

# Spread of hatch and delayed feed access affect post hatch performance of female broiler chicks up to day 5

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*It is not rare that newly hatched chicks remain without feed for about 24 to 48 h before they are placed on farms due to a series of logistic operations. Furthermore, the spread in hatching time can also mount up to 30 to 48 h for late v. early hatching. In other words, the practice is a complex combination of spread of hatch and delayed feed access. The present study was aimed to investigate the combined effects of hatching time with a delay in feed access of 48 h, starting from their hatch-time (biological age). When chicks had access to feed immediately after hatch, late hatching had a higher feed intake and relative growth rate up to day 5 compared with their early hatching counterparts. Feed deprivation during the first 48 h resulted in retarded early growth rate, which was further aggravated by an impaired feed intake after refeeding. In addition, the differential effects of hatching time on relative growth rate and feed intake observed in immediately fed chicks were eliminated by the 48 h feed delay. The yolk utilization after hatch was faster for the late hatching up to biological day 2 regardless of the feeding treatments. Hatching muscle glycogen content was higher in the late hatching compared with that of their early counterparts at hatch and at biological day 2 independent of feeding treatment. Moreover, the liver glycogen content of the late hatching was also higher at hatch. For the immediately fed chicks, the proportional breast muscle weight of the late hatching was higher at biological day 2 and 5. For the starved chicks, on the other hand, this effect was only observed after they had access to feed (biological day 5). The different plasma T<sub>3</sub> levels at hatch may have contributed to the different post hatch performance. It is concluded that the spread of hatch influenced post hatch performance, especially appetite and growth at least until day 5. Moreover, the delay in feed access interacted with the hatching time and caused adverse effects on the post hatch performance.*

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**Keywords:** hatching time, delayed feed access, feed intake, post hatch growth, broiler

## Implications

Incubation of chicken eggs has a duration of about 21 days. The first hatched chicks are 30 to 48 h ahead of the last ones. In addition, before the start of rearing on the farm, chicks are sometimes deprived of feed and water for 24 to 48 h. In the present study, it was aimed to investigate the effects of hatching time and to combine this with the often seen practice in the poultry industry: delay in feed access after hatch. The interaction and underlying mechanisms are not only an interesting scientific topic, but also provide useful insight for animal husbandry.

## Introduction

Early feeding after hatch improves the initiation of growth in neonatal chicks (Bigot *et al.*, 2003a), and this growth promoting effect lasts until marketing (Noy and David, 1999). However, it is not rare that newly hatched chicks remain without feed for up to 48 h before they are placed on farms due to a series of operations, such as sexing, vaccination and transportation. Furthermore, there is also an inevitable spread of hatch of 30 to 48 h for late v. early hatching. In other words, the early hatching will undergo even longer feed deprivation due to the spread of hatch. Hence, in practical situations, a complex combination of spread of hatch and delayed feed access is present.

Several studies have paid close attention to the spread of hatch. Previous study indicated that the spread of hatch

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resulted in chicks of different qualities (Tona *et al.*, 2003a). Careghi *et al.* (2005) studied the interaction of spread of hatch and feed delay, and introduced the concept of biological age (BA: age counted from the precise time of hatch), which is more relevant than the chronological age (CA: age counted from end of hatch of the whole batch) for comparison between early, middle and late hatching chicks. Interestingly, they found that the spread of hatch had effects on relative growth rate and plasma triiodothyronine ( $T_3$ ) levels up to BA day 7. A recent study demonstrated that the spread of hatch not only affected the post hatch growth (CA was employed in this study), but also had interaction with hatching systems (van de Ven *et al.*, 2011).

Early access to feed is of crucial importance. Delayed feed access and early feed restriction will cause detrimental effects on juvenile performance. Layer chicks with 48 h delayed feed access had a lower BW and decreased serum glucose, total protein and triglycerides concentration up to 56 days (Gaglo-Disse *et al.*, 2010). Newly hatched broiler chicks subjected to 14 days of feed restriction (feed provided on alternate days) had lower BW and lateral gastrocnemius weight accompanied by reduced serum  $T_3$  levels and changes of mRNA expression for growth-related genes in muscle (Li *et al.*, 2007).

Despite the notable progress, as yet, it is still elusive how the spread of hatch and early feed deprivation interact and what the effects are on chicken post hatch performance and metabolism. Therefore the aim of the present study was to investigate the effects of hatching time, combined with a delay in feed access of 48 h, on chicken development, especially on the post hatch performance up to day 5 and underlying causal physiological mechanisms.

