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Effect of hatching time on time to first feed intake, organ development, enzymatic activity and growth in broiler chicks hatched on-farm

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ABSTRACT

The conventional commercial hatcheries used today do not allow the newly hatched chicks to consume feed or water. Combined with natural variation in hatching time, this can lead to early hatched chicks being feed-deprived for up to 72 h before being unloaded at the rearing site. This study investigated the effects of hatching time on time to first feed intake and development of organs, digestive enzymes and productivity in terms of growth and feed conversion ratio in chicks hatched on-farm. Chicks were divided into three hatching groups (early, mid-term and late), and assessed over a full production cycle of 34 days. The results revealed that chicks remain inactive for a considerable amount of time before engaging in eating-related activities. Eating activity of 5% (i.e. when 5% of birds in each hatching group were eating or standing close to the feeder) was recorded at an average biological age (**BA**) of 25.4 h and a proportion of 50% birds with full crop was reached at an average BA of 30.6 h. Considering that the hatching window was 35 h in this study, the average chick probably did not benefit from access to feed and water immediately post-hatch in this case. At hatch, mid-term hatchlings had a heavier small intestine (30.1 g/kg bw) than both early (26.4 g/kg bw) and late (26.0 g/kg bw) hatchlings. Relative length of the small intestine was shorter in late hatchlings (735 cm/kg bw) than in mid-term (849 cm/kg bw) and early (831 cm/kg bw) hatchlings. However, the relative weight of the bursa fabricii was greater in mid-term (1.30 g/kg bw) than in early hatchlings (1.01 g/kg bw). At hatch, late hatchlings were heavier than early and mid-term hatchlings ($P < 0.05$), but by 3 days of age early hatchlings were heavier than mid-term and late hatchlings ($P < 0.01$). The only effect persisting throughout the study was a difference in the relative weight of the small intestine, where late hatchlings had heavier intestines than early hatchlings ($P < 0.05$). Thus, while there were differences between hatching groups, this study showed that the hatchlings seemed capable of compensating for these as they grew.

Implications

Prolonged time without access to feed and water has been proven to have negative effects on the subsequent growth of broiler chickens, but the time it takes for a newly hatched chick to engage in eating-related activities has not been determined. This study found that newly hatched chicks rest for a considerable time before seeking feed and water. This finding is important when planning studies focusing on chicks' early life. It is also relevant for the chicken industry when considering which management system to invest in (i.e. on-farm hatching systems).

Introduction

The conventional way of hatching broiler chickens may not be optimal from a biological point of view. Even though all eggs are put into the hatcher at the same time at the hatchery, the chicks hatch over a period depending on the biological variation and egg storage time. This period is often referred to as the hatching window and according to Tong et al. (2013) it ranges from 24 to 48 h. Powell et al. (2016) observed a hatching window of 37 h for Ross 308 chickens. Even though there are new hatching concepts in use allowing provision of feed and water for the newly hatched chick (Van der Pol et al., 2015) the transition to such hatchery practices has started only during the last couple of years. Therefore, during commercial conditions, a broad hatching window will increase the time to first feed and water intake at the rearing site. At pull, when the majority of the chicks have hatched, management routines at the hatchery and loading and transportation add to the delay (Van de Ven et al., 2013). According to Willemsen et al. (2010), some chicks may be feed-deprived for up to 72 h on arrival at the rearing site. Although the residual yolk supports the chick with nutrients immediately post-hatch (Noy and Sklan, 2001), delayed access to feed and water has been shown to have adverse effects on early chick growth (Noy and Sklan, 1999; Sklan et al., 2000), muscle cell proliferation

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(Powell et al., 2016), yolk sac utilisation (Noy and Sklan, 2001), development of the gastrointestinal tract (Lamot et al., 2014) and immune function (Bar Shira et al., 2004).

At hatch, the chick's digestive system has to undergo considerable changes to convert to digestion of exogenous feed rich in carbohydrates, instead of the lipids that constitute the majority of the yolk (Uni et al., 1998; Ravindran, 2003). The chick actually begins preparing for ingestion of exogenous feed during the neonatal state. During embryonic development, the pancreas starts to secrete digestive enzymes to the neonate chick's intestine. However, the digestibility of starch is low at hatch and increases with age (Marchaim and Kulka, 1967; Noy and Sklan, 1998; Ravindran, 2003).

To overcome the possible disadvantages of deprivation of feed and water post-hatch, different concepts for on-farm hatching have been developed in the Netherlands, where brooded eggs are transported from the hatchery to the rearing facilities at embryonic day 18. Chicks are then hatched during embryonic days 20–21 and provided with immediate access to feed and water (Van de Ven et al., 2009). Chicks hatched at different times during the hatching window have been shown to differ physiologically at hatch in e.g. organ weight, yolk uptake (Van de Ven et al., 2013) and feeding behaviour (Nielsen et al., 2010). However, to the best of our knowledge, no previous study has investigated whether these differences persist and are significant later in the growing period. The aim of the present study was thus to evaluate the effects of hatching time on time to first feed intake and development as regards organ size, secretion of digestive enzymes and growth in chicks hatched on-farm, in a trial ending at 34 days of age.

