10025-2 360°). Next to the nipples, a feeding line[3] was equipped with 1 feeding pan per 61 birds.

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Climate System. Outside air entered an air conditioning room (9 x 2 x 1 m) through an adjustable inlet at the front of the building (Figure 1). In this room, air could be mixed with exhausted air from the Patio house (internal circulation) or with preheated fresh air from an air-air heat exchanger (capacity of 15,000 m^3/h). In addition, the air in the air conditioning room could be heated by a water filled radiator system. From this conditioning room, air entered the insulated attic of the Patio house. For situations that no heating was required, a by-pass inlet directly allowed fresh air from outside to flow onto the attic. At the attic, air could be humidified by means of spray nozzles.
From the attic, the air entered the outer corridors of the Patio system through controllable openings in the ceiling (**F** in figures 1 and 2). The air could be heated by a proportionally controlled warm water heating system, installed along the bottom side of the Patio system (**G** in figure 2), thereby causing air movement and mixing in the outer corridors. Via air flow controlled balance valves (**H**), the air was distributed evenly over the Patio units. In this way, the temperature difference of the air entering the upper and lower Patio units was maximal 1 °C.
Through a steel grid at the side of the central corridor between the 2 rows (**I**), air left the Patio units. From the central corridor between the 2 rows, air was removed via fans (**J** in figures 1 and 2), thus creating negative pressure. In this way, air was drawn from the outer corridors over the birds towards the central corridor. The ventilation capacity was 180,000 m^3/h or about 6 m^3/h per bird, and the heating capacity was 120 kW or about 4 W per bird.
At high ventilation rates (indicating high inside temperatures), part of the air was drawn through the space between the upper and bottom side of the conveyor floor (**K** in figure 2), thereby cooling the litter on the conveyor floor from below. This airflow was also controlled by balance valves, which were positioned at the side of the central corridor (**L**). Through the exhaust fans, the air was either removed from the house (**M**) or directed towards the air conditioning room via a duct (**N**) with a maximum capacity of 25,000 m^3/h. From this duct, part of the air could be directed towards the heat exchanger to warm outside air entering the house, after which it was exhausted from the building.

Climate Control and Settings. Ventilation, heating and humidifying were controlled by a computer system[4] using measurement data from 6 airflow sensors in the ventilation shafts, and 1 CO_2 sensor, 4 temperature sensors and 1 relative humidity (RH) sensor positioned at a height of 4 m in the central corridor between the 2 rows (**D** in figure 1). During hatch, climate set points were an air temperature of 34.5°C (observed air temperature surrounding the eggs about 35°C) and a minimum RH of 35%. The air was internally circulated until the CO_2 level reached 2,000 ppm. From that moment on, a gradually increasing fraction of the air was taken from outside. After hatching, the temperature was decreased by 0.5°C per day during the first week, and a gradual further decrease according to the recommendations of the breeding company (Cobb-Vantress, 2008). Minimum RH was increased to 45% and ventilation was increased with the growth of the birds and/or when the temperature in the Patio system was higher than the set point.

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Management of eggs and chicks

For all 3 locations, eggs produced by breeder flocks aged between 29 and 59 wk were obtained from commercial hatcheries in the Netherlands. At 18 d of incubation, eggs were removed from the incubator for candling, and thereafter randomly assigned to be transferred to either a hatcher cabinet[5] or to the Patio system. This means that the origin of the eggs, regarding parent flock, days of lay, storage duration and apparent fertility was the same for both hatching systems. For eggs destined to hatch in the hatcher cabinet, conditions during candling followed standard procedures applied in the hatcheries. Infertile eggs were removed from the incubation trays, and apparently fertile eggs were transferred to hatcher baskets. In the event that high numbers of eggs were removed during candling, hatcher baskets were filled up with apparently fertile eggs from other trays, until they contained at least 120 eggs (at the hatcheries where control eggs for location 1 and 3 were hatched), or were not filled up (at the hatchery where control eggs for location 2 were hatched). After candling, the hatcher baskets with the eggs were placed in the hatcher cabinet for the last 3 d of incubation, during which a standard hatching program was used, with a set temperature starting at about 37°C and a minimum RH of about 50%.

For the eggs destined for Patio, the infertile eggs which were removed during candling, were replaced by apparently fertile eggs from other trays. Thus, incubator trays containing 150 apparently fertile eggs were transported to the Patio in a climate controlled truck at an air temperature of approximately 31°C. Upon receipt at the farm, the egg trays were inserted in the Patio system. The day of placement of the eggs in the Patio system was considered as day –3. Chicks started to hatch about 24 h after the eggs were inserted in the Patio. After hatch, chicks moved to the side of the egg tray or made their way through the opening in the egg tray underneath the eggshell and fell on the bedding, where feed and water were directly available. At d 0 or 1, egg trays with eggshells and unhatched eggs were removed from the system at the back (Figure 1). Chicks were raised at standard conditions of light and temperature, according to the guidelines of the breeding company. A commercially available broiler corn-wheat-soybean-based diet and water were provided ad libitum.

At an age of 7-14 d (location 1 and 2), or at slaughter age (location 3), birds were, level by level, removed from the system by moving the conveyor floor towards the back end of the system with a speed of circa 0.04 m/s. Here, the chickens moved onto a transverse conveyor belt (**O** in figure 1), while the manure and litter fell down on another conveyor belt, which transported it to the manure storage. Via the transverse belt, broilers were conveyed to a loading platform[6], where they were distributed over transport containers, either to be transported to another poultry house or to the slaughter house.

Data Collection

When egg trays were removed from the system, the unhatched eggs were counted, and the number of hatched eggs was calculated as the total number of apparently fertile eggs minus the

5 Petersime, Zulte, Belgium (for location 1 and 3); and HatchTech, Veenendaal, The Netherlands (for location 2 and 3)

6 Ciemme Calabria, Cazzago San Martino, Italy

number of unhatched eggs. Hatchability in the Patio was calculated as the ratio of the number of hatched eggs to the number of apparently fertile eggs. In the hatchery, hatchability was calculated as the number of first grade chicks divided by the number of apparently fertile eggs. As a result, second grade chicks (non-marketable chicks, showing physical anomalies, such as splayed legs, unhealed navels, unabsorbed yolk sac or lacking alertness) were not included in the calculation of hatchability in the hatchery.

In each of the 21 trials in the Patio system, the numbers of dead broilers in the flock were recorded daily by the animal caretaker as a routine procedure. Cumulative 7-d mortality was calculated from the total number of birds that had died until d 7 divided by the total number of chicks present at d 0. No data are available on 7-d mortality of the control flocks in the broiler house. In 3 trials at location 3, within 6 h after the moment of placement of the hatchery birds in the broiler house, individual weights were collected of both Patio birds and hatchery birds.

Statistical Analysis

Hatchability results of eggs hatched in the Patio system and in the hatchery were analyzed in a GLM procedure (SAS Institute, 2004). The model was

$$Y_{ijkl} = \mu + T_i + C_j\,(T_i) + \mathrm{S}_k + e_{ijkl},$$

where Y_{ijkl} = hatchability; μ = overall mean; T_i = location (i = 1-3); C_j = trial nested within location (j = 1-9); S_k = hatching system (k = Patio or hatchery); and e_{ijkl} = residual error term. Before the analysis, hatchability data were transformed to arcsin-square root. Hatchability data are presented as back-transformed Least Squares Means.

Chick weights (n = 680) collected at location 3 were analyzed in a GLM procedure (SAS Institute, 2004). The model was

$$Y_{ijk} = \mu + C_i + \mathrm{S}_j + e_{ijk},$$

where Y_{ijk} = individual chick weight at d 0; μ = overall mean; C_i = trial (i = 1-3), S_j = hatching system (j = Patio or hatchery) and e_{ijk} = residual error term. Data on chick weights are presented as Least Squares Means.

RESULTS AND DISCUSSION

Hatchability

Based on results of a total of 780,686 hatching eggs, hatchability in the Patio system was on average 1.45%, 1.83% and 1.86% higher at location 1, 2 and 3, respectively, compared to control hatches in the hatchery (Table 2). A number of factors may have contributed to this apparent difference in hatchability:

1) Part of the higher hatchability was probably due to the second grade chicks, which were removed in the hatchery before the number of chicks was computed by automated counting machines. In the Patio, hatchability was calculated as the ratio of the total number of hatched eggs to the total number of fertilized eggs, meaning that possible second grade chicks were included. To our knowledge, there is no scientific data available on mean percentages of second grade chicks at commercial hatcheries. Estimations of this percentage obtained through personal communication with several hatchery managers varied between 0.2-2.0%. It is known that the portion of second grade chicks varies with parental age, storage time, hatchery management and incubation conditions (Lourens, 2002; Lourens et al., 2005).

2) The hatching process in the Patio system was not terminated by human intervention as it was in hatchery practice, when chicks were removed from the hatchers after approximately 21.5 days of incubation, and non hatched eggs with potential viable chicks inside were wasted. In the Patio, egg trays were removed up to 6 h (location 1 and 3) or even 1 day later (location 2), and thus the higher hatchability may be partly due to chicks hatching after 21.5 days of incubation.

3) The hatchability from fertilized eggs really was higher in the Patio system compared to hatcher cabinets, which may be due to differences in climate conditions during hatching. The set point for air temperature during hatching of the control eggs in the hatchers was 36.5-37.0°C, whereas the temperature in the Patio was set at 34.5°C. During hatching, RH rose up to 90% in hatchers and remained around 40% in the Patio. Furthermore, in the hatchers, with capacities up to 28,800 chicken eggs, the volume of air per egg varied from 0.6 to 0.9 dm^3 depending on brand and type, whereas 4.4-7.3 (location 1 and 2) and 34.1 dm^3 (location 3) was available per egg in the Patio, depending on the Patio unit dimensions and the stocking density. In addition, air speed in the Patio was maximal 0.2 m/s, which is considered still air (Simmons et al., 2003). Air velocities in the hatchers were not determined, but it is known that in commercial incubation high air velocities are required in order to remove the heat from the eggs effectively (Van Brecht et al., 2003). During the last phase of incubation, eggs produce considerable amounts of heat and effective heat removal from the eggs is crucial to prevent overheating and subsequent decreases in hatchability and chick quality (Lourens et al., 2005; Hulet et al., 2007; Leksrisompong et al., 2007). Combined with the lower set air temperature, the greater air volume in the Patio system may have enabled the heat dissipation from the eggs, even at a low air speed. Another possible factor that may have contributed to a difference in hatchability is the vertical position of the eggs in the Patio, which may facilitate the hatching process as was found in quail eggs (Mao et al., 2007), as opposed to the horizontal position of the eggs in the hatcher baskets. In addition,

the relative silent environment in the Patio system may increase the possibility for embryos to communicate with each other, which has been shown to stimulate the hatching process in quail embryos (Vince, 1964).
Based on the results obtained in these trials, none of the factors can be excluded in the explanation of a possible difference in hatchability between the Patio and the hatchery. However the results show that good hatchabilities can be achieved in a combined hatching/brooding system.

Table 2: Hatchability of apparently fertilized eggs in the Patio system and in the hatchery and 7-d mortality of chicks in the Patio system. Control eggs originated from the same parent flock and were incubated simultaneously with eggs destined for the Patio system until d 18.

Location	Trials, n	Hatching eggs, n	Breed	Mean hatchability (%)[1]			7-d mortality in Patio (%)[2]
				Patio	Control	Difference	
1	9	415,820	Ross 308 /507/708	96.17 (95.58-96.71)	94.72 (94.05-95.36)	1.45**	1.27 (0.83 – 1.83)
2	9	246,966	Ross 308	97.60 (97.15-98.00)	95.76 (95.19-96.31)	1.83**	1.09 (0.72 – 1.73)
3	3	117,900	Cobb 500	95.53 (94.43-96.52)	93.67 (92.38-94.85)	1.86*	1.43 (0.91 – 2.34)
Total	21	780,686		96.49	94.75	1.73	1.21

[1] Hatchability figures are back-transformed Least Squares Means; 95% confidence limits in parentheses. The hatchability of Control eggs that hatched in the hatchery, was based on at least 15,000 eggs per trial, and was calculated after removal from second grade chicks.
[2] Raw mean 7-d mortality includes cull chicks; range in parentheses.
**P ≤ 0.05, **P ≤ 0.01.*

Body Weight

At location 3, chick weights were collected in the Patio system and the broiler house at d 0. Birds hatched in the Patio system were 7.3 (16.3%), 7.0 (15.4%), and 5.5 g (11.6%) heavier in trials 1, 2 and 3, respectively ($P < 0.001$). These findings are in accordance with earlier reports on weight loss during posthatch holding of chicks before first access to feed and water. In broilers, body weight loss up to 8% per 24 h occurs in this early posthatch period (Noy and Sklan, 1999a,b; Geyra et al., 2001; Bigot et al., 2003; Gonzales et al., 2003; Careghi et al., 2005). In hatchery practice, it may take up to 50 h until the first feed and water intake for the early hatched birds (Sklan et al., 2000; Careghi et al., 2005). The time until first feed and water intake for the chicks that hatched in the hatchery in the present study was not assessed, but it is likely that these birds had lost weight before being placed in the broiler house. In addition, the birds in the Patio system already had access to feed and water, and probably feed was present in their digestive

tract or body growth had occurred at the moment of weighing at d 0 or both. In broilers, a weight gain of 6.91-15.03% in the first 48 h after clearing from the eggs was demonstrated when given immediate feed and water access, depending on the moment of hatching within the hatch window (Careghi et al., 2005).
Based on the present results, it is not known to what extent the observed differences in chick weight between birds that hatched in the hatchery and those that hatched in the Patio system are a result from weight loss of the hatchery birds or weight gain of the Patio birds or both.

Chick Mortality

Mean cumulative 7-d mortality in the Patio was 1.27%, 1.09% and 1.43% at location 1, 2 and 3, respectively (Table 2). These data are in accordance with results from a large epidemiological research in the Netherlands during 2004-2006, in which the average mortality in the first week was 1.5% (Yassin et al., 2009). These figures agree with the mean 7-d mortality of 1.54% in a similar study on field data obtained from Norwegian broiler farms during 1996-1999 (Heier et al., 2002), and to the 1.55% mortality based on data collected from 38 broiler flocks at the research facilities of the University of Arkansas (Tabler et al., 2004). It can be hypothesized that the early mortality in the Patio is not different from that in traditional broiler houses, although second grade chicks were not removed in a standard procedure in the Patio system as occurred in the hatchery. A possible reason for this observation could be that from the moment of hatching, climate conditions in the Patio system to a great extent corresponded to the recommendations for day old chicks of the breeding company (Cobb-Vantress, 2008). The recommended conditions (temperature of 33°C, RH between 30-50% and still air) were in contrast to the climate conditions in which the control birds in the hatchery hatched and remained until removal from the hatcher. Conditions during subsequent chick handling and transportation procedures, but also after placement in the broiler house, may not have been optimal for newly hatched birds. After hatch, the thermoregulatory system of chickens is limited (Nichelmann and Tzschentke, 2002) and warmth is a critical need to young birds. Early mortality in chickens and poults has been related to suboptimal truck temperatures and longer duration of transport from the hatchery to the farm (Carver et al., 2002; Chou et al., 2004). Low temperatures in the brooding phase lead to increased early mortalities in broiler chicks (Bruzual et al., 2000) and improper brooding conditions are a major important factor for decreased flock performance (Cobb-Vantress, 2008). Another factor, which can possibly explain the absence of increased mortality in birds hatched in the Patio system, is the immediate access to feed and water compared to the delay to which chicks in hatchery practice were exposed. Delays in the moment of first feed and water supply for the birds hatched in the hatchery were related to increased mortality in broiler flocks (Kingston, 1979; Carver et al., 2002; Chou et al., 2004).

In conclusion, combining the hatching and brooding phase in one system, as in the Patio, has proved to function as a promising alternative for current hatching and brooding systems, with regard to hatchability, early growth and livability of broiler chicks. Further studies are required to

determine to what extent the difference in hatchability is due to second grade chicks and to late hatchers, and to an actual higher hatching percentage.

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chapter 3

Hatching system and time effects on broiler physiology and post hatch growth

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ABSTRACT

A multi-level housing system for broilers was developed, named Patio, in which the hatching and brooding phase are combined. In Patio, climate conditions differ from those provided in hatchers currently used. We compared physiology of broilers hatched in a hatcher or in Patio, and included effects of hatching time. Eggs from one breeder flock were incubated until d E18 in one setter, and subsequently placed in a hatcher or the Patio until the end of incubation. From each hatching system, 154 chicks were collected per hatching time: at 465h (Early), 480h (Midterm) and 493h (Late) of incubation, of which 24 chicks per group were decapitated for analyses of blood plasma and organ weights. The remaining 130 chicks of each group from both systems were individually labeled, and placed together in the Patio system. All chicks were given access to feed and water directly after hatch, and housed up to d 45 for monitoring of growth. From d E18 till the end of incubation, average ambient temperature and relative humidity were 38.1°C and 50.8% in the hatcher and 35.2°C and 29.7% in the Patio. Glucose and corticosterone were slightly higher in Hatcher chicks, while organ weights were not affected by hatching system. Although hatchling weights were lower in Hatchery chicks, growth from d 0-45 was not affected by hatching system. In both systems, glucose increased with hatching time, while lactate and T_3 levels decreased. Yolk weights decreased with hatching time, while absolute and relative weights of the yolk free body, intestines, stomach, lungs, and heart increased, indicating more advanced maturation of organs. Growth up to d 21 was depressed in Late chicks, which was possibly related to lower thyroid hormone levels at hatching.

We conclude that hatching system had minor effects on hatchling physiology, and post hatch growth and liveability were not affected. Because hatching time affected broiler physiology, it seems important to take hatching time into account in future studies related to hatchling physiology.

(*Key words*: broiler, hatching system, hatch window, hatchling physiology)

INTRODUCTION

Broiler hatching eggs are commonly incubated for 17-18 d in setters, after which they are candled and transferred to hatchers for the last 3-4 d of incubation. Chicks hatch over a time window of approximately 36-48 h and are removed from the hatchers only when the majority of the chicks has hatched (Careghi et al., 2005), leading to early post hatch periods of feed and water deprivation. Suboptimal conditions during subsequent chick handling and transport and further delays in chick placement, and thus first feed and water intake, are associated with higher early mortality in chicks and poults (Kingston, 1979; Carver et al., 2002; Chou et al., 2004) and impaired post hatch performance (Halevy et al., 2000; Gonzales et al., 2003). The magnitude of the response to these adverse conditions seems to be influenced by the moment of hatching within the hatch window (Careghi et al., 2005) and especially late hatching chicks seem to be vulnerable (Kingston et al, 1979).

An alternative system that can overcome negative effects of early post hatch deprivation of feed and water, is a system that combines the hatching process and post hatch phase, in which feed and water are provided immediately after hatch. In the period of 2002 to 2006, such a system, named Patio[1], was developed and has proved to function as an alternative to current hatching and brooding systems, with regard to hatchability, early growth, and liveability of broiler chicks (Van de Ven et al., 2009).

In contrast to conditions typically observed in hatchers in the last phase of incubation, air temperature, relative humidity, and air speed are lower in the Patio, whereas the volume of air per egg is higher (Van de Ven et al., 2009). In the last phase of incubation, thermal conditions can affect the development of several organs (Leksrisompong et al., 2007; Molenaar et al., 2010a), thermoregulation (Shinder et al., 2009), and muscle tissue (Piestun et al., 2008). Consequently, differences in climate conditions between the hatcher and the Patio system may lead to different post hatch physiology in the broiler, with possible long lasting effects on post hatch performance. Effects of hatching in the Patio system on broiler physiology and post hatch growth are not known, and may vary in chicks hatching at different moments within the hatch window.

We investigated the consequences of hatching in the Patio system versus hatching in a hatcher on organ development, plasma hormone concentrations, and growth until slaughter weight in broilers, taking into account the moment of hatching within the hatch window.

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MATERIALS AND METHODS

All procedures in this study were approved by the Animal Care and Use committee of Wageningen University, the Netherlands.

Incubation and chick management

Hatching eggs were obtained from a commercial Ross 308 breeder flock aged 47 weeks. Eggs were stored for 3d before being set in a HatchTech setter[2]. A standard single-stage incubation program was used in the setter with a gradually decreasing machine temperature from 38.1°C at embryo d 0 (d E0) to 37.5°C at d E18 of incubation. At d E18, eggs were candled and apparently fertilized eggs were randomly divided and transferred to 2 hatching systems: 15,185 eggs were transferred to a Petersime hatcher[3] and 16,200 eggs were transported to the Patio system for 30 min, in a climate conditioned truck at an air temperature of 30°C.

A standard hatching program was used in the hatcher, starting at a set point temperature of 37.2°C at d E18 which was gradually decreased to 36.4°C at d E21. In the Patio system, the set point of the air temperature was 34.5°C during the entire hatching process. At about d E21.5 (after 514h of incubation) the hatching process was ended in both hatching systems. For measurements on physiology and growth, chicks that still had some wet down, indicating they had just hatched, were randomly selected from both hatching systems. This was done at 3 moments during the hatching process: after 465 incubation h (Early), after 480 incubation h (Midterm), and after 493 incubation h (Late). In total 154 chicks per hatching time per hatching system (named a hatch group) were selected, from which 24 chicks were decapitated for blood collection and analyses of organ development. The remaining 130 chicks of each hatch group that hatched in the hatcher were transported for 30 min to the Patio system at an air temperature of 30°C. After arrival at the Patio system, both the 130 chicks from the hatchery and the 130 chicks that hatched in the Patio system were individually marked with wing clips, and placed in the Patio system, where they were given free access to water and feed. When the hatching process was ended, 390 chicks (130 Early + 130 Midterm + 130 Late chicks) from the hatcher and 390 chicks from the Patio were housed together, forming one group of 780 chicks, in a separated compartment of the Patio system. Chicks were fed ad libitum with a commercial available feed and raised at standard conditions according to the recommendations of the breeder company, until slaughter weight was reached at d 45.

Data collection

During the entire hatching process, in both hatching systems the temperature and relative humidity (RH) were logged every 5 min using data loggers (175-H2 Logger, Testo[4]). In the hatcher, 3 loggers were placed among the eggs in different hatching baskets. In the Patio system, 3 loggers were placed among the eggs on the egg trays.

2 HatchTech, Veenendaal, The Netherlands
3 Petersime, Zulte, Belgium
4 Testo, Almere, The Netherlands

Chick weights were determined right after chick collection and at different ages post hatch: at d E21.5 (from now on termed d 0, meaning the normal day of placement in the broiler house), d 7, 21, and 45. Chicks were weighed between 10.00-16.00h, in a random order. At the moment of weighing at d 0, the mean biological age of the Early chicks was 53h, of the Midterm chicks 38h and of the Late chicks 25h. Growth per h was calculated for different growth periods: hatch-d 0, d 0-d 7, d 7-d 21, and d 21-d 45. During the last weighing at d 45, sex of all birds was determined.

Blood plasma and organ analysis

For blood sampling, 24 chicks per hatch group were decapitated within 1.5h after collection from the hatching system. Until that time none of the chicks had access to feed or water. Blood samples were collected in 4 ml Fluoride tubes (BD Vacutainer[5]) to which 10 µl heparine was added, and put on ice immediately after collection. When 6 samples were collected, the tubes were centrifuged during 6 min at 3,700 rpm using a Rotofix 32 centrifuge[6]. Plasma was collected and stored in 0.2 ml Eppendorfs at -20°C until analysis.

T_3 and T_4 concentrations were measured in plasma samples by RIA as described by Darras et al. (1992) using antisera and T_3 and T_4 standards[7]. Corticosterone concentrations were analysed as described previously (Decuypere et al., 1983; Meeuwis et al., 1989), using a double-antibody RIA-kit[8]. Plasma glucose, lactate, and uric acid were analysed by colorimetric diagnostics using a biochemical analyzer (VetTest 8008[9]).

The residual yolk, heart, liver, stomach (proventriculus plus gizzard), intestines, and lungs were dissected and weighed, and the relative organ weight was calculated as the ratio of the organ weight to the live body weight. Sex of each chick was determined by visual inspection after dissection.

Statistical analyses

All data were analysed with the SAS 9.1 software package (SAS Institute, 2004). Individual chick measurements were treated as the experimental unit in all statistical analyses. Non-normal distributed data were log transformed before analyses. Data on plasma hormone concentrations, absolute and relative organ weights, chick weights, and growth per hour were analysed using the GLM procedure, according to the following model:

$$Y_{ijkl} = SEX_i + HT_j + SYS_k + \text{interaction terms} + e_{ijkl},$$

where SEX_i = sex of the chick, HT_j = hatching time (Early, Midterm, Late), SYS_k = system (Hatchery, Patio), and e_{ijkl} = residual error term. Data on mortality and sex distribution were analysed using the LOGISTIC procedure, using the following model:

$$Y_{ijk} = HT_i + SYS_j + \text{interaction term} + e_{ijk}.$$

In all analyses, *P*-values ≤ 0.05 were considered statistically significant and non-significant interaction terms were deleted from the model. When the means of the GLM were statistically different, means were compared using Least Squares means with Tukey's adjustment for multiple comparisons. Data are expressed as Least Squares means ± SE, unless otherwise stated.

5 Becton Dickinson, Franklin Lakes, USA
6 Hettich Zentrifugen, Tuttlingen, Germany
7 Byk-Belga, Brussels, Belgium
8 IDS Ltd, Boldon, UK
9 Indexx Laboratories Inc, Maine, USA

RESULTS

Registrations of temperature and RH in both hatching systems were used from 449h of incubation until the end of incubation. The mean ± SE, minimal, and maximal temperature was 38.1±0.02, 36.3, and 40.1°C in the hatcher and 35.2±0.02, 32.7, and 36.0°C in the Patio. The mean ± SE, minimal, and maximal RH was 50.8±0.54, 22.9, and 89.8% in the hatcher, and 29.7±0.17, 18.0, and 46.1% in the Patio.

Hatching Time

Female chicks hatched earlier than male chicks ($P < 0.01$; Figure 1). Chick weights at each age are presented in Table 1. There was no difference in hatch weight among hatching times, but weight gain per hour from hatch-d 0 decreased with hatching time ($P < 0.01$). At d 0, chick weights were higher in Early (52.9±0.3 g), compared to Midterm (51.0±0.3 g; $P < 0.01$) and Late (48.3±0.3 g; $P < 0.01$) chicks. From d 0-7, growth per h decreased with hatching time ($P < 0.01$; Figure 2). D 7 weights were higher in Early (168.4±1.4 g) compared to Midterm (162.7±1.3 g; $P < 0.01$) and Late chicks (151.8±1.3 g; $P < 0.01$). From d 7-21, Late chicks showed lower growth than Early ($P = 0.05$) and Midterm ($P = 0.02$) chicks. At d 21, Early (823±8 g) and Midterm (821±7 g) chicks were heavier than Late (788±7 g; $P < 0.01$) chicks. The effects of HT on chick growth were irrespective of SYS or sex. From d 21-45, growth was not affected by HT and d 45 weights did not differ among hatching times.

Organ weights are presented in Figure 3. All absolute and relative organ weights, except yolk weights, increased from the Early and Midterm to the Late chicks. Both absolute and relative yolk weights decreased with hatching time ($P < 0.01$). Yolk free body mass (YFBM) was calculated as body weight minus yolk weight, and was not affected by HT.

Concentrations of blood parameters at hatch are presented in Figure 4. Late chicks had higher glucose than Early and Midterm chicks ($P < 0.01$). Lactate was higher in Early than in Late chicks ($P < 0.01$), with Midterm chicks intermediate and not different from both other groups. There was no effect of HT on corticosterone level.

	Early				Midterm			
	Males		Females		Males		Females	
	HAT	PAT	HAT	PAT	HAT	PAT	HAT	PAT
Hatch	47.0 (0.6)	48.0 (0.7)	47.5 (0.4)	48.4 (0.4)	47.4 (0.5)	48.6 (0.5)	47.8 (0.4)	49.1 (0.4)
D 0	53.3 (0.8)	51.5 (0.9)	53.5 (0.5)	52.5 (0.5)	51.1 (0.6)	51.5 (0.6)	50.8 (0.6)	50.6 (0.6)
D 7	170.4 (3.6)	165.9 (4.2)	168.9 (2.0)	166.5 (2.2)	164.3 (2.7)	164.8 (2.7)	159.8 (2.5)	162.3 (2.5)
D 21	847 (18)	869 (21)	798 (10)	780 (11)	851 (13)	839 (13)	781 (12)	811 (12)
D 45	2772 (50)	2937 (57)	2462 (28)	2434 (30)	2805 (37)	2757 (37)	2452 (35)	2515 (35)

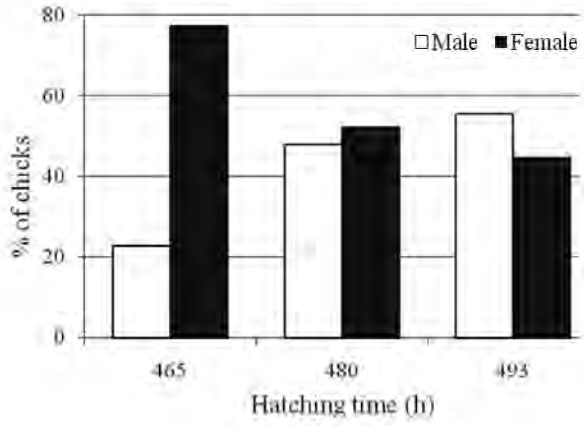

Figure 1. Sex distribution in chicks hatched Early (465 h), Midterm (480 h), or Late (493 h) in the hatching process.

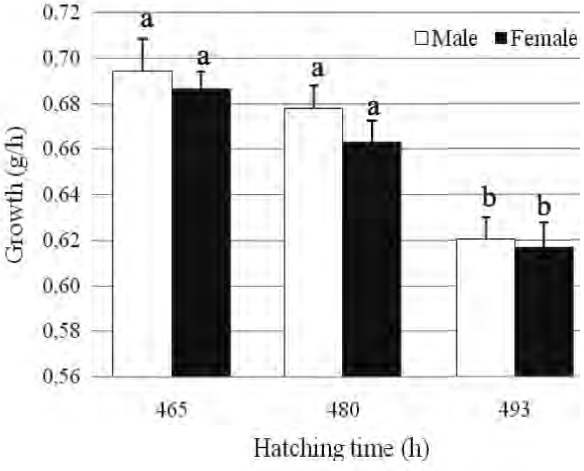

Figure 2. Growth per hour from d 0 until d 7 of chicks hatched Early (465 h), Midterm (480 h), or Late (493 h) in the hatching process in a hatcher or a Patio system. Data lacking a common letter are significantly different (P < 0.05).

	Late			
	Males		Females	
	HAT	PAT	HAT	PAT
	47.6 (0.4)	48.7 (0.5)	47.0 (0.5)	48.9 (0.5)
	48.1 (0.5)	48.4 (0.6)	48.1 (0.7)	48.3 (0.6)
	149.9 (2.4)	154.7 (2.6)	152.5 (3.0)	150.8 (2.8)
	799 (12)	820 (13)	775 (15)	761 (14)
	2731 (34)	2734 (37)	2467 (41)	2466 (39)

Table 1: Least Squares Means of weights in g of approximately 130 chicks hatched Early (465 h), Midterm (480 h) or Late (493 h) in the hatching process in a hatcher (HAT) or in a Patio system (PAT). Standard errors in parentheses.