## Material and methods

### *Incubation and hatch*

A total of 1200 eggs produced by a commercial flock of Ross 308 breeders were obtained from a local hatchery (Belgabroed, Merksplas, Belgium). All eggs were set in a forced-draft incubator at specific dry bulb temperature of 37.6°C and wet bulb temperature of 29°C. The eggs were turned every hour until day 18 of incubation, when the eggs were candled and those with evidence of living embryos were transferred from turning trays to hatching baskets. Between 480 and 524 h of incubation, the eggs were checked every 2 h, and hatched chicks were sexed, marked with leg tags and weighed individually. Only female chicks were employed in this study. Newly hatched female chicks were evenly divided into immediate feed access and 48 h feed delay groups forthwith after removing from the incubator.

### *Early, middle and late hatchers and spread of hatch*

After the total hatching period, a hatching curve of all the female chicks, approaching normal distribution, was obtained. Three hatching groups were defined according to

the standard deviation ( $\sigma$ ) and average hatching time ( $\mu$ ) of the hatching curve: early hatchers ( $\mu - 2\sigma \sim \mu - 1\sigma$ ), middle hatchers (around  $\mu$  but with  $\mu - 1\sigma \sim \mu + 1\sigma$  as limits), and late hatchers ( $\mu + 1\sigma \sim \mu + 2\sigma$ ). The duration of spread of hatch was calculated as the confidence interval of the hatching curve, namely spread of hatch =  $4 \times \sigma$ .

According to the hatching time and feeding status of chicks, six experimental groups were established: namely early hatcher access to feed immediately (EI), early hatcher 48 h feed delay (ED), middle hatcher access to feed immediately (MI), middle hatcher 48 h feed delay (MD), late hatcher access to feed immediately (LI) and late hatcher 48 h feed delay (LD).

### *Feed intake and BW*

The chicks ( $n = 45$  per group) were raised at standard conditions of light and temperature. A broiler corn-wheat-soybean-based starter diet (AVEVE, Merkssem, Belgium) was provided *ad libitum* immediately when they were removed from the incubator or *ad libitum* after the 48 h feed delay process based on BA. Chicks had always free access to water. For daily feed intake study, 25 chicks from each group were further divided into three pens ( $n = 8, 8$  and 9). Feed intake and BW were recorded daily exactly at their BA up to 5 days post hatch. These BWs together with the hatch weight were used to calculate relative growth rate or BW loss. Relative growth =  $100 \times (W_{t_5} - W_{t_0})/W_{t_0}$ ; Weight loss =  $100 \times (W_{t_0} - W_{t_2})/W_{t_0}$  where  $W_{t_0}$  is the weight at hatch,  $W_{t_2}$  the weight at BA day 2;  $W_{t_5}$  the weight at BA day 5.

### *Sampling*

Ten chicks per hatching time were sampled at BA day 0 (immediately after being removed from the incubator; early, middle and late time of hatch was estimated based on experience of the hatching process, and verified by the hatching curve after the termination of complete hatching process). On BA day 2 (before the feed-delay group had access to feed) and BA day 5, 10 chicks from each group were sacrificed. At each sampling time, blood samples from the vena jugularis, residual yolk sac (BA day 0 and 2), breast muscle, liver and hatching muscle (BA day 0 and 2) were collected. Blood was centrifuged (3000 r.p.m., 15 min, 4°C) to obtain plasma which was stored at  $-20^\circ\text{C}$  for further analysis. Liver and breast muscle were weighed to calculate the proportional organ weight (proportional organ weight (%) =  $100 \times \text{organ weight}/\text{BW}$ ). The absolute weight of residual yolk sacs (at hatch and BA day 2) was recorded for yolk utilization comparison. The present experiment was approved by the Ethical Commission for Experimental Use of Animal of the KU Leuven.

### *Radioimmunoassay (RIA) of plasma $T_3$*

Plasma  $T_3$  concentrations were measured by RIA as described by Darras *et al.* (1991). The antisera for  $T_3$  was purchased from Byk Belga (Brussels, Belgium).

### Plasma glucose determination

Plasma glucose concentration was determined by a commercial kit (no. 298-65701; WAKO Pure Chemical Industries Ltd., Osaka, Japan). The assay was carried out according to manufacturer's protocol.