Material and methods

Housing, birds and feed

The eggs, laid by a 40-week-old breeder flock, had been stored for 4 days prior to incubation and were incubated at 37.8 °C at the commercial hatchery SweHatch, Väderstad. At embryonic day 17.5, 400 Ross-308 eggs that had been automatically candled to confirm fertilisation were transported 309 km by car (approximately 3.5 h) to Lövsta Research Centre at Uppsala, Sweden. The eggshell temperature was checked regularly during transportation using an ear thermometer (Braun ThermoScan® 5, Braun GmbH, Kronberg, Germany).

When the eggs were placed at the research centre, the temperature in the animal facility was set to 33 °C for the first 3 days and was thereafter successively lowered until it reached 23 °C at 24 days and throughout the study. The relative humidity was around 40%. During the period of hatch at the animal facility, starting at embryonic day 19, the first third of the chicks to hatch were assigned to an 'early' hatching group ($n = 95$), the second third to a 'mid-term' hatching group ($n = 95$) and the remaining chicks to a 'late' hatching group ($n = 95$). Day 0 was defined as the day the peak of the hatching window took place, namely embryonic day 20. As soon as the feathers of a chick had dried, it was weighed and placed in one of five replicate modules assigned to the relevant hatching group. There were 15 modules in total.

Each module measured 1.5 m × 0.75 m and contained a feeder and three nipple drinkers to which the chicks had immediate access post-hatch. When the experiment started, there were 16.9 chicks/m^2, whereas the Swedish regulations allow a maximum of 25 chicks/m^2 (Swedish Board of Agriculture, 2019). At the end of the study the stocking density was 16 kg/m^2, whereas the maximum density according to European Union (**EU**) regulation is 33 kg which can be expanded to 39 or even 42 kg/m^2 if certain criteria are fulfilled (Council of the European Union, 2007). Wood shavings were used as litter material. Constant light was provided during hatch and for 2 days post-hatch. On day 3, the chicks were given 1 h of darkness between 23.00 h and midnight. Thereafter, the chicks were provided with 1 h of extra darkness per night until day 8. From day 8 until the end of the study, lights were off between 23.00 and 05.00 h. In the first days, chick body temperature was determined regularly following the recommendations given in the Ross broiler handbook (Aviagen, 2018) by recording the vent temperature of the chicks using the ear thermometer (Braun ThermoScan® 5, Braun GmbH, Kronberg, Germany). A body temperature of 39.4–40.5 was considered optimal (Aviagen, 2018).

The chicks were fed crumbled, sieved pellets as a starter feed and then switched to a grower feed at 10 days of age (both feeds Svenska Foder AB, Lidköping). All birds were given the same commercial starter and grower feeds (pellet diameter 3.5 mm). No coccidiostats were used. Feed samples were dried at 103 °C for 16 h for analysis of DM, while ash was analysed after incineration for 3 h at 550 °C (Jennische and Larsson, 1990). Crude protein (**CP**) content (N × 6.25) was determined by the Kjeldahl method (Nordic Committee on Food Analysis, 2003). Ether extract was determined according to the European Communities (EC) (1998). The analysed chemical composition of the feed was (g/kg DM): ash 57, CP 243, crude fibre 33 and ether extract 53 in the starter feed and ash 48, CP 229, crude fibre 43 and ether extract 64 in the grower feed. The calculated energy content (according to EU MJ) was 13.6 AME MJ/kg DM for the starter feed and 14.5 AME MJ/kg DM for the grower feed.

Recordings

See Table 1 for the number of chicks used at every sampling occasion.

Chick length and organ development

At hatch, live weight and chick length (from middle toe to beak in chicks placed belly down, measured by the same person) were recorded for 20 chicks per hatching group. These chicks were then euthanised by neck dislocation and dissected to determine the weight of the yolk sac, small intestine (with intestinal content), bursa fabricii, heart, liver, gizzard (as dissected and also after emptying and washing) and proventriculus (as dissected), and length of small intestine. At 6, 10, 20 and 34 days of age, two birds from each replicate module were euthanised (by a blow to the head followed by neck dislocation in young chicks and by an intravenous injection of pentobarbital sodium, 100 mg/ml, in chicks aged 20 and 34 days) and weight and length of organs were determined. Thus, at hatch there were 95 birds per hatching treatment (i.e. 19 chicks per module), whilst at the end of the study only 40 chicks per hatching treatment remained (i.e. 8 chicks per module) because of reduction of the number of birds due to both sampling and evening of groups (25 birds per hatching group were excluded from the experiment at day 10) (Table 1).

Enzymatic activity

Samples from the pancreas and small intestine were collected from every second bird used for organ sampling. The duodenal loop was identified and an approximately 5 cm tissue sample starting from the apex, including both intestine and pancreas, was taken and immediately frozen at −80 °C for later analysis of enzymatic activity.