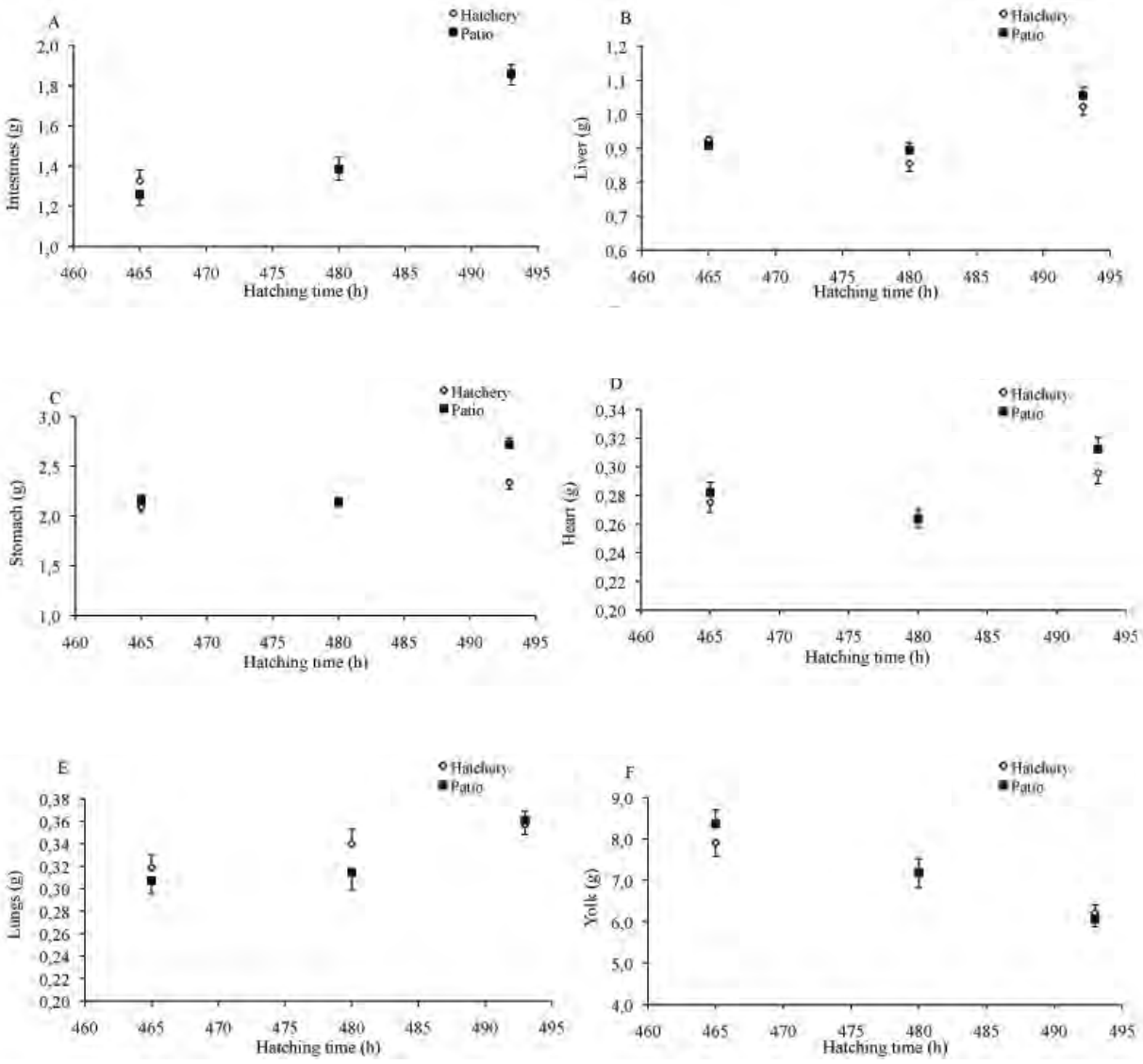

Figure 3 A-F. Least Squares Means of organ weights at hatch of chicks hatched Early (465 h), Midterm (480 h), or Late (493 h) in the hatching process in a hatcher or a Patio system; A) intestines; B) liver; C) stomach; D) heart; E) lungs; F) yolk

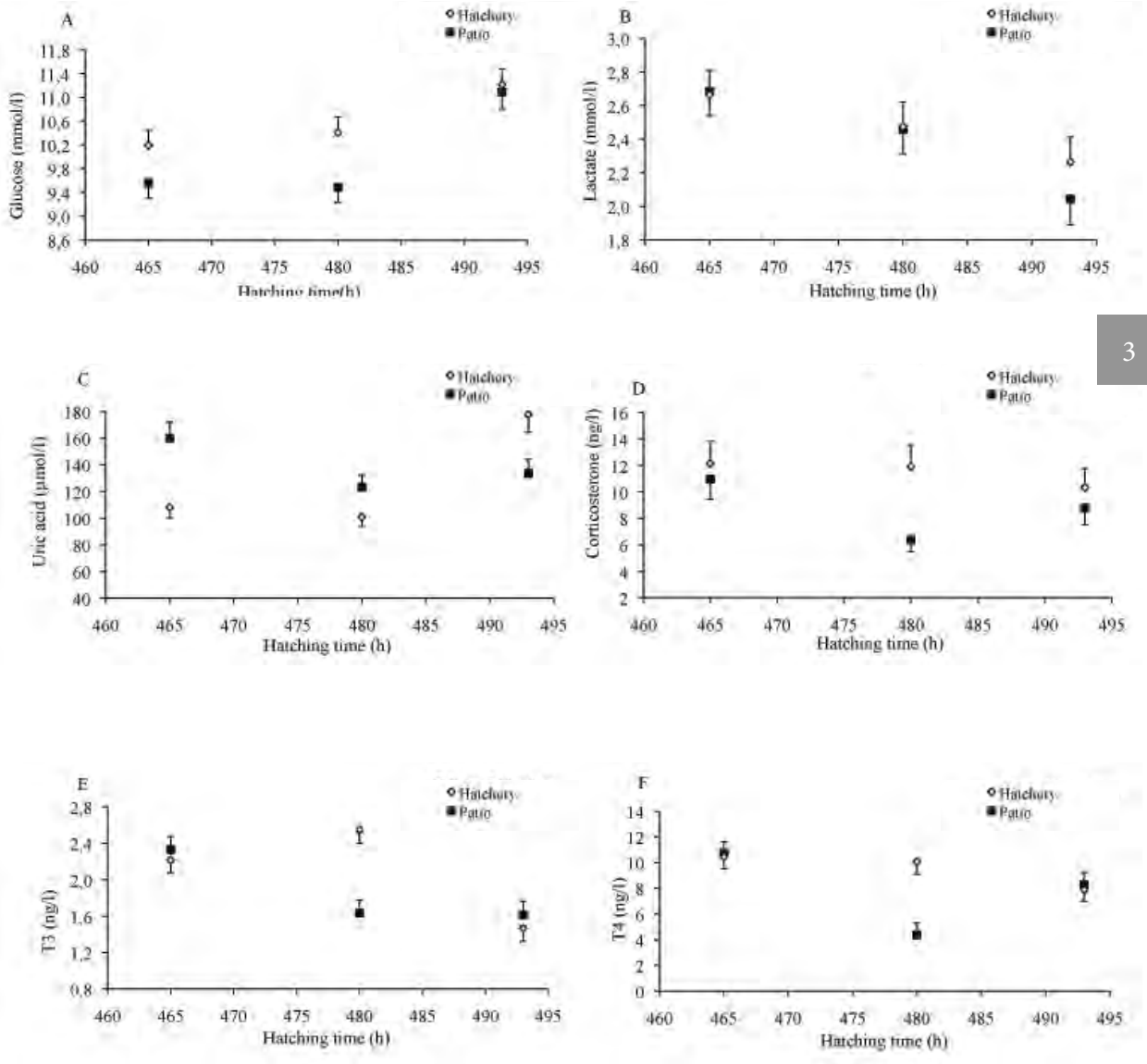

Figure 4 A-F. Least Squares Means of plasma hormone concentrations in chicks hatched Early (465 h), Midterm (480 h), or Late (493 h) in the hatching process in a hatcher or a Patio system; A) glucose; B) lactate; C) uric acid; D) corticosterone, E) T_3, F) T_4.

Hatching System

Sexes were distributed equally over the hatching systems. At hatch, Patio chicks were heavier (48.7±0.2 g) than Hatchery chicks (47.4±0.2 g; $P < 0.01$). From hatch-d 0, Hatchery chicks gained more weight per hour than Patio chicks ($P < 0.01$). Although given immediate feed access, the Late chicks in the Patio lost weight during the first 25h post hatch. From d 0 onward, chick weights or growth per hour were not affected by SYS anymore.

There were no main effects of SYS on any of the absolute or relative organ weights (Figure 3). Plasma glucose was higher in Hatchery than in Patio chicks ($P = 0.02$; Figure 4). For lactate level, there was no difference between Hatchery and Patio chicks. Corticosterone was higher in Hatchery than in Patio chicks ($P = 0.02$).

Hatching Time x Hatching System

Overall mortality at d 45 was 2.92% (n=23) and showed an interaction of HT x SYS ($P = 0.01$). For Hatchery chicks, d 45 mortality was 0.72, 0.77, and 6.98% for the Early, Midterm, and Late group, respectively, and for Patio chicks, d 45 mortality was 3.88, 2.96, and 2.36% for the Early, Midterm and Late group, respectively. Of the 6.98% mortality (n=9) in the Late group from the Hatchery, about half of the chicks (n=4) died before d 7, with no clear reason.

For both absolute and relative stomach weight, a HT x SYS interaction was observed ($P < 0.01$). In Late chicks, stomach weights were higher in Patio than in Hatchery chicks ($P < 0.01$), whereas there were no differences between the hatching systems in Early and Midterm chicks.

For uric acid, T_3, and T_4 an interaction of HT x SYS was observed ($P < 0.01$). Uric acid was higher in Late than in Midterm and Early chicks in Hatchery chicks ($P < 0.01$), whereas for Patio chicks there were no differences in uric acid level among hatching times. In Early chicks, uric acid was higher in Patio than in Hatchery chicks ($P < 0.01$). Plasma T_3 was higher in Hatchery than in Patio only in Midterm chicks ($P < 0.01$), whereas in Early and Late chicks, T_3 did not differ between the hatching systems. There was a general trend for decreasing T_3 with hatching time: For Hatchery chicks, plasma T_3 was lower in Late than in Early and Midterm chicks ($P < 0.01$) and for Patio chicks, T_3 was lower in Late and Midterm than in Early chicks ($P < 0.01$). A comparable interaction between HT x SYS was observed for T_4, which was higher in Hatchery than in the Patio only in Midterm chicks ($P < 0.01$). For Patio, Midterm chicks had lower T_4 levels than Early ($P < 0.01$) and Late chicks ($P = 0.05$). T_3:T_4 ratio was higher in Midterm than in Late chicks ($P < 0.01$) with Early chicks intermediate (data not shown).

Sex

Sexes were not distributed equally in the experimental chicks ($P < 0.01$); 58.16% of all chicks were female. There were no effects of sex on hatch weight, on growth from d 0-7 or on weight at d 7. From d 7-21 and d 21-45, males showed higher growth rates than females ($P < 0.01$). Males were heavier than females at d 21 (837±6 g vs. 784±5 g, respectively; $P < 0.01$) and at d 45 (2,786±17 g vs. 2,466±14 g, respectively; $P < 0.01$). Overall, weight gain per hour from hatch until d 45 was only affected by sex, with higher growth in males (2.45±0.02 g/h) than in females

(2.16±0.01 g/h). There were no effects of sex on absolute or relative organ weights or plasma hormone concentrations at hatch.

DISCUSSION

Hatching Time

Hatching time is known to be influenced by factors such as parental age, storage time and storage conditions, and incubation conditions (Tona et al., 2003; Careghi et al., 2005; Decuypere and Bruggeman, 2007). In the current study, these factors were standardized as much as possible: eggs were obtained from one single breeder flock, produced at the same day of lay, stored in one storage room and incubated simultaneously in the same single-stage incubator. However, there still was a spread of hatch of about 30 h in both hatching systems. Additional intrinsic factors influencing hatching time are order of an egg in a clutch and egg weight (Meijer and Siemers, 1993; Careghi et al., 2005), which were not controlled in the present study.

In agreement to findings of Careghi et al. (2005), chick weights at hatch were not affected by hatching time, but early growth was lower in Late chicks. In accordance to present data, decreased chick quality and performance to 7d in Late hatching chicks were linked to lower thyroid levels (Decuypere et al., 1990; Buys et al., 1998; Careghi et al., 2005; Decuypere and Bruggeman, 2005). The reason for low quality of late hatching chicks is not clear, but studies in other avian species pointed to a relation with yolk androgen levels from maternal origin, linked to order of an egg in a clutch (Eising et al., 2001; Müller et al., 2004). Because in the present study no data were available on yolk androgen levels or order of an egg in a clutch, such influence could not be examined.

The present data showed increasing organ weights and decreasing yolk weights with increasing hatching time, indicating more advanced maturation of organs with longer hatching time, as was proposed by Ricklefs (1987) in a comparison among avian species. The higher organ weights in Late chicks in the current study coincided with lower post hatch growth, indicating the relation between organ weights at hatch and organ functionality or growth potential post hatch is weak, and needs further investigation.

In the current study, plasma glucose at hatch increased and lactate decreased with hatching time. Christensen et al. (2000, 2001) demonstrated that time of hatch, but also the duration of the hatching process itself, show a large variation, which was associated with differences in energy availability in this phase. Energy required for hatching activities comes from glucose provided from glycogen in liver and muscle tissues (Freeman, 1969), resulting in an increase in plasma glucose between pipping and hatch (Freeman, 1965, 1969; Christensen et al., 2001). As hatching progresses, oxygen becomes limiting and energy comes from anaerobic glycolysis leading to increases in plasma lactate (Tazawa et al., 1983; Høiby et al., 1987; Moran Jr., 2007). Hypoxic conditions end at the moment of external pipping, and as soon as oxygen availability is restored, birds can recycle lactate back into glucose in the liver (De Oliveira et al., 2008). The combination

of high glucose and low lactate in Late hatchers may point at an increased interval between external pipping and hatching, enabling Late chicks to recycle lactate back into glucose before emergence from the egg. Alternatively, Late hatchers may have used less glucose and produced less lactate during the hatching process because of a lower metabolism. In the current experiment, lower thyroid levels were found in Late hatchers, suggesting a lower metabolism (Decuypere et al., 1990), which could be linked to increased external pipping-hatching intervals (Tona et al., 2007; Everaert et al., 2008).

Hatching System

During hatching, air temperature and relative humidity were higher in the hatcher than in the Patio system, but air speed was lower in Patio which makes it difficult to quantify sensible and latent heat losses. However, hatch weights were lower in Hatchery than in Patio chicks, which could point at an increased dissipation of latent heat from the embryos from the Hatchery. Wineland et al. (2006) also found higher hatch weights in chicks incubated at a lower temperature (36°C) than in chicks incubated at high (39°C) temperature from d E17-E21. From hatch-d 0, Hatchery chicks gained more weight than Patio chicks, which may be related to a higher need to compensate the moisture loss in the last incubation phase, by a higher water intake. Chick weights at d 0 and growth from d 0-45 were not affected by hatching system.

Despite differences in climate conditions, and in hatchling weight and early growth between the hatching systems, no clear differences were found in absolute or relative organ weights. In previous studies, high temperatures (> 38.8°C) in the last week of incubation lead to lower weights of the chicks' body (Leksrisompong et al., 2007; Lourens et al., 2007), heart (Givisiez et al., 2001; Leksrisompong et al., 2007; Lourens et al., 2007; Molenaar et al., 2010a), gizzard, proventriculus, and small intestines, and to higher yolk sac weights (Leksrisompong et al. 2007, Molenaar et al., 2010a) compared to controls which were incubated at 37.8-38.2°C. The difference between these studies and the present study can possibly be explained by the timing and duration of exposure to high temperatures. In the present study eggs were exposed during 2-3 d from d E18 until hatching, whereas in previous studies, exposure was from d E9-15 onward. In addition, temperatures applied in the high temperature groups in previous studies were higher than the mean temperature measured in the hatcher in the current study (38.1°C), which may explain the different findings. It can be speculated that during d E18-21, embryos are able to maintain normal organ development at air temperatures in the range of 35.2-38.2°C, as applied in the present study.

Plasma glucose and corticosterone at hatch were slightly higher in Hatchery than in Patio chicks. Higher corticosterone could point at a more energy demanding hatching process (Piestun et al., 2008) resulting in increased gluconeogenesis (Joseph and Ramachandran, 1992) and higher plasma glucose at hatch.

Hatching Time x Hatching System

The interaction between hatching time and hatching system on uric acid and thyroid hormone levels suggests that although the length of the hatch window was not greatly affected, the hatching peak may have been delayed in the Hatchery chicks. High temperatures in the last incubation phase increase energy use from the anaerobic system by the embryo and suppress embryo development (Lourens et al., 2006). In Hatchery conditions, chicks that were termed 'Midterm' may have hatched relatively early within the hatch window, and consequently resemble Early chicks, while 'Midterm' chicks in Patio may have hatched relatively late within the hatch window, and physiologically resemble Late chicks.

In Hatchery chicks, uric acid was higher in Late than in Midterm and Early chicks, while for Patio chicks, there were no differences in uric acid level among hatching times. Uric acid is a major nitrogenous waste product resulting from protein catabolism (Harr, 2002) and was found to increase in broilers subjected to heat stress at d 21 post hatch (Yalcin et al., 2009). It can be speculated that in the last incubation phase, prolonged exposure to a higher temperature may be related to a higher need for gluconeogenesis (Christensen et al., 2007) to fuel anaerobic metabolism using amino acids as a substrate (Lourens et al., 2006; Molenaar et al., 2010b), resulting in higher uric acid levels in the Late chicks compared to the Early and Midterm chicks.

Sex

At hatch, there were no effects of sex on organ weights or any of the plasma hormone concentrations, corresponding to findings of Lu et al. (2007). Also, no hatch weight differences were found between sexes, which agrees with earlier literature (Burke et al., 1992; Reis et al., 1997; Joseph and Moran Jr, 2005; Leksrisompong et al., 2007). Females showed earlier hatching times than males, which is consistent with previous findings in chicken (Burke, 1992; Reis et al., 1997). The reason for sex-linked differences in hatching time in chickens remains unclear. In the present study, sex affected growth from d 7 onward and this effect became more pronounced with ageing, with males showing higher growth rates than females. Higher growth rates in broiler males were linked to higher plasma growth hormone (GH) concentrations and better GH receptor occupancy (Kühn et al., 1996), and are in accordance to performance expectations provided by the breeder company.

In conclusion, present findings show a large effect of hatching time on physiology of newly hatched broilers. Lower early growth was observed in chicks that hatch late in the hatch window compared to chicks that hatch early or at the peak of the hatch window. This physiological variation related to age differences in one batch of 'day-old' chicks is rarely considered, but it can be advised to take hatching moment into account in future studies on hatchling physiology. Although there were marked differences between climate conditions in the hatcher and the Patio system, physiological differences in chicks at hatch were limited and post hatch growth and liveability were not affected.

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chapter 4

Perinatal broiler physiology between hatching and chick collection in two hatching systems

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ABSTRACT

Little is known about physiological responses of early vs. late hatching chicks to early posthatch conditions in broiler practice. We investigated effects of hatching time on perinatal broiler physiology in two hatching systems, differing in conditions: a conventional hatcher, where chicks are deprived of feed and water between hatching and the moment of chick pulling (d E21.5), and a Patio system, in which the hatching and brooding phase are combined, and chicks have immediate posthatch feed and water access. Climate conditions in Patio also differ with about 3°C lower temperature and 20% lower RH compared to conventional hatchers. At E18, fertile eggs were transferred to either a hatcher or the Patio until the end of incubation. From each system, 50 newly hatched chicks were collected at 3 hatching times: at 468h (early), 483h (midterm) and 498h (late) of incubation, of which 25 chicks were decapitated for analyses of physiological parameters. The other 25 chicks were returned to the hatching system for analyses after 515h of incubation (E21.5). At hatch, weights of the heart, lungs, stomach, and intestine increased with hatching time, concurrent with a decrease in residual yolk weight, regardless of hatching system, and indicating that later hatching chicks 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. At E21.5, plasma glucose and T_3 had increased with time after hatch in Patio chicks, whereas levels were similar among hatching times and lower in Hatcher chicks. Early feed and water access seems to enable early hatching chicks to compensate for their apparent disadvantage in development at hatching, whereas chicks subjected to fasting show metabolic adaptations to preserve nutrients. Chick physiology at chick pulling time was shown to vary with time after hatching and posthatch conditions, especially feed access.

(*Key words*: broiler, hatching time, hatching system, hatchling physiology, early feeding)

INTRODUCTION

Broiler hatching eggs are commonly incubated for 17 to 18 d in setters, after which they are candled and the vital eggs are transferred to hatchers for the last 3 to 4 d of incubation. Several studies demonstrated effects of different climate conditions during incubation on chick quality and physiology. In these studies, chicks were examined either at the moment of hatching (Givisiez et al., 2001; Molenaar et al., 2010a; Willemsen et al., 2010) or at the moment of chick collection from the hatcher ('chick pulling'), usually after about 21.5 d of incubation (Hulet et al., 2007; Leksrisompong et al., 2007; Lourens et al., 2007). Chicks hatch over a time window of 24-36 h (Decuypere et al., 2001), leading to a variation in biological age among chicks in one batch of only several hours up to 2 d when they are removed from the hatcher. This means that in the period between hatching and the moment of chick collection, chicks of different hatching times remain in the hatching system for a variable period of time.

In common practice, chicks do not have access to feed and water during the early posthatch period, until they are removed from the hatcher, counted, transported and placed in the broiler house. This early period of feed and water deprivation was associated with higher early mortality and impaired posthatch performance (Kingston, 1979; Halevy et al., 2000; Gonzales et al., 2003). An alternative hatching system was developed, named Patio, in which the hatching and brooding phase are combined (Van de Ven et al., 2009), thereby enabling direct posthatch access to feed and water. In previous trials it was shown that Patio functions as a hatching and brooding system, based on good hatchability of fertile eggs and livability of broiler chicks (Van de Ven et al., 2009). Next to earlier feed and water access, climate conditions in the Patio system differ from those in traditional hatching systems, e.g. with lower temperature, relative humidity, air speed, and larger volume of air per egg (Van de Ven et al., 2009, 2011). In a previous study, the physiology of chicks hatched in a hatcher or a Patio system was investigated right after hatching, and found to differ slightly between the systems. However, large variation in chick physiology was observed, which was related to hatching time within the hatch window (Van de Ven et al., 2011).

The effects of different climate conditions and moment of first feed and water access in conventional hatchers or the Patio system on chick physiology in the early posthatch phase are unknown. As the metabolism of chicks hatching early or late in the hatch window seems to differ (Iqbal et al., 1989; Careghi et al., 2005; Van de Ven et al., 2011), they may respond differently to early posthatch conditions. In the current study, the physiological development of broiler chicks in the early posthatch period was investigated, in relation to hatching time and hatching conditions including feed and water access. The objective of this study was to reveal the physiological status of chicks right after hatching and at the moment of chick collection from two hatching systems, differing in environmental conditions. Organ weights and metabolic blood variables were used as indicators for hatchling physiology.

MATERIALS AND METHODS

All procedures in this study were approved by the Animal Care and Use committee of Wageningen University, the Netherlands.

Incubation and chick management

Hatching eggs were obtained from a commercial Ross 308 breeder flock aged 40 weeks. Eggs were stored for 2-3d before being set in a Petersime[1] setter. A standard single-stage incubation program was used in the setter with a gradually decreasing machine temperature from 38.0°C at embryo d 0 (d E0) to 36.5°C at d E18 of incubation. At d E18 (after 440 h of incubation), eggs were candled and apparently vital eggs were randomly divided and transferred to 1 of 2 hatching systems: 16,200 eggs were transferred to a Petersime hatcher and 15,875 eggs were transported during 30 min to the Patio system, in a climate conditioned truck at an air temperature of 30°C. From both systems, a sample of eggs (n=50) was weighed directly after egg transfer. Mean egg weight was 57.6±0.4g, and similar for both hatching systems.

A standard hatching program was used in the hatcher, starting at a set point temperature of 37.2°C at d E18 which was gradually decreased to 36.4°C at d E21. In the Patio system, the set point of the air temperature was 35.0°C during the entire hatching process. Relative humidity was set at 50% in the hatcher and 30% in the Patio system, and allowed to rise above these set points. The set points for climate conditions in Patio were based on results from preliminary (unpublished) trials, where they resulted in highest hatchability.

For measurements on physiological parameters, newly hatched chicks, which still had some wet down, were randomly selected from both hatching systems. This was done at 3 moments during the hatching process: after 468 incubation h (early), after 483 incubation h (midterm), and after 498 incubation h (late). Fifty chicks per hatching time per hatching system (named a hatch group) were selected, of which 25 chicks were weighed, and decapitated for blood collection and analyses of organ development. The remaining 25 chicks of each hatch group that hatched in the Hatcher, or in the Patio, were individually marked and placed back in the hatching system where they had hatched. At day E21.5 (after 515 h of incubation), which can be considered the typical moment of chick collection in hatchery practice, the remaining 25 chicks per hatch group were collected from both hatching systems, weighed, and decapitated for blood collection and organ analyses.

Blood Plasma and Organ Analysis

Data collection on blood plasma and organ development occurred at 2 time points: 1) within 1.5 h after hatch and 2) at d E21.5. At the moment of sampling at d E21.5, the mean biological age of the early chicks was approximately 47 h, of the midterm chicks 32 h and of the late chicks 17 h.

1 Petersime, Zulte, Belgium

After decapitation, blood samples were collected in 4 ml Fluoride tubes (BD Vacutainer[2]) to which 10 µl heparine was added, and put on ice immediately after collection. When 6 samples were collected, the tubes were centrifuged during 6 min at 3,700 rpm using a Rotofix 32 centrifuge[3]. Plasma was collected and stored in 0.2 ml Eppendorfs at -20°C until analysis.

T_3 and T_4 concentrations were measured in plasma samples by RIA as described by Darras et al. (1992) using antisera and T_3 and T_4 standards[4]. Corticosterone concentrations were analysed as described previously (Decuypere et al., 1983; Meeuwis et al., 1989), using a double-antibody RIA-kit[5]. Plasma glucose, lactate, and uric acid were analysed by colorimetric diagnostics using a biochemical analyzer (VetTest 8008[6]).

After bleeding of the chicks, livers were immediately dissected and frozen in liquid nitrogen until further analyses. Hepatic glycogen analyses occurred following the protocol described by Molenaar et al. (2010a).

After removal of the liver, the remainder of the chicks' body was stored at -20°C until dissection for organ weights. After defrosting in a water bath at 37°C, the residual yolk, heart, spleen, bursa of Fabricius, stomach (proventriculus plus gizzard), intestines, and lungs were dissected and weighed, and the relative organ weight was calculated as the ratio of the organ weight to the yolk-free body mass (YFBM). Length of the intestines, from stomach to cloaca, was measured. Sex of each chick was determined by visual inspection after dissection.

Statistical analyses

Data were analysed with the SAS 9.1 software package (SAS Institute, 2004). The individual chick was treated as the experimental unit in all analyses. Distribution of the means and residuals were examined to check model assumptions, and non-normal distributed data (Uric acid, T_3 level, T_3:T_4 ratio, and hepatic glycogen content) were log transformed before analyses.

Preliminary analyses showed no effects of sex on any of the parameters measured, and sex was therefore excluded from the analyses. Data on plasma hormone concentrations, absolute and relative organ weights, and chick BW were analysed for two sampling moments separately: first for the moment of hatching and second for d E21.5. The GLM procedure was used according to the following model:

$$Y_{ijk} = SYS_i + HT_j + (SYS \times HT)_{ij} + e_{ijk},$$

where SYS_i = system (Hatcher, Patio), HT_j = hatching time (early, midterm, late), and e_{ijk} = residual error term.

In order to examine the changes in physiological parameters between hatch and d E21.5, data of both chicks at hatch and chicks at d E21.5 were used, and the model was extended with the factor Age and interactions with the other factors, where Age refers to the age of the chick at the moment of sampling (at hatch, or at d E21.5).

2 Becton Dickinson, Franklin Lakes, USA
3 Hettich Zentrifugen, Tuttlingen, Germany
4 Byk-Belga, Brussels, Belgium
5 IDS Ltd, Boldon, UK
6 Idexx Laboratories Inc, Maine, USA

Main factors and interactions were analyzed for significance at $P \leq 0.05$. When a factor or interaction was statistically significant, least squares means were compared after Tukey's adjustment for multiple comparisons. Data are expressed as least squares means ± SEM.

RESULTS

First the physiological status of chicks just after hatch is presented, then the changes in the period between hatch and d E.21.5 are described, followed by a summary of the physiological status of chicks at d E21.5.

Physiological parameters at hatch

Body and organ weights. Because results on relative organ weights were very similar to those of absolute organ weights, only the findings of absolute organ weights are presented. BW, YFBM, and absolute organ weights are presented in Table 1. At hatch, there were no differences in BW or YFBM among hatch groups. For intestine and liver weights a SYS x HT interaction was observed: weights increased with increased hatching time in Patio chicks, while in Hatcher chicks there were no differences among hatching times, resulting in higher weights in late Patio than in late Hatcher chicks. Length of intestines increased from early to midterm to late in Patio chicks, while in Hatcher chicks, the length was higher in early than midterm with late chicks intermediate.

Residual yolk weight at hatch decreased with hatching time, whereas weights of the heart, lungs, stomach, bursa of Fabricius, intestines, and length of intestines increased with hatching time. Weights of the heart, liver, stomach, and intestines were higher in Patio than in Hatcher chicks, and lung weight showed a similar tendency ($P = 0.079$). Data on hepatic glycogen are presented in Table 2. At hatch, there was a trend towards higher glycogen levels in Patio compared to Hatcher chicks ($P = 0.071$).

Blood plasma parameters. Plasma hormone and metabolite levels are presented in Table 3. For T_3 and lactate, a SYS x HT interaction was observed: T_3 concentration in Hatcher chicks increased from early to midterm and late ($P < 0.001$), whereas in Patio chicks there were no differences among hatching times. Lactate concentration in Hatcher chicks was higher in midterm than in early and late chicks ($P < 0.001$), whereas in Patio chicks, there were no differences among hatching times. In midterm chicks, plasma lactate concentration was higher in Hatcher than in Patio chicks ($P < 0.001$).

Glucose was higher in midterm and late than in early chicks ($P < 0.001$), and higher in Patio than in Hatcher chicks ($P = 0.005$). Plasma T_4 concentration was higher in late than in early and midterm chicks ($P < 0.001$), and higher in Hatcher than in Patio chicks ($P = 0.013$). No significant effects of HT or SYS were found for uric acid and corticosterone concentration at hatch.

Table 1: Least Squares Means and SEM of organ weights and intestinal length of chicks hatched Early, Midterm, or Late in the hatching process in a hatcher or a Patio system, determined directly after hatch (468 h for Early, 483 h for Midterm, or 498 h for Late chicks).

Treatment	n	BW (g)	YFBM (g)	Yolk (g)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Bursa (g)	Stomach (g)	Intestines (g)	Intestines (cm)
HT												
Early	50	45.40	38.66	6.74^{a}	0.27^{ab}	0.24^{b}	0.91	0.02	0.03^{b}	1.93^{b}	1.19	32.0
Midterm	50	45.85	39.69	6.16^{a}	0.26^{b}	0.25^{ab}	0.96	0.02	0.04^{ab}	2.13^{a}	1.32	35.1
Late	50	45.25	39.82	5.43^{b}	0.28^{a}	0.27^{a}	0.95	0.02	0.04^{a}	2.15^{a}	1.38	35.4
Pooled SEM		0.53	0.44	0.19	0.01	0.01	0.02	0.00	0.00	0.04	0.03	0.5
SYS												
Hatcher	75	45.75	39.51	6.27	0.26^{b}	0.24	0.89^{b}	0.015	0.036	2.03^{b}	1.23	33.8
Patio	75	45.26	39.30	5.95	0.28^{a}	0.26	0.98^{a}	0.016	0.038	2.11^{a}	1.36	34.6
Pooled SEM		0.44	0.36	0.16	0.00	0.01	0.01	0.001	0.001	0.03	0.03	0.4
SYS x HT												
Hatcher x Early	25	44.90	38.41	6.61	0.25	0.24	0.88^{c}	0.015	0.030	1.91	1.17^{b}	31.6^{b}
Hatcher x Midterm	25	46.33	39.92	6.40	0.26	0.24	0.93^{bc}	0.015	0.036	2.10	1.29^{b}	35.7^{a}
Hatcher x Late	25	46.00	40.21	5.80	0.27	0.25	0.87^{c}	0.015	0.041	2.08	1.24^{b}	34.1^{ab}
Patio x Early	25	45.89	39.01	6.88	0.29	0.24	0.94^{abc}	0.016	0.036	1.95	1.20^{b}	32.5^{b}
Patio x Midterm	25	45.38	39.46	5.92	0.26	0.25	0.98^{ab}	0.016	0.038	2.17	1.34^{ab}	34.5^{ab}
Patio x Late	25	44.50	39.43	5.06	0.29	0.29	1.03^{a}	0.016	0.039	2.22	1.53^{a}	36.7^{a}
Pooled SEM		0.76	0.63	0.27	0.01	0.01	0.02	0.001	0.003	0.05	0.05	0.8
Source of variation												
HT		0.703	0.164	<0.001	0.038	0.006	0.082	0.928	0.025	<0.001	<0.001	<0.001
SYS		0.426	0.678	0.155	0.005	0.079	<0.001	0.232	0.272	0.043	0.002	0.218
SYS x HT		0.226	0.522	0.164	0.070	0.227	0.050	0.918	0.371	0.505	0.013	0.043

^{a,b}LSMeans followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

Table 2. Least squares means of total hepatic glycogen of chicks collected right after hatch or after 515 h of incubation (d21.5 of incubation) from chicks hatched at 468h (Early) at 483h (Midterm) or at 498h of incubation (Late) in hatcher or in Patio conditions.

Treatment	n	Total hepatic glycogen (mg)		
		At hatch	At day E21.5	Delta
HT				
Early	50	8.3 ± 0.7	14.8 ± 3.3	+ 6.5
Midterm	50	8.6 ± 0.8	9.2 ± 2.0	+ 0.6
Late	50	8.7 ± 0.8	7.8 ± 1.6	- 0.9
SYS				
Hatcher	75	7.8 ± 0.5	1.8 ± 0.3	- 6.0
Patio	75	9.4 ± 0.7	58.0 ± 9.6	+ 48.6
SYS x HT				
Hatcher x Early	25	7.2 ± 1.5	1.3 ± 1.3[c]	- 5.9
Hatcher x Midterm	25	8.3 ± 1.8	1.2 ± 0.3[c]	- 7.1
Hatcher x Late	25	7.9 ± 1.7	3.6 ± 0.8[c]	- 4.3
Patio x Early	25	9.5 ± 2.1	165.1 ± 35.8[a]	+ 155.6
Patio x Midterm	25	8.9 ± 1.9	69.0 ± 14.9[a]	+ 60.0
Patio x Late	25	9.6 ± 2.1	17.1 ± 3.7[b]	+ 7.5
Source of variation				
HT		0.926	0.1033	
SYS		0.071	<0.001	
SYS x HT		0.710	<0.001	

[a-c]LSMeans followed by different superscripts within a column and factor are significantly different (P ≤ 0.05).

Table 3: Least Squares Means and SEM of plasma hormone and metabolite concentrations of chicks hatched Early, Midterm, or Late in the hatching process in a hatcher or a Patio system, determined directly after hatch (468 h for Early, 483 h for Midterm, or 498 h for Late chicks).

Treatment	n	Glucose (mg/dL)	Lactate (ng/ml)	Uric acid (ng/ml)	Corticosterone (ng/ml)	T_3 (ng/ml)	T_4 (ng/ml)	T_3:T_4 ratio
HT								
Early	50	179.52[b]	43.81	5.15	23.72	1.19	5.35[b]	0.25
Midterm	50	190.29[a]	50.08	5.51	20.88	1.60	5.63[b]	0.31
Late	50	192.73[a]	38.46	5.23	18.80	1.88	7.72[a]	0.27
Pooled SEM		1.92	1.31	0.20	1.49	0.09	0.39	0.02
SYS								
Hatcher	75	184.35[b]	45.85	5.33	21.46	1.56	6.81[a]	0.26
Patio	75	190.67[a]	42.38	5.26	20.81	1.50	5.66[b]	0.30
Pooled SEM		1.57	1.07	0.16	1.21	0.07	0.32	0.02
SYS x HT								
Hatcher x Early	25	173.8	42.39[b]	5.16	26.25	1.07[c]	5.50	0.22
Hatcher x Midterm	25	190.4	57.49[a]	5.73	21.61	1.68[ab]	6.17	0.29
Hatcher x Late	25	188.9	37.68[b]	5.12	16.52	2.13[a]	8.75	0.27
Patio x Early	25	185.2	45.22[b]	5.14	21.19	1.32[bc]	5.19	0.28
Patio x Midterm	25	190.2	42.67[b]	5.31	20.15	1.53[b]	5.09	0.33
Patio x Late	25	196.6	39.24[b]	5.33	21.08	1.67[ab]	6.70	0.28
Pooled SEM		2.71	1.86	0.28	2.10	0.12	0.55	0.03
Source of variation								
HT		<0.001	<0.001	0.417	0.070	<0.001	<0.001	0.148
SYS		0.005	0.024	0.759	0.705	0.535	0.013	0.154
SYS x HT		0.099	<0.001	0.551	0.077	0.012	0.298	0.574

[a-c]*LSMeans followed by different superscripts within a column and factor are significantly different (P ≤ 0.05).*

Physiological changes between hatch – d E21.5

Body and organ weights. Relative changes in body and organ weights are presented in Figure 1. In the period between hatch and d E21.5, BW of early Hatcher chicks decreased ($P = 0.007$), whereas the BW of early Patio chicks increased ($P < 0.001$). In midterm and late chicks, BW did not change significantly. The YFBM did not change in this period in Hatcher chicks of any of the hatching times, while the YFBM of Patio chicks, including possible feed and water residues in the intestines and stomach, increased in early (+ 10.1 g), midterm (+ 5.6 g), and in late chicks (+ 2.4 g). Between hatch and d E21.5, residual yolk weight decreased in all groups, by 4.5 g in early, by 3.2 g in midterm and by 2.0 g in late chicks.