### Glycogen determination

A protocol based on the method of Dreiling *et al.* (1987) was employed to determine the glycogen concentration in the hatching muscle or liver. In short, tissue was homogenized in 7% HClO<sub>4</sub> (1 µl/mg tissue). Homogenates were centrifuged at 4°C at 14 000 × g until a clear supernatant was obtained. The supernatant was washed with 1 ml of petroleum ether and stored at -20°C. An iodine colour reagent (0.39 ml of an iodine solution (0.104 g I<sub>2</sub>, 1.04 g KI<sub>2</sub> in 4 ml of MQ water) + 30 ml of 10% CaCl<sub>2</sub>) was added to standards or tissue extracts in a microtiter plate. After mixing and a reacting period of 10 min, the absorbance was measured at 450 nm (Victor 1420 Multilabel counter; PerkinElmer, MA, USA). Tissue glycogen concentration was calculated using a standard curve prepared with rabbit liver glycogen (Sigma, Bornem, Belgium).

### Statistical analysis

All statistical analyses were performed with SAS version 9.2 (SAS Institute Inc., Cary, NC, USA). Comparisons between groups were performed using one-way ANOVA or a general linear model, with access to feed (yes/no) and time of hatching (early, middle and late) and their interaction as factors. The level of significance was set at  $P < 0.05$ . When the means of the general linear model were statistically different, means were compared using least squares means with Tukey's adjustment for multiple comparisons. All data are shown as average ± s.e.m.

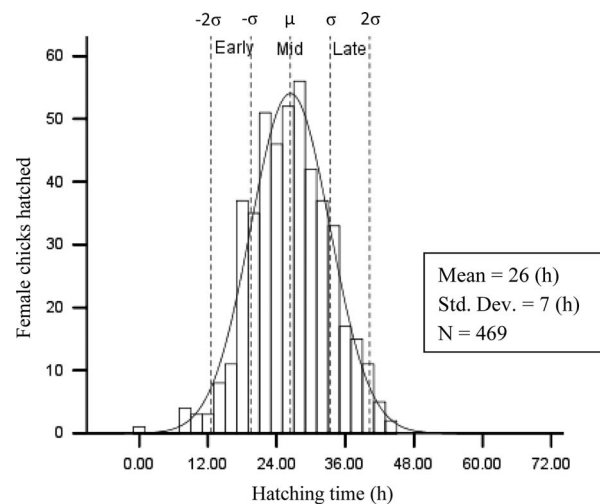
## Results

### The hatching time and spread of hatch

Based on the hatching time of all the female chicks, a hatching curve approaching normal distribution was obtained (Figure 1). The  $\sigma$  of the hatching curve of female chicks was 6 h 55 min. The hatching time (early, middle and late) of female chicks was divided by the  $\mu$  and  $\sigma$ . The complete hatching period of female chicks lasted 44 h, but the spread of hatch of female chicks was 27 h 40 min ( $4 \times \sigma$ ).

### Daily feed intake

The interaction of feed delay and hatching time on daily feed intake was significant on BA day 3 (BA 48 to 72 h,  $P = 0.01$ ) and BA day 4 (BA 72 to 96 h,  $P = 0.03$ ), and almost significant on day 5 (BA 96 to 120 h,  $P = 0.05$ ; Table 1). Furthermore, spread of hatch had a significant effect on feed intake on BA day 3 ( $P = 0.03$ ) and day 4 ( $P = 0.01$ ), but not on day 5. LI chicks had markedly higher daily feed intake than the EI and MI chicks on day 3 and day 4 ( $P < 0.01$ ). At BA day 5 feed intake of LI chicks was also 14% and 17%



**Figure 1** Hatching curve, early, middle and late hatching and spread of hatch of female chicks ( $n = 469$ ). Distance between two close dotted lines is  $\sigma$ .

higher than that of the EI ( $P = 0.15$ ) and MI ( $P = 0.07$ ) groups, although these differences were not significant. After 48 h delay in feed access, the feed intake of the three hatch groups was similar. The effect of feeding treatment was significant on day 4 ( $P < 0.001$ ) and day 5 ( $P < 0.001$ ), but not on day 3.

### BW and relative growth rate

The three hatch groups had similar BW at hatch (Figure 2). The effect of hatching time, delay in feed access and their interaction were all significant from BA day 2 up until day 5 ( $P < 0.01$ ). When chicks were fed immediately after hatch, the BW of late hatching was higher than their early and middle counterparts from day 2 up to day 5 ( $P < 0.01$ ). Moreover, after the 48 h fasting, the weight loss of each hatch group was not different, and the BW among the feed delay chicks was equal up to BA day 5 (data not shown).