For α-amylase activity assays, intestinal and pancreatic samples from days 6, 10, 20 and 34 were thawed separately, washed with ice-cold phosphate buffer saline, individually cut into small pieces and suspended in 20 volumes of ice-cold malic acid buffer (pH 5.4) and homogenised in an electrical homogeniser (Ultra turrax tube dispenser, IKA Werke GMBH & Co.KG, Staufen, Germany). The homogenate was centrifuged for 10 min at 15 800 × *g* and aliquots of the supernatant were stored at −80 °C for later analysis. The protease inhibitor phenylmethylsulfonyl fluoride (PMSF; 0.5 mM; Sigma no. P7626, Sigma–Aldrich Sweden AB, Stockholm, Sweden) was added before the homogenate was analysed for α-amylase activity. For day 0 samples, intestine plus early pancreas tissue were homogenised together, due to

Table 1
Number of chickens euthanised and used in different recordings at hatch, day 6, 10, 20 and 34. Remaining number of chicks per hatching group (HG) after each sampling occasion is also presented.

Recording	At hatch	Day 6	Day 10	Day 20	Day 34
Organ weights and lengths	20 chicks per HG	2 birds per replicate module, i.e. 10 chicks per HG[1]	2 birds per replicate module, i.e. 10 chicks per HG	2 birds per replicate module, i.e. 10 chicks per HG	2 birds per replicate module, i.e. 10 chicks per HG
Small intestine measurements	10 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG
Amylase activity	5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG	1 bird per replicate module, i.e. 5 chicks per HG
Evening of groups			5 birds per replicate module, i.e. 25 chicks per HG		
Remaining chicks per hatching group after sampling	95	85	50	40	0[1]

[1] The experiment was ended.

lack of material. Apart from this, the procedure was the same for all samples.

Level of α-amylase activity was determined with a commercial kit (Ceralpha Method, AOAC Official Method 2002.01., Megazyme International Ireland Ltd., Wicklow, Ireland) using benzylidene end-blocked *p*-nitrophenyl-α-D-maltoheptaoside as substrate. Test tubes containing the homogenate and amylase high-range reagent solution were incubated for 20 min at room temperature. The reaction was stopped by adding stopping solution (alkaline solution) and absorbance was recorded at 405 nm.

Crop fill assessment

Approximately 4 h after completion of hatchling placement, four randomly pre-selected focal chicks in each module were gently picked up and their crop was examined by palpation (Aviagen, 2018) to determine whether it was empty, half-full or full. This was done every 4 h for 36 h.

Behaviour observations

Behaviour observations commenced within 3 h after completed placement in the early hatching group and were extended to the two later hatching groups after completed placement of chicks in those groups. Chick behaviour was studied by scan sampling every hour, to determine time to first feed intake. Three persons in total took terms quietly walking down the stable aisles, recording the observed behaviours. These persons had beforehand synchronised the methodology to be able to perform the scan sampling as equal as possible. The number of chicks performing either of the following behaviours was recorded: (a) eating from the feeder while standing on the floor or on the feeder or (b) standing close to the feeder or on top of the feeder. A maximum distance of 5 cm from the feeder was considered close. Behaviour observations continued for 52 h.

Production performance

Bird live weight and feed intake were recorded weekly for each replicate module. All chicks in each module were weighed together in a basket placed on a scale outside the module. Weights were then divided by the number of chicks in the module at the weighing occasion. Feed conversion ratio (kg feed consumed divided by kg growth, i.e. FCR) was calculated from these results. Mortality was recorded daily.

Statistics

Growth, digestive enzyme and organ weight data were analysed using the Procedure Mixed (PROC MIXED), in the statistical program SAS (version 9.4), with hatching treatment and age as fixed factors and module as a random factor. The behaviours 'eating' and 'standing close to the feeder' were combined and defined as 'eating activity'. The proportion of chicks performing eating activity was analysed with the statistical software R, using a mixed logistic regression model with module as a random effect and a smooth spline component with respect to time from observation start. The model was used to estimate proportions with 95% confidence intervals and test differences in eating activity with respect to biological age (**BA**), defined for each hatching group as time elapsed in hours since hatch of the median chick in that group. Crop fill measurements were analysed with the statistical software R, using a mixed ordinal regression model assuming proportional odds, with module as random effect and observation time as a categorical variable. The model was used to estimate odds ratios (**OR)** with respect to BA, where a ratio > 1 indicates higher probability of the numerator (first-mentioned factor) than the denominator (second factor), and a ratio < 1 the reverse.

For growth, FCR, eating activity and crop fill, module was considered the experimental unit, giving five replicates per hatching treatment. Organ development and enzyme activity was analysed with individual animal as experimental unit.

Results

The first third of chicks (early hatching group) hatched within 476–496 h post start of incubation, the second third (mid-term group) hatched within 496–504 h and the remaining third (late group) within 505–511 h. The length of the hatching window for all chicks hatched was thus 35 h.