All organ weights increased in all hatch groups between hatch and d E21.5, but the weight gain differed among hatching times and between hatching systems. The weight gain in liver, lungs, and spleen, and increase in intestine length were higher in Patio than in Hatcher chicks. The increase in heart weight was similar for both hatching systems and all hatching times. Liver weight gain, and length of intestines decreased from early to midterm to late chicks. Similarly, but irrespective of hatching system, weight gain of the bursa of Fabricius decreased from early and midterm to late chicks. Because intestine and stomach weight included feed residues in Patio chicks at d E21.5, weights of these organs were not analysed.

Between hatch – d E21.5, hepatic glycogen levels decreased in early and midterm Hatcher chicks, whereas in Patio chicks, glycogen levels increased in chicks of each of the hatching times (Table 2).

Blood plasma parameters. Relative changes in plasma hormone and metabolite levels are presented in Figure 2. From hatch – d E21.5, glucose levels showed higher increases in early (+ 19.3%) compared with midterm (+ 10.5 %) and late chicks (+ 4.6%), and higher increases in Patio (+ 14.9%) than in Hatcher chicks (+ 7.5%). Lactate levels in early and midterm Hatcher chicks decreased and did not change in late chicks, whereas in Patio chicks, lactate levels did not change significantly in any of the hatch groups. Uric acid levels increased in early chicks (+ 20.0%) and decreased in midterm (- 5.5%) and late chicks (- 6.7%), irrespective of hatching system. Between hatch – d E21.5, corticosterone levels increased in Hatcher chicks (+ 15.3%) and decreased in Patio chicks (- 36.5%). T_3 levels decreased in all hatch groups, but with larger magnitude in late and midterm than in early chicks, and more pronounced in Hatcher than in Patio chicks. In Hatcher chicks, T_4 levels did not change in early chicks, increased in midterm and decreased in late chicks, whereas in Patio chicks, T_4 did not change in chicks of any of the hatching times. From hatch – d E21.5, T_3:T_4 ratio showed a higher decrease in Hatcher (- 0.2 or - 72.2%) than in Patio chicks (- 0.1 or - 34.5%) (data not shown).

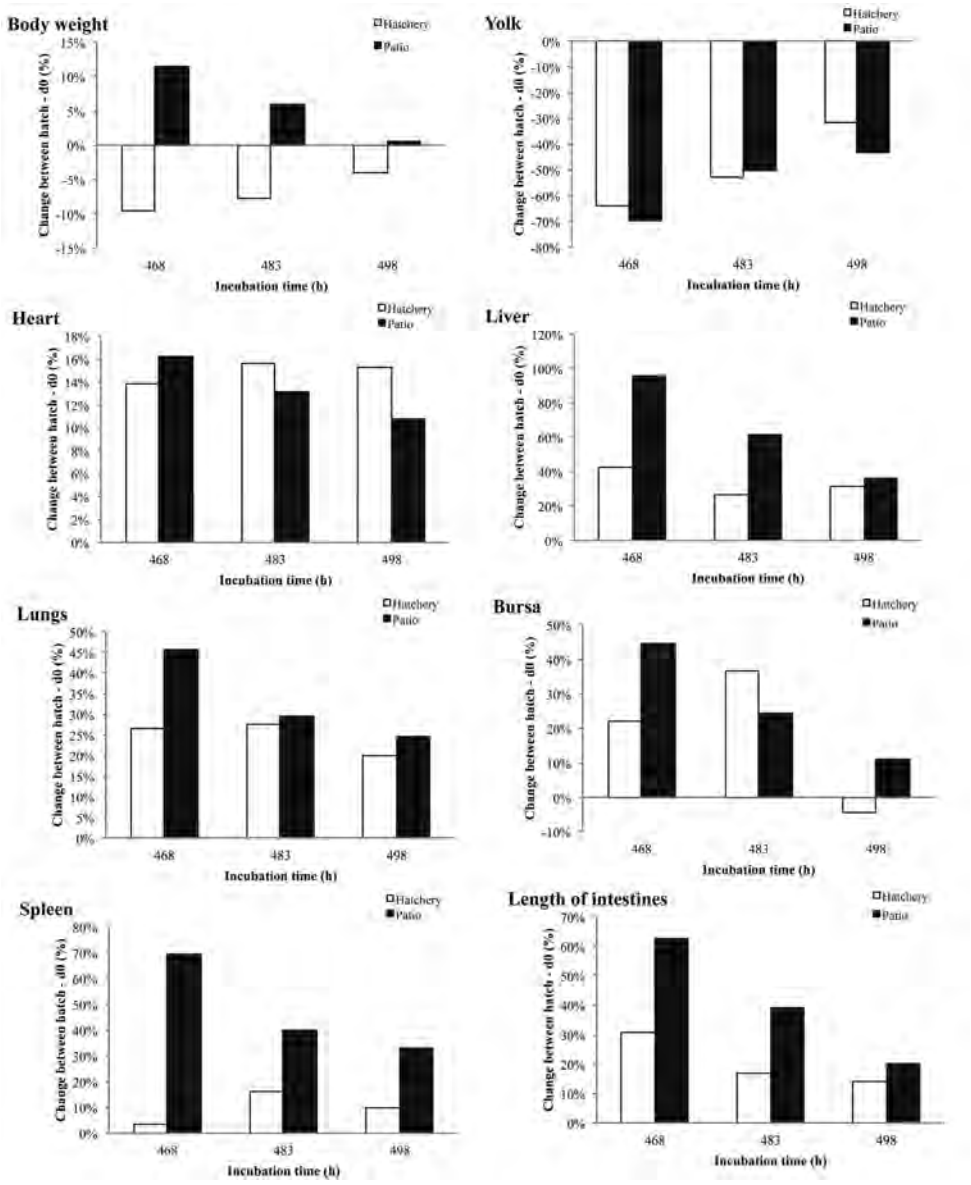

Figure 1. Least Squares Means of relative weight changes of the whole body, yolk, heart, liver, lung, Bursa of Fabricius, spleen, and length of intestines of chicks hatched Early, Midterm, or Late in the hatching process in a hatcher or a Patio system, determined directly after hatch (468 h for early, 483 h for midterm, or 498 h for late chicks), or at the end of incubation (515 h for all three hatch groups).

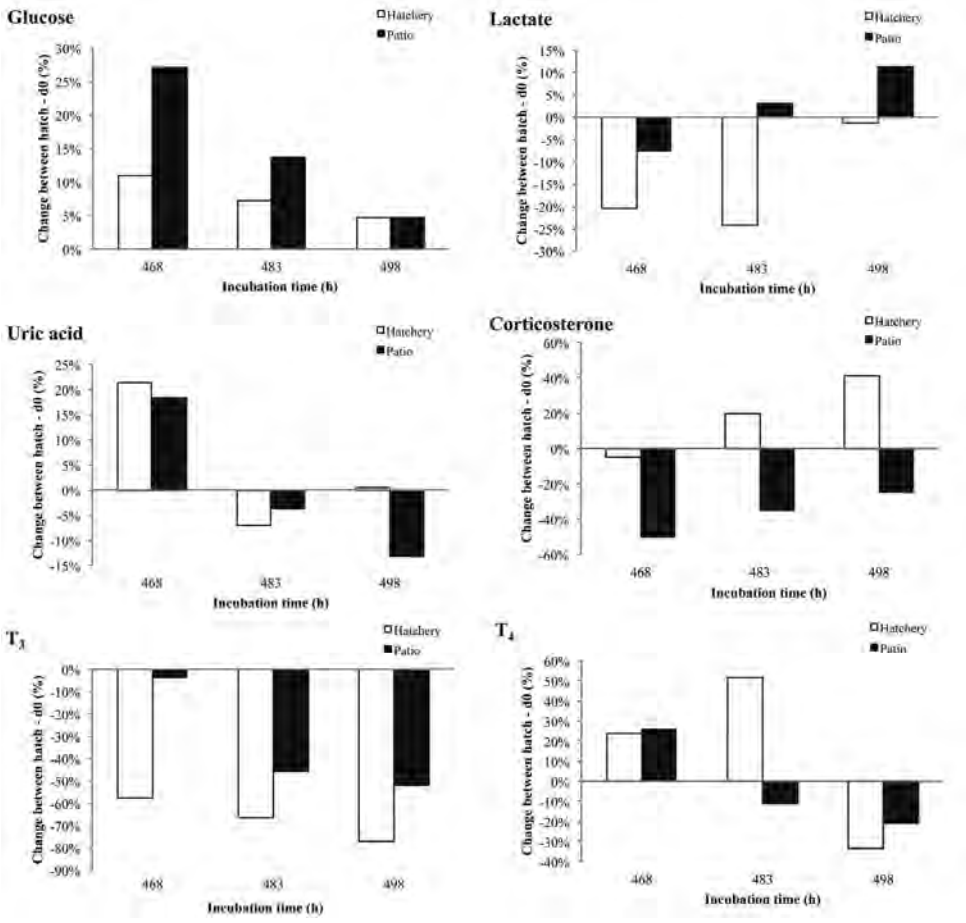

Figure 2. Least Squares Means of relative changes in plasma concentrations of glucose, lactate, uric acid, corticosterone, T_3, and T_4, in chicks hatched early, midterm, or late in the hatching process in a hatcher or a Patio system, determined directly after hatch (468 h for early, 483 h for midterm, or 498 h for late chicks), or at the end of incubation (515 h for all three hatch groups).

Physiological parameters at day E21.5

Body and organ weights. BW and absolute organ weights at d E21.5 are summarized in Table 4. For all variables significant SYS x HT interactions were found, except for residual yolk weights. Early and midterm chicks hatched in Patio showed higher BW and YFBM than early and midterm Hatcher chicks ($P < 0.001$), while for late chicks, the difference was not significant. The SYS x HT interaction for absolute weights of the heart, liver, bursa, spleen, and intestine length was due to higher values in early compared with midterm and late chicks in Patio, while in Hatcher chicks no differences were found among hatching times. Consequently, differences between Patio and Hatcher chicks in these organs were highest and significant in early chicks, and smaller in midterm and late chicks. Absolute heart weights were higher in Patio than in Hatcher chicks ($P = 0.017$), but relative heart weights were higher in Hatcher than in Patio chicks ($P = 0.002$), and

Table 4: Least Squares Means and SEM of organ weights and intestinal length of chicks hatched Early, Midterm, or Late in the hatching process in a hatcher or a Patio system, determined at the end of incubation (515h for all three hatch groups).

Treatment	n	BW (g)	YFBM (g)	Yolk (g)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Bursa (g)	Intestines (cm)
HT										
Early	50	45.88	43.64	2.24^{b}	0.31	0.32	1.54	0.021	0.044	47.0
Midterm	50	45.39	42.40	2.99^{a}	0.30	0.32	1.38	0.020	0.049	44.8
Late	50	44.44	41.02	3.42^{a}	0.32	0.33	1.27	0.019	0.041	41.5
Pooled SEM		0.59	0.52	0.17	0.01	0.01	0.03	0.001	0.002	0.8
SYS										
Hatcher	75	42.50	39.38	3.12^{a}	0.30	0.31	1.19	0.016	0.042	40.7
Patio	75	47.97	45.35	2.64^{b}	0.32	0.35	1.61	0.024	0.048	48.3
Pooled SEM		0.48	0.43	0.13	0.00	0.01	0.03	0.001	0.002	0.6
SYS x HT										
Hatcher x Early	25	40.59^{d}	38.20^{d}	2.39	0.28^{b}	0.30^{b}	1.25^{cd}	0.016^{c}	0.037^{c}	41.4^{bc}
Hatcher x Midterm	25	42.75^{cd}	39.73^{cd}	3.02	0.30^{b}	0.31^{b}	1.18^{d}	0.017^{bc}	0.049^{ab}	41.7^{bc}
Hatcher x Late	25	44.17^{c}	40.22^{cd}	3.95	0.32^{ab}	0.31^{b}	1.15^{d}	0.016^{c}	0.039^{bc}	38.9^{c}
Patio x Early	25	51.17^{a}	49.15^{a}	2.09	0.33^{a}	0.35^{ab}	1.84^{a}	0.027^{a}	0.052^{a}	52.8^{a}
Patio x Midterm	25	48.03^{ab}	45.07^{b}	2.96	0.30^{b}	0.32^{ab}	1.58^{b}	0.022^{ab}	0.048^{abc}	48.0^{b}
Patio x Late	25	44.71^{bc}	41.83^{c}	2.88	0.32^{ab}	0.36^{a}	1.40^{c}	0.021^{b}	0.044^{abc}	44.2^{b}
Pooled SEM		0.83	0.74	0.23	0.01	0.01	0.05	0.001	0.003	1.1
Source of variation										
HT		0.210	0.002	<0.001	0.071	0.288	<0.001	0.066	0.046	<0.001
SYS		<0.001	<0.001	0.013	0.017	<0.001	<0.001	<0.001	0.010	<0.001
SYS x HT		<0.001	<0.001	0.081	0.003	0.046	0.001	0.018	0.018	0.011

[a-d]LSMeans followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

Table 5: Least Squares Means and SEM of plasma hormone and metabolite concentrations of chicks hatched Early, Midterm, or Late in the hatching process in a hatcher or a Patio system, determined at the end of incubation (515h for all three hatch groups).

Treatment	n	Glucose (mg/dl)	Lactate (ng/ml)	Uric acid (ng/ml)	Corticosterone (ng/ml)	T_3 (ng/ml)	T_4 (ng/ml)	T_3:T_4 ratio
HT								
Early	50	214.18	37.76	6.18^{a}	17.81	0.76	6.67	0.12
Midterm	50	210.19	43.78	5.21^{b}	19.48	0.69	6.95	0.11
Late	50	201.67	40.44	4.88^{b}	19.64	0.62	5.56	0.12
Pooled SEM		2.64	1.28	0.23	1.53	0.04	0.29	0.01
SYS								
Hatcher	75	198.20	38.16^{b}	5.56	24.74^{a}	0.50^{b}	7.33	0.07
Patio	75	219.16	43.16^{a}	5.24	13.21^{b}	0.95^{a}	5.46	0.19
Pooled SEM		2.16	1.04	0.18	1.25	0.04	0.24	0.01
SYS x HT								
Hatcher x Early	25	192.81^{c}	33.67	6.27	25.01	0.45^{d}	6.80^{b}	0.07^{b}
Hatcher x Midterm	25	204.20^{bc}	43.56	5.32	25.87	0.57^{cd}	9.37^{a}	0.06^{b}
Hatcher x Late	25	197.58^{c}	37.24	5.14	23.34	0.48^{d}	5.81^{bc}	0.09^{b}
Patio x Early	25	235.54^{a}	41.84	6.09	10.61	1.27^{a}	6.54^{b}	0.21^{a}
Patio x Midterm	25	216.19^{b}	44.01	5.10	13.10	0.84^{b}	4.52^{c}	0.21^{a}
Patio x Late	25	205.76^{bc}	43.65	4.63	15.93	0.80^{bc}	5.31^{bc}	0.17^{a}
Pooled SEM		3.73	1.80	0.32	2.16	0.07	0.42	0.02
Source of variation								
HT		0.003	0.005	<0.001	0.641	0.085	0.004	0.843
SYS		<0.001	<0.001	0.227	<0.001	<0.001	<0.001	<0.001
SYS x HT		<0.001	0.088	0.779	0.230	0.002	<0.001	0.038

$^{a-d}$LSMeans followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

higher in late than in early and midterm chicks in both hatching systems. At d E21.5, weights of the lungs, liver, spleen, and bursa of Fabricius were higher in Patio than in Hatcher chicks ($P < 0.001$).
Residual yolk weights were lower in early compared with midterm and late chicks. At d E21.5, yolk weight was higher in Hatcher than in Patio chicks ($P = 0.013$).
At d E21.5, a SYS x HT interaction was found for hepatic glycogen. Glycogen was higher in early than in midterm and late chicks in Patio, while it did not differ among hatching times in Hatcher chicks.

Blood plasma parameters. Plasma hormone and metabolite levels at d E21.5 are presented in Table 5. At d E21.5, a SYS x HT interaction was found for concentrations of glucose, T_3, T_4 and T_3:T_4 ratio. In Patio, glucose and T_3 concentrations decreased from early to midterm to late chicks, while in Hatcher chicks levels were similar among hatching times. In Hatcher chicks, T_4 was higher in midterm than in early and late chicks, whereas in Patio, T_4 was higher in early than in midterm chicks, with late chicks intermediate. In midterm chicks, T_4 was higher in Hatcher than in Patio chicks whereas for early and late chicks there were no differences due to SYS. For T_3:T_4 ratio, a SYS x HT interaction was found, but when least squares means were compared using post hoc Tukey's adjustment, no significant differences were observed. T_3:T_4 ratio's were higher for Patio than for Hatcher chicks.
Lactate concentration was higher in midterm than in early chicks with late chicks intermediate and not significantly different from the others. Uric acid was higher in early than in late and midterm chicks. Corticosterone concentration was higher in Hatcher than in Patio chicks, and lactate was higher in Patio than in Hatcher chicks.

DISCUSSION

Similar to our previous findings (Van de Ven et al., 2011), the spread of hatch was at least 30 h in both hatching systems as early chicks were collected at 468 h and late chicks at 498 h of incubation. Egg transfer to the hatching systems occurred at 440 h of incubation, and chick removal from the hatcher at 515 h of incubation. Consequently, the late prehatch period and the early posthatch period that was spent in either hatching system lasted about 28 h and 47 h for early, 43 h and 32 h for midterm, and 58 h and 17 h for late chicks, respectively. During this period, embryos and chicks in both hatching systems were subjected to different climate conditions, and to either feed and water deprivation in the hatcher or given immediate access to feed and water in the Patio system, conform commercial broiler practice in the two hatching systems. Although in the present experiment it was attempted to approach the situation in broiler practice, it must be noted that presently 33% of the sampled chicks were collected as midterm chicks, whereas in broiler practice, a majority of the chicks would belong to this group.

Physiology at hatch

Hatching Time x Hatching System. Plasma glucose levels were higher in chicks hatching late vs early in the hatch window, corresponding previous findings in newly hatched chicken (Van de Ven et al., 2011) and poults (Fairchild and Christensen, 2000). However, in our earlier study the rise in plasma glucose was accompanied by a decrease in lactate, whereas presently, a slight decrease in lactate was only found in Patio chicks, and a high peak in lactate was found in midterm Hatcher chicks. Plasma lactate levels rise when oxygen becomes limiting during hatching, and energy comes from anaerobic glycolysis (Tazawa et al., 1983; Høiby et al., 1987; Moran Jr., 2007). Hypoxic conditions end at the moment of external pipping, and as soon as oxygen availability is restored, birds can recycle lactate back into glucose in the liver (De Oliveira et al., 2008). Based on the higher glucose in late chicks in our previous study, it was hypothesized that a possible longer interval between external pipping and hatching may have enabled these chicks to recycle lactate back into glucose before emergence from the egg, or that late chicks may have used less glucose and produced less lactate during hatching because of a lower metabolism. Because in the present study the rise in glucose was not accompanied by a decrease in lactate, these results may indicate that variations in lactate levels are explained by variable production rather than by variations in removal of lactate.

Hatching Time. At hatch, no difference was found in BW or YFBM among chicks of different hatching times, corresponding to previous findings (Fairchild and Christensen, 2000; Careghi et al., 2005; Van de Ven et al., 2011). The increase in organ weights with hatching time, concurrent with a decrease in residual yolk weight, is in agreement with earlier data on newly hatched poults and chicks (Fairchild and Christensen, 2000; Van de Ven et al., 2011), and points to an increased organ maturation in chicks hatching later in the hatch window.

Corresponding to previous findings in broilers, liver glycogen stores were low at hatch, (Kornasio et al., 2011; Molenaar et al., 2011) and no differences were found among early, midterm, or late chicks, which agrees with findings of Fairchild and Christensen in poults (2000). Liver glycogen concentration peaks around d E18 and then decreases during the energy demanding hatching process (Freeman, 1965, 1969; Willemsen et al., 2010; Molenaar et al., 2010b). Variations in glycogen stores, and thus energy reserves for hatching were found due to different incubation conditions and were associated with changes in hatching time (Willemsen et al., 2010; Molenaar et al., 2010b). Because glycogen stores at the beginning of the hatching process were not measured in the present study, it is unclear whether early, midterm or late chicks used different amounts of energy for hatching.

Increased yolk uptake during embryo development of later hatching chicks might explain the higher plasma glucose compared to earlier hatching chicks, as the yolk sac was shown to be a major glucose synthesizing organ, using amino acids and glycerol through the gluconeogenesis pathway, and possibly releasing free glucose into the blood (Yadgari and Uni, 2012). A trend for higher T_3 and T_4 levels with increasing hatching time was observed, in contrast to previous findings, where thyroid levels at hatch tended to be lower in late compared with earlier hatching chicks, which was linked to a lower metabolic rate (Iqbal et al., 1989; Careghi et al., 2005; Van de

Ven et al., 2011). The background of these opposite results in the present study is unclear, but may reside in the background for later hatching.

Hatching System. At hatch, higher weights were presently found of heart, liver, stomach, intestines, and lungs in Patio compared with Hatcher chicks, whereas no organ weight differences were noted in the previous study (Van de Ven et al., 2011). Based on these earlier results, it was hypothesized that in the range of 35.2 and 38.1°C, which were the average temperatures measured during hatching in Patio and the hatcher respectively, normal organ development could occur. However presently, Hatcher embryo physiology appeared to respond to the higher temperatures, which seems in agreement with earlier reports where higher temperatures (>38.8°C) during late incubation lead to lower heart, lung, stomach, liver, and intestine weights at hatch (Molenaar et al., 2010a; 2011) or heart weights after 21d of incubation (Wineland et al., 2000; Leksrisompong et al., 2007; Lourens et al., 2007; Molenaar et al., 2011). Effects of high temperatures on hatchling BW, liver, spleen, gizzard, proventriculus and intestine weights in broilers appear inconsistent among studies or within studies (Givisiez et al., 2001; Hulet et al., 2007; Leksrisompong et al., 2007; Lourens et al., 2007), and appears difficult to explain but may be related to interfering factors such as parent age or conditions during the incubation period preceding the hatching phase.

Energy required for hatching activities comes from glucose provided from glycogen in liver, yolk sac membrane, and muscle (Freeman, 1965, 1969; Christensen et al., 2001; Yadgary and Uni, 2012), resulting in an increase in plasma glucose between pipping and hatch (Christensen et al., 2001). At hatch, plasma glucose was slightly higher in Patio than in Hatcher chicks, in contrast to previous findings where the reverse effect was found and higher glucose levels in hatcher chicks were linked to higher corticosterone levels, possibly indicating a more energy demanded hatching process (Van de Ven et al., 2011). The background for these opposite results is not clear. A continuous lower incubation temperature (35°C vs. 38°C) was shown to stimulate the expression of a metabolic regulator PGC-1α in the liver of chicken embryos, which may activate gluconeogenesis (Walter and Seebacher, 2007). Hence, a lower temperature in Patio may have resulted in the increased glucose levels at hatch.

Early posthatch period

Hatch Time x Hatching System. BW gain in fed Patio chicks and BW loss in deprived Hatcher chicks in the early posthatch period were comparable to findings in newly hatched broilers that were fed or fasted (Noy and Sklan, 1999). In this period, the YFBM did not change in Hatcher chicks, whereas YFBM in Patio chicks increased with time after hatch. At d E21.5, yolk weights were higher in Hatcher than in Patio chicks, whereas no differences were found in residual yolk weight at hatch. These data suggest that Hatcher chicks used slightly less yolk than Patio chicks, which is probably due to the earlier access to feed and water for the latter, in agreement to previous studies (Noy et al., 1996; Noy and Sklan, 1999). These results seem to contrast the findings of Gonzales et al. (2003), Bigot et al. (2003) and Van den Brand et al. (2010), who found similar rate of yolk uptake in fed and deprived broiler hatchlings. However, the feed deprived chicks in these

studies were provided with water (Bigot et al., 2003; Gonzales et al., 2003; Van den Brand et al., 2010), in contrast to the Hatcher chicks in the present study, which were deprived of feed and water. Higher yolk uptake in fed chicks was associated to increased activity of the gastrointestinal tract, and to increased physical pressure in the abdominal cavity (Noy and Sklan, 1999; 2001). Water intake may have stimulated yolk absorption through the same mechanisms. Intestinal length increased in both fed and deprived birds, but at a lower rate in the latter, corresponding to earlier reports (Noy and Sklan, 1999; Bigot et al., 2003; Van den Brand et al., 2010).

In the early posthatch period, lactate levels decreased in early and midterm Hatcher chicks, and although these chicks had no access to feed and water, they still showed a rise in plasma glucose. The increase in glucose together with a decrease in lactate suggests that lactate was recycled back into glucose in the liver. However this may have occurred some time after hatch, because the drop in lactate was not found in late chicks. Additional glucose was probably generated by glycogenolysis, because hepatic glycogen content decreased in Hatcher chicks from all hatching times to minimum values at d E21.5. In contrast, liver glycogen content in Patio chicks increased in the early posthatch period, concurrent with an increase in plasma glucose level, which was probably related to nutrient uptake from the residual yolk (Rinaudo et al., 1982), and from exogenous feed (Edwards et al., 1999). In addition, conversion of lactate into glucose in Patio chicks may have coincided with lactate formation due to glucose uptake from feed, because it was shown that up to 37% from glucose absorbed during feed intake in chicken is first converted to lactate before entering the circulation (Riesenfeld et al., 1982).

At E 21.5, T_3:T_4 ratios were lower than at hatch for all hatch groups, and 2-3 times higher in fed Patio than in deprived Hatcher chicks, whereas at hatch there were no differences among hatch groups. This was due to a more pronounced decrease of T_3 levels in Hatcher than in Patio chicks in the early posthatch period, which seems in agreement with the decreasing plasma T_3 levels in the first 1-3 d posthatch found in fasted chicks (Noy and Sklan, 2001; Careghi et al., 2005). T_3 stimulates the use of metabolic fuels such as glucose and free fatty acids (Noy and Sklan, 2001), and lowering circulating T_3 may be considered a physiological adaptation to maintain nutritional reserves (Decuypere and Kühn, 1984), which could possibly be related to the slightly lower yolk uptake in the Hatcher chicks. In fed chicks, T_3 levels in the first 1-3 d were previously found to increase (Noy and Sklan, 2001) or to remain constant (Careghi et al., 2005), which seems in contrast to decreased T_3 levels in the fed midterm and late Patio chicks. After hatching Patio chicks stayed in the system, where a temperature of 35°C was provided during the first days posthatch until d E21.5, which differs from the procedure of Noy and Sklan (2001) who transported chicks to the broiler facilities immediately following hatch. Conditions during transport or in the facilities were not mentioned, but temperature may have been lower than 35°C which was considered thermoneutral for newly hatched chicks (Freeman et al., 1967), thereby stimulating the production of T_3 (McNabb, 2006) and glucose utilization for heat production. In older chicken, the decrease in T_3 levels during fasting was accompanied by an increase in T_4 levels, which was at least partly due to decreased peripheral conversion of T_4 to T_3 (Decuypere and Kühn, 1984). This seems in agreement with the observations in midterm, and to a lesser

extent, early Hatcher chicks, but not for late Hatcher chicks, which may be related to the shorter period of fasting for the latter.

Hatching Time. Because the uptake of residual yolk increased with time after hatch, early chicks had the lowest residual yolk weights at d E21.5, whereas the reverse was found at hatch. These data emphasize that analysing the physiological status of hatchlings at chick pulling may result in different findings compared to analysing chicks at hatch. Organ weights increased in the immediate posthatch period, corresponding to previous results (Molenaar et al., 2011). Although Hatcher chicks had no access to feed and water, the weight gain of some organs was almost similar to the fed Patio chicks. Heart weights even showed equal increases in both hatching systems. These results may confirm the high priority of the development of supply organs early in life (Katanbaf et al., 1988).

At d E21.5, uric acid levels were higher than at hatch in early, but lower than at hatch in midterm and late chicks in both hatching systems, suggesting an initial decrease in uric acid level and increase thereafter. The increase in uric acid level in early chicks between hatch and the age of 47 h was 20%, similar to the 23% increase in uric acid level from 12-48 h posthatch reported by Molenaar et al. (2011). The rise of uric acid in early chicks from both hatching systems points at protein catabolism (Mori and George, 1978), and might be due to gluconeogenesis from glucogenic amino acids derived from the yolk sac (Rinaudo et al., 1982; Yadgari and Uni, 2012), from exogenous feed in Patio chicks, and possibly from tissue proteolysis in the Hatcher chicks, which were subjected to prolonged fasting (Jenni-Eiermann and Jenni, 1998).

Hatching System. Higher plasma corticosterone levels at d E21.5 in Hatcher compared to Patio chicks may be associated with a condition of chronic stress, but changes in corticosterone secretion may also be related to the metabolic effect of feed restriction as corticosterone is involved in the regulation of blood glucose levels (Mench, 2002), and increased after severe feed restriction in older meat type chicken (Rajman et al., 2006). However, corticosterone did not seem to increase with time after hatching, and thus prolonged fasting, as levels were similar for early, midterm and late Hatcher chicks. Gonzales et al. (2003) found no differences in corticosterone in the early posthatch period between fed chicks and chicks that were deprived from feed, but not from water, up to 36 h after placement in the farm. It can be suggested that corticosterone levels, which were shown to increase in the last incubation phase of chicken embryos and peak around hatching (Tona et al., 2003), decrease in the early posthatch period when given immediate access to feed and water, as in the Patio system.

In summary, chicks hatching early in the hatch window seem less matured at hatch, based on lower organ weights compared to later hatching chicks. The lower yolk uptake and T_3 levels in the early posthatch period in Hatcher chicks point at metabolic adaptations to preserve nutritional reserves during fasting. In Patio chicks, increased body and organ weights, yolk uptake, glucose, and T_3 levels indicate an advanced metabolic rate and physiological development, probably as a result of early feeding, and these developments were more pronounced in earlier hatching chicks. As a consequence, in Patio earlier hatching chicks appear physiologically more developed at the

moment of chick pulling at d E21.5, whereas the early chicks in the hatcher seem less developed than later hatching chicks that were fasted for a shorter period of time. Present data emphasize that at the moment of chick pulling, the physiological status of chicks is affected both by hatching time and by the length of exposure to posthatch conditions, especially feed and water deprivation, or access.

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chapter 5

Effects of hatching time, first feed access, and hatching system on broiler growth

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ABSTRACT

A spread of hatch leads to variation in chick age of 24-48 hours at the moment of chick removal from the hatcher. Chicks commonly have no feed and water access until placement in the farm, meaning that early hatching chicks are deprived from feed and water for a longer period than late hatching chicks. Early and late chicks may respond differently to post-hatch fasting, but also to other environmental conditions between hatching and chick collection. In 3 experiments, post-hatch growth of chickens was investigated in relation to hatching time and post-hatch fasting. This was done in 2 hatching systems with different climate conditions: a conventional hatcher and a Patio system, in which chicks are usually directly fed post-hatch. At incubation day 17 or 18, broiler eggs were assigned to 1 of the 2 hatching systems. At 3 moments during the hatching process (early, midterm, late), chicks were collected from each system, and BW was determined at hatch, d0, d7, and at slaughter age. In chicks that were directly fed post-hatch, growth from d0-7 was higher in early and midterm compared to late chicks, which was likely due to the shorter period of feed and water access for the latter. Also in chicks that were fasted till d0, as in hatchery practice, a slight advanced growth in early vs. later hatching chicks up to d7 was observed. Higher growth in deprived early chicks may be attributed to higher motivation for eating and drinking due to the longer period of fasting, compared to the later hatching chicks. Growth in chicks that were directly fed was increased up to slaughter age, with 2.2%-7.8% higher weights at slaughter age compared with fasted chicks. Except for the period hatch – d7, growth was not affected by hatching system. We conclude that the present practice of depriving chicks of feed and water until d0, reduces growth post-hatch, and although these effects become less pronounced over time, they last until slaughter age.

(*Key words*: broiler, hatching time, feed deprivation, hatching system)

INTRODUCTION

Chicks hatch over a time window of 24-36 h and are only removed from the hatcher when the majority of the chicks has hatched (Hager and Beane, 1983; Decuypere et al., 2001), usually after about 21.5 days of incubation. This means that at the moment of chick collection from the hatcher ('chick pulling'), there is a large variation in chick age, with early hatching chicks being up to 2d old and late chicks only several hours. In common practice, chicks do not have access to feed and water until placement in the broiler house. Several researchers have investigated the effects of early post-hatch fasting and observed impaired post-hatch performance and higher early mortality (Kingston, 1979; Stamps and Andrews, 1995; Halevy et al., 2000; Gonzales et al., 2003). A majority of these studies compared chicks that were directly fed after placement at the farm with chicks that were deprived for a period of additional 24-48h after placement (Stamps and Andrews, 1995; Dibner et al., 1998; Vieira and Moran, 1999; Halevy et al., 2000; Gonzales et al., 2003). This means that the early post-hatch period between hatching and the moment of chick pulling, and the additional time needed for chick handling and transfer to the farm, was not considered, nor was the variation in chick age at the moment of placement (Careghi et al., 2005). The metabolism of early vs. late hatching chicks was found to differ (Iqbal et al., 1989; Careghi et al., 2005; Van de Ven et al., 2011) and these chicks may also respond differently to early post-hatch fasting (Hager and Beane, 1983; Careghi et al., 2005). Careghi et al. (2005) investigated the interaction between hatching time and period of feed and water deprivation on chick growth, by subjecting chicks that hatched early, midterm and late in the hatch window to similar periods (48 h) of feed and water deprivation. The authors concluded that late chicks benefited more from immediate post-hatch access to feed than did earlier hatching chicks. The set up of their study (Careghi et al., 2005) was in contrast with hatchery practice where early hatchers are deprived from feed and water for a longer period than late hatchers. Therefore, in the present study we investigated the effects of hatching time and period of feed and water deprivation on post-hatch growth, but the fasting period was different for chicks of different hatching times, as in hatchery practice.

The consequences of post-hatch fasting on growth are probably not only dependent on the duration of fasting, but may also be affected by the environment during late incubation and the early post-hatch phase until chick pulling. It was shown that high temperatures during late incubation depress growth in broilers post-hatch (Hulet et al., 2007; Leksrisompong et al., 2009). An alternative system for the hatching phase is the Patio system, a multi-tiered housing system in which the hatching and brooding phase are combined (Van de Ven et al., 2009). In the Patio system, the air temperature in the hatching phase was found to be 2.9°C lower, and relative humidity was about 21.1% lower compared with conditions measured in the hatcher (Van de Ven et al., 2011). In addition, in Patio access to feed and water is provided immediately post-hatch.