The effect of hatching time and feeding treatment on the relative growth rate up to day 5 was significant ( $P < 0.01$ ;  $P < 0.001$ ), and they showed a pronounced interaction ( $P < 0.001$ , Figure 3). More specifically, the LI chicks had greater relative growth rate compared with the EI and MI chicks ( $P < 0.01$ ), which were similar in this respect. When feed access was delayed, there was no difference in relative growth rate between the three hatching groups.

### Proportional breast muscle and liver weights

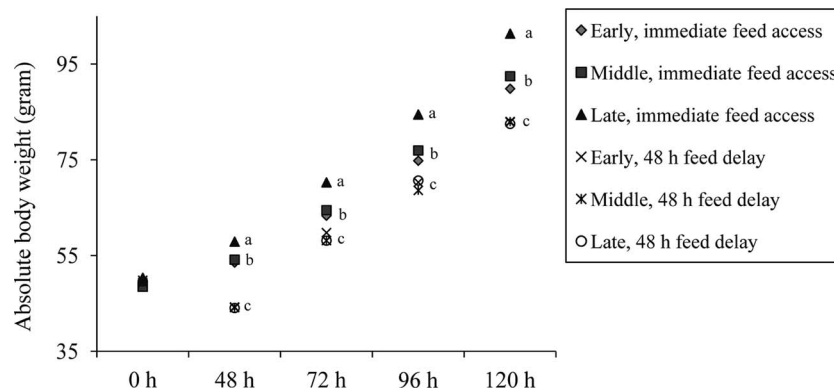
The hatching time did not affect the proportional breast muscle weight at hatch (data not shown). However, hatching time affected the proportional breast muscle on BA day 2 ( $P = 0.02$ , Table 2) and day 5 ( $P < 0.001$ , Table 3). The effect of the feeding treatment was significant on BA day 2 ( $P < 0.01$ ) and day 5 ( $P < 0.001$ ). Moreover, the interaction of hatching time and delay in feed intake was significant on BA day 2 ( $P = 0.03$ , Table 2), and almost significant on day 5 ( $P = 0.07$ , Table 3). When chicks were fed immediately after hatch, the late hatching had a higher proportional breast

**Table 1** Effects of hatching time and delay in feed intake on daily feed intake from BA 48 h up to BA 120 h

Age (hours)	Feeding treatment	Hatching period			s.e.m.	P-value		
		Early	Middle	Late		HT	DF	HT × DF
48 to 72	Immediate	8.82 <sup>b</sup>	8.96 <sup>b</sup>	11.04 <sup>a</sup>	0.22	0.03	0.89	<0.01
	Delayed	9.93 <sup>ab</sup>	9.30 <sup>b</sup>	9.47 <sup>b</sup>				
72 to 96	Immediate	11.90 <sup>bc</sup>	12.92 <sup>b</sup>	14.99 <sup>a</sup>	0.41	<0.01	<0.001	0.03
	Delayed	10.66 <sup>c</sup>	10.57 <sup>c</sup>	11.23 <sup>c</sup>				
96 to 120	Immediate	16.69	16.29	19.02	0.52	0.15	<0.001	0.05
	Delayed	13.71	14.08	13.60				

BA = biological age; HT = hatching; DF = delay in feed intake; Immediate = fed immediately; Delayed = delay in feed intake.

<sup>a-c</sup>Values within the same age with different superscripts differ significantly at  $P < 0.05$ .



**Figure 2** Effect of hatching time and feeding treatments (immediate access to feed and 48 h delay in feed access) on absolute BW up to biological age day 5 ( $n = 25$ ). Data sharing no common letter are different between hatching times within the same feeding treatment ( $P < 0.05$ ).

muscle weight on BA day 2 compared with their early ( $P = 0.03$ ) and middle ( $P < 0.01$ ) hatched counterparts, however this parameter did not differ between the fasted chicks (Table 2).

The spread of hatch did not influence the proportional liver weight at hatch. No effect of hatching time and feeding treatment or interactions were observed on BA day 2 and 5 (data not shown).

#### Absolute residual yolk weights

There was no effect of spread of hatch on the residual yolk weight at hatch (Table 4). However, the hatching time significantly affected the yolk weight on post hatch day 2 ( $P < 0.01$ , Table 2): the late hatchers had lower yolk sac weights than their early and middle counterparts. The feeding treatment had no effect on the residual yolk weight on BA day 2, nor was there an interaction with the hatching time.