Body weight and organ development at hatch

Data collected from a sample of chicks ($n = 20$ per hatching group) immediately after hatch showed that there were no differences between hatching groups with regard to BW, yolk-free body mass (**YFBM**), yolk sac, chick length, heart, liver, gizzard and proventriculus weighed together, or gizzard alone (as dissected) (Table 2). However, early and late hatchlings had a lighter small intestine at hatch than mid-term hatchlings (Table 2). Length-wise, at hatch late hatchlings had a shorter small intestine in relation to BW than both early and mid-term hatchlings (Table 2). Moreover, there was a difference in bursal weight between hatching groups, with the mid-term group having relatively heavier bursa fabricii than the early hatching group.

Growth, feed conversion ratio and organ development during the growing period

There were no differences in FCR between hatching groups throughout the experimental period (Table 3). However, there was a difference in BW between hatching groups at 0 and 3 days of age. At hatch, late hatchlings were heavier than both early and mid-term hatched chicks, but by 3 days of age the early hatchlings were heavier than both

Table 2
Body, yolk sac and organ weight at hatch in chicks hatched early, mid-term and late in the hatching window. Organ weights are expressed as a proportion of BW.

Variable	Hatching group			Pooled SEM	*P*-value
	Early [1]$n = 20$ [2]$n = 10$	Mid-term [1]$n = 20$ [2]$n = 10$	Late [1]$n = 20$ [2]$n = 10$		Hatching group
BW (g)	[3]43.53	43.97	45.34	0.40	ns
Yolk sac (g)	6.22	5.95	6.86	0.16	ns
YFBM[5] (g)	37.3	38.0	38.5	0.36	ns
Chick length (cm)	18.4	18.8	18.5	0.07	ns
Yolk sac (g/kg BW)	142.5	134.9	151.9	3.39	ns
Small intestine[2] (g/kg BW)	[4]26.4[b]	30.1[a]	26.0[b]	0.65	*
Small intestine[2] (cm/kg BW)	831[a]	849[a]	735[b]	14.7	**
Bursa fabricii (g/kg BW)	1.01[b]	1.30[a]	1.14[ab]	0.05	*
Heart[2] (g/kg BW)	8.51	8.80	8.59	0.18	ns
Liver[2] (g/kg BW)	20.3	21.5	18.8	0.43	ns
Proventriculus and gizzard[2] (g/kg BW)	45.2	47.8	44.9	0.90	ns
Gizzard[2] (g/kg BW)	38.4	39.0	37.6	0.73	ns

$^{**}P < 0.01$; $^{*}P < 0.05$; ns $P > 0.05$.
[1] A total of 20 chicks from each hatching group were euthanised and weight, length, yolk sac weight and bursal weight were recorded for all birds.
[2] Small intestine, heart, liver, proventriculus and gizzard measurements were performed on every second bird.
[3] Values are least-squares means (LSM).
[4] LSM values within rows lacking a common superscript are significantly different ($P < 0.05$).
[5] Yolk free body mass (YFBM).

mid-term and late hatchlings. The early hatching chicks had numerically greater weight throughout the study, but from 10 days of age there were no significant differences in BW (Table 3).

As regards weight of organs during the growing period (Table 4), the small intestine was the only parameter differing between hatching groups, with early hatched chicks having significantly lower relative intestinal weight than late hatchlings and a tendency for lower intestinal weights than mid-term hatchlings ($P = 0.0747$, data not shown). At 20 days of age, the yolk sac was completely utilised and not detectable during dissection.

An effect of age was also observed for all organs studied (Table 4). Weight or size decreased with age when considered as a proportion of the total BW for yolk sac, small intestine (g and cm), heart, liver, proventriculus and gizzard. The relative weight of the bursa fabricii was significantly greater at 20 days of age than at 6, 10 and 34 days of age. At 34 days of age, early hatchings were heavier than both mid-term and late hatching chicks, resulting in an interaction between hatching group and age.

Table 3
Feed conversion ratio – g/g growth (FCR) and BW – g (BW) at seven different ages (days) in chicks hatched early, mid-term and late in the hatching window.

	Hatching group			Pooled SEM	*P*-value
	Early $n = 5$	Mid-term $n = 5$	Late $n = 5$		
FCR					
Day 0	–	–	–	–	–
Day 3	[1]0.93	0.97	1.00	0.018	ns
Day 10	1.09	1.09	1.06	0.011	ns
Day 17	1.61	1.63	1.59	0.030	ns
Day 24	1.52	1.54	1.55	0.024	ns
Day 31	1.51	1.60	1.52	0.031	ns
Day 34	1.56	1.57	1.56	0.008	ns
BW					
Day 0	[2]44.8[b]	44.9[b]	46.3[a]	0.19	*
Day 3	77.1[a]	67.9[b]	64.3[b]	1.11	**
Day 10	307.1	286.0	278.7	10.09	ns
Day 17	718.3	651.2	655.9	23.80	ns
Day 24	1 273.5	1 221.7	1 158.9	32.14	ns
Day 31	1 928.7	1 889.7	1 826.2	48.65	ns
Day 34	2 232.9	2 190.3	2 136.3	56.50	ns

$^{**}P < 0.01$; $^{*}P < 0.05$; ns $P > 0.05$.
[1] Values are least-squares means (LSM).
[2] LSM values within rows lacking a common superscript differ ($P < 0.05$).