Based on the hypothesis that effects of hatching time and moment of first feed and water access depend on the environment during the hatching phase, the hatching system was included in the present study. In 3 experiments, we studied effects of hatching time, moment of first feed and water access, and hatching system on broiler growth up to slaughter age.

MATERIALS AND METHODS

Experimental design

Three experiments were conducted, in which the post-hatch growth was examined of chicks that hatched at different moments within the hatch window (early, midterm, late). For each of the experiments, hatching eggs were obtained from commercial Ross 308 prime age breeder flocks of various ages (Table 1). Prior to incubation, all eggs were stored for 2-3 days. Eggs for experiment 1 were incubated in a HatchTech setter[1] and the eggs for experiments 2 and 3 were incubated in a Petersime setter[2]. At d E18 (experiments 1 and 3), or at d E17 (experiment 2), eggs were candled and apparently fertilized eggs were randomly divided and transferred to a Petersime hatcher or transported to the Patio system during 30 min, in a climate conditioned truck at an air temperature of 30°C. For all three experiments, a standard hatching program was used in the hatcher, starting at a set point temperature of 37.2°C which was gradually decreased to 36.4°C at d E21. In the Patio system, the set point of the air temperature was 35.0°C during the entire hatching process in all three experiments.

Table 1. Overview of treatments in three experiments in which effects of hatching time, time of first feed access, and hatching system were examined on growth of broiler chicks.

Experiment	Breeder age (weeks)	Hatching system		Feeding treatment hatch - day 0	N	Processing age (days)
		Egg transfer – hatch	Hatch – day 0			
1	47	Hatcher	Patio	Fed	130	45
		Hatcher	Patio	Deprived	130	
		Patio	Patio	Fed	130	
		Patio	Patio	Deprived	130	
2	37	Hatcher	Hatcher	Deprived	110	34
		Hatcher	Patio	Deprived	110	
		Patio	Patio	Deprived	110	
3	40	Hatcher	Hatcher	Deprived	50	41
		Patio	Patio	Fed	50	

Experiment 1. In the first experiment, the effects of time of first feed and water access on post-hatch growth were studied in chicks that hatched in a hatcher or in Patio, but were housed in Patio shortly after hatch. Hence, the environment in the period between hatch and chick pulling time was the same for the Hatcher and Patio chicks.

During the hatching process, chicks that still had some wet down, indicating they had just hatched, were randomly selected from both hatching systems at three time points: at 465h of

1 HatchTech, Veenendaal, The Netherlands
2 Petersime, Zulte, Belgium

incubation (early chicks), at 480h of incubation (midterm chicks), and at 493h of incubation (late chicks). At each of the time points, chicks were collected from several locations across both hatching systems. The newly hatched chicks were weighed, individually labeled, and the Hatcher chicks were transferred to the Patio system during 30 min in a climate conditioned truck. Upon arrival at the Patio farm, Hatcher chicks were randomly distributed over 2 groups which were placed in separated compartments: one group was given direct feed and water access (fed), while for the other group no feed or water was provided (deprived). Likewise, the chicks of each hatching moment that hatched in the Patio system were also distributed over 2 groups (fed and deprived), and placed in the same 2 compartments together with the Hatcher chicks. After 514 h of incubation, the hatching process was ended in both hatching systems. At that time, the separation wall between the 2 compartments was removed, and the fed and deprived chicks were mixed. Thus, from that moment on, all chicks were given access to feed and water. All experimental chicks from the hatcher and the Patio stayed in the separated compartment of the Patio system until slaughter weight was reached at d45.

Experiment 2. In the second experiment, effects of hatching system during the last incubation phase and the early post-hatch phase were studied in chicks that were all deprived from feed and water until chick pulling time. Chicks from the hatcher and the Patio system remained in the hatching system where they had hatched until the moment of chick pulling. Additionally, a third treatment group consisted of chicks that hatched in the hatcher, but were transferred to the Patio system shortly after hatch.

The early, midterm, and late chicks were collected from both hatching systems at 469h, 484h, and 497h of incubation. After collection, chicks were weighed and individually labeled. The chicks that were collected from the hatcher were randomly divided in 2 groups: one group was returned to the hatcher, and the other group was transferred to the Patio system (termed the HaPa chicks), where they were housed together with the Patio chicks in a separated compartment in the Patio system. After 512 h of incubation, the experimental Hatcher chicks were removed from the hatcher, and transferred to the Patio system. None of the chicks from the three treatment groups (Hatcher, HaPa, and Patio chicks) had access to feed and water until the Hatcher chicks arrived. All experimental chicks were housed together from that moment on, until slaughter weight was reached at d34.

Experiment 3. In the third experiment, we followed the post-hatch growth of chicks from both hatching systems, but chicks remained in the hatching system until the moment of chick pulling. Thus, chicks from the Hatcher were deprived from feed and water until chick pulling, and chicks from the Patio had immediate post-hatch feed and water access, which is conform practice in the two hatching systems. Early, midterm, and late chicks were collected from the hatcher and the Patio system at 468h, 483h, and 498h of incubation, weighed, individually labeled, and returned to the hatching system in which they had hatched. The chicks were collected from the hatcher after 515 h of incubation, transferred to the Patio system, and housed together with the Patio chicks in a separated compartment of the Patio system, where they had access to feed and water. Chicks were raised until slaughter weight was reached at d41.

The time of egg transfer to the hatching system, the moments of sampling of the early, midterm, and late chicks, and time of chick pulling from the hatcher in each of the experiments are summarized in Table 2.

Table 2. Time of egg transfer from setter to hatcher, time of sampling of the early, midterm, and late chicks, time of chick pulling from the hatcher, and age of chicks at chick pulling in three experiments.

Experiment	Time of egg transfer (incubation h)	Chick collection (incubation h)			Time of chick pulling (incubation h)	Age at chick pulling (hours post-hatch)		
		Early	Midterm	Late		Early	Midterm	Late
1	441	465	480	493	514	49	34	21
2	420	469	484	497	512	43	28	15
3	440	468	483	498	515	47	32	17

Bird management

Chickens were raised at standard conditions according to the recommendations of the breeder company, until slaughter weight was reached. Bird density in each of the experiments was comparable, approximately 17 birds/m^2. Chickens were fed a commercially available crumbled starter diet (12.75 MJ of ME/kg, 22.6% CP) up to d10, a pelleted grower diet (12.90 MJ of ME/kg, 22.5% CP) from d10-30, and a finisher diet from d30 up to slaughter age (13.00 MJ of ME/kg, 23.5% CP). From d0 onward, feed and water was available ad libitum throughout the experiments for all chickens.

Data collection

During the hatching process in each of the experiments, the temperature and relative humidity (RH) were measured and logged every 5 min using data loggers (175-H2 Logger, Testo[3]) in both hatching systems. In the hatcher, 3 loggers were placed among the eggs in different hatching baskets. In the Patio system, 3 loggers were placed among the eggs on the egg trays.

Chick weighing occurred within 1.5h after hatch and approximately 4h after chick pulling at d E21.5, from now on termed d0. The mean age of early, midterm, and late chicks at the moment of chick pulling in each of the experiments is presented in Table 2. Thereafter, individual chick weights were taken at d7 and at slaughter age, which was the end of the experiment. Growth rate per h was calculated for different growth periods: hatch - d0, hatch – d7, d0 - d7, d7 – slaughter age. During the last weighing, sex of all birds was determined.

Statistical analyses

Per experiment, data were analysed with the SAS 9.1 software package (SAS Institute, 2004). The individual chick was treated as the experimental unit in the analyses. Data on chick weights and growth per hour were analysed using the GLM procedure. The model for analyses of the data was:

$$Y_{ijkl} = SEX_i + HT_j + SYS_k + \text{interaction terms} + e_{ijk},$$

3 Testo, Almere, The Netherlands

where SEX_i = sex of the chick, HT_j = hatching time (Early, Midterm, Late), SYS_k = system (Hatcher, Patio), and e_{ijk} = residual error term.

In experiment 1, the factor feeding treatment (FT: fed, deprived) and the interaction terms were added to the model. In experiment 2, a third treatment group was included in the factor SYS, which were chicks that hatched in the Hatcher and were transferred to Patio shortly after hatch (HaPa chicks).

In all analyses, *P*-values ≤ 0.05 were considered statistically significant and non-significant interaction terms were stepwise deleted from the model. When the means of the GLM were statistically different, means were compared using Least Squares means with Tukey's adjustment for multiple comparisons. Data are expressed as least squares means ± SEM.

Table 3. Mean and standard error of temperature and relative humidity registrations during hatching of broiler eggs in 3 experiments in a Hatcher or the Patio system.

Exp.	Hatching system	Registration period (incubation h)	Temperature (°C)			Relative humidity (%)		
			Mean ± SE	Min	Max	Mean ± SE	Min	Max
1	Hatcher	449 – 514 h	38.1 ± 0.02	36.3	40.1	50.8 ± 0.54	22.9	89.8
	Patio		35.2 ± 0.02	32.7	36.0	29.7 ± 0.17	18.0	46.1
2	Hatcher	423 – 512 h	37.6 ± 0.01	33.9	39.0	54.3 ± 0.37	30.9	84.6
	Patio		35.7 ± 0.03	31.8	37.2	41.4 ± 0.07	27.0	54.1
3	Hatcher	448 – 515 h	-	-	-	-	-	-
	Patio		35.4 ± 0.03	32.9	38.3	30.2 ± 0.10	25.5	37.5

RESULTS

Climate conditions are summarized in Table 3. Registrations of temperature and RH in both hatching systems were used from the moment the temperature had stabilized, until the end of incubation. Due to incorrect functioning of the climate data loggers in experiment 3, climate registrations in the hatcher were lost. Mean temperature was 2.9 °C and 1.9°C higher in the hatcher than in the Patio system, and mean relative humidity was 21.1% and 12.9% higher in the hatcher than in the Patio system in experiment 1 and 2, respectively.

Experiment 1

Chick BW at hatch, d0, d7, and at slaughter age in relation to hatching time are presented in Figure 1. A significant HT x SYS interaction ($P = 0.025$) was found for BW at hatch: hatchling weights did not differ among hatching times in Patio, while in Hatcher chicks, midterm chicks were heavier than late chicks (48.3±0.3 vs. 47.2±0.3g; $P = 0.02$) with early chicks intermediate (47.6±0.3g). Between hatch – d0, BW loss per hour was similar in deprived Hatcher and Patio

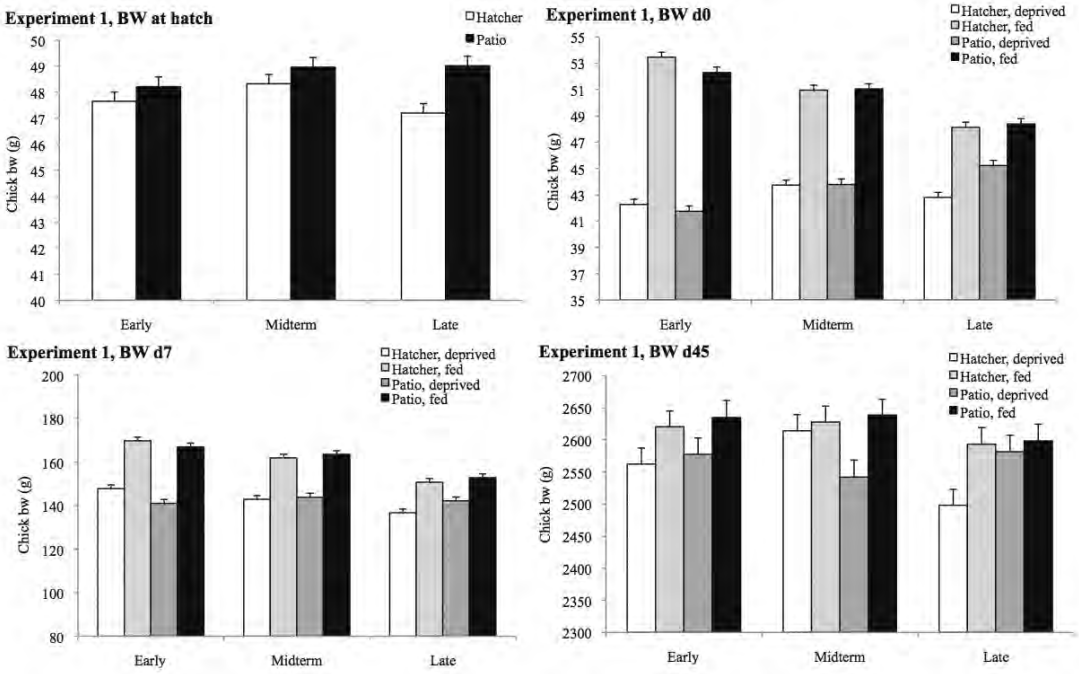

Figure 1. Least squares means and standard error of chick weights in experiment 1. Chicks hatched early, midterm, or late during the hatching process in a Hatcher or in a Patio system, and were either fed immediately after hatch, or deprived from feed and water until d E21.5 / d0. Data are pooled for both sexes.

chicks, but in fed chicks, BW gain per hour was higher in Hatcher than in Patio chicks (0.07±0.00 g/h vs. 0.03±0.00 g/h; $P < 0.001$). Weight loss in the deprived chicks was highest in late (0.16±0.01 g/h) and lowest in early chicks (0.11±0.01 g/h) with midterm chicks intermediate, and in fed chicks weight gain was higher in early (0.09±0.01 g/h) than in midterm (0.07±0.01 g/h) chicks, whereas in late chicks, no BW change was observed.

At d0, fed chicks were heavier than deprived chicks. The difference between these groups were comparable for both hatching systems, and decreased with hatching time, from 10.9g (25.9% of deprived BW) in early, to 7.2g (16.5% of deprived BW) in midterm, to 4.3g (9.7% of deprived BW) in late chicks. Late Hatcher chicks weighed less at d0 than midterm and early Hatcher chicks ($P < 0.001$ for both).

Growth from hatch till d7 did not differ among hatching times or between hatching systems in fed chicks. In deprived chicks, growth was depressed in early compared to midterm and late Patio chicks, while there were no significant differences among hatching times in Hatcher chicks. For the period d0-7, growth was higher in fed than in deprived chicks, regardless of hatching system and hatching time. A SYS x HT interaction was observed for growth from d0-7: growth was higher in early than in midterm and late chicks from both hatching systems, but these differences were more pronounced in Hatcher than in Patio chicks. D7 weights in the fed chicks decreased from early (168±1g) to midterm (163±1g; $P = 0.015$) and from midterm to late (152±1g; $P < 0.001$), whereas in the deprived chicks, only the BW of early and late chicks differed (144±1g vs. 139±1g; $P = 0.043$), with midterm chicks intermediate (143±1g), and not significantly different

from the others. The weight difference between fed and deprived chicks at d7 was 23.8g (16.5% of deprived bw), 19.4g (13.5% of deprived BW) and 12.3g (8.8% of deprived BW) for early, midterm, and late chicks, respectively. At d7, weights in Hatcher chicks were higher in Early (159±1 g) than in Midterm (152±1 g) and Late (144±1 g) chicks, and in Patio chicks, higher in Early and Midterm chicks (both 154±1 g) than in Late chicks (148±1g). From d7 onward, growth was not affected by hatching system anymore.

Growth from d0 till slaughter age was affected by a HT x SEX interaction (P = 0.003), in males, early chicks grew faster (2.57±0.02g/h) than late chicks (2.47±0.02g/h; P = 0.006), with midterm chicks intermediate and not significantly different from the others, while in females there were no differences due to HT. Growth from d0 till slaughter age was higher in fed chicks than in deprived chicks (2.39±2.39 vs. 2.34±0.01g/h; P < 0.001) and higher in males than in females (2.43±0.01 vs. 2.13±0.01 g/h; P < 0.001). D45 males were heavier than females (2,765±12g vs. 2,434±10g; P < 0.001), and early chicks were heavier than late chicks (2,621±15 vs. 2,569±13g; P = 0.024) with midterm chicks intermediate (2,607±13g) and not statistically different from both other hatching times.

Experiment 2

Chick BW at different ages in relation to hatching time are presented in Figure 2. At hatch, late chicks were heavier (47.5±0.2g) than early and midterm chicks (both 46.5±0.2g; P < 0.001), and Hatcher chicks were heavier than Patio chicks (P = 0.030).

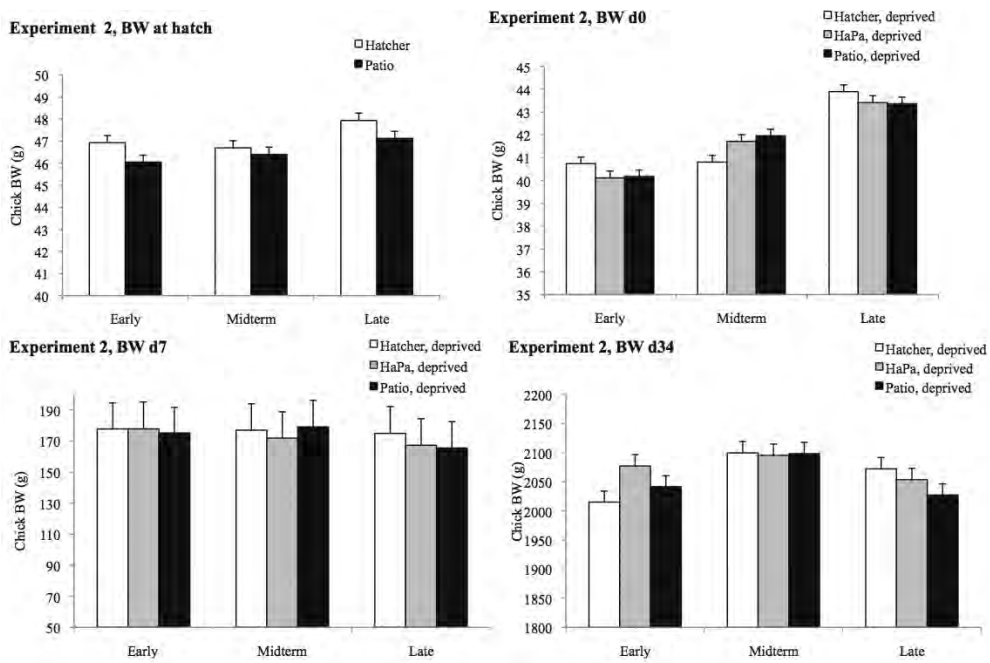

Figure 2. Least squares means and standard error of chick weights in experiment 2. Chicks hatched early, midterm, or late during the hatching process in a Hatcher or in a Patio system. Half of the Hatcher chicks was transferred to the Patio system immediately posthatch (HaPa chicks) and the other half was transferred at d E21.5. All chicks were deprived from feed and water until d E21.5 / d0. Data are pooled for both sexes.

Between hatch – d0, BW loss per hour was highest in late (0.21±0.00g/h) and lowest in early chicks (0.13±0.00g/h), with midterm chicks intermediate (0.15±0.00g/h), both in Hatcher, HaPa, and Patio chicks. Patio chicks showed smaller weight loss per hour (0.15 g/h±0.00) than Hatcher and HaPa chicks (both 0.17±0.00g/h; $P < 0.001$). At d0, early chicks had lower BW than midterm and late chicks in all three treatment groups. Growth from hatch till d7 was affected by a significant HT x SYS interaction ($P < 0.001$): growth increased from early to midterm and late chicks, but this effect was more pronounced in Hatcher than in HaPa and Patio chicks, because in late chicks, growth was higher in Hatcher than in HaPa ($P = 0.011$) and Patio chicks ($P = 0.002$). When growth was considered from d0-7, a HT x SYS interaction was found ($P = 0.007$): overall, early and midterm chicks grew faster than late chicks, but differences among hatch times were smaller in Hatcher than in HaPa and Patio chicks. Growth from d0 – 7 was higher in late Hatcher chicks (0.79±0.01g/h) than in late HaPa (0.74±0.01g/h) and Patio chicks (0.75±0.01g/h), and at d7, late Hatcher chicks were heavier than late HaPa and Patio chicks. D7 weights of early (177±1g) and midterm chicks (176±1g) were higher than those of late chicks (169±1g; $P < 0.001$). After d7, no effects of SYS were observed on chick BW or growth rate.

From d0-34, midterm chicks (2.53±0.01g/h) grew faster than early (2.47±0.01g/h; $P = 0.003$) and late chicks (2.46±0.01g/h; $P = 0.008$), and growth was higher in males (2.67±0.01g/h) than in females (2.30±0.02g/h; $P < 0.001$). At d34, midterm chicks were heavier (2,097±11g) than early (2,044±11g) and late chicks (2,050±11g), and male chicks were heavier than female chicks (2,215±10g vs. 1,913±9g, $P < 0.001$).

Experiment 3

Chick BW at different ages in relation to hatching time are presented in Figure 3. A significant HT x SYS interaction was found for BW at hatch: hatchling weights did not differ among hatching times in Patio, while in Hatcher chicks, midterm and late chicks (both 47.4±0.5g) were heavier than early chicks (44.8±0.5g; $P = 0.02$). Between hatch – d0, BW loss per hour in Hatcher chicks was highest in late (-0.18±0.01g/h), and lowest in early chicks (-0.10±0.01g/h), with midterm chicks intermediate (-0.14±0.01g/h). For fed Patio chicks, growth in this period was similar for early (0.09±0.01g/h) and midterm chicks (0.07±0.01g/h), whereas late chicks lost weight in this period (-0.04±0.01g/h). At d0, differences between fed Patio and deprived Hatcher chicks were 9.9g in early chicks (or 24.7% of Hatcher chick BW), 4.9g in midterm chicks (or 11.5% of Hatcher chick BW) and 0.4g in late chicks (or 1% of Hatcher chick BW). At d0, BW increased from late to midterm to early chicks in Patio chicks, and decreased from late to midterm to early chicks in Hatcher chicks.

Growth from hatch till d7 in Hatcher chicks was lower in early (0.34±0.01g/h) than in midterm (0.37±0.01g/h) and late chicks (0.38±0.01g/h), while in Patio late chicks had lower growth rate (0.45±0.01g/h) compared with early and midterm chicks (both 0.49±0.01g/h), although differences were not statistically significant. For the period d0-7, growth was higher in early and midterm chicks than in late chicks, both in Hatcher and in Patio chicks. Growth from d0-7 was higher in Patio than in Hatcher chicks (0.56±0.01g/h vs. 0.46±0.01g/h; $P < 0.001$). At d7, BW increased from late (128±2.8g) to midterm (144±2.8g) and early chicks (152±2.8g) in Patio, and no differences

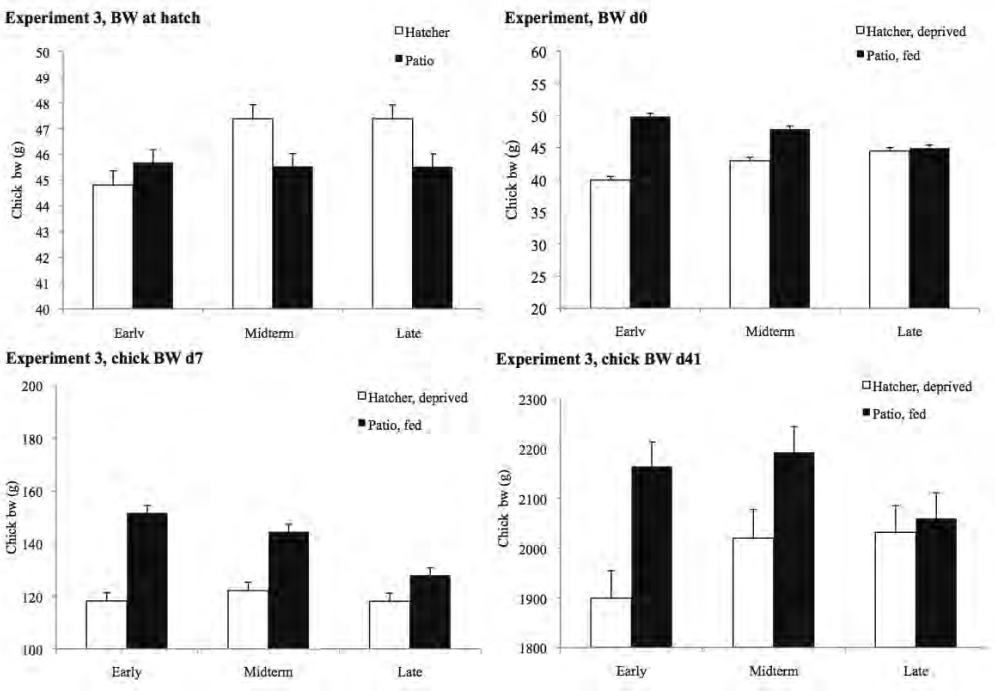

Figure 3. Least squares means and standard error of chick weights in experiment 3. Chicks hatched early, midterm or late during the hatching process in a Hatcher or in a Patio system. The Patio chicks were fed immediately after hatch, and the Hatcher chicks were deprived from feed and water until d E21.5 / d0. Data are pooled for both sexes.

among hatching times were observed in Hatcher chicks. At d7, weight differences between Patio and Hatcher chicks were 33.4g (28.3% of Hatcher chick BW), 22.3g (18.3% of Hatcher chick BW) and 9.9g (8.4% of Hatcher chick BW) for early, midterm and late chicks, respectively.

From d0-41, a tendency for a HT x SYS interaction was found (P = 0.092): in Patio chicks, growth in early (2.15±0.05g/h) and midterm chicks (2.18±0.05g/h) was higher than in late chicks (2.05±0.05g/h), while in Hatcher chicks, growth was higher in midterm (2.00±0.06g/h) and late chicks (2.01±0.05g/h) than in early chicks (1.89±0.06g/h). Growth from d0 till slaughter age was higher in males (2.18±0.04g/h) than in females (1.91±0.03g/h; $P < 0.001$). At slaughter age, Hatcher chicks (1,984±32.2g) weighed less and Patio chicks (2,139±29g; $P < 0.001$), and males showed higher weights (2,195±35g) than females (1,929±28g; $P < 0.001$).

DISCUSSION

First, the data from all three experiments were used to discuss the effects of hatching time on post-hatch growth. Next, the effects of time of first feed and water access were discussed based on the results from the fed and deprived chicks from both hatching systems from experiment 1, and from the deprived Hatcher and the fed Patio chicks from experiment 3. Effects of hatching system were discussed using the data from experiment 1, where chicks were hatched in the hatcher or in

Patio, but all housed in the same environment shortly after hatch, and from experiment 2, where data were collected of chicks that stayed in the Hatcher or stayed in the Patio system during the period between hatching and chick collection, without feed and water.

Hatching Time

Body weights at hatch were not clearly affected by hatching time. Whereas BW were similar among hatching times in Patio chicks in experiment 1 and 3, BW increased with hatching time in chicks from both systems in experiment 2, and in Hatcher chicks only in experiment 3. Previously, BW at hatch were comparable in chicks of different hatching times (Careghi et al., 2005) and poults of different hatching times (Fairchild and Christensen, 2000).

Between hatch – d0, BW loss per hour in deprived chicks was highest in late, and lowest in early chicks in all 3 experiments. These data indicate that weight loss in the initial post-hatch hours is higher than later on, which is probably due to evaporation of the water in the wet down of newly hatched chicks. In fed chicks from experiments 1 and 3, BW gain per hour was higher in early than in midterm chicks and in late chicks no BW gain was observed, which may be related to a relative large impact of the initial post-hatch hours during which the down dries and chicks probably consume little feed or water. In addition, growth is only initiated approximately 24 h after the first intake of feed and water (Noy and Sklan, 1999), and late chicks were only approximately 25 and 21 hours old at the moment of weighing at d0 in experiment 1 and 3, respectively. In the deprived chicks from all 3 experiments, early chicks had lower BW at d0 than midterm and late chicks due to the prolonged period without feed and water. The reverse was found in the fed chicks from experiments 1 and 3, where BW increased from late to midterm to early chicks, probably as a result of a longer period of feed and water access for the latter.

Growth from hatch till d7 was lower in early than in midterm and late chicks in deprived groups from all 3 experiments, although not significantly in experiment 3, which was likely explained by the smaller sample sizes because absolute differences in growth rate among hatching times were similar among the 3 experiments. Lower growth in early deprived chicks was probably due to the prolonged period without feed and water before d0 for these chicks during which weight loss occurred. Indeed, in fed chicks from experiment 1 and 3, no differences were observed in growth rate among hatching times. These findings are in agreement to data of Careghi et al. (2005), who found comparable growth between immediately fed early, midterm, and late chicks at the chronological age of 7d, which was the age calculated from end of hatch of the whole batch. Hence, in the study of Careghi et al. (2005) early chicks were older (7d and 21h) than late hatching chicks (7d and 0h) at the moment of weighing, as in the current experimental set-up. These authors also evaluated growth of early, midterm, and late hatching chicks that were all deprived for 48 h post-hatch, and found depressed growth in the later hatchers at the chronological age of 7d. It was speculated that unknown intrinsic factors depressed growth in late hatchers, which was aggravated by delaying the first feed access (Careghi et al., 2005). However, it could also be suggested that the depressed growth in deprived late hatching chicks is related to the relatively large impact of the 48-h period of fasting on growth up to d7, during which weight loss occurs,

and hence growth potential may be similar in chicks of different hatching times. This suggestion seems in agreement to the data of chick weighing at the biological age of 7d of Careghi et al. (2005). Chicks of different hatching times were weighed at their biological age of exactly 7d, and thus weighing of the late chicks was delayed by the same period as their time of hatch was delayed compared to early hatching chicks (21h). At the biological age of 7 d, growth was increased in the late compared to the early chicks (Careghi et al., 2005).

For the period from d0 till d7, growth was higher in early and midterm chicks than in late chicks in all 3 experiments, corresponding to the findings of Tona et al. (2003), which indicated that earlier hatching improved relative growth from d0 up to d7. It was suggested that lower growth in later hatching chicks could be associated with lower intrinsic quality and lower plasma T_3 concentrations of late hatching chicks (Tona et al., 2003; Decuypere and Bruggeman, 2007). However, the present results and the data on chick weights at biological d7 of Careghi et al. (2005) suggest that the lower growth in late chicks from d0-7 is explained by the relatively large impact of the early post-hatch period for late hatching chicks, and that growth potential may be similar or even higher than those of earlier hatching chicks. Nonetheless, at d7 the fed early and midterm chicks were heavier than late chicks in the present experiments, which was likely due to the longer access time to feed in early hatchers, although also in the deprived chicks of experiments 1 and 2, early chicks were heavier at d7 than later hatching chicks, despite a shorter period of feed deprivation for the latter. A longer fasting period for the earlier hatching chicks probably resulted in a higher motivation for feed and water uptake compared to later hatching chicks. Indeed, a higher growth in the first 2d after access to feed was observed in delayed chicks compared with fed chicks in their first 2d after access to feed (Bigot et al., 2003; Careghi et al., 2005).

Growth from d0 till slaughter age in experiment 1 was affected by a HT x SEX interaction, due to higher growth in early males compared with late males, while in females there were no differences due to HT. In experiment 2, midterm chicks grew faster than early and late chicks, regardless of sex, and in experiment 3, early and midterm chicks tended to grow faster than late fed Patio chicks, whereas the reverse seemed true for the deprived Hatcher chicks. From the present results, effects of hatching time on the growth period from d0 up to slaughter age were not clear.

It can be concluded that in fed chicks, growth in the first week post-hatch is advanced in early compared to later hatching chicks, which appears to be related to the younger age of the late chicks at the moment of weighing at d7. In earlier hatching chicks that were deprived from feed and water for an extended period, a higher motivation for eating and drinking may lead to enhanced growth in the early days after feed access.

Feed and water access

In experiment 1 and 3, chicks with immediate feed and water access after hatch showed higher BW at d0 than deprived chicks, and the differences between fed and deprived chicks became smaller with increasing hatching time and thus shorter time of access to feed and water for the fed chicks.

Growth from d0 up to slaughter age was higher in fed than in deprived chicks, but became relatively smaller with increasing age, which is in line with earlier studies on early post-hatch feed deprivation (Hager and Beane, 1983; Stamps and Andrews, 1995; Vieira and Moran, 1999; Gonzales et al., 2003; Joseph and Moran, 2005; Henderson et al., 2008: Kornasio et al., 2011). At slaughter age, the differences between fed and deprived chicks in the present study were 56.3g (2.2% of deprived BW) and 155.4 g (7.8% of deprived BW) in experiments 1 and 3, respectively. Higher early growth rate in chicks that were fed shortly after hatch compared to chicks that were fasted post-hatch was previously attributed to a more advanced intestinal development and better utilization of nutrients (Noy and Sklan, 1999; Bigot et al., 2003). Furthermore, the higher slaughter weight in birds that were fed immediately post-hatch was also due to a longer period of access to feed and water. In the present experiments, ages of the chicks at the time of chick collection from the hatcher were between 15 – 49h, depending on the time of hatching in the hatch window. Hence the chicks that were immediately fed post-hatch, had at least 15 – 49h longer access to feed and water, compared to chicks that were deprived until chick collection. Growth rates in modern broilers may increase up to 90 g/d in the 6th week of life (Aviagen, 2007), and it thus may be expected that an extra day of access to feed and water chicks with direct feed and water access results in approximately 90 g higher slaughter weight.

Hatching System

Registrations of temperature and relative humidity in both hatching systems were used from the moment the temperature had stabilized, until the end of incubation. In both systems, the temperature of the air among the eggs was higher than the set temperature, which was expected, because in this phase of incubation eggs produce considerable amounts of heat (Lourens et al., 2005; Hulet et al., 2007). Air temperatures were higher in the hatcher than in the Patio, but because air velocity was not measured, the difference in temperature experienced by the embryo and the newly hatched chick could not be quantified. The difference in relative humidity between the hatching systems was probably of minor importance for the embryo, because the contribution of air humidity to heat exchange of an egg with its environment is not significant at temperatures operated in the present experiments (Van Brecht et al., 2005). In addition, differences in relative humidity (20 or 80%) were shown to have very little effect on the metabolism of one-day-old chicks (Misson, 1976), and was probably less important to post-hatch growth.