#### Liver glycogen content

At hatch, even though the effect of spread of hatch on the liver glycogen content was not statistically significant ( $P = 0.09$ , Table 4), the liver glycogen content of the late hatchers was 111% higher than that of the early hatchers, and the middle hatchers had an intermediate value. The liver glycogen of LI chicks was 25% lower than that of MI and 18% lower than EI on BA day 2, but they were not statistically different (data not shown). In the chicks

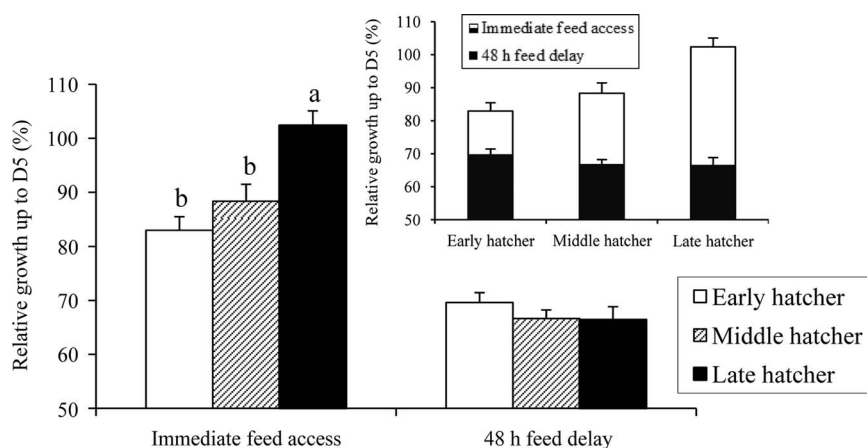
subjected to 48 h of feed delay, on the other hand, the liver glycogen level was below the detection limit on BA day 2. The previous starvation significantly decreased the liver glycogen content on BA day 5 ( $P = 0.02$ ). No effect of hatching time, nor the interaction with the feeding treatment on the liver glycogen content was observed on BA day 5 (Table 3).

#### Hatching muscle glycogen contents

At hatch, the effect of spread of hatch on hatching muscle glycogen content was almost statistically significant ( $P = 0.07$ , Table 4). The glycogen content in the hatching muscle was 69% higher for late hatchers than that of the early hatchers, and the middle hatchers had an intermediate value. The hatching muscle glycogen content was decreased at BA day 2 compared with that at hatch (Table 2). Delayed feed intake resulted in a lower hatching muscle glycogen content ( $P < 0.001$ ). The effect of the hatching time was also significant on BA day 2 ( $P = 0.02$ ): the late hatchers had higher hatching muscle glycogen content than the early hatchers ( $P = 0.02$ ), and the middle hatchers had an intermediate value. On BA day 2, no interaction between spread of hatch and feeding treatment was seen on this parameter.

#### Plasma glucose levels

The plasma glucose levels of newly hatched chicks were not affected by the hatching time (Table 4). The 48 h starvation period dramatically decreased the blood glucose level on BA



**Figure 3** Effect of hatching time and feeding treatments (immediate access to feed and 48 h delay in feed access) on relative growth up to biological age day 5 ( $n = 25$ ). Data sharing no common letter are different between hatching times within the same feeding treatment ( $P < 0.05$ ). HT = hatching time; DF = delay in feed intake.

**Table 2** Effects of hatching time and delay in feed intake on proportional breast muscle weight, residual yolk, hatching muscle glycogen content, plasma glucose and  $T_3$  levels on BA day 2

Performance parameters	Feeding treatment	Hatching period			s.e.m.	P-value		
		Early	Middle	Late		HT	DF	HT × DF
Proportional BM (%)	Immediate	0.62 <sup>bc</sup>	0.62 <sup>c</sup>	0.74 <sup>a</sup>	0.01	0.02	<0.01	0.03
	Delayed	0.72 <sup>ab</sup>	0.71 <sup>abc</sup>	0.71 <sup>abc</sup>				
Residual yolk (g)	Immediate	3.40	3.38	2.44	0.13	<0.01	0.92	0.78
	Delayed	3.58	3.16	2.56				
HM glycogen (µg/g)	Immediate	115.0 <sup>ab</sup>	149.7 <sup>ab</sup>	197.2 <sup>a</sup>	11.6	0.02	<0.001	0.30
	Delayed	29.6 <sup>c</sup>	99.4 <sup>bc</sup>	75.1 <sup>bc</sup>				
Glucose (mg/dl)	Immediate	229.5 <sup>a</sup>	227.3 <sup>a</sup>	217.0 <sup>ab</sup>	3.0	0.04	<0.001	0.99
	Delayed	197.0 <sup>bc</sup>	192.3 <sup>c</sup>	185.7 <sup>c</sup>				
Plasma $T_3$ (ng/ml)	Immediate	1.89 <sup>a</sup>	1.70 <sup>a</sup>	1.68 <sup>a</sup>	0.07	0.28	<0.001	0.53
	Delayed	0.93 <sup>b</sup>	0.97 <sup>b</sup>	0.82 <sup>b</sup>				