Enzymes at hatch and during the growing period

No effect of hatching group on α-amylase activity (U/g sample, where U is μmol hydrolysed per minute) was observed when analysing mixed or separated intestinal and pancreatic samples, either at hatch or later in the study (Table 5).

Intestinal α-amylase in relation to intestinal content was higher at 6 days of age than at 20 and 34 days of age (Table 5). Moreover, there was an interaction between hatching group and age with regard to α-amylase (U/g sample) activity in the intestine. This interaction arose because there was no effect of age within the early and late hatching groups, whereas the mid-term chicks had higher α-amylase activity in the intestine at 6 days of age compared with 10, 20 and 34 days of age.

In the pancreas, α-amylase activity (U/g sample) was lower at 6 and 10 days of age than at 20 and 34 days (Table 5).

Eating activity

The percentage of chicks showing active eating behaviour and related confidence intervals at BA 20, 30, 35 and 40 h is shown in Table 6. There were differences between hatching groups in their eating activity in relation to BA. Comparisons of confidence intervals between hatching groups (Table 6) revealed that eating activity was higher in the late hatching group than in the early hatching group at BA 20. There was also a tendency for a difference ($P = 0.062$, data not shown) between the late and mid-term hatching groups at the same BA. At BA 30, eating-related activity was highest in the mid-term group and lowest in the early group, whereas the late group was intermediate and not different from either the early or mid-term group. At BA 35 and 40, eating activity was higher in the early group and mid-term group compared with the late group, but there were no differences between the early and mid-term groups. An eating activity level of 5% (i.e. when 5% of the birds were either eating or standing close to the feeder) was reached at BA 21.7 h in the late hatching group, 25.1 h in the mid-term hatching group and 29.5 h in the early hatching group, hence, a 5% eating activity was observed first at a mean BA of 25.4 h (data not shown).

Table 4
Organ weight (as proportion of BW) at four different ages in chicks hatched early, mid-term and late in the hatching window. Values for hatching groups are averages for the 34 days growth period.

Variable	Hatching group			Age (days)				Pooled SEM	*P*-value		
	Early $n = 40$ [1]$n = 20$	Mid-term $n = 40$ [1]$n = 20$	Late $n = 40$ [1]$n = 20$	6 $n = 30$ [1]$n = 15$	10 $n = 30$ [1]$n = 15$	20 $n = 30$ [1]$n = 15$	34 $n = 30$ [1]$n = 15$		Hatching group	Age	Hatching group * age
Weight (g)	[2]955.4	862.7	863.8	143.3[d]	294.8[c]	890.7[b]	2 246.9[a]	21.2	ns	***	*
YFBM[4] (g)	231.4	214.4	210.9	143.1[b]	294.8[a]			6.8	ns	***	ns
Yolk sac (g/kg BW)	0.63	0.72	0.76	1.31[a]	0.11[b]			0.13	ns	***	ns
Small intestine[1] (g/kg BW)	[3]77.5[b]	84.2[ab]	85.4[a]	105.4[a]	90.2[b]	76.1[c]	57.8[d]	1.22	*	***	ns
Small intestine[1] (cm/kg BW)	286.5	341.1	341.0	678.6[a]	395.8[b]	152.3[c]	64.8[d]	14.8	ns	***	ns
Bursa fabricii (g/kg BW)	1.68	1.78	1.68	1.44[b]	1.66[b]	2.05[a]	1.71[b]	0.04	ns	***	ns
Heart (g/kg BW)	7.06	6.85	7.04	8.64[a]	8.07[a]	6.05[b]	5.17[b]	0.17	ns	***	ns
Liver (g/kg BW)	38.7	38.6	41.2	50.1[a]	44.3[b]	34.5[c]	29.1[d]	0.55	ns	***	ns
Proventriculus (g/kg BW)	41.6	43.7	42.1	72.7[a]	46.3[b]	32.2[c]	18.5[d]	1.05	ns	***	ns
Gizzard full (g/kg BW)	33.4	35.5	33.5	61.2[a]	37.0[b]	24.8[c]	13.6[d]	0.88	ns	***	ns
Gizzard empty (g/kg BW)	21.4	23.2	22.4	36.1[a]	25.4[b]	17.8[c]	10.1[d]	0.71	ns	***	ns

***$P < 0.001$; *$P < 0.05$; ns $P > 0.05$.
[1] Small intestine measurements were performed on every second bird.
[2] Values are least-squares means (LSM).
[3] LSM values within rows lacking a common superscript differ ($P < 0.05$).
[4] Yolk free body mass (YFBM).