Apart from the higher weight gain from hatch – d0 in fed Hatcher compared to fed Patio chicks in experiment 1, which was possibly related to a higher need for compensation for moisture loss due to the higher temperature, growth was not significantly affected by different conditions in the hatcher or the Patio system in the last incubation phase. In addition, different conditions in the early post-hatch phase did not have any effect on post-hatch growth aside from slightly lower growth in late Patio and Hapa compared to Hatcher chicks from d0 – 7. On the other hand, a more pronounced difference between the fed and deprived chicks in experiment 3 (7.8% of deprived BW) compared with experiment 1 (2.2% of deprived BW) may suggest that conditions in the hatcher in the early post-hatch phase aggravated the effects of the delay in feed access, because chicks in experiment

1 were all housed in Patio after hatch, while Hatcher chicks remained in the hatcher until chick pulling in experiment 3. The background of the more pronounced difference in growth between fed Patio and deprived Hatcher chicks in experiment 3 vs. experiment 1 is not clear.
Present findings seem in contrast to previous studies where broiler chicks that were subjected to higher temperature (39.4-40.7°C) from day E16 till the end of incubation, showed lower post-hatch growth compared to control temperature (37.6°; Hulet et al., 2007; Leksrisompong et al., 2009). However, the exposure time to different temperatures in the present study was shorter, and the hatcher temperature in experiments 1 and 2 was considerably lower than the high temperature treatment in the previous studies (Hulet et al., 2007; Leksrisompong et al., 2009). In addition, Willemsen et al. (2010) concluded that an increase of 3°C compared to control temperature (37.6°C) during E16-E18.5 had more dramatic effects on hatchling physiology than a decrease of 3°C. Thus, present results seem to confirm that in the last incubation phase, broiler embryos are not really sensitive to lower temperatures and consequences to later growth performance are limited.
It must be noted that in the present experiments, standard hatchery procedures such as chick counting, packaging and transportation by a chick truck, were omitted. No studies can be found on the effects of chick handling in the hatchery on growth post-hatch, but it was suggested that contamination of chicks can occur by pathogens that may be found on surfaces of chick handling equipment (Knowles et al., 2004), and hence post-hatch growth may be affected.

It can be concluded that the present practice of depriving chicks of feed and water until d0 reduces growth post-hatch, and although these effects become less pronounced over time, they last until slaughter age. Effects of hatching time on the growth period from d0 up to slaughter age were not clear. Except for the early post-hatch period, growth in broilers was not affected by hatching system.

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chapter 6

Effects of egg position during late incubation on hatching parameters and chick quality

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ABSTRACT

Chicken eggs are commonly incubated for 17-18d in setters, after which they are transferred to the hatcher for the last 3-4d of incubation. While eggs are positioned vertically with the air cell up during the first incubation phase, they are placed horizontally for the hatching phase. It is unknown whether egg position in the last phase of incubation is of importance to the hatching process and chick quality. An experiment was conducted to investigate effects of egg position in the last 4d of incubation on the hatching process and chick quality. The experiment consisted of 2 identical trials, where 300 fertile eggs per trial were transferred to a hatching cabinet at E17. Eggs were placed in one of 3 positions: with the air cell up (ACU), with the air cell down (ACD), or horizontally (HOR). Starting at E18, the following data was collected for each egg at 3h intervals: time of internal pipping (IP), external pipping (EP), hatching, and position of EP. Approximately 6h after hatch, body weight, chick length, and chick quality based on the Pasgar©score, were determined for each chick. In addition, residual yolk weight and yolk-free-body-mass (YFBM) were determined in every 4th chick that hatched. Time of IP was not affected by egg position, but EP occurred 5h later in ACU eggs, and thus the IP-EP interval was increased by 3-4h in this group compared to the other egg positions. Hatching occurred 1-2h earlier in HOR eggs than in the other 2 positions. Body weight, yolk weight, and YFBM were not affected by egg position. Chick length was 1-2 mm shorter and Pasgar©score was slightly lower in ACD eggs compared to ACU and HOR eggs, mainly caused by a high incidence of poor navel quality, red hocks, and red beaks. Hatchability was not affected by egg position. We concluded that egg position in the last phase of incubation affects the duration of the hatching process, and has small effects on chick quality.

(*Key words*: hatching system, egg position, chick quality, hatching time)

INTRODUCTION

Hatching eggs are commonly incubated for 17-18d in setters during which they are positioned in a setter tray with the large end of the egg up. Because during this period eggs are turned regularly at an angle of 90° (Tona et al., 2005), they rest with their longitudinal axis at an angle of 45°. For the last 3-4d of incubation, eggs are transferred to hatcher baskets and placed in hatchers. In the hatcher baskets, eggs generally lay in a horizontal position (Bauer et al., 1990). Recently, an alternative hatching system was developed, named Patio[1] (Van de Ven et al., 2009). The Patio is a multi-tiered housing system, which was developed to combine the hatching and brooding phase, so after hatch, chicks stay in this system for the remainder of the growing period. When using the Patio system, eggs at d17 or 18 of incubation are not transferred to hatcher baskets, but remain in the upright position in the setter trays, which are placed in the Patio system. Consequently, in contrast to the horizontal egg position in traditional hatching systems, chicks in Patio hatch from eggs positioned with the large end up.

Egg position during the first part of incubation has been shown to influence hatchability in chicken and quails (Byerly and Olsen, 1931; Cain and Abbot, 1971; Bauer et al., 1990; Wilson et al., 2003; Mao et al., 2007; Moraes et al., 2008), primarily by affecting the proportion of embryos that have the head located in the large end of the egg, right under the air cell, which is considered the optimum position for hatching (Oppenheim, 1972). Positioning eggs with the small end up results in a higher percentage of embryos with the heads located in the small end of the egg, leading to lower hatchability compared to eggs which are placed horizontally or with the large end up (Byerly and Olsen, 1931; Cain and Abbot, 1971). In addition, positioning eggs with the small end up leads to lower chick quality at hatch (Bauer et al., 1990).

The studies mentioned above focused on effects of different egg positions during the first phase of incubation only, after which the eggs were transferred to a horizontal position for the hatching phase (Byerly and Olsen, 1931; Cain and Abbot, 1971; Bauer et al., 1990; Moraes et al., 2008), or the experiment was ended (Wilson et al., 2003). Effects of different egg positions in the last days of incubation are unknown, but may affect embryo movement and thus the hatching process. In the current study we investigated the effects of egg position in the last 4d of incubation on hatchability, hatching time, and chick quality.

MATERIALS AND METHODS

All procedures in this study were approved by the Animal Care and Use committee of Wageningen University, the Netherlands.

Experimental design

The experiment consisted of two identical trials, conducted one week after each other, with hatching eggs from the same commercial Cobb 500 broiler breeder flock. The breeder flock aged

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33 wks and 34 wks when eggs were obtained for trial 1 and 2, respectively. Eggs were stored for 6d in trial 1, and 5d in trial 2, and then incubated for 17 days in a multi-stage incubator[2] at a constant machine temperature of 37.6°C and relative humidity of 53%. Eggs were placed in a vertical egg position with the air cell up, and turned hourly at an angle of 90°. After candling at d E17 (after 416h of incubation), fertile eggs were collected from 1 setter trolley, weighed and only eggs in the range of 51-56 g were transferred to a Plexiglas hatching cabinet (1.2 x 2.3m) which provided room for 3 setter trays. Per trial, 300 eggs were randomly distributed over 3 setter trays (100 eggs per tray). Per tray, all eggs were placed in the same position, which were: 1) vertical with the air cell up (**ACU**); 2) vertical with the air cell down (**ACD**); or 3) horizontal (**HOR**). The setter trays were placed next to each other in the hatching cabinet, about 30 cm above the floor of the cabinet, which was covered by wood shavings. The position of the egg trays in relation to one another was changed between the trials.

From d E17-21.5, eggs were not turned, the set point of the air temperature remained constant at 35.0°C, and air speed was lower than 0.2 m/s, which is considered still air (Simmons et al., 2003). These conditions were based on set points commonly applied in the Patio system, and were shown to result in the highest hatchability in preliminary (unpublished) trials.

Data collection

From the moment the eggs were placed in the cabinet until the trial was ended at d E21.5, the temperature was logged every 5 min using 3 data loggers with an accuracy of 0.1°C (175-H2 Logger, Testo[3]) that were placed among the eggs on each of the setter trays, at a distance of approximately 1 cm from the eggs. In addition, 1 of the 3 loggers also measured relative humidity every 5 min. The data loggers were the same for both trials and were calibrated before the experiments started.

After 442h of incubation (d E18), the measurements started at intervals of 3h until hatching was finished at 505h and 496h of incubation in trial 1 and 2, respectively. At each interval, all eggs were examined to determine the moment of internal pipping (IP), by use of a candling light, external pipping (EP), and hatching. In addition, the position of EP was registered and categorized in ring shaped areas A, B, C, D, E, or F, each having the same width. Area A was located at the outermost large end of the egg, containing the air cell, and F was located at the small end of the egg.

When a chick had hatched, it fell down through one of the openings in the egg tray onto the wood shavings on the floor of the cabinet, which was divided into 3 separated compartments, one below each egg tray. Here, chicks were kept separately per treatment until measurements were performed 6h later. At that moment, from each chick the body weight, chick length, measured from the top of the beak to the tip of the middle-toe excluding the nail (Hill, 2001), and chick quality were scored. Chick quality was measured using the Pasgar©score (Boerjan, 2002). Based on this score, the quality of each chick was evaluated based on 5 criteria: 1) Low alertness; 2) Suboptimal navel condition; 3) Red hocks (red or swollen hocks); 4) Abnormal beak; and 5)

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Large size of the residual yolk sac. For each of the 5 criteria, one point was subtracted from 10, with chicks scoring 10 being free of any abnormality and 5 being the lowest score.
In addition to the above described measurements, every 4th chick that hatched was sacrificed for measurements on residual yolk and yolk-free-body-mass (YFBM). Hatchability was determined as total number of eggs hatched divided by the total number of fertile eggs x 100.

Statistical analyses

Data were analysed with the SAS 9.1 software package (SAS Institute, 2004). Measurements on each individual egg or chick were treated as experimental unit in all analyses. Normally distributed data (body weight, yolk weight, YFBM, chick length, time of IP, EP, and hatch) were analysed using the GLM procedure using the following model:

$$Y_{ij} = EP_i + Trial_j + \text{interaction term} + e_{ijk},$$

where EP_i = egg position (ACU, ACD, HOR), $Trial_j$ = trial (1 or 2), and e_{ijk} = residual error term. Data on Pasgar©score and hatchability were analysed using the LOGISTIC procedure, using the same model (SAS User's Guide, 2004). Variation in hatching time parameters was tested for treatment effects using Levene's test for homogeneity of variance (hovtest). Data are expressed as means ± SE, and differences were considered statistically significant at $P \leq 0.05$.

RESULTS

Egg weight at the moment of transfer to the hatching cabinet was 53.5 ± 0.06g, and did not differ among treatments. Mean temperature was 34.5±0.02°C during trial 1 and 34.8±0.02°C during trial 2. Relative humidity was 22.7±0.05% in trial 1, and 26.5±0.05% in trial 2.

Hatching parameters

Mean hatchability was 97.2%, and not affected by trial or egg position. The 17 unhatched eggs (11 from trial 1 and 6 from trial 2) were opened for macroscopic examination. Eleven embryos had already died before the start of the trial, 2 embryos showed malformations, and 4 embryos were malpositioned. No effect of egg position was noted on these parameters.
IP, EP and Hatch occurred about 10h earlier in trial 2 than in trial 1 (Table 1). Egg position did not affect time of IP but EP occurred approximately 5h later in ACU eggs compared to ACD and HOR eggs ($P < 0.001$), which were not different from each other (Table 1). In line with these results, the IP–EP interval was increased in ACU eggs compared to ACD and HOR eggs ($P < 0.001$), while this interval did not differ between ACD and HOR eggs. Variation of time of EP and duration of the IP-EP interval differed among egg positions ($P = 0.028$ and $P = 0.018$, respectively), and was greatest in ACU eggs. Of all chicks, 99.5% pipped the eggshell at the large side of the egg (area A, B or C). There was a small effect of egg position on the distribution of EP-position ($P = 0.030$); in ACD eggs, a higher proportion of chicks pipped at area B (89.8%), compared to ACU (84.4%) and HOR (81.6%) eggs.

Table 1. Means ± SE of the moment of internal pipping (IP), external pipping (EP), hatching, and the interval between these moments in hours, determined in eggs positioned air cell up (ACU), air cell down (ACD), or horizontal (HOR), during the last 4 d of incubation. Data are expressed in hours.

	n	IP	EP	Hatch	IP–EP	EP–Hatch	IP-Hatch
Trial							
Trial 1	289	461 ± 0.5[a]	478 ± 0.4[a]	492 ± 0.3[a]	17 ± 0.5[a]	14 ± 0.3[a]	31 ± 0.5[a]
Trial 2	294	452 ± 0.3[b]	468 ± 0.4[b]	481 ± 0.3[b]	16 ± 0.4[b]	13 ± 0.3[b]	29 ± 0.3[b]
Egg position							
Air cell up	194	457 ± 0.7	476 ± 0.6[a]	487 ± 0.6[a]	19 ± 0.6[a]	12 ± 0.3[b]	31 ± 0.5[a]
Air cell down	194	456 ± 0.6	471 ± 0.5[b]	486 ± 0.5[ab]	16 ± 0.5[b]	15 ± 0.3[a]	30 ± 0.5[ab]
Horizontal	195	456 ± 0.6	471 ± 0.6[b]	485 ± 0.5[b]	15 ± 0.6[b]	14 ± 0.3[a]	29 ± 0.5[b]
Trial x Egg position							
Trial 1 x ACU	97	462 ± 0.9	481 ± 0.8	493 ± 0.6[a]	19 ± 1.0	12 ± 0.5	31 ± 0.8[a]
Trial 1 x ACD	95	460 ± 0.9	476 ± 0.7	491 ± 0.6[a]	16 ± 0.8	15 ± 0.4	31 ± 0.8[a]
Trial 1 x HOR	97	460 ± 1.0	476 ± 0.6	491 ± 0.5[a]	16 ± 0.9	15 ± 0.4	31 ± 0.9[a]
Trial 2 x ACU	97	451 ± 0.6	470 ± 0.6	481 ± 0.4[b]	19 ± 0.7	11 ± 0.5	30 ± 0.6[a]
Trial 2 x ACD	99	452 ± 0.5	467 ± 0.6	482 ± 0.4[b]	15 ± 0.6	15 ± 0.5	29 ± 0.6[ab]
Trial 2 x HOR	98	452 ± 0.5	466 ± 0.6	479 ± 0.4[c]	14 ± 0.6	13 ± 0.4	27 ± 0.5[b]
Source of variation							
Trial		$P < 0.001$	$P < 0.001$	$P < 0.001$	$P = 0.019$	$P = 0.025$	$P = 0.044$
Egg position		$P = 0.508$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$	$P < 0.001$
Trial x egg pos.		$P = 0.068$	$P = 0.304$	$P = 0.006$	$P = 0.304$	$P = 0.103$	$P = 0.025$

[a-c]*Different superscript letters indicate a significant difference between treatment groups (P ≤ 0.05).*

The EP–hatch interval was smaller in ACU eggs than in ACD and HOR eggs ($P < 0.001$), which were not different from each other. The IP–hatch interval was not affected by egg position in trial 1, but in trial 2 HOR eggs showed shorter IP-hatch intervals compared to the ACU ($P < 0.001$) and ACD eggs ($P = 0.002$). In trial 1, the moment of hatch did not differ among egg positions, but in trial 2, hatch occurred earlier in HOR eggs compared to ACU eggs ($P = 0.004$) and ACD eggs ($P = 0.002$).

Chick quality

Body weight, yolk weight, and chick length were lower in trial 1 than in trial 2, while YFBM was higher in trial 1 than in trial 2 (Table 2). There were no effects of egg position on body weight, yolk weight, or YFBM. Chick length was slightly shorter in chicks from ACD eggs compared to the ACU and HOR eggs ($P = 0.006$). The Pasgar©score was lower in trial 2 than in trial 1, mainly explained by a higher incidence of Red hocks in all egg positions (18% in trial 1 vs. 36% in trial

2). Chicks from ACD eggs had a lower Pasgar©score than chicks from ACU eggs and HOR eggs ($P = 0.008$). The lower Pasgar©score in ACD chicks was largely due to a high incidence of Suboptimal navel score (36% in ACU, 51% in ACD, and 48% in HOR eggs), Red hocks (29% in ACU, 29% in ACD, and 23% in HOR eggs), and Abnormal beak score (2% in ACU, 9% in ACD, and 4% in HOR eggs).

Table 2. Means ± SE of body weight, yolk-free-body-mass (YFBM), yolk weight, chick length and mean ± SE Pasgar©score of chicks hatched from eggs positioned air cell up (ACU), air cell down (ACD), or horizontal (HOR), during the last 4 d of incubation.

	Body weight (g)	YFBM (g)	Yolk weight (g)	Chick length (cm)	Pasgar©score
Trial					
Trial 1	42.7 ± 0.08^{b}	37.5 ± 0.15^{a}	5.2 ± 0.11^{b}	19.1 ± 0.03^{b}	9.3 ± 0.04^{a}
Trial 2	43.2 ± 0.07^{a}	36.9 ± 0.12^{b}	6.2 ± 0.10^{a}	19.4 ± 0.02^{a}	9.1 ± 0.05^{b}
Egg position					
Air cell up	43.0 ± 0.10	37.2 ± 0.16	5.6 ± 0.13	19.2 ± 0.03^{a}	9.3 ± 0.05^{a}
Air cell down	42.8 ± 0.10	37.0 ± 0.16	5.8 ± 0.12	19.1 ± 0.03^{b}	9.1 ± 0.06^{c}
Horizontal	43.0 ± 0.10	37.3 ± 0.17	5.7 ± 0.13	19.3 ± 0.03^{a}	9.2 ± 0.05^{b}
Trial x Egg position					
Trial 1 x ACU	42.9 ± 0.15	37.8 ± 0.25^{a}	5.2 ± 0.20	19.1 ± 0.05	9.4 ± 0.07
Trial 1 x ACD	42.5 ± 0.14	36.9 ± 0.30^{ab}	5.1 ± 0.14	19.0 ± 0.04	9.2 ± 0.07
Trial 1 x HOR	42.6 ± 0.14	37.6 ± 0.20^{a}	5.2 ± 0.22	19.1 ± 0.05	9.4 ± 0.06
Trial 2 x ACU	43.1 ± 0.12	36.6 ± 0.23^{b}	6.0 ± 0.17	19.4 ± 0.04	9.2 ± 0.08
Trial 2 x ACD	43.1 ± 0.13	37.0 ± 0.21^{ab}	6.4 ± 0.16	19.3 ± 0.04	9.0 ± 0.08
Trial 2 x HOR	43.3 ± 0.13	37.0 ± 0.21^{ab}	6.3 ± 0.16	19.4 ± 0.04	9.1 ± 0.07
Source of variation					
Trial	$P < 0.001$	$P = 0.002$	$P < 0.001$	$P < 0.001$	$P = 0.003$
Egg position	$P = 0.401$	$P = 0.341$	$P = 0.731$	$P = 0.006$	$P = 0.008$
Trial x egg pos.	$P = 0.226$	$P = 0.026$	$P = 0.458$	$P = 0.818$	$P = 0.442$

^{a,b}*Different superscript letters indicate a significant difference between treatment groups ($P \leq 0.05$).*

DISCUSSION

Hatching parameters

Before preparations for hatching start, avian embryos commonly lie on their left side with the neck curved under the air cell and the beak and anterior head region buried in the yolk between the legs (Oppenheim, 1972). Around d 17-18, embryos lift their head out of the yolk, position their

head under the right wing, and bring the beak and right shoulder towards the air cell, which is considered the optimum hatching position (Oppenheim, 1972). In the present study, hatchability was high and not affected by egg position during the last 4d of incubation. In addition, there were no effects of egg position on the occurrence of malpositions, and egg position did not greatly affect the position of external pipping, since all but 3 chicks (0.5%) pipped the eggshell at the region of the air cell. It was suggested that obtaining the right embryo position for hatching is mainly influenced by the need for oxygen and gravity (Byerly and Olsen, 1931), which was based on high incidences of malpositioned embryos in eggs placed with the air cell down, and in eggs placed air cell up but with the porous air cell region sealed with paraffin. From present data it seems that gravity does not play a significant role after E17, because the embryo seems to be able to obtain the right hatching position irrespective of egg position during E17-21.

The IP-EP interval presently found was about 5-9h longer compared to previous findings in eggs obtained from the same broiler breed, of parent flocks of 38-48 wks old, while the EP–hatch interval was comparable to these studies (Tona et al., 2003; De Smit et al., 2008; Everaert et al., 2008; Willemsen et al., 2010). The IP-EP interval was found to increase with longer egg storage time (Tona et al., 2003), however, despite a shorter storage time in the present study (5-6d) compared to the 18-d stored eggs in the study of Tona et al. (2003), the IP-EP interval was about 5h longer in the present study. Another possibility is that the longer IP-EP interval in the present study was related to a lower incubation temperature in the last phase of incubation (34.5-34.8°C) compared with the previous studies, where the set point temperature was about 37.6°C in this phase (Tona et al., 2003; De Smit et al., 2008; Everaert et al., 2008; Willemsen et al., 2010). A lower incubation temperature during late incubation was associated with a lower embryonic metabolism resulting in a lower build-up of CO_2 in the air cell (Willemsen et al., 2011), which was shown to increase the length of the IP-EP interval (Visschedijk, 1968).

The IP-EP interval was 3-4h longer in eggs positioned with the air cell up which may indicate that embryos in these eggs encountered greater difficulty lifting their head from the yolk towards the air cell against gravity, compared to embryos in the other egg positions.

The EP–hatch interval was 2-3h shorter in eggs positioned with the air cell up. It was suggested that the length of the EP–hatch interval is influenced by the availability of energy in this phase, such as yolk lipids or liver glycogen (Christensen et al., 2000; Everaert et al. 2008). There were no differences noted in residual yolk weight among egg positions, and no measurements on energy use were performed, so a relation between length of the EP-hatch interval and energy availability could not be assessed in this study. Possibly, embryos in ACU eggs found less difficulty moving their head during the process of external pipping compared to embryos in ACD eggs, and to a lesser extent in HOR eggs, which probably encountered more pressure of their own body weight during this process.

The hatching process started about 10h earlier in trial 2 compared to trial 1. Factors that influence time of hatch are breeder age, storage time, and egg size (Decuypere and Bruggeman, 2007), which were kept similar in both trials. Incubation temperature is another important factor that affects hatching time (Decuypere and Bruggeman, 2007). Based on the assumption that the

chick embryo needs a fixed amount of heat for full development, as suggested by Decuypere and Michels (1992), an air temperature difference of 0.3°C between the trials during the period from transfer until hatch (76h in trial 1, 65h in trial 2) was unlikely to explain the difference of 10h in hatching time. For both trials, eggs were incubated for 16d in a multi-stage setter, at a constant set point temperature of 37.6°C. Despite equal machine set points, temperature differences could occur at different positions within a setter (Van Brecht et al., 2003), and eggs for the 2 trials were obtained from different setter trolleys. The shorter hatching time in trial 2 suggests that these eggs could have been subjected to a higher temperature during d E0-16 at level of the setter trolley.

Chick quality

Egg position did not affect body weight, yolk weight, or YFBM. The Pasgar©score was highest in chicks hatched from eggs with the air cell up, and lowest in chicks hatched from eggs with the air cell down. The incidence of poor navel quality was highest in chicks from eggs with the air cell down, followed by chicks from horizontally placed eggs. In the current experiment, after emergence from the eggs, chicks had to crawl away from the egg and fall down through openings in the setter trays onto the litter. Chicks that hatched from eggs that were positioned with the air cell down, emerged from the egg at the region of the air cell, facing the floor of the hatching cabinet. Possibly, remnants of the chorioallantoic arteries were torn as the chick fell down directly after it fractured the eggshell, thereby increasing the risk for unhealed navels and leaving a small scab of blood, which is the most common type of unhealed navels (Fasenko and O'Dea, 2008). High incidences of red hocks and red beaks in chicks from ACD eggs also point at a demanding hatching process. The length of chicks from ACD eggs was 1-2 mm shorter in comparison to chicks from the other egg positions. It must be noted that although differences in Pasgar©score and chick length were statistically significant, they are small and relevance of these differences for later life is not clear.

Interestingly, despite equal initial egg weights, both body and yolk weights of chicks in trial 1 were 0.5g and 1.0g lower than in trial 2, while the YFBM was 0.6g higher. These data point in the same direction as the earlier hatching time in trial 2, and suggest a higher incubation temperature from E0-16 for eggs in trial 2, as higher temperatures lead to lower YFBM and higher yolk weights at hatch (Molenaar et al., 2010). The Pasgar©score was lower in trial 2, due to an increase in red hock incidence in chicks from each of the egg positions. Red hocks were associated to prolonged pushing of the hocks against the eggshell during the hatching process (Wilson, 2004). However, both the IP–EP interval and the EP–Hatch interval were about 1h shorter in trial 2 than in trial 1, thus it seems unlikely that prolonged pushing of the hocks against the eggshell was the cause of the increased red hock incidence in trial 2. If the temperature from E0-16 was higher in trial 2, as suggested by the earlier hatching time and lower YFBM, then possibly the metabolism was increased in these embryos, and thereby the CO_2 pressure in the air cell at the end of incubation, stimulating the time of IP (Visschedijk, 1968). Thus these embryos may have been struggling more for quick access to the air cell, thereby pushing the hocks against the eggshell.

It is concluded that egg position in the last 4d of incubation does not affect hatchability, but seems to affect the duration of the hatching process and chick quality.

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chapter 7

Significance of chick quality score in broiler production

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ABSTRACT

The quality of day old chicks is crucial for profitable broiler production, but a difficult trait to define. In research, both qualitative and quantitative measures are used with variable predictive value for subsequent performance. In hatchery practice, chick quality is judged on a binomial scale, as chicks are divided into first grade (Q1- saleable) and second grade (Q2) chicks right after hatch. Incidences and reasons for classifying chicks as Q2, and potential of these chicks for survival and post-hatch performance have hardly been investigated, but may provide information for flock performance. We conducted an experiment to investigate 1) the quality of a broiler flock and the relation with post-hatch flock performance based on a qualitative score (Pasgar©score) of Q1 chicks and based on the incidence of Q2 chicks; and 2) the reasons for classifying chicks as Q2, and the potential of these chicks for survival and post-hatch growth. The performance was followed of Q1 and Q2 chicks obtained from 2 breeder flocks that hatched in 2 different hatching systems (a traditional hatcher or a combined hatching and brooding system, named Patio). Eggs were incubated until embryo day 18, when they were transferred to one of the two hatching systems. At embryo day 21 / post-hatch day 0, all chicks from the hatcher (including Q2 chicks) were brought to Patio, where the hatchery manager marked the Q2 chicks from both flocks and hatching systems and registered apparent reasons for classifying these chicks as Q2. Chick quality was assessed of 100 Q1 chicks from each flock and hatching system. Weights of all chicks were determined at day 0, 7, 21 and 42. There were no correlations between mean Pasgar©score and post-hatch growth or mortality, and suboptimal navel quality was the only quality trait associated with lower post-hatch growth. Growth was clearly affected by breeder flock and hatching system, which could not be linked to mean Pasgar©score or incidence of Q2 chicks. Q2 chicks showed lower post-hatch growth compared to Q1 chicks but effects on flock performance at slaughter weight were limited because early mortality in Q2 chicks was high (62.50% at 7 days). We concluded that chick qualitative scores and the incidence of Q2 chicks may be informative for the quality of incubation, but are not predictive for post-hatch flock performance. Culling Q2 chicks after hatch is well-founded in terms of both animal welfare and profitability.

(*Key words*: chick quality, second grade chicks, hatching system, broiler performance)

IMPLICATIONS

Day old chick quality is crucial for profitable broiler production but a difficult trait to define. In hatchery practice, chick quality is judged on a binomial scale as first grade chicks are placed at farms and second grade chicks are culled after hatch. This study investigates reasons for classifying chicks as second grade, and confirms that the selection of second grade chicks seems justified, based on high mortality in these chicks on farm. In first grade chicks, navel quality is the only physical trait influencing growth, of the five traits evaluated in a practical chick quality score.

INTRODUCTION

High quality of day old chicks is crucial for profitable broiler production (Decuypere and Bruggeman, 2007), but has been found a difficult trait to define (Willemsen *et al.*, 2008). Chick length, chick weight, and qualitative scores were used in previous research with variable predictive value for later performance (Willemsen *et al.*, 2008). In the hatchery, chick quality is currently judged on a binomial scale: right after hatch, a batch of chicks is divided into first grade or saleable chicks and second grade chicks (Decuypere and Bruggeman, 2007). There are limited data on the percentage of second grade chicks in hatchery practice, since these data are not collected routinely, and there is no uniform definition nor method to qualify chicks into first or second grade. Information on the occurrence of second grade chicks as such may be valuable to the hatchery industry because these chicks are culled right after hatch, and therefore of direct economic importance. These data may, however, also be indicative for subsequent flock performance. In scientific research, the percentage of second grade chicks in a flock has been used as an indicator of flock quality and found to vary with egg storage duration (Tona *et al.*, 2004; Reijrink *et al.*, 2010), pre-incubation treatments during storage (Reijrink *et al.*, 2009), and incubation temperature profile (Lourens *et al.*, 2005), and large variation (0.0%-20.6%) was found among these studies. Chicks were classified as second grade when they "were not able to stand straight up or showed visible signs of suboptimal incubation conditions, such as red hocks or rough navels" (Lourens *et al.*, 2005), or as all chicks that were not a first grade chick, "being clean, dry and free from deformities, completely sealed navel, and no yolk sac or residual membrane protruding from the navel" (Tona *et al*, 2004; Reijrink *et al.* 2009, 2010).

Little is known on the reasons for classifying chicks as second grade in hatchery practice, and it has not been investigated whether these chicks actually have smaller chances for survival and perform suboptimally. Therefore, we conducted a study to investigate 1) the quality of a flock of broiler chicks based on a qualitative score of first grade chicks, and based on the incidence of second grade chicks, and to assess the relation with post-hatch flock performance; and 2) the reasons for classifying chicks as second grade, and the potential of these chicks for survival and post-hatch growth. An experiment was conducted in which we followed the performance of first and second grade broiler chicks obtained from two breeder flocks, that hatched in two different

hatching systems. The latter two factors were introduced to investigate whether the relationship between chick quality and post-hatch performance is affected by background of the chicks or different hatching conditions.

MATERIALS AND METHODS

Incubation and chick management

Hatching eggs were obtained from two commercial Ross 308 breeder flocks of different ages: flock A aged 35 wk and flock B aged 53 wk. Eggs were stored for two to three days before being set in a Microclimer 57600 setter (HatchTech, Veenendaal. The Netherlands). A standard single-stage incubation program was used in the setter during which the set point temperature was gradually decreased from 38.1°C at embryo day (**E**) 0 to 37.5°C at d E18. At d E18, eggs were candled and apparently fertilized eggs were randomly divided and transferred to two hatching systems (**SYS**): 10,623 eggs of flock A and 6,554 eggs of flock B were transferred to hatcher baskets and placed in a H192 hatcher (Petersime, Zulte, Belgium), and 10,578 eggs of flock A and 6,527 eggs of flock B remained positioned on the setter trays and were transported to the Patio system. The Patio system is a multi-tiered broiler housing concept consisting of two system rows (Vencomatic BV, Eersel, the Netherlands), where the hatching and rearing phase are combined (Van de Ven *et al.*, 2009). Transport of the eggs to the Patio system occurred in a climate conditioned truck at an air temperature of 30°C, and took 30 min. Upon arrival in the Patio house, eggs on the setter trays were equally distributed over six tiers of one of the two system rows.

A standard hatching program was used in the hatcher, starting at a set point temperature of 37.2°C at d E18 and gradually decreasing to 36.4°C at d E21. In Patio, the set point temperature from d E18 to d E21 was 35.0°C. In preliminary (unpublished) trials, this temperature was found to lead to highest hatchability in Patio. At d E21.5, equivalent to day 0 post-hatch (d0; meaning the normal day of placement in the broiler house), chicks were collected from the hatcher, and subjected to (Dutch) standard hatchery procedures (separation from eggshells and unhatched eggs, counted, and put into transport boxes), however the selection of second grade chicks was omitted. Thus, all chicks that hatched in the hatcher, including second grade chicks, were transported to the Patio system for 30 min in an air conditioned truck and distributed equally over the six tiers of the empty system row upon arrival.

For Patio chicks, the moment of access to feed and water was immediately after hatching, while the Hatchery chicks had access from the moment they were placed in the Patio system at d0, corresponding to common broiler practice. Chicks were given a commercially available diet and raised at standard conditions according to the recommendations of the breeder company, until slaughter weight was reached at d42.

Data collection

At the end of incubation (at d E21.5/d0), hatchability per flock was calculated as the ratio of the

number of all chicks hatched over the number of fertilized eggs transferred to each hatching system at d E18. After placement of the Hatchery chicks in the Patio system at d0, the hatchery manager responsible for selection of second grade chicks in the hatchery (Belgabroed, Veldhoven, the Netherlands), was invited to the Patio house to select all second grade chicks (**Q2**) from both flocks hatched in the two hatching systems. In addition, a sample of approximately 100 first quality chicks (**Q1**) was randomly selected from both flocks and both hatching systems (N=414). The sample of Q1 and all Q2 chicks of both flocks and both hatching systems were tagged with individual numbers, and housed together in one group in a separated compartment (2.3m x 17.3m) in the Patio system, at a stocking density reflecting commercial standards.

The quality of each Q1 chick was assessed using the Pasgar©score (Boerjan, 2002). Based on this score, the quality of each chick was evaluated based on five criteria: 1) Navel condition (black button or leaky navel); 2) Yolk sac (large size of the residual yolk sac); 3) Red hocks (red or swollen hocks); 4) Abnormal beak (red beak or nostrils contaminated with albumen); and 5) Low alertness. For each of the five criteria, one point was subtracted from 10, with chicks scoring 10 being free of any abnormality and five being the lowest score.

For each Q2 chick, the apparent reason for being classified as second grade was registered. Reasons for classifying chicks as second grade were grouped in six categories which are described in Table 1. Chicks that were clearly not viable, were registered and culled directly after examination. Individual weights of all Q1 and Q2 chicks were taken at d0, 7, 21, and 42, and mortality was registered daily.

Table 1. Description of categories of second grade (Q2) chicks.

Category	
1. Physical anomaly	Chicks showing physical anomalies, such as an open skull, crossed beak, four legs
2. Abnormal down	Wet, sticky or short white down
3. Leg deformation	Cripple chicks resulting from leg deformity, or from being trapped in an egg tray
4. Weak appearance	Small or unstable chicks
5. Low quality score	Either multiple criteria were scored based on Pasgar©score, indicating low chick quality without a clear single abnormality, or no obvious reason could be identified for classifying as second grade
6. Dead before examination	Chicks that emerged from the egg, but died before examination of the chicks took place

Data and statistical analyses

Data were analysed with the SAS 9.1 software package (SAS Institute, 2004). In all analyses, the experimental unit was the individual egg (data on hatchability) or chick (data on Q2 incidence, Pasgar©score, chick weights, mortality).