BA = biological age; HT = hatching; DF = delay in feed intake; BM = breast muscle; Immediate = fed immediately; Delayed = delay in feed intake; HM = hatching muscle; Glucose = plasma glucose.

<sup>a-c</sup>Values within the same parameter with different superscripts differ significantly at  $P < 0.05$ .

**Table 3** Effects of hatching time and delay in feed intake on proportional breast muscle weight, liver glycogen content and plasma glucose level on BA day 5

Performance parameters	Feeding treatment	Hatching period			s.e.m.	P-value		
		Early	Middle	Late		HT	DF	HT × DF
Proportional BM (%)	Immediate	1.47 <sup>b</sup>	1.72 <sup>a</sup>	1.88 <sup>a</sup>	0.04	<0.001	<0.001	0.07
	Delayed	1.16 <sup>c</sup>	1.43 <sup>b</sup>	1.36 <sup>bc</sup>				
Liver glycogen (µg/mg)	Immediate	4.13	4.96	7.76	0.58	0.62	0.02	0.16
	Delayed	3.96	2.73	2.40				
Glucose (mg/dl)	Immediate	190.2 <sup>b</sup>	197.4 <sup>b</sup>	205.2 <sup>ab</sup>	2.9	0.02	<0.01	0.49
	Delayed	208.5 <sup>ab</sup>	204.1 <sup>ab</sup>	226.2 <sup>a</sup>				

BA = biological age; HT = hatching; DF = delay in feed intake; BM = breast muscle; Immediate = fed immediately; Delayed = delay in feed intake; Glucose = plasma glucose.

<sup>a-c</sup>Values within the same parameter with different superscripts differ significantly at  $P < 0.05$ .

day 2 ( $P < 0.001$ ). Moreover, the effect of spread of hatch was significant ( $P = 0.04$ ): the plasma glucose level was higher in early hatchers than the late hatchers ( $P = 0.05$ ),

and the middle hatchers had an intermediate value (Table 2). No interaction of hatching time and feeding treatment on the blood glucose level was observed on BA day 2.

**Table 4** Effect of hatching time on residual yolk weight, liver glycogen content, hatching muscle glycogen content, plasma glucose and T<sub>3</sub> levels at hatch (BA day 0)

Performance parameters	Hatching period			s.e.m.	P-value
	Early	Middle	Late		
Residual yolk (g)	8.47	8.08	8.98	0.38	0.64
Liver glycogen (µg/mg)	1.82	3.04	3.84	0.37	0.09
HM glycogen (µg/g)	179.1	279.1	301.8	23.3	0.07
Plasma glucose (mg/dl)	205.4	208.6	209.4	3.0	0.87
Plasma T <sub>3</sub> (ng/ml)	4.68 <sup>a</sup>	2.69 <sup>b</sup>	2.90 <sup>b</sup>	0.37	0.04

BA = biological age; HM = hatching muscle.

<sup>a,b</sup>Values within the same parameter with different superscripts differ significantly at  $P < 0.05$ .

On BA day 5, the blood glucose level was significantly higher in the chicks fed with a delay than their immediately fed counterparts ( $P < 0.01$ ). The spread of hatch significantly influenced the blood glucose on BA day 5 ( $P = 0.02$ ): the late hatchers had a higher level than the early hatchers ( $P = 0.03$ ), and the middle hatchers had an intermediate value (Table 3). There was no interaction between hatching time and feeding treatments on blood glucose level on BA day 5.

#### Plasma T<sub>3</sub> levels

The plasma T<sub>3</sub> levels at hatch were significantly higher in the early hatchers than the middle and late hatchers ( $P = 0.02$ ,  $0.04$ , Table 4). However, on BA day 2, the spread of hatch had no effect on the blood T<sub>3</sub> levels. The feed delay decreased the plasma T<sub>3</sub> levels on day 2 compared with chicks that had access to feed immediately ( $P < 0.001$ , Table 2). No interaction of hatching time and feeding treatment was observed on BA day 2. The effect of hatching time, feeding treatment and their interaction on T<sub>3</sub> level were not significant on BA day 5 (data not shown).