Crop fill

As illustrated in Fig. 1, the BA at which all four focal birds (100%) from each module had either half-full or full crop differed between hatching groups, decreasing from 40.6 h in the early hatching group to 32.4 h in the mid-term hatching group and 30.5 h in the late hatching group. This indicates that chicks in late and mid-term groups started to eat earlier post-hatch than the early hatching chicks. The three hatching groups reached a proportion of 50% of birds with full crop at approximately the same BA (32.6, 28.6 and 30.5 h in the early, mid-term and late hatching group, respectively) (Fig. 1). According to OR the mid-term group had a higher proportion of full crops than the early group at BA 30 h (OR = 6.3) and 35 h (OR = 4.5) (Table 7). A similar pattern was seen at BA 35 h for the mid-term hatching group compared with the late hatching chicks (OR = 3.6), while a tendency for an effect was observed at BA 40 h (OR = 3.0). At BA 40 h, the early hatching group tended to have a higher proportion of full crops than the late group (OR = 0.3) (Table 7). The increase from a proportion of 50% birds with full crop to 90% took an extra 7 h for the early hatching group and 3 h for the mid-term group. In the late group, only 65% of the birds had a full crop at BA 43 h, when the measurements ended (Fig. 1).

Table 6
Proportion of chicks active in eating-related behaviours and confidence intervals between chicks hatched early, mid-term and late in the hatching window. Biological age (BA) is defined for each hatching group as time (h) since hatch of the median chick in that group.

	Estimate	95% Confidence intervals
BA 20		
Early	[2]0.7%[b]	[1]0.3–1.4%
Mid-term	1.4%[ab]	0.8–2.4%
Late	3.4%[a]	1.6–6.9%
BA 30		
Early	5.7%[b]	4.1–7.9%
Mid-term	14.7%[a]	12.0–17.9%
Late	9.3%[ab]	4.8–17.2%
BA 35		
Early	13.7%[a]	10.6–17.6%
Mid-term	14.7%[a]	12.0–17.7%
Late	4.0%[b]	2.0–8.0%
BA 40		
Early	13.6%[a]	10.7–17.2%
Mid-term	14.6%[a]	11.9–17.7%
Late	2.3%[b]	1.0–5.0%

[1] Confidence intervals that are not overlapping within a BA group differ significantly ($P < 0.05$).
[2] Different superscripts within a BA group indicate significant differences ($P < 0.05$).

Table 5
Activity of α-amylase in chicks hatched early, mid-term and late in the hatching window, at hatch and at 6, 10, 20 and 34 days of age. At hatch, α-amylase activity was analysed in samples containing mixed intestine and pancreas. During the growing period, α-amylase activity was analysed in separate intestinal and pancreas samples. Values for hatching groups are averages for the 34 days growth period.

Variable	Hatching group			Age (d)				Pooled SEM	*P*-value		
Mixed pancreas and intestine, α-amylase activity at hatch	Early $n = 5$	Mid-term $n = 5$	Late $n = 5$						Hatching group	–	–
α-Amylase [2]U/g sample	[1]1 874.4	2 834.6	1 577.7	At hatch				253.51	ns	–	–
Intestinal α-amylase activity	Early $n = 20$	Mid-term $n = 20$	Late $n = 20$	6 $n = 15$	10 $n = 15$	20 $n = 15$	34 $n = 15$		Hatching group	Age	Hatching group * age
α-Amylase U/g sample	98.5	120.1	99.3	[3]157.3[a]	114.1[ab]	64.4[b]	88.1[b]	7.93	ns	***	*
Pancreatic α-amylase activity	Early $n = 20$	Mid-term $n = 20$	Late $n = 20$	6 $n = 15$	10 $n = 15$	20 $n = 15$	34 $n = 15$		Hatching group	Age	Hatching group * age
α-Amylase U/g sample	335.0	355.4	398.3	249.4[b]	271.5[b]	501.9[a]	428.9[a]	16.8	ns	***	ns

***$P < 0.001$; *$P < 0.05$; ns $P > 0.05$.
[1] Values are least-squares means (LSM).
[2] U is defined as μmol hydrolysed per minute.
[3] LSM values within rows lacking a common superscript differ ($P < 0.05$).