Correlations between Pasgar©score and chick weights at various ages were determined per flock and hatching system combination, using the CORR procedure. When data were normally distributed, Pearson correlations were used; otherwise, Spearman correlations were used. Effects

of single Pasgar©score-criterion and of Q2 category on Q1 and Q2 chick weights, respectively, were assessed using the GENMOD procedure, according to the following model:

$$Y_{ij} = \text{intercept} + \text{Pasgar©score-criterion or Q2-category}_i + e_{ij}$$

Where Y was chick weight at d0, 7, 21, or 42.

Main effects of Hatching system (SYS) and breeder flock (Flock), and the interaction term were analysed using the same procedure, according to the following model:

$$Y_{ijk} = \text{intercept} + \text{Flock}_i + \text{SYS}_j + \text{interaction term} + e_{ijk}$$

where Y was % hatchability, total incidence of Q2 chicks, incidence per Q2 category, % cumulative mortality at d0, 7, and 42, mean Pasgar©score, % scores of individual Pasgar©score-criteria, and chick weights at d0, 7, 21, and 42.

For normally distributed data (chick weights at d0, 7, 21, and 42) the Identity link was used, for binomial data (% hatchability, total incidence of Q2 chicks, incidence per Q2 category, % cumulative mortality, incidence of individual Pasgar©score-criteria) the Logit link function was used; for multinomial data (mean Pasgar©score (5-10)), the Cumulative logit link was used (SAS Institute, 2004). *P*-values ≤ 0.05 were considered statistically significant.

RESULTS

Hatchability

Results on hatchability are summarized in Table 2. For hatchability, a Flock x SYS interaction was observed ($P<0.01$): in flock A, hatchability was higher in Patio than in Hatchery eggs, whereas no significant difference was found in hatchability between systems for flock B.

Table 2. Number of eggs and chicks, hatchability of fertile eggs, and incidence of second grade chicks, of eggs obtained from a breeder flock aged 35 weeks (A) and a flock aged 53 weeks (B), hatched in Hatchery or in Patio conditions.

Flock	System	No. of eggs	No. of chicks	Hatchability	% Q2 chicks
A	Hatchery	10,623	10,126	95.32%[b]	0.96%
	Patio	10,578	10,157	96.02%[a]	1.40%
B	Hatchery	6,554	6,287	95.93%[ab]	0.99%
	Patio	6,527	6,219	95.28%[b]	1.21%
Total		34,282	32,789	95.64%	1.15%

[a, b] *Within columns, values with different superscripts differ significantly (P≤0.05).*

Q1 chicks

Quality x performance. Data on the mean Pasgar©score of the Q1 chicks and incidences of each of the criteria is summarized in Table 3; chick weights at each age are shown in Figure 1. At d7, a total of four chicks had died (0.97%), and at d42, total cumulative mortality was eight chicks (1.93%). Because mortality was low, an association with mean Pasgar©score or any of the Pasgar©score criteria could not be assessed.

Table 3. Mean±SE of Pasgar©scores and incidences of five criteria used in the Pasgar©score of about 100 first grade chicks (Q1) from a breeder flock aged 35 weeks (A) and a flock aged 53 weeks (B), hatched in Hatchery or in Patio conditions.

	Flock	A		B	
	System	Hatchery	Patio	Hatchery	Patio
Criterium		N=107	N=106	N=101	N=100
Pasgar©score		9.72 ± 0.05	9.20 ± 0.10	9.52 ± 0.06	9.27 ± 0.06
Navel condition		24.30%	33.80%	28.71%	39.00%
Yolk sac		1.87%	7.04%	11.88%	12.00%
Red hocks		0.00%	38.03%	1.98%	7.00%
Abnormal beak		0.93%	0.00%	0.99%	1.00%
Low alertness		0.93%	1.41%	3.96%	14.00%

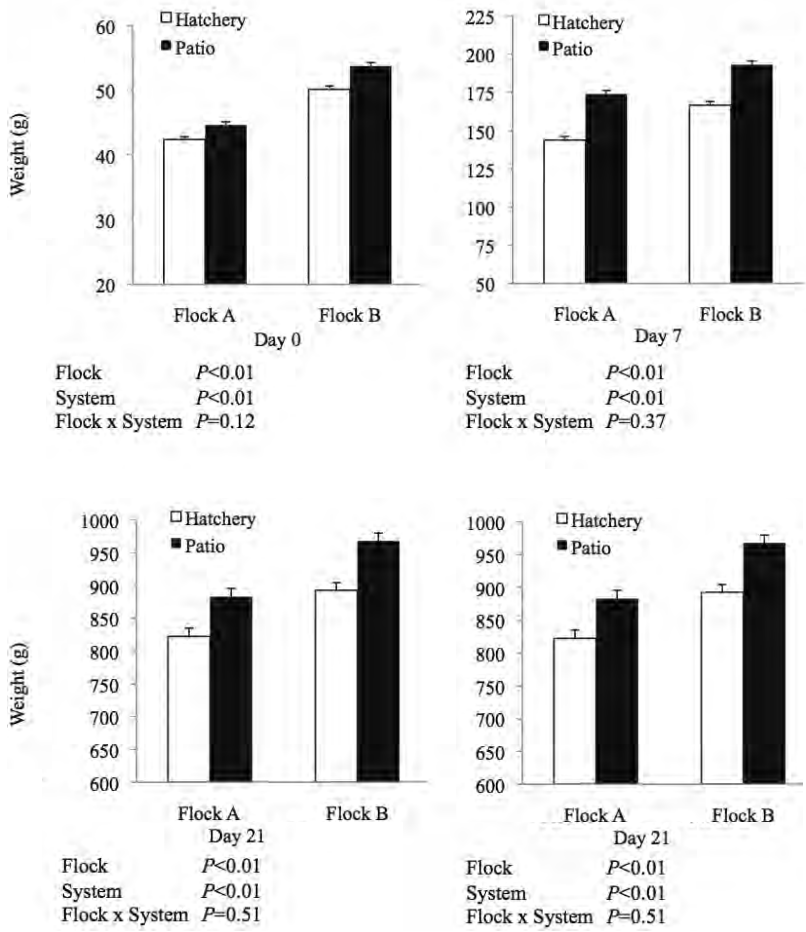

Figure 1. Least Squares Means (and SE) of body weights of first grade chicks (Q1) obtained from a breeder flock aged 35 weeks (A) and a breeder flock aged 53 weeks (B) at four chick ages: day 0, 7, 21 and 42.

Mean weight of all Q1 chicks at d42 was 2,528g. Correlations among mean Pasgar©score and chick weights were not significant at any of the ages tested, irrespective of hatching system or flock (Table 4). Correlations among body weights at various ages were all positive and decreased with increasing time lapse between the measurements.

Table 4. Correlations among Pasgar©score, and chick body weights at day 0, 7, 21 and 42, of about 100 first grade chicks from a breeder flock aged 35 weeks (A) and a flock aged 53 weeks (B), hatched in Hatchery or in Patio conditions.

	Pasgar©score		BW day 0		BW day 7		BW day 21	
Parameter	Hatcher	Patio	Hatcher	Patio	Hatcher	Patio	Hatcher	Patio
BW day 0								
Flock A	-0.05	-0.09						
Flock B	-0.11	-0.10						
BW day 7								
Flock A	-0.05	0.02	0.42**	0.62**				
Flock B	-0.08	-0.13	0.66**	0.61**				
BW day 21								
Flock A	0.04	-0.03	0.33**	0.28*	0.73**	0.68**		
Flock B	0.07	-0.15	0.47**	0.36**	0.70**	0.71**		
BW day 42								
Flock A	0.18	0.06	0.10	0.02	0.20*	0.39**	0.56**	0.75**
Flock B	0.18	-0.15	0.28**	0.19	0.30**	0.44**	0.77**	0.80**

**Correlations significantly different from 0; P≤0.05*
***Correlations significantly different from 0; P≤0.01*

Analyses of effects of individual Pasgar©score-criteria on growth revealed that Yolk sac influenced chick weight at d0 ($P<0.01$): chicks with this abnormality were 4.0g heavier than chicks without this abnormality. There was no effect of Yolk sac on body weight at the other ages tested. Chicks which were scored for Low alertness were 2.7g heavier than chicks without this score ($P=0.03$) at d0, and 8.3g heavier at d7 ($P=0.04$). At later ages, this effect was not apparent. Chick weight at d42 was only affected by Navel condition: chicks scored for suboptimal Navel condition weighed 80g less than chicks without this condition ($P=0.03$). Although the interaction was not significant ($P=0.09$), this effect was remarkably greater in Hatchery chicks (131g) than in Patio chicks (28g). Beak and Red hock score showed no clear effects on growth.

SYS and Flock effects. There were no effects of SYS or Flock on cumulative mortality at d42. The mean Pasgar©score was lower in Patio than in Hatchery chicks (9.25±0.06 vs. 9.63±0.04; $P<0.01$).

There were no SYS x Flock interactions observed for incidences of any of the Pasgar©score-criteria, except for Red hock-score (P<0.01). Incidence of Red hocks was higher in Patio than in Hatchery chicks, and this effect was more pronounced in flock A than in flock B. In chicks from both flocks, the highest incidence was found for Navel condition (31.13%), and was higher in Patio than in Hatchery chicks (36.84% vs. 26.44%; P=0.04). Incidences of Large yolk sacs and Low alertness were higher in flock B than in flock A (P<0.01 for both).
At d0, 7, 21, and 42 bird weights were, respectively, 2.9, 28.3, 68 and 86g higher in Patio than in Hatchery chicks (P<0.01 at all ages). In addition, at d0, 7, 21, and 42 weights were, respectively, 8.4, 21.0, 78 and 159g higher in flock B than in flock A chicks (P<0.01 at all ages). Successive inclusion of d0, 7, and 21 weights as a covariate in the model, showed that growth up to d42 was affected by SYS, but not by Flock.

Q2 chicks

Quality x performance. Of all 32,789 chicks hatched, a total of 376 chicks (1.15%) were classified as Q2 chicks (Table 2). Incidences of each of the six categories of Q2 chicks are summarized in Table 5. Of all Q2 chicks, 123 (32.71%) died at d0, including 36 culled chicks. At d7, a total of 235 chicks had died (62.50%) and between d7-42, another 10 chicks died, resulting in a total cumulative mortality of 65.16%. Mortality from d0-42 was high in all categories (Table 5), with a large range from 26.32% in the chicks with Abnormal down to 97.06% in the chicks with Leg deformities.

Table 5. Incidence of 376 second grade (Q2) chicks per Q2 category (1-6) and day 42 mortality of these chicks, obtained from a breeder flock aged 35 weeks and a flock aged 53 weeks, hatched in Hatchery or in Patio conditions.

		Incidence			Day 42 mortality	
		n	% of all chicks hatched	% of Q2 chicks	N	%
1.	Physical anomaly	33	0.10%	8.78%	29	87.88%
2.	Abnormal down	57	0.17%	15.16%	15	26.32%
3.	Leg deformation	34	0.10%	9.04%	33	97.06%
4.	Weak appearance	46	0.14%	12.23%	26	56.52%
5.	Low quality	119	0.36%	31.65%	55	46.22%
6.	Dead*	87	0.27%	23.14%	87	100.0%
Total		*376*	*1.15%*	*100.00%*	*245*	*65.16%*

**Chicks in category 6 were dead before they were examined.*

Body weights of Q2 chicks at d0, 7, 21, and 42 are shown in Figure 2. Mean weight of all Q2 chicks alive at d42 was 2,270g. The relation between Q2-category and post-hatch growth could not be established because the number of chicks alive in most categories was low after d7.

SYS and Flock effects. Mortality at d0 was higher in flock B than in flock A (40.15% vs. 28.45%; *P*=0.02). Mortality from d0-42 was not affected by SYS or Flock. The incidence of Q2 chicks was higher in Patio than in Hatchery chicks (1.33% vs. 0.97%; *P*<0.01). Overall, based on all chicks hatched, there were more chicks with Physical anomalies (0.14% vs. 0.06%; *P*=0.03), and more chicks of Low quality (0.50% vs. 0.23%; *P*<0.01) in Patio than in Hatchery. The incidence of Physical anomalies was higher in flock A than in flock B (0.14% vs. 0.04%; *P*=0.02), while the incidence of Dead before examination was higher in flock B than in flock A (0.38% vs. 0.19%; *P*<0.01). Incidences of the other categories of Q2 chicks were not affected by SYS or Flock.
SYS did not significantly affect weights of Q2 chicks at any of the ages tested. Q2 chicks of flock B were heavier than chicks of flock A at 0, 7, and 21 days of age (*P*<0.01). At d42, the effect of Flock was not significant anymore (*P*=0.08).

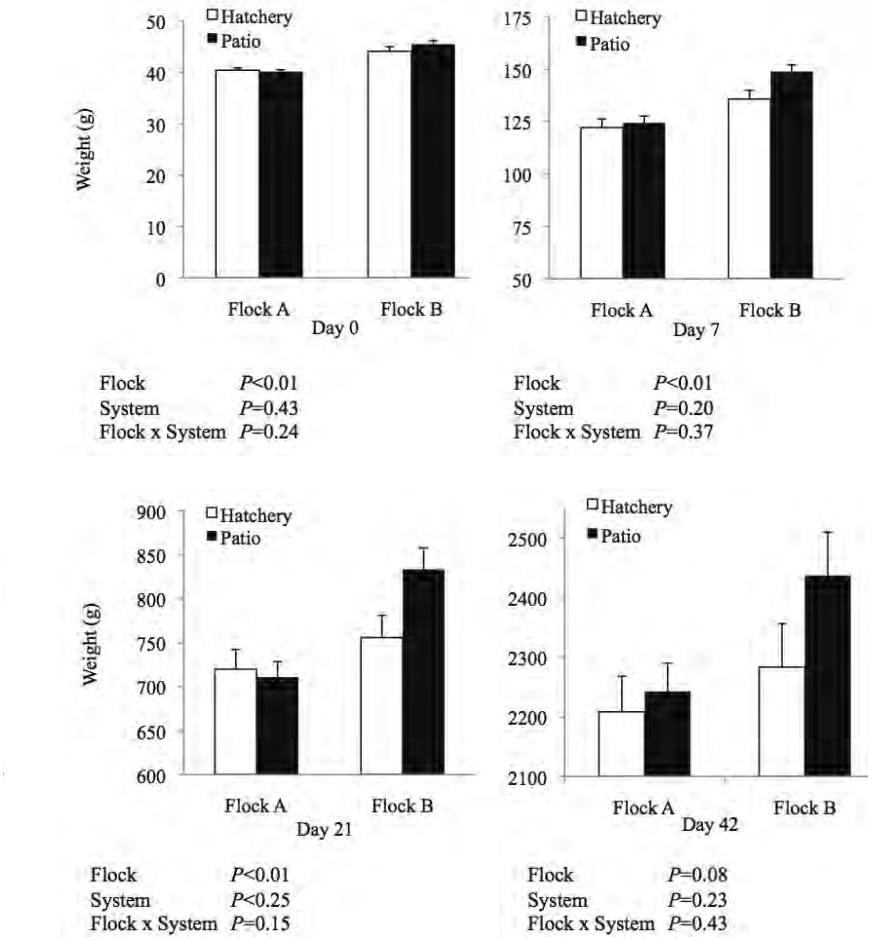

Figure 2. Least Squares Means (and SE) of body weights of second grade (Q2) chicks obtained from a breeder flock aged 35 weeks (A) and a breeder flock aged 53 weeks (B) at four chick ages: day 0, 7, 21 and 42.

DISCUSSION

Q1 chicks

Quality x performance. Overall, there were no correlations between mean Pasgar©score and chick weight at any of the ages tested, irrespective of hatching system or flock. Present findings confirm the conclusions of Willemsen *et al.* (2008) using the Tona-score, that the correlation between qualitative chick scores and post-hatch performance is not meaningful, unless a considerable percentage of second grade chicks is included. However, because second grade chicks are normally removed from the flock at the hatchery, it can be questioned whether chick quality indicators based on physical characteristics are useful as a predictor for post-hatch performance in hatchery practice.

The only Pasgar©score-criteria affecting weight of Q1 chicks after d7 was Navel condition. Of the five criteria evaluated in the Pasgar©score, the incidence of suboptimal Navel condition was highest, in agreement with previous studies where qualitative chick scoring was used (Tona *et al.*, 2004, 2005; Willemsen *et al.*, 2008). Present findings of lower post-hatch growth in chicks with navel conditions at hatch correspond to results of Fasenko and O'Dea (2008), who found 97g and 108g lower body weights at d41 in chicks with Leaky navels or Button navels, respectively, compared to chicks lacking a navel score. It was hypothesized that depressed growth of chicks with navel conditions may be a result of subclinical yolk sac infections (Fasenko and O'Dea, 2008). Furthermore, chicks with navel conditions at hatch were also shown to have reduced duodenal and ileal villi height in the early post-hatch period (Kwalilak *et al.*, 2010). In chicks with immediate access to feed and water after hatch, intestinal development was stimulated compared to chicks that were deprived for 48h (Noy and Sklan, 1999). It can be speculated that the direct post-hatch feed and water access for Patio chicks may have partly overcome the negative effects of suboptimal navel quality on intestinal development and post-hatch growth, since the impact of navel conditions on d42 weight presently found was more pronounced in Hatchery (131g) than in Patio chicks (28g).

SYS and Flock effects. Mean Pasgar©scores were not affected by breeder flock but were lower in Patio than in Hatchery chicks. Navel condition was clearly affected by hatching system, with about 10 percentage points higher incidences in Patio than in Hatchery chicks in both flocks. Suboptimal navel quality in newly hatched chicks was linked to very high (Decuypere and Bruggeman, 2007; Leksrisompong *et al.*, 2007), as well as to very low temperatures (Wilson, 2004) in the hatching phase. Higher incidences of navel conditions in the Patio may be related to lower temperatures than optimum, considering the lower set point temperature in the Patio compared to the hatcher. Red hocks were observed more frequently in Patio than in Hatchery chicks, especially in flock A. Red hocks are assumed to result from prolonged pushing on the eggshell during pipping and hatching (Wilson, 2004), and were related to high temperatures in the last incubation phase (Decuypere and Bruggeman, 2007; Leksrisompong *et al.*, 2007). However, the latter seems unlikely to be the reason for a high Red hock incidence in Patio. Alternatively, low temperature in the final days of incubation (35°C) can lengthen the hatching process compared

to a higher temperature (38°C) (Szdzuy *et al.*, 2008). Thus, it can be speculated that a lower temperature in Patio prolonged the duration of pushing with the hocks on the eggshell during hatching, leading to higher incidences of red hocks.
Although average Pasgar©scores were similar, post-hatch growth was higher in chicks from flock B than from the younger flock A, which seems to correspond to previous findings on breeder age (Ulmer-Franco *et al.*, 2010). In addition, despite a lower mean Pasgar©score, which was mainly explained by higher incidences of Navel conditions and Red hocks, weights were higher in Patio chicks from both flocks at all ages tested. Chicks that hatched in the hatchery were deprived from feed and water until placement in the Patio house at d0, while the chicks that hatched in Patio had immediate access to feed and water. Present results confirm the negative effects of delayed access to feed and water on post-hatch growth in broilers (Gonzales *et al.*, 2003; Careghi *et al.*, 2005).
Similar to the Tona-score (Tona *et al.*, 2003), the Pasgar©score is based on physical quality criteria which are used in hatchery practice to evaluate and optimize incubation conditions (Boerjan, 2002). Present data seem to confirm that different hatching conditions affect these physical criteria, but it is not clear in what way. This should be kept in mind when this type of criteria is used to evaluate incubation conditions.

Q2 chicks

Quality x performance. The mean incidence of 1.15% of second grade chicks presently found agrees with own observations on 20 hatches of two broiler breeds in a commercial hatchery in the Netherlands (a total of 539,493 chicks hatched), where a large variation of second grade chicks (0.25%-2.22%) was found with a mean of 1.06%. The evaluation of second grade chicks in the present study therefore seems to correspond with the procedure applied in hatchery practice. Mortality in Q2 chicks showed similar patterns in both hatching systems and both flocks. Of all Q2 chicks, 34.84% was still alive at d42, meaning that at d42, 0.40% of the total flock (1.15% x 34.84%) consisted of Q2 chicks, which on average weighed 258g less than Q1 chicks on d42. This means that the impact of Q2 chicks on flock level, expressed in underperformance compared to Q1 chicks, was about 33.8 kg (0.40% x 32,789 chicks x 258g) of live weight, or 0.04% of the total live weight of the flock. Thus, the impact of keeping second grade chicks in the flock on post-hatch growth on flock level can be considered negligible, in both flocks and hatching systems. This may be a concern when using the Patio system, where the removal of second grade chicks as a standard management practice is omitted, so all chicks that hatch remain in the flock (Van de Ven *et al.*, 2009). There may however be other reasons for culling second grade chicks, such as risk for infections and negative impacts on animal welfare, feed conversion ratio and flock uniformity. Moreover, leaving second grade chicks in the flock affects the percentage of mortality on farm. Based on present results, total mortality after d0 increased by 0.75% on flock level (1.15% second grade chicks x 65.16% total mortality), compared to the practice where second grade chicks are removed right after hatch, before placement in the broiler house. Increased mortality on farm is intolerable with respect to ethical aspects but also in view of the current European Union directive, which dictates that

mortality in broiler farms should be below 1% + 0.06% multiplied by the slaughter age in days (European Union, 2007).

SYS and Flock effects. The percentage of second grade chicks did not differ between the two flocks of different age, corresponding to findings of Ulmer-Franco *et al.* (2010). In chicks from both flocks, there was a higher incidence of second grade chicks in the Patio than in the Hatchery, which was mainly explained by a higher incidence of chicks with a Physical anomaly and chicks of Low quality in the Patio. Physical anomalies observed in the current study included chicks with four legs, a crossed beak or lacking one or two eyes. These anomalies originate from an early stage in embryonic development (Romanoff, 1960; Wilson, 2004), and are thus unlikely to result from different conditions during the last 3d of incubation.

A higher incidence of Low quality in Patio chicks may be related to different climate conditions during hatching. In a previous study where climate conditions in Patio were compared to the same type of hatcher with the identical set points as used in the present experiment, temperature at egg level in Patio was almost 3°C lower and relative humidity was about 21% lower (Van de Ven *et al.*, 2011). However, signs typical of low temperature in this incubation phase, such as higher incidence of unhealed navels (Wilson, 2004), and of low humidity, such as closed eyes or down stuck to the eyes (Wilson, 2004), were not observed more frequently in Low quality chicks in Patio than in the Hatchery. The reasons for the higher incidence of Low quality chicks in Patio remains unclear.

In conclusion, a qualitative score of first grade chicks or the incidence and background of second grade chicks may be used to evaluate conditions during incubation or hatchery management procedures, but neither of the two methods predicted the overall post-hatch performance of broiler chicks, irrespective of breeder flock or hatching system.

The high (early) mortality in second grade chicks indicates that the common practice of culling of these chicks at the hatchery is justified. Although the impact of leaving second grade chicks in the flock is negligible in terms of growth, it is necessary to apply the practice of culling second grade chicks also in the Patio system for ethical reasons, and in view of European welfare legislation.

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chapter 8

General discussion

INTRODUCTION

Poultry meat consumption is expected to increase by 60% over the next 20 years and will be the most important meat category worldwide by 2030. In 2010, over 53 billion meat type chicken or broilers, were produced globally (Food and Agriculture Organisation, 2010). The success of broiler production is influenced by the quality of day old chicks produced by the hatchery to start with (Tona et al., 2003; Decuypere and Bruggeman, 2007). Additionally, because broiler chicks reach their slaughter weight in a decreased period of time, the importance of a good start grows. It seems that chick quality and performance in terms of liveability and growth potential may not be optimal in current incubation systems and hatchery management procedures. Chicks hatch over a time window of 24-36 hours, and are only removed from the hatcher when the majority of the chicks have hatched, leading to delay in time of first feed and water access for most of the chicks, and associated effects on posthatch growth (Gonzales et al., 2003), immune functioning (Dibner et al., 1998), thermoregulation (Van den Brand et al., 2010), and intestine development (Geyra et al., 2001). In addition, climate conditions may not be optimal during hatching, and it can therefore be questioned whether the broiler chicks' requirements can be met in current hatchery practice.
An alternative hatching system was developed, named Patio, in which the hatching and brooding phase are combined, thereby enabling direct posthatch access to feed and water in broiler practice. Next to earlier feed and water access, hatching conditions in the Patio system differ from those in traditional hatching systems, e.g. with lower temperature, relative humidity, air velocity, larger volume of air per egg, and different egg position during hatching (Chapter 2).
Different conditions in the hatcher and the Patio system in the late prehatch and early posthatch phase, especially early feed and water access, may influence chick quality, physiology, and posthatch growth of broiler chickens. Furthermore, because chicks hatching at different moments in the hatch window are exposed to feed and water deprivation and a different environment for a variable period of time, the spread in hatching time may lead to a variation in physiological status at the moment of chick collection from the hatcher. Moreover, it seems that physiological responses of chicks to delayed access in feed and water varies with age at hatching (Careghi et al., 2005), a factor that was not taken into account in most of the previous studies on delayed feeding, or in hatchery practice (Decuypere and Bruggeman, 2007). Consequently, it was hypothesized that both the length of the hatch window and the moment of hatch within this window lead to a variation in physiological status among chicks of both hatching systems at the typical moment of chick pulling, after 21 days of incubation.

In this thesis, the first aim was to evaluate effects of hatching in the Patio system on hatchability, chick quality, and posthatch growth. The second aim was to determine the physiological status of chicks that hatch at different moments in the hatcher and the Patio system, at hatch, and at the moment of chick collection, at 21.5 d of incubation, using blood plasma hormone and metabolite levels, and organ development as indicators. In addition, effects of hatching time and moment of first feed and water access on posthatch growth were studied.

In this discussion, data from the previous chapters are combined with data collected during additional field trials in the period 2008-2012 to examine the effects of hatching in Patio on true hatchability, and to analyze the different hatching conditions in the hatcher and the Patio system. Next, effects of hatching time and the different conditions in a hatcher and the Patio system, with emphasis on early feed and water access, on perinatal broiler physiology, chick quality, and posthatch growth are discussed. Finally, the conclusions and recommendations of this thesis are given.

HATCHABILITY AND LIVABILITY

In chapter 2, based on the first trials at three locations from 2006-2008, it was concluded that the Patio system as a hatching and brooding system, results in good hatchability, early growth, and livability of broilers. Based on these trials, it was concluded that apparent hatchability was 1.45-1.86% higher in the Patio compared to control eggs of the same parent flock that hatched in the hatcher which could be due to three reasons. First, in the calculation of the hatchability in Patio, possible second grade chicks were included, whereas these were excluded in the calculation of hatchability in the hatchery. Second, in the hatchery, the hatching process was disrupted earlier than in the Patio system meaning that possible late hatching chicks remained in the flock in the Patio, but not in the hatchery. Third, it is possible that the hatchability was truly higher in Patio, which could be related to different climate conditions provided in the two hatching systems, the different egg position during hatching, namely horizontal in the hatcher and vertical in Patio, or other different conditions during hatch.

During the years 2008-2012, another 24 cycles were followed in the Patio system which was described in chapter 2 as location 3. Data were collected on hatchability and the percentage of second grade chicks from eggs of the same breeder flock, which were simultaneously incubated in the same incubator until dE17-18, but hatched in the hatcher or in the Patio system. In addition, during these trials the (unhatched) eggs in the Patio were removed from the system around the same time as in the hatchery. Results of these trials are summarized in Table 1.

Table 1. Summary of trials during 2008-2012 where data of hatchability in the hatcher and Patio were compared, using eggs of the same breeder flock, incubated simultaneously in the same incubator until d E17-18.

	Hatcher	Patio	Difference
Trials	24	24	
Eggs	Unknown	646,291	
Apparent hatchability of fertile eggs	93.04%	-	
Second grade chicks (% of all chicks hatched)	0.99%	-	
Total hatchability of fertile eggs (incl. 2nd grade chicks)	94.03%	95.06%	1.03%
7d mortality of chicks in the farm, excl. 2nd grade chicks[1]	1.32%	1.45%[2]	0.13%
Total 7d mortality of chicks, incl. 2nd grade chicks[1]	2.31%	1.45%[2]	0.86%

[1] *7d mortality was based on data of 22 flocks*
[2] *In Patio, no distinction was made between 7d mortality among first or second grade chicks*

From these data it can be concluded that the hatchability was 1.03% higher in the Patio system, and thus the difference between the systems was smaller than first mentioned in chapter 2 due to the inclusion of second grade chicks in the Patio. A true higher hatchability in the Patio system may have resulted from different conditions during the hatching process.
Mortality at 7d was 0.13% higher in Patio compared to hatcher chicks, which was likely due to the inclusion of second grade chicks in the Patio flocks. In chapter 7, an average incidence of 1.31% of second grade chicks was found in chicks of two parent flocks in the Patio (1.40% in the young and 1.21% in the old flock), and it was shown that 62.50% of these chicks died in the first week. These results indicate that an extra 7d mortality in Patio can be expected of about 1.31% x 62.50% = 0.81%. Different conditions in the early posthatch period may explain why 7 d mortality in Patio was not increased further, even though the removal of second grade chicks as a standard practice directly after hatch is omitted. In the next paragraph the different hatching conditions in the hatcher and the Patio system are summarized, based on the data from previous chapters, and on measurements during additional field trials.

HATCHING CONDITIONS

Climate

A number of papers were published on climate conditions during the first 18 days of incubation in setters (French, 1997; Van Brecht et al., 2003; Elibol and Brake, 2008), however few data is available on climate conditions during hatching in commercial hatchers. During 4 trials, among which 1 was described in chapter 3, the temperature and relative humidity during hatching was simultaneously measured in different types of hatchers, and the Patio system (Figure 1).

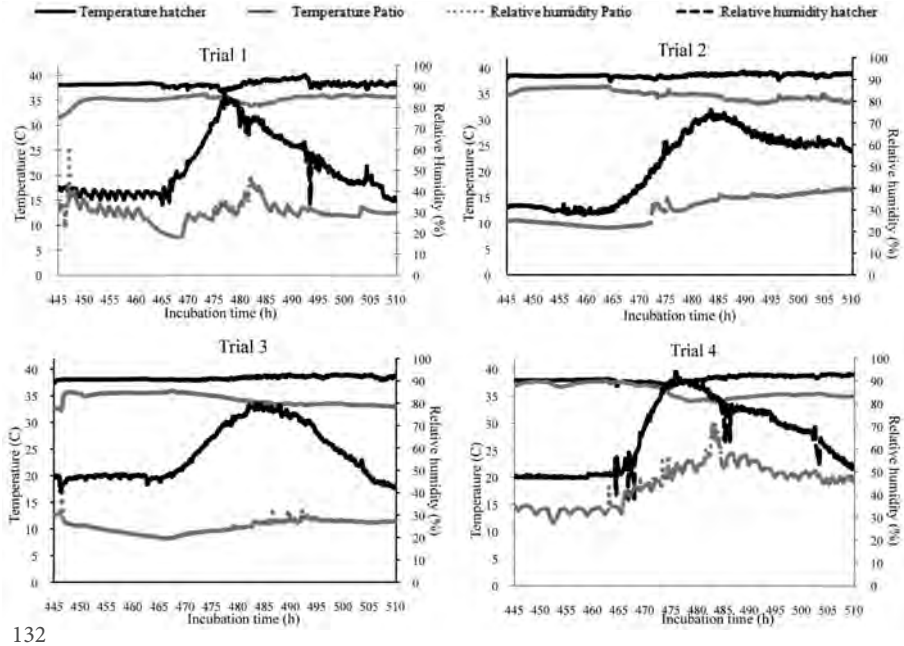

Figure 1. Course of the temperature and relative humidity registered by loggers every 5-10 min during 4 trials in a hatcher (Trial 1, 3 and 4: Petersime, trial 2: HatchTech), and the Patio system, where eggs of the same broiler breeder flock hatched simultaneously.

In each of the trials, eggs in both hatching systems were obtained from the same breeder flock, and incubated in the same incubator until incubation day 18. Measurements of temperature and relative humidity were made every 5-10 min, by 3 loggers (175-H2 Logger, Testo[1]) at various positions in the hatcher and the Patio system. The average air temperature at level of the eggs during the 4 trials depicted in Figure 1 was 38.3°C and 35.2°C in the hatchers and the Patio, respectively. As can be seen in Figure 1, relative humidity in the hatcher peaks around 480 h of incubation in each of the trials. During trial 4, data on the air velocity and CO_2 level were measured in the two hatching systems. Air velocity was recorded every 10 min using thermal anemometers (425 Anemometer, Testo[1]). The probe of the sensor was positioned horizontally with the extreme end penetrating about 5 cm inside the hatcher basket. In Patio, the probe was positioned at approximately 5 cm above one egg tray in Patio. Every 10-15h the probes were placed in a different hatcher basket in the hatcher and above a different egg tray in Patio to get insight in differences in air velocity at different positions within the systems. The CO_2 level was determined every 5 min using a CO_2 logger (Gasman CO_2, Crowcon[2]) in the hatcher and a CO_2 sensor in Patio (MF420-IR-CO2, J. Dittrich Electronic[3]). Before measurements started, the CO_2 measurement equipments were calibrated. Average, minimum and maximum values of air velocity and CO_2 level during this trial are listed in Table 2.

Table 2. Air velocity and CO_2 level measured in a hatcher and the Patio system during hatching of broiler eggs.