## Discussion

### Spread of hatch

The spread of hatch is a factor that potentially contributes to the different chick qualities and physiological traits in one batch of hatched chicks (Decuypere *et al.*, 2001; Careghi *et al.*, 2005). Therefore, the effect of spread of hatch on post hatch performance and its potential mechanisms are of interest and importance.

Hatching time is known to be influenced by factors such as parental age, egg storage time and conditions, and incubation conditions (temperature, humidity, air velocity, etc.) and gender (Tona *et al.*, 2003b; Careghi *et al.*, 2005; Decuypere and Bruggeman, 2007). To limit the number of affecting factors, eggs from the same young flock, that were laid on the same day were chosen, with very short storage period (1 to 2 days) before incubation and were incubated in one incubator but switched eggs location in the incubator daily. After hatch, only female chicks were employed in the

present study. Hence, genetic and nutritional variation between eggs might be two major factors that affected the spread of hatch in this experiment. Additionally, the standard deviation was used as a criterion to partition the different hatching time, which resulted in a quantifiable and repeatable design to perform experiments investigating the spread of hatch.

The present study showed that the spread of hatch did not affect the hatch weight, which is consistent with observation of Careghi *et al.* (2005) and van de Ven *et al.* (2011). In addition, no difference in residual yolk weight was found at hatch, which agrees with Careghi *et al.* (2005). van de Ven *et al.* (2011), on the other hand, observed a lower residual yolk weight at hatch for the late hatchers, whereas in the present study this was only seen on BA day 2.

The process of hatching is dependent on the proper development of the supporting musculature, especially the hatching muscle (*Musculus complexus*), and the available energy storage (Decuypere *et al.*, 1990). The higher liver and hatching muscle glycogen at hatch of the late hatchers suggests that the late hatchers stored more energy during incubation, and/or consumed less which brought about the later hatching of these birds. As embryonic metabolism and the hatching time are known to be determined by regulators of thyroid hormone availability and action (Beck *et al.*, 2005; Van Herck *et al.*, 2013), differences concerning these factors between eggs of one batch might also have induced differences in the spread of hatch.

It is well known that thyroid hormones affect the time of hatching and hence the length of incubation and play a crucial role during chicken post hatch development (Decuypere *et al.*, 1990). In accordance with the study of van de Ven *et al.* (2011), the early hatchers had higher levels of plasma T<sub>3</sub> in the present study, which also agrees with findings of Decuypere *et al.* (1990) showing that treatment of embryos with T<sub>3</sub> on embryonic day 19 advanced hatching time. As it was reported that T<sub>3</sub> could decrease the liver and skeletal muscle glycogen (Potenza *et al.*, 2009; Ribeiro *et al.*, 2012), through glycogen synthase kinase 3  $\beta$  (GSK 3  $\beta$ ) (Kuzman *et al.*, 2005), the higher plasma T<sub>3</sub> level of early hatchers at hatch is consistent with the lower glycogen in liver and hatching muscle.

When chicks had access to feed immediately after hatch, the late hatchers grew faster than chicks that hatched earlier. This improved growth performance of the late chicks, when measured on their BA, is in agreement with Careghi *et al.* (2005). However, it is notable that when the CA was used to measure the BW in former investigations, no difference or even a decreased growth performance was observed for the late hatchers (Careghi *et al.*, 2005; van de Ven *et al.*, 2011). Interestingly, despite the higher BW of the late hatchers on BA day 2 and 5, the proportion of the breast muscle to BW of the late chicks was still higher than the other two fed hatchers.

It is worth noting that the augmented metabolic rate caused by T<sub>3</sub> is normally accompanied by an increased feed intake (Kong *et al.*, 2004). In the present study, however, the early hatched chicks were characterized by higher plasma T<sub>3</sub>

levels yet had lower feed intake at the beginning of their post hatch life, indicating that factors other than  $T_3$  are involved in manipulating the energy intake.