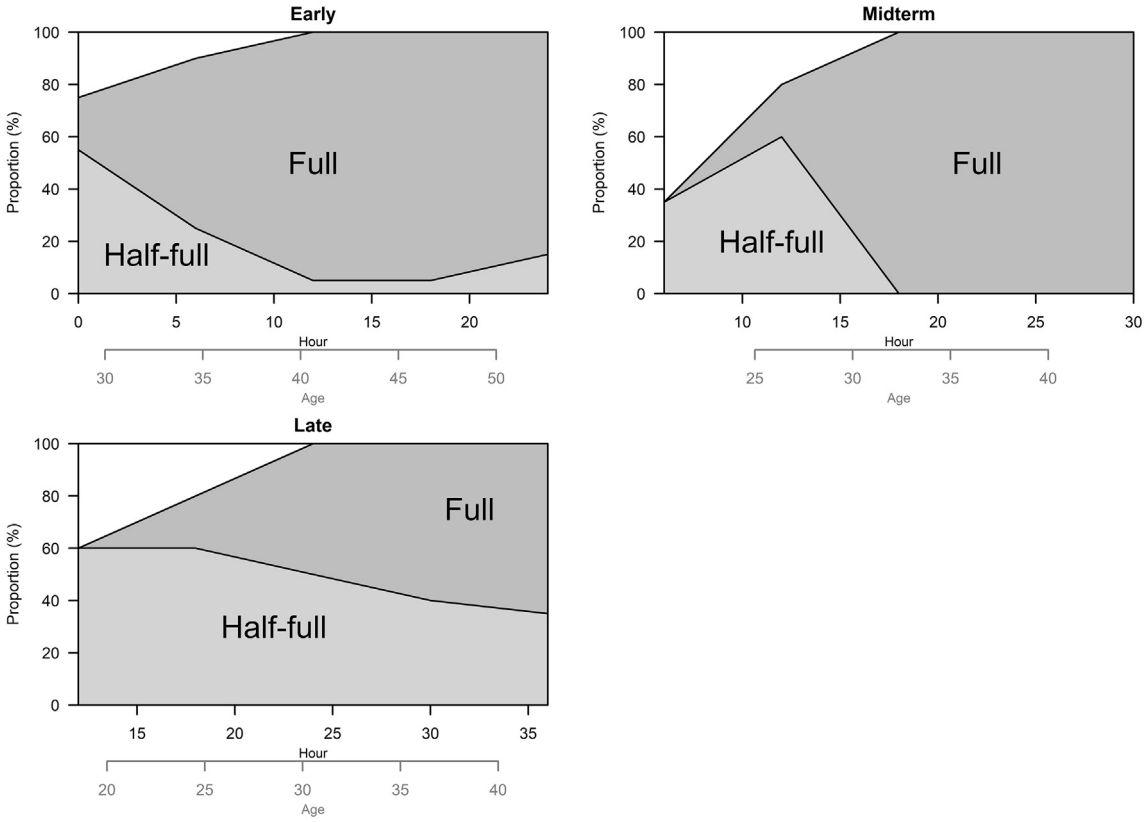

Fig. 1. Level of crop fill in newly hatched chicks. The primary x-axis (black) shows time from examination start and the secondary x-axis (grey) biological age (BA, defined for each hatching group as time (h) since hatch of the median chick). Four individually marked chicks out of 19 chicks per module were examined every 4 h, observed proportions are shown.

Discussion

Many studies have emphasised the importance of immediate access to feed and water in broiler chicks post-hatch (Sklan et al., 2000; Lamot et al., 2014, among others). However, although chicks were offered feed and water from the moment they hatched in this study, 5% eating activity was observed first at a mean BA of 25.4 h. Moreover, it took on average 30.6 h post-hatch before 50% of the birds examined had a full crop. The hatching window in the study was 35 h in accordance with the 37 h long interval reported by Powell et al. (2016), whereas in hatchery practice it would probably have been shorter due to the set pull time. Aviagen, the company that developed the Ross 308 genotype, states that the hatching window for its broiler (time from 1 to 99% hatched chicks) is around 30 h (Tullett, 2009). In the present study, less than 5% of the focal birds in all hatching groups were engaged in eating-related behaviour at the end of the hatching window. In other words, it appears to take some time post-hatch before the chicks are motivated to engage in feed-seeking activities at all.

However, it should be pointed out that, because of the small number of chicks in the present study and the calm environment in the research facility compared with a hatchery, it is possible that chicks hatched in a hatchery would have been stimulated to start eating-related activities earlier. Moreover, the scan sampling methodology takes spot scans, and thus does not cover the birds' activities at all times.

Considering the new knowledge obtained in this study on time to first feed intake, on-farm hatching as a housing system might have greater impacts on production and welfare parameters at farms located far from the hatchery. Despite shorter distances between hatcheries and farms in the Netherlands than in Sweden, benefits for welfare parameters and production performance in on-farm hatched chicks compared with their conventional counterparts have been reported (De Jong et al., 2017). However, these differences may be dependent on factors other than lack of access to feed and water under conventional conditions, such as bacterial load at the hatchery and stress due to climate in the hatchery, handling and transportation. Further studies comparing conventional and on-farm hatching practices should include our findings on time to first feed intake, to obtain reliable decision support for the chicken industry.

Many factors affect the time from the start of incubation to hatch for individual eggs. For example, incubation duration increases with egg size (Wilson, 1991) and the weight of the newly hatched chick has been shown to correlate with the weight of the egg at setting (Tona et al., 2003). This could possibly explain the greater weight of the late-hatched chicks in the present study compared with early and mid-term hatchlings. Age of broiler mother flock and storage time of fertilised eggs also affect the duration of hatch (Sklan et al., 2000).

Table 7
Pair-wise comparisons of crop fill between chicks hatched early, mid-term and late in the hatching window, based on results of an ordinal regression model. Biological age (BA) is defined for each hatching group as time (h) since hatch of the median chick in that group.

	Odds ratio	*P*-value
BA 30		
Mid-term – Early	6.3	0.002
Mid-term – Late	2.5	0.12
Late – Early	2.5	0.16
BA 35		
Mid-term – Early	4.5	0.018
Mid-term – Late	3.6	0.032
Late – Early	1.3	0.73
BA 40		
Mid-term – Early	0.8	0.80
Mid-term – Late	3.0	0.078
Late – Early	0.3	0.094

Biological variation, incubation conditions and hatching synchronisation through species-specific vocalisation also play a part (Tong et al., 2013).