	Air velocity, m/s			CO_2, ppm		
System	Mean ± SE	Min	Max	Mean ± SE	Min	Max
Hatcher	0.57 ± 0.01	0.12	1.58	3,586 ± 123	1,200	6,400
Patio	0.08 ± 0.00	0.02	0.20	896 ± 10	720	1,151

These data indicate that the thermal conditions at level of the eggs in both hatching systems differed considerably. According to French (1997), the temperature experienced by the embryo depends on three factors: 1) the incubator temperature, 2) the ability of heat to pass between the incubator and the embryo, and 3) the metabolic heat production of the embryo itself.
In the last days of incubation, the heat produced from broiler eggs of 60-65 g was estimated to be approximately 140 mW (Lourens et al., 2007; Molenaar et al., 2010b). Heat produced by the embryo is dissipated from the egg as sensible heat and as latent heat. At an average egg weight loss of 0.4 g/d (based on 12% egg weight loss between d E0-18 recommended by Aviagen, 2012), latent heat loss for a 60-g egg is about 11.2 mW (French, 1997). Thus the major part of heat produced by the embryo is lost as sensible heat, which may occur by convection, conduction, and radiation. In commercial incubators, heat loss predominantly occurs through convection, which depends largely on the air velocity over the eggs (French, 1997; Lourens, 2001; Van Brecht et al., 2005; Elibol and Brake, 2008). Although it was assumed that radiation is of minor

1 Testo, Almere, The Netherlands
2 Crowcon, Rotterdam, The Netherlands
3 J. Dittrich Electronic, Baden-Baden, Germany

importance in incubators (Van Brecht et al., 2005), and neglected in present calculations, it must be noted that in Patio conditions radiation may contribute to heat loss because materials surrounding the eggs (parts of the Patio system) may have a lower temperature than the eggs.
Because it was previously shown that different incubator temperatures, leading to different eggshell temperatures (37.8 vs. 38.9°C), did not result in significantly different heat production at E16-19 (Lourens et al., 2007), it was assumed that heat production from the eggs in both hatching systems was similar. The eggshell temperature, reflecting the embryo temperature, can be estimated using the equations proposed by Van Brecht et al. (2005):

$$Q_{convection} = h \times A_{eggshell} \times (T_{eggshell} - T_{air})$$

Where h is the convective heat transfer coefficient, calculated as $30.28 \times V^{0.460}$ for vertical air flow over eggs (in hatcher baskets), and $22.55 \times V^{0.387}$ for horizontal air flow over eggs (in incubator trays in Patio; where V is air velocity, for which mean air velocities from Table 2 were used), $A_{eggshell}$ is the surface area of the eggshell, approached based on the equation proposed by Paganelli et al. (1974) and using the average egg weights for the experiments in chapters 3 (58.4g) and 4 (57.6g), $T_{eggshell}$ is the mean surface temperature of the egg, which was to be calculated, and T_{air} is the reference air temperature (38.3°C in the hatcher; 35.2°C in the Patio). Based on these equations, the eggshell temperature in the hatcher was approximately 39.1°C, which is 0.8°C higher than the air temperature, with a range of 38.8 – 40.0°C at the maximum and minimum measured air velocity, respectively. These findings on differences between incubator air temperature and eggshell temperature in the last incubation phase, are in line with field observations of Lourens (2001), French (1997), Elibol and Brake (2008) and Van Brecht et al. (2005) on eggshell temperatures in commercial setters.
Following the same equations, the eggshell temperature in the Patio system was approximately 37.5°C, which is 2.3°C higher than the air temperature, with a range of 36.8 – 39.2°C at maximum and minimum measured air velocity, respectively. To verify these theoretical assumptions, eggshell temperatures were monitored during one trial in the hatcher on 6 eggs in different hatcher baskets during the first 36 h after egg transfer (unpublished data). Average eggshell temperature was 38.8°C, with minimum and maximum values of 35.6 and 40.1°C, respectively. During the same trial, mean eggshell temperature in the Patio system based on 450 manual measurements was 37.1°C, with minimum and maximum values of 34.0 and 38.9°C, respectively. Summarizing, these data suggest that temperatures at egg level during hatching were approximately 1.7°C higher in the hatcher than in the Patio system.
The differences in relative humidity are probably of minor importance in the comparison of thermal conditions in the hatcher and the Patio, because the contribution of air humidity to heat exchange of an egg with its environment is not significant at temperatures operated in the present trials (Van Brecht et al., 2005). The average CO_2 level was almost four times higher in the hatcher than in the Patio, based on data from trial 4. A peak of 6,400 ppm (Table 2) was registered at day 20 of incubation in the hatcher, while in Patio, the CO_2 level remained rather constant. In a previous study (Buys et al., 1998), an elevated ambient CO_2 level (4,000 ppm) from d E14-19 increased thyroid hormone levels at the end of incubation compared to control eggs incubated at

2,000 ppm, and advanced hatching time in an ascites sensitive broiler line, but not in an ascites resistant line. Based on these findings, it can be suggested that the increased CO_2 level in the hatcher compared to the Patio system may have affected chick physiology, although the duration of exposure to different CO_2 levels was short compared to the study of Buys et al. (1998).
Summarizing, late prehatch conditions differ considerably between the hatcher and the Patio system, but due to higher air velocity in the hatcher, the temperatures at eggshell level are smaller than differences in air temperature. Relative humidity is probably of minor importance, but higher CO_2 levels in the hatcher might affect chick physiology at hatch.
In the period between hatch and the moment of chick pulling, different temperatures in the hatching systems were likely to affect the physiology of chicks. Newly hatched unfed chicks were shown to have a narrow thermoneutral zone of 34 – 37°C (Misson, 1976). Higher temperatures led to increases in evaporative heat loss, and water loss increases from 80 mg / h at 37°C to 300 mg / h at 40°C, and it may thus be expected that the stay in the hatcher or the Patio system in the early posthatch period affects BW development in newly hatched chicks. However increased air velocity helps broilers maintaining their body temperature in high temperatures (Drury and Siegel, 1966), and at the mean air velocity of 0.57 m/s in the hatcher (Table 2), a windchill effect of approximately 1°C can be expected for young chicks (Czarick and Lacy, 1999). Relative humidity (20 or 80%) had very little effect on the metabolism of one-day-old chicks (Misson, 1976), and was probably of minor importance when comparing conditions in the hatcher and Patio. In the paragraph 'Effects of hatching system and early feed and water access' the influences of the late prehatch and early posthatch conditions in the two hatching systems on physiology and posthatch growth are discussed.

Feed and water access

In common hatchery practice, no feed or water is supplied to chicks in the hatching baskets, leading to delay in first access to feed and water until placement in the farm. In the experiments of chapter 5, early hatched chicks were collected between 465-469 h and late hatched chicks between 493-498 h of incubation in both the hatcher and the Patio system. Consequently, at the moment of chick collection from the hatcher, which occurred around 512-515 h, a variation in chick age was found between 15-49 h. Adding the time for chick counting, vaccination, packaging and transportation to the farm of about 4-6 h (Hatchery managers, personal communication), this means that in hatchery practice, early chicks would be deprived for approximately 54 h, and late chicks for 20 h before access to feed and water. As described in chapter 2, chicks find access to feed and water as they fall on the bedding from the incubation trays after hatching in the Patio system. Chick growth normally commences approximately 24 h after the first intake of feed and water (Noy and Sklan, 1999b), thus differences in BW may be expected between the two hatching systems at the time of chick collection.

Other conditions

As concluded in chapter 2, apart from climate conditions, several other conditions during hatching

differed between the hatcher and the Patio system. These conditions are briefly described here and summarized in Table 3.

- ***Egg position***. During hatching, eggs remained in a vertical position in Patio with the air cell up, whereas in the hatcher eggs were placed horizontally. From the data in chapter 6, it can be concluded that egg position in this phase of incubation does not influence hatchability, but positioning eggs vertically with the air cell up results in a 3-4 h longer IP-EP interval, and hatch occurs 2h later compared with eggs placed horizontally.
- ***Airborne fluff and dust.*** The differences in air velocity and air volume in the hatcher and the Patio system, mentioned in Table 2, may have led to a difference in bacterial load. It was shown that large amounts of airborne fluff and dust are generated during hatching in hatching cabinets, and this was found to be one of the primary sources for Salmonella contamination of broilers (Bailey et al., 1992). High air velocities in the hatcher carry the dust generated during hatch along with pathogens that may be present on or inside the eggs and recirculate them throughout the cabinet during the last 2 d of incubation (Mitchell et al., 2002). Consequently, due to a lower air velocity, bacterial load in the Patio may have been lower in comparison to the hatcher.
- ***Air volume***. The volume of air in the hatching system is approximately 4-34 times higher in Patio than in conventional hatchers (Chapter 2). Very little data can be found on effects of different air volume on embryo or chick development, but it can be expected that indirect effects of larger air volume in the Patio system are the lower CO_2 level, and the possibility to operate lower air temperature and velocity during hatching.
- ***Lighting.*** Whereas in the Patio system the lights are on during d E18-21, hatching in hatchers commonly occurs in darkness. In a recent study, no effects were observed of providing continuous light, or 12 h light daily, on hatchability, growth, mortality, and other production parameters in broilers compared to broilers that were incubated in darkness, but subtle effects on feeding behavior were found (Archer et al., 2009). In other studies, provision of light in the last phase of incubation led to asymmetrical stimulation of the eyes due to the position of the embryo, covering its left eye with its body and thus exposing only the right eye to light (Rogers, 2008). The asymmetrical stimulation was shown to result in brain lateralization and was related to different social cognition in chicks (Daisley et al., 2009; Wichman et al., 2009).
- ***Background noise.*** In the hatcher, there is background noise of fans and engines at a loudness of approximately 85 db, whereas in Patio a background noise of approximately 56 db can be heard (unpublished observations). It was hypothesized that a louder background noise could mask the clicking sounds that can be heard due to rapid breathing by hatching embryos, and were shown to synchronize hatching behavior (Vince, 1970; 1984). In 2 experiments, in which the levels of the background noise in the two hatching systems were mimicked, hatching time was slightly delayed and the hatch window was larger in the eggs that hatched in presence of the hatcher noise (Van de Ven et al., 2010).

- ***Chick handling.*** Although conditions studied in the experiments in this thesis were as similar to practical conditions as possible, it must be noted that the chicks evaluated in chapters 3, 4, and 5 were not subjected to standard hatchery procedures such as chick handling, counting, or transport in the chick trucks. In chapter 7, chicks from the hatcher were subjected to standard chick handling procedures, except for the selection of second grade chicks. In a survey in large modern hatcheries in the UK, it was concluded that consequences for welfare of day old chicks that are transferred over automatic chick handling systems are limited (Knowles et al., 2004). The maximum height of drops from one chick conveyor belt to another in the systems studied was in a range of 35-50 cm, depending on the hatchery, which is similar to the drop that Patio chicks experience when falling from the setter trays onto the litter, approximately 45 cm below (Chapter 2). The height of the drop did not significantly affect the degree of disorientation among the chicks, and the authors suggested that due to the light body weight and the cushioning effect of the chicks' fluff, the impact on the chick is limited (Knowles et al., 2004).

Table 3. Summary of different conditions during hatching in a hatcher or in the Patio system.

Variable	Hatcher	Patio
Climate		
Air temperature, average	38.3°C	35.2°C
Relative humidity, average	55.6%	32.0%
Air velocity, average	0.57 m/s	0.08 m/s
CO_2 level, average	3,586 ppm	896 ppm
Air volume per egg	0.6-0.9 dm^3	4.4-34.1 dm^3
Time of feed and water access	19 – 53 h posthatch	Directly posthatch
Egg position	Horizontal	Vertical
Other conditions		
Light	Off	On
Background noise	85 dB	56 dB
Chick handling	Yes	No
2^{nd} grade chicks	Excluded	Included

In addition to these different conditions it must be noted that for the experiments in this thesis, chicks in the hatcher were not subjected to disinfection by formaldehyde gas. In commercial hatcheries, hydrogen peroxide or formaldehyde gas is typically infused into hatching cabinets to reduce airborne pathogens (Mitchell and Waltman, 2003). The use of formaldehyde during hatching is damaging to the respiratory epithelium of lungs of newly hatched chicken (Sander

et al., 1995), and affects posthatch performance (Zulkifi et al., 1999). Because its use for this purpose will probably be prohibited in the Netherlands in the near future, it was omitted in our experiments.

From the data presented here, it becomes clear that apart from the hatching climate, conditions during the late prehatch and early posthatch phase differ considerably between conventional hatchery practice and procedures in the Patio system. The experiments and discussion of this thesis mainly focus on the effects of the climate, the moment of first feed and water access, and egg position in the two hatching systems on hatchability, chick quality, physiology, and posthatch growth. It may be speculated that apart from effects on these parameters, consequences for chicken health and social behavior may be expected.

EFFECTS OF HATCHING TIME

The period between collection of the early and the late chicks in the experiments of chapters 3, 4, 5, and 6, indicated a hatch window of approximately 28 – 30h, despite the fact that eggs were obtained from a single breeder flock, stored for 2-3 days, and incubated in one incubator. In the following paragraphs, the physiological variation in chicks of different hatching times, and consequences for posthatch performance are discussed.

Perinatal physiology

Several studies demonstrated differences in liveability and posthatch growth in chicks hatching at different times in a batch of eggs (Williams et al., 1951; Kingston, 1979; Hager and Beane, 1983; Careghi et al., 2005). However, the differences in chick physiology and chick quality related to this variation in hatching time were hardly investigated. Iqbal et al. (1989) and Careghi et al. (2005) demonstrated differences in levels of thyroid hormones in newly hatched chicks of different hatching moments, and Fairchild and Christensen (2000) found different organ development and plasma glucose levels in newly hatched poults, as determined right after hatch. In this thesis, physiological variation due to different hatching times was demonstrated in chapters 3 and 4, and slightly different posthatch growth was shown in chapter 5.

In the experiments of chapters 3 and 4, the yolk-free body mass did not differ significantly among hatching times, but organ weights increased with hatching time, concurrent with a decrease in residual yolk weights. Fairchild and Christensen (2000) also observed an increase in heart and liver weights with increased hatching time. In a comparison among avian species, increasing organ weights and decreasing yolk weights with increasing incubation periods was suggested to point at a more advanced maturation of organs (Ricklefs, 1987). It could be speculated that having more developed organs at hatch would be beneficial for later life, but from the results of chapter 3 and 5, it seems this is not related to higher potential for posthatch growth.

Next to differential organ development, plasma hormone concentrations and metabolites also

varied with hatching time. Christensen et al. (2000, 2001) demonstrated that the time of hatch, but also the duration of the preceding hatching process itself, showed a large variation, which was associated with differences in energy availability in this phase. Consequently, it was speculated that later hatching chicks experience a prolonged process of hatching, and that these chicks would have lower energy stores prior to hatching (Christensen et al., 2000; Everaert et al., 2008; Willemsen et al., 2010b). The data on pipping and hatching times of the experiments in chapter 6 were used to analyse the association between length of the internal pipping (IP) to external pipping (EP) interval, and the EP to hatch interval, and the time of hatching (Figure 2).

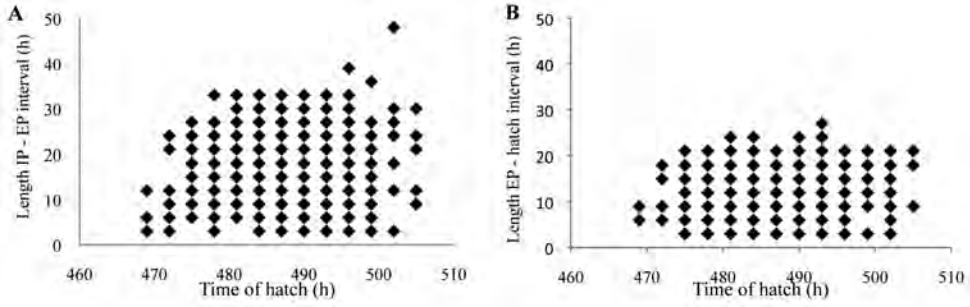

Figure 2. Relationship between the length of the interval between internal pipping (IP) and external pipping (EP), and time of hatch (A), and association between the length of the interval between EP and hatching, and time of hatch (B), based on results of chapter 5.

Pearson correlation coefficients were analysed using the CORR procedure (SAS Institute, 2004), and appeared low between the intervals and time of hatch (R^2 for IP-EP interval and hatch = 0.053; R^2 for EP-hatch interval and hatch = 0.010). Consequently, later hatching chicks in a batch of eggs do not seem to experience increased intervals between external pipping and hatching, which agrees with observations in naturally incubated Red Junglefowl eggs, where EP-hatch intervals for early and late hatching chicks were 11.0 and 11.2h, respectively (Meijer and Siemers, 1993).

Despite similar IP-EP and EP-hatch intervals it may still be possible that chicks hatching at different moments in the hatch window showed different energy use for hatching. Indicators of energy metabolism measured in the present thesis were plasma glucose, lactate, uric acid, and liver glycogen content. Hatching activities are mainly fueled by glucose provided from glycogen in the liver, muscles (Freeman, 1969), and in the yolk sac (Yadgari and Uni, 2012). Glycogen reserves increase during embryonic development, and peak around d E18 (Willemsen et al., 2010b; Molenaar et al., 2010b; Yadgari and Uni, 2012), prior to the start of the hatching process. In chapter 4, no differences in glycogen levels were found among hatching times shortly after hatch, corresponding to data of Fairchild and Christensen (2000), who found similar glycogen levels in liver and heart among poults of different hatching times. The mobilization of glycogen during hatching results in an increase in plasma glucose between pipping and hatch (Freeman,

1965, 1969; Christensen et al., 2001). Higher plasma glucose levels were observed in chicks hatching late in the hatch window (Chapters 3 and 4), which agrees to findings in poults of Fairchild and Christensen (2000). These authors concluded that the similar glycogen levels in poults from early and late hatching times indicated that the lower blood glucose in early hatched poults was not due to a lack of resources.

As hatching progresses, oxygen becomes limiting and energy comes from anaerobic glycolysis leading to increases in plasma lactate (Tazawa et al., 1983; Høiby et al., 1987; Moran Jr., 2007). Hypoxic conditions end at the moment of external pipping (EP), and as soon as oxygen availability is restored, birds can recycle lactate back into glucose in the liver (De Oliveira et al., 2008). Higher blood glucose in late hatching chicks may indicate that these chicks used less glucose during the hatching process and produced less lactate, as shown in chapter 3, because of a lower metabolism. On the other hand, in chapter 4 a high peak in plasma lactate was observed in midterm chicks from the hatcher, and otherwise no trends were observed. It was previously (Chapter 3) suggested that the combination of high glucose and low lactate in late chicks may point at an increased EP-hatch interval, enabling late chicks to recycle lactate back into glucose before emergence from the egg, but as the EP-hatch interval seemed similar for chicks of different hatching times, variations in lactate levels are more likely to be explained by variable production rather than by variations in removal of lactate. The background for the observed trends in lactate remains unclear.

Possibly, prolonged incubation times and associated increases in yolk absorption in late hatching chicks might explain the higher plasma glucose compared to earlier hatching chicks, as the yolk sac was shown to be a major glucose synthesizing organ, using amino acids and glycerol through the gluconeogenesis pathway, and possibly releasing free glucose into the blood (Yadgari and Uni, 2012).

In chapter 3 of this thesis, lower thyroid levels and a lower T_3:T_4 ratio were found in late hatching chicks, in agreement to earlier findings in chicks hatching late in the hatch window of the same hatch (Decuypere and Kühn, 1988; Iqbal et al. 1989; Careghi et al., 2005). It was suggested that an increased conversion of T_4 to T_3 is required during the pipping phase as a stimulus for hatching, and that lower T_3 levels were responsible for delays in hatching (Iqbal et al., 1989). However, in the experiment of chapter 4 the opposite was found: in late hatching chicks from both hatching systems higher T_3 and T_4 levels were observed, whereas no differences in T_3:T_4 ratio were observed among hatching times. The background of these opposite results is not known, but from these data it can be speculated that although lower T_3 levels were previously associated with delayed hatching, they are not the reason for delayed hatching.

It must be noted that in the experiments of chapters 3, 4, and 5, chicks were evaluated from one batch of eggs from a single breeder flock, but these batches were, as in hatchery practice, very large. Moreover, in both studies, eggs were incubated from E0-18 in large scale setters (capacity of 76,800 eggs). Van Brecht et al. (2003) noticed temperature gradients of approximately 1.1°C during E0-16 in a similar type of setter, and hence differences in hatching time in the present experiments can be related to temperature gradients in the setter during the first 18 days of

incubation. It is not known to what extent the present variation in hatching time was due to different environmental conditions during incubation. Additional factors that may influence variation in hatching time are egg handling procedures prior to incubation at the breeder farm or at the hatchery. For instance, frequency of egg collection in the breeder house, especially at house temperatures above the physiological zero when embryonic development occurs, may lead to variation in embryo development (Fasenko et al., 1991; Fasenko et al., 1999). Moreover, additional intrinsic egg factors influence the time of hatch. For example, when incubated at the same temperature, heavier eggs require more time for incubation than lighter eggs (Meijer and Siemers, 1993), first eggs in a clutch tend to contain more advanced embryos than subsequent eggs (Fasenko et al., 1992), and, possibly due to the same mechanism, eggs laid in the afternoon tend to hatch earlier than morning eggs (Yannakopoulos et al., 1988).

In conclusion, within a batch of eggs from the same origin, intrinsic factors influence hatching time, which can unlikely be controlled in hatchery practice. Because of these factors, and due to the temperature gradients in modern incubators, a spread in hatch of about 30 h in commercial incubation as in the current experiments seems inevitable. Based on these findings, it is clear that variation in hatching time in hatchery practice leads to physiological variation at the time of hatch, and that late hatching chicks seem more matured, based on smaller residual yolk and higher organ weights.

Growout period

Several studies showed that chicks of different moments within the hatch window have different posthatch growth potential (Williams et al., 1951; Kingston, 1979; Hager and Beane, 1983; Careghi et al., 2005). The results of the 3 experiments described in chapter 5 confirm that, compared with earlier hatching chicks, late hatching chicks show slightly reduced growth when considered from the moment of chick pulling (d0) up to d7, regardless of direct posthatch presence of feed and water. In previous studies where delayed hatching was linked to lower growth up to d7, it was suggested that unknown intrinsic factors, expressed in different (thyroid) hormone levels, lead to lower chick quality and growth potential (Tona et al., 2003; Careghi et al., 2005; Decuypere and Bruggeman, 2007). However, the current findings indicate that the lower growth from d0-7 in later hatching chicks may be explained by the relatively large impact of the initial posthatch hours. In these hours weight loss occurs, both in fed and in unfed chicks, probably due to evaporation of moisture from the down right after hatch (Chapters 4 and 5), and furthermore growth is initiated only 24 h after the first feed and water intake (Noy and Sklan, 1999b).

On the other hand, after d7 early male chicks showed higher growth up to slaughter age than later hatching males in experiment 1 of chapter 5, and in the other two experiments, advanced growth up to slaughter age was observed in midterm chicks, in comparison to early and late hatching chicks. These data may suggest that there is some variation in growth potential in relation to time of hatching in the hatch window. In other avian species, it was suggested that mother birds allocate androgens to eggs depending on the sequence within the clutch, thereby influencing the

competitiveness of chicks hatching from these eggs, and consequently modifying the hierarchy of siblings within one brood (based on the 'brood-reduction hypothesis' by Lack, 1947). In the black-headed gull, a semi-precocial species, increasing yolk testosterone was found to accelerate embryonic development and hence decrease incubation time, and increase posthatch growth (Eising et al., 2001; Eising and Groothuis, 2003), but in the precocial (Leghorn) chicken, no differences in yolk testosterone were observed within clutches of 6 eggs (Elf and Fivizzani, 2002). It was however suggested that differential androgen deposition in eggs within a clutch may depend on food availability for the mother, because this influences the need for brood reduction and sibling hierarchy (Groothuis et al., 2005). The chickens in the study of Elf and Fivizzani (2002) were fed ad libitum, and it can be speculated that the common practice of feed restriction in broiler breeders (De Jong et al., 2002) may affect androgen allocation to eggs and differences in growth potential among broiler chicks of different hatching times. In the present study, there was no information on the background of eggs regarding the sequence in a clutch, and studies on this matter with broiler eggs are not known.

EFFECTS OF HATCHING SYSTEM AND EARLY FEED AND WATER ACCESS

It was previously concluded that climate conditions in the last incubation phase differed considerably between the hatcher and the Patio system. Apart from lower weights of several organs in Hatcher compared to Patio chicks in the experiment of chapter 4, no clear differences in physiology at hatching were observed between the two hatching systems in the present experiments. Previously it was shown that an air temperature of 3°C lower than considered optimal (37.6°C) during the period d E16 – 18, had little effect on broiler chick physiology at hatch (Willemsen et al., 2010b). Based on the experiments of chapter 5, posthatch growth after d7 was not affected by the different conditions in the two hatching systems between egg transfer and hatching, and it can be concluded that the temperatures provided in both systems are within acceptable limits for the embryo. It must also be noted that the first chicks hatched approximately 25h after egg transfer (Chapters 3, 4, and 5), and considering that internal pipping occurs about 30h earlier (using data of chapter 6), a majority of the chicks probably started the pipping phase shortly after egg transfer. As it was shown that embryos in the pipping stages already possess a limited degree of thermoregulatory capacity (Szdzuy et al., 2008), effects of different temperatures in this stage may be smaller than in studies where temperature treatments were applied in earlier stages as in the study of Willemsen et al. (2010b). On the other hand, it is possible that the lower hatchability in the hatcher is related to higher temperatures than optimum, which may occur at spots where air velocity is low. Willemsen et al. (2010b) showed depressed hatchability at 40.6°C air temperature.

Although effects of the two hatching systems on chick physiology appeared limited at the moment of hatch, chick physiology and chick quality between the two hatching systems differed

considerably at the typical moment of chick pulling from the hatcher which typically occurs at d E21.5. The variation in chick physiology seemed mainly related to the variation in chick age at that time, and the presence or absence of feed in the Patio system and the hatcher, respectively.

Perinatal physiology

The results from the experiment in chapter 4 showed conclusively that at the time of chick pulling, large variation exists between chicks of various hatching times that hatched in a hatcher or in the Patio system. It must be noted that in this experiment, effects of early posthatch climate conditions in both hatching systems on chick physiology could not be separated from effects of first access to feed and water. As expected, because feed and water was provided, the weights of Patio chicks increased between hatching and d E21.5. Early Patio chicks gained 5.3g in 47h, similar to the 5.0g increase in 48 h in neonatal chicks shown by Noy and Sklan (1999b), which concurred with an intake of 6.5g feed. The BW in early hatcher chicks, deprived of feed and water, decreased by 4.3g in early chicks which was accompanied by a decrease in residual yolk weights by 4.2g. In deprived broiler chicks, Noy and Sklan (1999b) observed a reduction in body weight of 3.5g and in yolk weight of 3.8 g during 48 h. These authors found that the fat and protein that was used from the yolk corresponded to 5.3 kcal energy / day, and concluded that this value represents the energy use for tissue growth and metabolism of the feed-deprived chick. In the present experiments, late chicks hatched at about 495h of incubation and chick pulling occurred around 515h of incubation, and hence chicks stayed in the hatching system for at least 20h posthatch. In order to minimize energy and water loss and increase yolk use for body development, it can be suggested that conditions during the early posthatch period until chick pulling must be matched to requirements of the hatchling. Minimal metabolism of unfed newly hatched chicks occurs at 35°C, and because water loss due to evaporation increases above 37°C (Misson, 1976), it may be suggested that hatcher temperatures should be lowered, although it must be kept in mind that the higher air velocity alleviates the effects of the higher temperature (Czarick and Lacy, 1999).

Although organ development occurred both in deprived hatcher and fed Patio chicks, the increase in organ weights was higher in Patio chicks. The sum of the increase of all organ weights (heart, lung, stomach, intestine, spleen, and Bursa of Fabricius) together was 2.0, 1.8, and 1.6 g in early, midterm, and late deprived hatcher chicks, and 6.6, 4.5, and 2.8g in early, midterm, and late fed Patio chicks. It was suggested that the decreased yolk content in the early posthatch phase accounts for most of the changes observed in overall body composition (Noy and Sklan, 2001). In chicks from both hatching systems, the early organ weight increases were predominantly due to stomach and intestine weight gain, which accounted for 83.2% and 83.5% of the organ growth in the Hatcher and Patio chicks, respectively. Preferential growth of the small intestine was also observed by Noy and Sklan (1999b).

Residual yolk uptake was slightly increased in Patio compared to Hatcher chicks. Previously, it was suggested that yolk absorption is stimulated through the presence of exogenous material in the gastrointestinal tract, the physical pressure on the yolk opposed by bulk in the intestine, and

the stimulation of peristaltic activity of the intestine (Noy and Sklan, 1999b; 2001). Additionally, higher yolk uptake in the fed Patio birds may be related to increased thyroid hormone levels, which stimulate the use of lipids and free fatty acids.
Hepatic glycogen decreased in the early posthatch period in hatcher chicks to minimum values of 1.3, 1.2, and 3.6 mg for early, midterm, and late chicks, respectively, which is in line with the 1.5 mg observed by Molenaar et al. (2011b) at 48 h posthatch in broiler chicks. Present findings in the feed deprived hatcher chicks indicate that hepatic glycogen is mobilized, probably to be converted to glucose (Miova et al., 2008) and used as a metabolic fuel. The fact that hepatic glycogen levels were similar among early and midterm hatcher chicks suggests that after 32h of deprivation to which the midterm chicks were subjected, alternative sources were used for energy. In the early chicks which were deprived for 47 h, increased plasma uric acid levels were found, similar to findings of Molenaar et al. (2011b), and pointing at additional gluconeogenesis of glucogenic amino acids, or that the amino acid skeleton was used for immediate ATP production. Apart from the yolk sac (Yadgari and Uni, 2012), a source of amino acid to be used for gluconeogenesis in fasted hatcher chicks may be from tissue proteolyses, whereas in fed Patio chicks, substrates may come from exogenous feed intake. The lower yolk uptake and T_3 levels in the early posthatch period in hatcher chicks point at metabolic adaptations to preserve nutritional reserves during fasting, and the minimum hepatic glycogen levels may emphasize the need to match the environmental conditions to the newly hatched chicks, in order to prevent energy use by muscle catabolism. In Patio chicks, increased body and organ weights, yolk uptake, glucose, and T_3 levels indicate an advanced metabolic rate and physiological development, which is likely due to early feeding, and these developments were more pronounced in earlier hatching chicks. As a consequence, at the moment of chick pulling at d E21.5, early chicks in Patio seem at advantage compared to later hatching chicks, whereas early hatcher chicks appear less developed than later hatching chicks that were fasted for a shorter period of time.

Summarizing, chicks that hatched in Patio, having immediate access to feed and water, showed increased physiological development compared with chicks that hatched in a hatcher. Hatcher chicks seem to retard organ development in the absence of feed, resulting in large differences at the time of chick pulling between both systems, which are most pronounced in the earlier hatching chicks. At hatch, later hatching chicks seem more developed based on higher organ weights and lower residual yolk weights, but the opposite was found at chick pulling time in Patio chicks. In the hatcher, early hatching chicks are subjected to feed and water deprivation for a longer period of time, therefore these chicks seem at disadvantage compared to later hatching chicks.
It is clear that by evaluating physiology of chicks at the time of chick pulling rather than right after hatch, effects of hatching time and length of exposure to posthatch conditions are confounded. This is a factor that has often been ignored in studies in this field till now.

Chick quality

In chapter 7, it was concluded that chick quality score was lower in Patio than in the hatcher,

but no relation was found between the average quality score and posthatch growth or mortality. Of all single traits evaluated using the Pasgar©score, the only trait affecting posthatch growth was navel quality Suboptimal navel quality showed higher incidence in Patio chicks. It was not completely clear how conditions in the Patio resulted in a poor navel quality, because an increased incidence of bad navel quality was previously linked both to higher than optimum incubation temperatures (>38.9°C; Leksrisompong *et al.*, 2007; Molenaar et al., 2011a), and to too low temperatures (temperature not specified; Wilson, 2004) in the hatching phase. Furthermore, as was hypothesized in chapter 5, by falling of the chick from the setter tray shortly after emergence from the egg, remnants of the chorioallantoic arteries were possibly torn, thereby increasing the risk for unhealed navels and leaving a small scab of blood, which is the most common type of unhealed navel (Fasenko and O'Dea, 2008). In the hatchers, chicks stay in the basket after hatch, and there is little risk for sudden tearing of the chorioallantoic arteries.

Consequences of bad navel quality for growth were more pronounced in hatcher than in Patio chicks. Previously, it was hypothesized that depressed growth of chicks with navel conditions may be a result of subclinical yolk sac infections (Fasenko and O'Dea, 2008). Unhealed navels were suggested to be a port of entry for pathogens, resulting in yolk sac infections, but this mechanism was not proven. It was shown that bacterial load in hatchers is high (Mitchell et al., 2002; Mitchell and Waltman, 2003), and the risks for transmission of pathogens may have been increased due to the high air speeds, in comparison to conditions in Patio (Table 2). It must be noted that in the experiments of this thesis, no formaldehyde was applied during hatching in the hatcher, which probably lead to higher bacterial load in the hatcher than in common hatchery practice, where formaldehyde is commonly used for disinfection. Possibly, omitting the disinfection procedure aggravated the effects of poor navel quality.

Growout period

In chapters 3, 5, and 7, growth was examined of chickens that hatched in a hatcher or in Patio, but were all housed in the Patio system from d0 onward. Therefore, only effects of the late prehatch and early posthatch conditions, up to chick pulling at d0, were evaluated. From the results of chapters 3 and 5, it was concluded that the different climate conditions in the perinatal phase of broilers in a hatcher or the Patio do not lead to differential growth posthatch after d7. In previous studies, higher incubation temperatures (>38.8°C) led to depressed posthatch growth in broilers (Lourens et al., 2005; Hulet et al., 2007; Leksrisompong et al., 2009) compared to chicks incubated at normal temperature (37.6-37.8°C). It may be concluded that the temperatures experienced by the chicks in the hatcher and the Patio were within acceptable limits for posthatch performance, or that temperatures in both systems were not optimal.

The early feed and water access for Patio chicks resulted in different broiler weights that persisted up to slaughter age (Chapters 5 and 7). In agreement to previous literature, differences in weight between fed and deprived birds became smaller over time (Hager and Beane, 1983; Stamps and Andrews, 1995; Vieira and Moran, 1999; Gonzales et al., 2003; Joseph and Moran, 2005; Henderson et al., 2008: Kornasio et al., 2011). It was shown that growth rates between chicks

that were directly fed and chicks fasted for 24h after placement in the farm were similar after d21 (Vieira and Moran, 1999). Several previous studies on early post-hatch feed deprivation subjected the deprived treatment group to a 24-48h period of feed deprivation after placement at the farm (Dibner et al., 1998; Stamps and Andrews, 1995; Vieira and Moran, 1999; Gonzales et al., 2003), which means that part of the deprived chicks in these studies were subjected to feed and water deprivation for extreme long periods. Results from the experiments in this thesis indicate that in common hatchery practice in the Netherlands, chicks are exposed to periods of 23 – 54h of feed deprivation, depending on the moment of hatch in de hatch window, and including chick handling and transportation time to the farm of about 4-6h.

The enhanced growth in chickens that were fed directly posthatch was previously ascribed to increased early posthatch development of the digestive tract (Bigot et al., 2003), thereby increasing absorption capacity. Furthermore, an advanced development of the immune system due to direct feed access, as was demonstrated in broiler chicks (Dibner et al., 1998; Bar Shira et al., 2005), may have contributed to improved posthatch growth.