The different hatching time did not affect the blood glucose level at hatch, which disagrees with van de Ven *et al.* (2011) who observed a higher plasma glucose for the late hatchers compared to their earlier hatched counterparts, suggesting that more glucose was available for the late hatchers. In our study, as the late hatchers started their post hatch life with a higher hepatic glycogen reserve and had equal (or even somewhat lower) levels of hepatic glycogen on day 2, they consumed more glucose than their early counterparts, as also implicated by the higher feed intake but a lower plasma glucose concentration on BA day 2. The available glucose on these first two post hatch days might have stimulated the utilization of yolk lipids, as the intermediate of the TCA cycle is then used for lipolysis (Bergman and Kon, 1964; Ørngreen *et al.*, 2009), resulting in a lower residual yolk of the late hatched chicks on day 2. This glucose and yolk consumption hypothesis was also true for the fasted chicks. Consequently, all these factors together with the higher feed intake and increased yolk utilization more than probably caused the higher BW (from day 2 onwards) of the fed late hatchers.

#### *Delay in feed access*

In practice, the first feed of chicks is commonly delayed due to the logistics of commercial production (Kornasio *et al.*, 2011). The 48 h of feed delay resulted in retarded early growth which is in agreement with reports from Bigot *et al.* (2003a) and Careghi *et al.* (2005). According to Noy and Sklan (2001), feed deprivation results in a slower yolk consumption in the newly hatched chicks, which is caused by a poor stimulation of the development of the gastrointestinal tract. Nevertheless, in this experiment no effect of fasting was observed on yolk sac utilization, which is analogous to some previous observations (Careghi *et al.*, 2005; Gaglo-Disse *et al.*, 2010).

Interestingly, when these chicks had access to feed, the feed intake was lower than their immediately fed counterparts, which implies that delayed feed access had impaired the feed intake which potentially further aggravated the growth retardation. In addition, the effects of hatching time on feed intake and relative growth observed in immediately fed chicks were eliminated by the 48 h feed delay. The reduction in relative growth rate caused by the delayed feed access was larger in the late hatchers than the other two hatching time groups (Figure 3, the smaller figure), which indicated that the 48 h feed delay had more impact on the late hatchers. Indeed, the advantage of the late hatchers, whether caused by nutrient availability or genetic potential, disappeared due to the 48 h feed delay. In the same line, Bigot *et al.* (2003a) found that post hatch starvation reduced interfamily variation in BW that is expressed in chicks given immediate access to feed, which may mask the expression of the genetic potential. Surprisingly, the increased yolk consumption of late hatchers from hatch to day 2 compared with

their earlier counterparts, was also observed for the feed deprived chicks, however, without any effect on BW (loss).

In accordance with previous studies (Decuypere and Kühn, 1984; Careghi *et al.*, 2005), decreased plasma  $T_3$  levels were observed immediately after the feed delay, which might be an adaptation in order to reduce oxygen consumption and metabolism.

Very similar to a former report (Decuypere and Kühn, 1984), the blood glucose level of previous starved chicks was significantly higher than their immediately fed counterparts after refeeding (BA day 5), although their feed intake was markedly lower at that time.

The proportional breast muscle weight on day 2 was higher when chicks were starved for the first 48 h of their life, while it was proportionally lower on day 5 compared with their fed counterparts. As the intestines are barely developing during the early post hatch starvation, the breast muscle takes a good proportion of the BW, while on day 5, when the starved chicks had access to feed, their intestines were most probably developing fast, which resulted in a proportionally lower breast muscle weight compared with their fed counterparts. The retardation in muscle development has been shown by several authors. Bigot *et al.* (2003b) concluded that muscle ribosomal S6 kinase 1 (S6K1), a key element in the control of protein synthesis, is activated only when food is available, without an altered improvement on the response of the S6K1 pathway after post hatch starvation. Additionally, another study suggested that early nutritional status affected the percentage of muscle fiber type and changed the mRNA expression for the growth-related genes in muscle (Li *et al.*, 2007). Interestingly, Kornasio *et al.* (2011) reported that delayed feed access (36 h post hatch) declined the number of the breast muscle cells and myofiber diameter in post hatch chicks.

In conclusion, when chicks were immediately fed after hatch, the spread of hatch influenced post hatch performance: chicks hatched later benefitted from a better appetite and faster growth at least until day 5. In this experimental set-up, the spread of hatch was due to their genetic potential, or the availability/consumption of less nutrients during embryonic development. The different  $T_3$  levels at hatch may have contributed to the distinct post hatch performance, although other factors might be involved in the differential feed intake, which is under further investigation in our lab. Delayed feed intake diminished the 'non-uniformity' caused by the different hatching time, but induced growth retardation compared with the chicks immediately fed after hatch, which was more pronounced for the late hatchers.

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