In experiment 1 of chapter 5, the BW difference between deprived Hatcher and fed Patio midterm chicks that all stayed in Patio shortly after hatch was 56 g (2.2%) at d45. In experiment 3 of chapter 5 and in the two flocks followed in chapter 7, where chicks remained in the hatching system where they had hatched until chick pulling, the differences were 155g at d41 (7.8%; exp. 3 of chapter 5), 86 g at d42 (3.6%; in the young flock in chapter 7) and 85g at d42 (3.3%; in the old flock in chapter 7), respectively. Combining these results, it may be suggested that effects in the early posthatch period in the hatching cabinet aggravate the consequences of feed and water deprivation on growth in the growout period. On the other hand no effects were observed on growth posthatch of the stay in the early posthatch period in the hatcher or the Patio when none of the chicks had access to feed and water (experiment 2 of chapter 5). As previously noted, chicks in the experiments of chapter 5 were not subjected to the chick handling procedures in the hatchery, which may influence pathogen pressure and lead to different growth posthatch.

CONCLUSIONS

Based on findings of the present thesis, the following conclusions can be drawn:

- By evaluating physiology of chicks at the time of chick pulling rather than right after hatch, effects of hatching time and length of exposure to posthatch conditions are confounded.
- Chicks with early feed access, as in Patio, show enhanced physiological developments, resulting in larger organ weights, higher hepatic glycogen reserves, and lower corticosterone levels at the moment of chick pulling, compared with feed deprived chicks, as in the hatcher.
- Improved posthatch growth in Patio chicks compared to hatcher chicks was largely due to earlier access to feed and water.
- Despite considerable differences in climate and other environmental factors, the effects of hatching system on physiology of broiler chickens at hatch and growth performance up to

slaughter age are limited.
- Apart from higher growth from d0-7 in early and midterm vs late hatching chicks, effects of hatching time on growth were not clear from this thesis.
- Navel quality is the most important trait of qualitative day old chick scores because it is the only trait evaluated to affect growth up to slaughter age.
- Using a chick qualitative score based on physical traits and the incidence of second grade chicks, chick quality was lower in Patio than in hatcher chicks.
- Chick qualitative scores and the incidence of second grade chicks may be informative for the quality of incubation, but are not predictive for post-hatch flock performance.
- Based on a very high 7-d mortality, the selection of second grade chicks in hatchery practice is defendable in terms of welfare.
- After adjustment for second grade and late hatching chicks, hatchability is still improved in the Patio system compared to eggs hatched in the hatcher, which may be related to different climate conditions.

RECOMMENDATIONS

Based on the findings of this thesis, several implications for present practice in the hatchery and the Patio farm can be formulated:
- The findings from this thesis confirm that early access to feed and water is crucial for optimal posthatch growth. Several solutions have been proposed to allow earlier feeding in the hatching system by providing hatchling supplements at the hatchery (reviewed by Willemsen et al., 2010a), which was shown beneficial for posthatch performance (Noy and Sklan, 1999a). Similarly, combining the hatching and the brooding period as in Patio also enables direct feed and water access after hatch. A different alternative may be through the use of in ovo feeding, which involves the injection of an isotonic nutrient solution into the embryonic amnion at embryo day E17-18, and allows embryos to consume externally administered nutrients together with the amniotic fluid absorbed just prior to pipping (Uni and Ferket, 2004). Furthemore, for present hatchery practice, it may be possible to advance the time of chick pulling in hatchery practice. In the present experiments chick pulling occurred 15 to 21 hours after the last chicks had hatched, hence a batch of chicks may be considered ready for chick pulling approximately 9-15h earlier, taking into account a period of 6 h for drying of the last hatching chicks.
- Navel quality was the only quality trait leading to lower body weight at slaughter age (Chapter 7), and may lead to high costs in broiler practice. Because the use of formaldehyde for disinfection during hatching may prohibited in the Netherlands in the near future, risks for yolk sac infections may increase in hatcheries. At the same time the use of antibiotics in broiler production must decrease (CBS, PBL, Wageningen UR, 2011), which may emphasize the need for optimizing navel quality even further. Previous studies demonstrated that

temperatures higher than optimum lead to increased incidences of poor navel quality, but the exact causes for lower navel quality are not clear (Molenaar et al., 2010a). In addition, the present results indicate that by improving conditions in the posthatch phase, e.g. reducing bacterial load in the hatcher, or enabling direct feed and water access, negative effects of poor navel quality can be reduced.

- Little research has been conducted till now on the conditions in commercially used hatchers. From present data, it appears that the thermal environment for eggs in a hatcher varies due to differences in air temperature and velocity, and temperature at level of the eggs may become too high at some spots. A break out analysis of unhatched eggs is a practical method to get insight in the background of suboptimal hatchability, similar to the study of Elibol and Brake (2008) on effects of different egg locations in setters. By increasing uniformity of air flows in hatchers the climate may be optimized, though this may be difficult when the capacity of the hatchers is fully used and the density of eggs per quantity of air is high. In addition, because after approximately 495 incubation hours the last chicks have hatched, conditions may be adjusted to match the hatchlings' needs (e.g. lowering the temperature) in order to prevent energy use due to evaporation of heat.
- The selection of second grade chicks in hatchery practice, which was found to be approximately 1%, seems reasonable based on the high 7-d mortality observed in chapter 7. However, for modern hatcheries, producing over 1 million birds per week (Knowles et al., 2004), the occurrence of second grade chicks is a costly problem and a welfare issue. Research should reveal if the incidence of second grade chicks can be decreased by adjusting climate conditions or management procedures. Furthermore, it is recommended to Patio farm managers to select the second grade chicks, or chicks that appear not viable, soon after hatching for welfare reasons.
- It can be advised to include the moment of hatching in the hatch window in studies on perinatal broiler physiology, because earlier or later hatching may mask the effects of treatments studied. Maternal androgen levels in yolk may provide further information on the background of the variation in hatching times.
- As summarized in this discussion, several conditions differ between the hatcher and the Patio system, that were not investigated in this thesis, but may be important for chick physiology, health or performance in later life. Especially the air quality during hatching with regard to dust and fluff contents may be important for the health of broiler flocks.

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Summary

SUMMARY

Good broiler performance up to slaughter age can only be achieved with high quality chicks. It seems that chick quality and performance in terms of livability and growth potential may not be optimal in current incubation systems and hatchery management procedures. Because chicks hatch over a time window of 24-36 hours and are only removed from the hatcher when the majority of the chicks has hatched, they are subjected to delay in time of first feed and water access, with associated negative effects on chick development and posthatch growth. In addition, climate conditions may not be optimal during hatching and in the period between hatching and chick removal from the hatcher. It can therefore be questioned whether the broiler chicks' requirements can be met in current hatchery practice. An alternative hatching system was developed, named Patio, in which the hatching and brooding phase are combined, thereby enabling direct posthatch access to feed and water in broiler practice. Next to earlier feed and water access, hatching conditions in the Patio system differ from those in traditional hatching systems, e.g. with lower temperature, relative humidity, air velocity, higher volume of air per egg, and a different egg position during hatching. Different conditions in the hatcher and the Patio system in the late prehatch and early posthatch phase of broiler chickens, especially early feed and water access, may influence chick quality, physiology, and growth. Furthermore, chicks hatching at different moments in the hatch window are exposed to feed and water deprivation and a different environment for a variable period of time, which may lead to a variation in physiological status at the typical moment of chick collection from the hatcher. In this thesis, the first aim was to evaluate effects of hatching in the Patio system on hatchability, chick quality, and posthatch growth. The second aim was to determine the physiological status of chicks of different hatching moments in the hatcher and the Patio system, at hatch, and at the moment of chick collection (21.5 d of incubation), using blood plasma hormone and metabolite levels, and organ development as indicators. Effects of hatching time and moment of first feed and water access on posthatch growth were also included in this thesis.

In chapter 2, based on the first trials from 2006-2008 (780,686 eggs), it was concluded that hatching and brooding in the Patio system results in good hatchability of fertile eggs, early growth, and livability of broilers. In these trials, apparent hatchability was 1.45-1.86% higher in the Patio compared to control eggs of the same parent flock that hatched in the hatcher. This apparent higher hatchability could be due to 1) the inclusion of possible second grade chicks in Patio, which were excluded in the calculation of hatchability in the hatchery, or 2) to an earlier disruption of the hatching process in the hatchery, meaning that possible late hatching chicks remained in the flock in the Patio but not in the hatchery. In the years 2008-2012, another 24 cycles (646,291 eggs) were evaluated for data on hatchability in the two hatching systems, with correction for these two factors: the hatching process was ended at the same time, and hatchability was adjusted for second grade chicks in the hatchery. From these data it was concluded that the hatchability was 1.03% higher in the Patio system, which can probably be explained by different conditions between the hatcher and the Patio system during the hatching

process. Different hatching conditions in the hatcher and the Patio system were examined during several experiments and field trials (Chapter 3 and 5, General discussion). Apart from lower temperature, relative humidity, CO_2 concentration and air velocity in Patio, eggs hatch from a vertical position in Patio, and are placed horizontally in baskets in the hatcher. In 2 experiments (Chapter 6) it was shown that egg position in the last incubation phase did not affect hatchability, and thus the difference in hatchability is most probably due to different climate conditions.

In 2 experiments (Chapter 3 and 4), perinatal broiler chick physiology was investigated in the hatcher and the Patio system, of chicks that hatched early, midterm, or late in the hatch window. Time of hatching in the hatch window affected physiology at hatch: in both experiments, longer incubation times led to increased organ weights, concurrent with a decrease in residual yolk weight, suggesting a higher level of maturation in late hatching chicks. Furthermore, plasma thyroid levels decreased with hatching time in chapter 3, indicating an decreased metabolism, whereas the opposite was found in chapter 4. Few data in literature are available on the physiological background of chicks hatching of different hatching times in chicken. In the available studies lower thyroid levels were associated with delayed hatching, but present data suggest they are not the reason for later hatching. Plasma glucose levels increased with hatching time in both experiments, which could be due to increased yolk absorption in the late chicks because the yolk sac was shown to be a major gluconeogenic organ. Growth from d0-7 was lower in late compared to early and midterm chicks, both in chicks that had direct posthatch feed access and in chicks that were deprived until d21.5 of incubation, but after d7 no clear effects on growth were observed of hatching time.

Hatching system had no clear effect on broiler chick physiology at hatch. In chapter 3, no effects were observed on organ development, but in chapter 4, most absolute and relative organ weights evaluated were smaller in hatcher than in Patio chicks, which could be related to the higher temperature in the hatcher, and which corresponds to previous literature on higher incubation temperatures (>38.9°C) in the second half of incubation. At the typical time of chick collection at d21.5 of incubation, large variation was observed between chicks of various hatching times that hatched in the hatcher or in the Patio system. Chicks that hatched in Patio, having immediate access to feed and water, showed larger body and organ weights, higher hepatic glycogen reserves, higher plasma glucose and T_3 levels, and lower corticosterone levels compared with the hatcher chicks which were fasted during the period between hatching and chick collection. Slightly lower yolk uptake and T_3 levels in the hatcher chicks pointed at metabolic adaptations to preserve nutritional reserves during the early period of fasting.

Growth posthatch was hardly influenced by hatching system (Chapter 3 and 5), when the factor time of feed access was excluded. These results seem in contrast to previous studies, where posthatch growth was depressed after exposure to high incubation temperatures as found in the hatcher vs. the Patio. However, the exposure time to the higher temperature in the hatcher vs. the lower temperature in Patio was shorter (2-3d) than in most other studies, and the temperature in the hatcher was lower than the high temperature treatments in other studies. It

appears that the temperatures in both hatching systems were within acceptable limits for the embryo and newly hatched chick with regard to posthatch growth.
It was hypothesized that the different climate conditions in the hatcher and the Patio system could affect chick quality at the moment of chick collection at 21.5 d of incubation. An additional question was whether omitting the selection of second grade chicks as a standard procedure as in the Patio would increase mortality in the posthatch phase. In chapter 7, chick quality was evaluated based on the Pasgar©score and the incidence of second grade chicks from chicks of 2 parent flocks that hatched in a hatcher or the Patio system. Using the Pasgar©score, chicks are evaluated based on 4 physical characteristics, and on alertness of the chick, hence 5 traits in total. In chicks from both parent flocks, the Pasgar©score was lower and the percentage of second grade chicks was higher in Patio than in the hatcher chicks, suggesting lower chick quality in the Patio system. Despite lower quality scores, posthatch growth was higher in Patio chicks, due to earlier access to feed and water, and mortality was not affected by hatching system. Within the hatching systems, mean Pasgar©score was not related to posthatch growth, and the only quality trait affecting growth posthatch was navel condition. Chicks with a poor navel quality at hatch, showed approximately 80g lower BW at slaughter age, and this effect appeared more pronounced in hatcher than in Patio chicks. Among second grade chicks, 7-d mortality was 62.5% and it was concluded that the culling of second grade chicks as a standard procedure in the hatchery, is defendable in terms of welfare.
Other conditions that differ during hatching in the hatcher and the Patio system were not investigated in present thesis, but could affect chick development, behavior and growth posthatch. Amongst others, these conditions are the exposure to light during hatching and to lower levels of background noise in Patio, whereas hatcher chicks hatch in darkness with a higher level of background noise. Furthermore, levels of dust and fluff in the air are probably different between the hatching systems. Future studies may evaluate the effects of these different hatching conditions.

From this thesis, it can be concluded that despite considerable differences in climate and other environmental factors, the effects of hatching in a hatcher or in Patio on broiler chick physiology at hatch are limited. Chicks with early feed access, as in Patio, show enhanced physiological developments in the early posthatch phase, resulting in larger organ weights, higher hepatic glycogen reserves, and higher plasma glucose and T_3 levels at the moment of chick collection, compared with feed deprived chicks, as in the hatcher. It became clear that by evaluating physiology of chicks at the time of chick collection rather than right after hatch, effects of hatching time and length of exposure to posthatch conditions are confounded.
The improved posthatch growth in Patio chicks compared to hatcher chicks demonstrated in several experiments is largely due to earlier access to feed and water. Apart from higher growth from d0-7 in early and midterm vs late hatching chicks, effects of hatching time on posthatch growth were not clear from the experiments in this thesis.
Using a chick qualitative score based on physical traits and the incidence of second grade chicks,

chick quality is lower in Patio than in hatcher chicks. Although these scores may be informative for the quality of incubation, they are not predictive for post-hatch flock performance. Navel quality is the most important trait of current qualitative day old chick scores because it affects growth up to slaughter age. Furthermore it can be concluded that the selection of second grade chicks in hatchery practice is defendable in terms of welfare, based on a very high 7-d mortality. Finally, after adjustment for second grade and late hatching chicks, hatchability is still improved in the Patio system compared to eggs hatched in the hatcher, which is not due to different egg position, but probably to different climate conditions.

Samenvatting

SAMENVATTING

Goede diertechnische resultaten van een koppel vleeskuikens tot aan slachtleeftijd kan enkel bereikt worden met hoge kwaliteit eendagskuikens. De kwaliteit en het technisch presteren van de kuikens in termen van vitaliteit en groei lijken echter niet optimaal in huidige broedsystemen en broederij management procedures. Het uitkomen van kuikens in een uitkomstkast vindt plaats over een periode van 24-36 uur (de 'hatch window'). Omdat de kuikens pas uit de uitkomstkast verwijderd worden wanneer een overgrote meerderheid is uitgekomen, over het algemeen na broeddag 21,5, worden ze blootgesteld aan uitstel van voer- en watertoegang, met daaraan gerelateerde negatieve effecten op kuikenkwaliteit en postnatale groei. Daarnaast lijken klimaatsomstandigheden niet optimaal in de periode tussen uitkomst uit het ei en het verwijderen van kuikens uit de uitkomstkast. Het is de vraag of aan de behoeften van het vleeskuiken voldaan kan worden in de huidige broederijpraktijk. Een alternatief uitkomstsysteem is ontwikkeld, genaamd Patio. Hierin worden de uitkomst- en opfokperiode gecombineerd, waardoor toegang tot voer en water direct na uitkomst mogelijk wordt gemaakt. Naast de vroege voer- en watertoegang zijn de condities zoals luchttemperatuur, relatieve luchtvochtigheid en luchtsnelheid, volume lucht per ei, en de positie van het ei tijdens het uitkomstproces anders dan die in traditionele uitkomstsystemen. Deze verschillende omstandigheden tijdens de late prenatale en vroege postnatale fase van vleeskuikens, met nadruk op de eerste voer- en watertoegang, kunnen de kuikenkwaliteit, fysiologie en groei beïnvloeden. Bovendien worden kuikens die op verschillende momenten uit het ei komen voor een verschillende tijdsduur blootgesteld aan voer- en watertoegang of onthouding, en aan de verschillende klimaatsomstandigheden, wat zou kunnen leiden tot een variatie in de fysiologische status op het typische moment van kuikenverzameling, na broeddag 21,5. Het eerste doel van dit onderzoek was om de effecten te evalueren van het uit laten komen van eieren in een Patiosysteem op het uitkomstpercentage, de kuikenkwaliteit, en de postnatale groei. Het tweede doel was om de fysiologische status van kuikens te bepalen die op verschillende momenten uitkomen in de uitkomstkast en in het Patiosysteem. De fysiologische status is beoordeeld op het moment van uitkomst en op het moment van kuikenverzameling uit de uitkomstkast (na 21,5 dag broeden), waarbij hormoon en metaboliet niveaus in het bloedplasma en orgaanontwikkeling als indicatoren zijn gebruikt. Daarnaast zijn in dit onderzoek de effecten van uitkomstmoment en moment van eerste voer- en watertoegang op postnatale groei geëvalueerd.

In hoofdstuk 2 werd op basis van de eerste testrondes in de jaren 2006-2008 (780.686 eieren) geconcludeerd, dat het uitkomen en opfokken van vleeskuikens in het Patiosysteem resulteert in een goed uitkomstpercentage van de eieren, en een goede vroege groei en vitaliteit van de vleeskuikens. In deze testrondes was het ogenschijnlijke uitkomstpercentage in het Patiosysteem 1,45-1,86% hoger vergeleken met de referentie eieren, die van hetzelfde moederdierkoppel afkomstig waren, en uitgekomen waren in de uitkomstkast. Het ogenschijnlijk hogere uitkomstpercentage in Patio zou verklaard kunnen worden door 1) een aantal 2^{e} soort kuikens

in het Patiosysteem, welke in de broederij in een standaardprocedure verwijderd worden voordat het uitkomstpercentage vastgesteld wordt, hetgeen in het Patiosysteem niet standaard gebeurt; 2) een vroegere onderbreking van het uitkomstproces in de broederij in vergelijking met de Patio, waardoor laat uitkomende kuikens wel in het Patiosysteem blijven maar niet in de broederij. In de periode 2008-2012 zijn nog 24 rondes (646.291 eieren) geëvalueerd voor data van uitkomstpercentages in de twee uitkomstsystemen, waarbij voor deze factoren werd gecorrigeerd. Het uitkomstpercentage werd gecorrigeerd voor de 2^{e} soort kuikens en het uitkomstproces werd op hetzelfde moment beëindigd. Uit deze data werd geconcludeerd dat het uitkomstpercentage 1,03% hoger lag in het Patiosysteem, wat waarschijnlijk verklaard kan worden door de verschillende omstandigheden tijdens het uitkomstproces in de uitkomstkast en het Patiosysteem. De verschillende uitkomstomstandigheden zijn onderzocht tijdens enkele experimenten en praktijkproeven (Hoofdstuk 3 en 5, General discussion). Naast een lagere luchttemperatuur, relatieve luchtvochtigheid, CO_2 concentratie en luchtsnelheid in de Patio, komen eieren uit vanuit een verticale positie in het Patiosysteem, terwijl ze horizontaal zijn geplaatst in uitkomstbakken in de uitkomstkast. In 2 experimenten (Hoofdstuk 6) werd aangetoond dat eipositie in de laatste fase van het broeden geen effect heeft op het uitkomstpercentage, en dus kan het verschil in uitkomstpercentage waarschijnlijk gerelateerd worden aan verschillende klimaatsomstandigheden.

In 2 experimenten (Hoofdstuk 3 en 4) werd de perinatale fysiologie bestudeerd van kuikens die vroeg, midden, of laat tijdens de hatch window uit het ei kwamen in de uitkomstkast en in het Patiosysteem. Het tijdstip van uitkomst binnen de hatch window beïnvloedde de kuikenfysiologie bij uitkomst: in beide experimenten leidde een langere broedduur tot een toename in orgaangewichten en een afname in het resterend dooiergewicht, wat een indicatie is van een hoger niveau van maturatie in de laat uitkomende kuikens. Daarnaast ging een langere broedduur gepaard met lagere plasma schildklierhormoon niveaus in het experiment van hoofdstuk 3, wat een lager metabolisme in de late kuikens suggereert, terwijl het tegenovergestelde gevonden werd in het experiment van hoofdstuk 4. Uit de literatuur zijn weinig data bekend over de fysiologische achtergrond van kuikens die op verschillende momenten binnen de hatch window uit het ei komen. In de beschikbare studies worden lagere schildklierhormoon niveaus geassocieerd met later uitkomen, maar de huidige data suggereren dat zij niet de oorzaak zijn van het later uitkomen. Plasma glucose niveaus namen toe met de broedduur in beide experimenten, wat gerelateerd zou kunnen zijn aan de toegenomen dooieropname in de late kuikens, omdat aangetoond is dat de dooierzak een belangrijk gluconeogeen orgaan is. De groei van de kuikens van dag 0-7 was in vergelijking met de vroege en midden kuikens, lager bij de late kuikens. Dit betrof zowel kuikens die direct toegang hadden tot voer en water als de kuikens die gevast hadden tot broeddag 21,5. Na dag 7 werd geen duidelijk effect meer waargenomen van uitkomsttijdstip op groei.

Het uitkomstsysteem had op het moment van uitkomst geen duidelijke effecten op de kuikenfysiologie. In het experiment van hoofdstuk 3 werden geen effecten op orgaanontwikkeling aangetoond, echter in het experiment van hoofdstuk 4 was het

merendeel van de bestudeerde absolute en relatieve orgaangewichten kleiner in de kuikens uit de uitkomstkast dan in de Patiokuikens. Dit zou verband kunnen hebben met de hogere luchttemperatuur in de uitkomstkast, wat overeen komt met eerdere literatuur over effecten van hogere broedtemperaturen (>38,9°C) tijdens de tweede helft van het broedproces.
Op het moment van kuikenverzameling na 21,5 dag broeden werd een grote variatie aangetoond in kuikenfysiologie tussen de verschillende uitkomstmomenten in de uitkomstkast en in het Patiosysteem. Kuikens die uitkwamen in het Patiosysteem en daarmee direct voer- en watertoegang hadden, hadden hogere lichaams- en orgaangewichten, hogere leverglycogeen reserves, hogere plasma glucose en T_3 niveaus, en lagere corticosteronniveaus vergeleken met de broederijkuikens die hadden gevast tijdens de periode tussen uitkomst en kuikenverzameling. Een enigszins lagere dooieropname en lagere T_3 niveaus in de broederijkuikens wijzen op metabole aanpassingen om nutritionele reserves te handhaven tijdens de vroege periode van vasten.
Postnatale groei werd nauwelijks beïnvloed door uitkomstsysteem (Hoofdstuk 3 en 5) wanneer het eerste moment van voer- en wateropname gelijk werd gehouden voor de kuikens. Deze resultaten lijken in contrast met eerdere literatuur, waarbij postnatale groei negatief beïnvloed werd na blootstelling aan hoge ten opzichte van lagere broedtemperaturen, zoals in de uitkomstkast ten opzichte van het Patiosysteem. Echter, de duur van blootstelling aan de hogere temperatuur in de uitkomstkast vs. de lagere temperatuur in het Patiosysteem was korter (2-3 dagen) dan in de meeste eerdere studies. Ook was de gemiddelde temperatuur in de uitkomstkast lager dan in de hoge temperatuur behandelingen in eerdere onderzoeken. De temperaturen in beide uitkomstsystemen lijken dus, voor wat betreft de postnatale groei, binnen acceptabele grenzen te blijven voor het embryo en het pas uitgekomen kuiken.
De hypothese werd gesteld dat de verschillende klimaatsomstandigheden in de uitkomstkast en het Patiosysteem de kuikenkwaliteit zouden beïnvloeden op het moment van kuikenverzameling na 21,5 dag broeden. Een vraag daarbij was of het achterwege laten van de selectie van 2^e soort kuikens als een standaardprocedure in het Patiosysteem zou leiden tot een hogere sterfte in de postnatale fase. In het experiment van hoofdstuk 7 werd kuikenkwaliteit geëvalueerd op basis van de Pasgar©score en de incidentie 2^e soort kuikens, bij kuikens afkomstig van 2 moederdierkoppels, die uitgekomen waren in de uitkomstkast of in het Patiosysteem. Bij de Pasgar©score worden kuikens op 5 kenmerken geëvalueerd: op de alertheid van het kuiken en op 4 fysieke kenmerken. In kuikens van beide moederdierkoppels was de Pasgar©score van de Patiokuikens lager, en het percentage 2^e soort kuikens hoger dan in de broederijkuikens, wat duidt op een lagere kuikenkwaliteit in het Patiosysteem. Ondanks een lagere kuikenkwaliteit was de postnatale groei, door een vroegere toegang tot voer en water, hoger bij de Patiokuikens, en was er geen verschil in kuikensterfte tussen de uitkomstsystemen. Binnen de uitkomstsystemen kon de gemiddelde Pasgar©score niet gerelateerd worden aan postnatale groei, en enkel de navelkwaliteit bleek van invloed op de groei. De kuikens met een slechte navelkwaliteit hadden ongeveer 80 gram lagere lichaamsgewichten op slachtleeftijd, en dit effect was meer uitgesproken in kuikens uit de uitkomstkast dan in Patiokuikens. Onder de 2^e soort kuikens was de 7-daagse sterfte 62,5%, en was vergelijkbaar bij kuikens uit beide uitkomstsystemen.

Geconcludeerd werd dat het verwijderen van 2e soort kuikens als een standaard procedure in de broederij verdedigbaar is in termen van welzijn.
Een aantal andere omstandigheden die verschillen tijdens het uitkomstproces in de uitkomstkast en het Patiosysteem zijn niet bestudeerd in het huidige onderzoek, maar zouden kuikenontwikkeling, gedrag en postnatale groei kunnen beïnvloeden. Deze omstandigheden zijn onder andere de blootstelling aan licht en aan lagere niveaus van achtergrondgeluid in Patio, en aan een donkere omgeving en een hoger niveau van achtergrondgeluid in de uitkomstkast. Daarnaast zijn waarschijnlijk de concentraties aan stof en dons in de lucht verschillend tussen de twee uitkomstsystemen. Toekomstige studies zouden de effecten van deze verschillende uitkomstomstandigheden kunnen evalueren.

Op basis van het huidige proefschrift kan geconcludeerd worden dat ondanks aanzienlijke verschillen in klimaatscondities en andere omstandigheden, de effecten van het uitkomen in een uitkomstkast of in het Patiosysteem op kuikenfysiologie op het moment van uitkomst uit het ei beperkt zijn. Kuikens met directe toegang tot voer en water, zoals in Patio, hebben een hoger niveau van fysiologische ontwikkeling in de vroege postnatale fase, wat resulteert in hogere orgaangewichten, hogere leverglycogeenreserves, en hoger plasma glucose en T_3 niveaus op het moment van kuikenverzameling, vergeleken met de kuikens die aan voer- en wateronthouding zijn blootgesteld in de vroege periode, zoals in de uitkomstkast. Het werd duidelijk dat door het evalueren van kuikenfysiologie op het moment van kuikenverzameling, in plaats van direct na uitkomst, de effecten van uitkomsttijdstip en blootstellingsduur aan postnatale omstandigheden door elkaar lopen.
De verbeterde postnatale groei in Patiokuikens ten opzichte van broederijkuikens, zoals in verschillende experimenten aangetoond werd, is grotendeels te wijten aan de vroege toegang tot voer en water. Naast de hogere groei van dag 0-7 in vroege en midden vs. laat uitkomende kuikens, waren effecten van uitkomsttijdstip op postnatale groei niet duidelijk uit de experimenten in dit proefschrift. Op basis van een kwalitatieve kuikenscore gebaseerd op fysieke kuikenkenmerken, en op de incidentie van 2e soortkuikens, was de kuikenkwaliteit lager in Patio- dan in broederijkuikens. Hoewel deze scores informatief kunnen zijn voor de kwaliteit van het broedproces, zijn zij niet voorspellend voor de postnatale groei van de kuikens. Navelkwaliteit is het belangrijkste kenmerk van huidige kwalitatieve kuikenscores omdat het de groei tot aan slachtleeftijd beïnvloedt. Daarnaast kan geconcludeerd worden dat de selectie van 2e soort kuikens in de broederijpraktijk verdedigbaar is in termen van welzijn, gebaseerd op een hoge 7-daagse sterfte. Na correctie voor de 2e soortkuikens en de laat uitkomende kuikens, is het uitkomstpercentage nog altijd verbeterd in het Patiosysteem ten opzichte van eieren die uitkomen in de uitkomstkast, wat niet te wijten is aan verschillende eipositie, maar waarschijnlijk aan verschillende klimaatsomstandigheden.

Curriculum Vitae

CURRICULUM VITAE

English

Lotte van de Ven was born on the 26th of March 1980 in Eersel, where she also spent her childhood. In 1998 she graduated from high school Rythoviuscollege in Eersel, after which she started with her study Animal Sciences at Wageningen University. During her study she specialized in Animal Production Systems and in Animal health and Reproduction. For the specialization Animal Production Systems, she investigated the health status of laying hens in several production systems. For the specialization Animal health and Reproduction, she compared the embryonic development of broiler and laying hen breeds, which was an assignment of hatchery equipment manufacturer Pas Reform in Zeddam. She spent her first internship at the Rowett Research Institute in Aberdeen, Scotland, and for her second internship she set up a draft quality handbook for Poultry veterinary practice Plantema in Hardenberg, the Netherlands. After her graduation for her MSc in November 2003, Lotte started to work at the R&D department of Pas Reform, and later worked as a project manager for this company. In the autumn of 2007 she stopped working for Pas Reform, and made a journey through Bolivia for 3 months. In January 2008 she started as a PhD student at the department of Adaptation Physiology at Wageningen University, in collaboration with Vencomatic in Eersel.
After finishing her PhD, Lotte will continue working for Vencomatic.

CURRICULUM VITAE

Nederlands

Lotte van de Ven werd geboren op 26 maart 1980 in Eersel, waar ze ook opgroeide. In 1998 behaalde zij haar VWO-diploma aan het Rythoviuscollege in Eersel, waarna zij met de studie Zoötechniek startte aan Wageningen Universiteit. Tijdens haar studie koos zij voor de specialisaties Dierlijke Productiesystemen en Gezondheidsleer en Reproductie. Voor de specialisatie Dierlijke Productiesystemen voerde zij onderzoek uit naar de gezondheidsstatus van leghennen in verschillende productiesystemen. Voor de specialisatie Gezondheidsleer en Reproductie deed zij een vergelijkend onderzoek naar de embryonale ontwikkeling van vleeskuiken- en leghenrassen, in opdracht van broedmachineproducent Pas Reform in Zeddam. Haar eerste stageopdracht heeft zij uitgevoerd bij het Rowett Research Institute in Aberdeen, Schotland, en voor haar tweede stageopdracht zette zij een concept kwaliteitshandboek op voor Pluimveepraktijk Plantema in Hardenberg, Nederland. Na haar afstuderen in november 2003 ging Lotte aan de slag als medewerkster op de R&D afdeling van Pas Reform, en daarna als projectmanager bij dit bedrijf. In het najaar van 2007 stopte zij haar baan bij Pas Reform, en maakte een reis van 3 maanden door Bolivia. In januari 2008 begon zij als promovenda bij de vakgroep Adaptatiefysiologie van Wageningen Universiteit aan een promotieonderzoek in samenwerking met Vencomatic.
Na het afronden van het promotieonderzoek blijft Lotte werkzaam bij Vencomatic.

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Training and Supervision Plan of the Graduate School WIAS

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EDUCATION AND TRAINING

	year	credits*
The Basic Package		
WIAS Introduction Course	2009	1,5
Course on philosophy of science and ethics	2010	1,5
Scientific Exposure		
International conferences		
International Symposium on Avian Endocrinology 2008, Leuven, Belgium	2008	1,1
Workshop Fundamental physiology of the European working group of physiology, and perinatal development in poultry, Bratislava, Slovakia	2009	0,6
WPSA European Poultry Conference, Tours, France	2010	1,2
Seminars and workshops		
WIAS Science Day, Wageningen, the Netherlands	2008, 2009, 2010	0,9
Workshop of Fertility and Incubation Research Group	2010	0,3
Workshop "The embryonic life of chickens, factors that influence development", Wageningen, the Netherlands	2010	0,3
Presentations		
Oral presentation Workshop Fundamental physiology of the European working group of physiology and perinatal development in poultry, Bratislava, Slovakia	2009	1,0
Oral presentation WIAS Science Day, Wageningen, the Netherlands	2010	1,0
Oral presentation WPSA European Poultry Conference, Tours, France	2010	1,0
Oral presentation "The embryonic life of chickens, factors that influence development", Wageningen, the Netherlands	2010	1,0
Oral presentation Danish Poultry Congress, Braedstrup, Denmark	2012	1,0
In-Depth Studies		
Disciplinary and interdisciplinary courses		
Epigenesis and epigenetics, Wageningen, the Netherlands	2009	0,8
Interpretation of animal stress responses, Denmark	2011	1,2
Advanced statistics courses		
Design of Animal experiments, Wageningen, the Netherlands	2008	1,0
Statistics for life science, Wageningen, the Netherlands	2009	2,0
PhD students' discussion groups		
Welfare Discussion Group	2010	1,0
Statutory Courses		
Use of Laboratory Animals	2008	3,0
Professional Skills Support Courses (minimum 3 credits)		
CrestCom Bullet Proof Manager Training (www.crestcom.com)	2007	2,0
Course Techniques for Scientific Writing	2009	1,2
Research Skills Training (optional)		
Preparing own PhD research proposal (maximum 6 credits)	2008	6,0
External training period (one month or more is 2 credits)		
Special research assignments (apart from PhD project)		
Subtotal Research Skills Training		
Didactic Skills Training (optional)		
Supervising theses		
Myrthe Gilbert (Major MSc thesis)	2008	2,0
Malou Gosselink (Major MSc thesis)	2009	2,0
Lieneke Baller (Minor BSc thesis)	2010	1,0
Pieter de Gouw (Major MSc thesis)	2010	2,0
Anet van de Wouw (MSc Major thesis)	2011	2,0
Management Skills Training		
Organisation of seminars and courses		
Organisation of Seminar Scientific Research in Animal Welfare: Do we make a difference?	2011	1,0
Organisation of Workshop Fundamental physiology of the European working group of physiology and perinatal development in poultry, Wageningen	2011	1,0
Education and Training Total		**41,6**

COLOPHON

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