Many studies have concluded that chicks hatched in different parts of a hatching window differ from each other physiologically (Van de Ven et al., 2013; Lamot et al., 2014). Some behavioural differences related to eating have also been observed (Nielsen et al., 2010). Our findings that early hatched chicks were lighter than late-hatched chicks at hatch, but heavier than both late and mid-term hatchings at 3 days of age, correspond well with Lamot et al. (2014) who found that early hatched chicks seemed to have compensated for their low hatching weight by 4 days of age, at which time they had a greater BW than mid-term and late hatchlings. Moreover, Nielsen et al. (2010) observed a minor weight advantage in early hatchlings at 3 days of age. These findings contradict those of Van de Ven et al. (2013), who found no differences due to hatching time with regard to BW or YFBM at hatch. Body weight is a commonly used parameter when assessing chick quality, but BW at hatch may not be a good predictor of post-hatch growth and 1-d BW (i.e. after access to feed and water) has been shown to have higher predictability (Lindholm et al., 2017). Yolk free body mass is also commonly used for assessing chick quality and has the advantage that it corrects for the weight of the residual yolk (Sozcu and Ipek, 2015).

In a study by Dibner et al. (1998), denying chicks access to feed on the day of hatch and the following day resulted in a more pronounced decrease in relative weight of bursa fabricii compared with other organs, an effect that persisted for 21 days. In contrast, early feeding increased bursal weight and also proliferation of B-cells (Dibner et al., 1998). In the present study, the early hatching group had lower relative bursal weight than the mid-term hatching group at hatch. Even if it took longer for the early hatchlings to start to eat compared with the mid-term and late groups, the birds chosen for post-hatch dissection were still euthanised before they had the chance to eat or drink. Therefore the explanation for the difference in organ weight presented by Dibner et al. (1998) is not applicable here.

Relative intestinal weight was greater in the mid-term group than in the early and late groups at hatch. The late group also had greater relative intestine weight than the early group when considering the whole experimental period. The greater relative length of intestine in the early and mid-term groups compared with the late group at hatch is also worth noting. Intestinal growth by elongation has been shown to be regulated by contraction of the smooth muscle cells already in the embryonic state. As embryogenesis progresses, differentiation of these smooth muscle cells depresses elongation (Khalipina et al., 2019). Varying effectiveness of this process during embryogenesis might be responsible for the differences in intestinal length at hatch, and needs further investigation.

No effect of hatching group on intestinal or pancreatic α-amylase activity was observed either at hatch or later during the study. In a study comparing poults 24 h post hatch, decreased activity of pancreatic α-amylase were observed in poults supplemented with a liquid nutrient mix composing glucose, starch and oil compared with poults kept feed-restricted post-hatch (Pinchasov, 1994). This indicates that the presence of feed in the intestine may not be of crucial importance for the onset of enzymatic activity (Pinchasov, 1994). It could well be the reason why differences in onset of feed intake and foraging observed between hatching groups in the present study did not result in any differences in α-amylase activity in the intestine or pancreas. On the other hand, Gracia et al. (2003) highlight that the early development of the gastrointestinal tract is stimulated by feed intake and also the importance of early synthesis of pancreatic enzymes to counteract negative effects on growth post-hatch. Moreover, Svihus (2014) states in a review that broiler chicks, when fed early post-hatch, have a high amylase activity.

Pancreatic α-amylase activity increased with age, confirming results by Noy and Sklan (1995). Intestinal α-amylase activity decreased with age, in contrast with earlier findings (Nir et al., 1993; Noy and Sklan, 1995). Why the age-dependent increase in pancreatic α-amylase did not bring about a corresponding increase in intestinal α-amylase activity in our study is not known. However, Nitsan et al. (1991) observed a decrease in intestinal α-amylase from 9 to 15 days of age, which is more in line with our results. Moreover, in a study comparing fast-growing broilers and slow-growing layer cockerels, Zelenka and Čerešňáková (2005) found that overall starch digestibility was linearly decreasing with age in the broilers.

In conclusion, the time of hatch affected some of the parameters studied. The observed differences in organ weights at hatch did however not persist throughout the production cycle. Neither did the differences in organ weights reflect themselves in the BW differences, because the BW differences were no longer apparent at 10 days of age. Even though there were some differences in early eating behaviour and crop fill in early life, the chicks seemed capable of compensating for these during the grow-out period.

Ethics approval

The experimental set-up was approved by Uppsala Animal Experiment Ethics Board (application reference number C 36/16).

Data and model availability statement

The statistical software SAS 9.4 and R version 3.4.0 were used for analysis of data. None of the data were deposited in an official repository. Models are available upon request.

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Declaration of interest

The authors have no conflict of interest to declare.

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References

Aviagen, 2018. Broiler management handbook. Retrieved on 8 July 2020 from. http://eu.aviagen.com/assets/Tech_Center/Ross_Broiler/Ross-BroilerHandbook2018-EN.pdf.

Bar Shira, E., Sklan, D., Friedman, A., 2004. Impaired immune response in broiler hatchling hindgut following delayed access to feed. Veterinary Immunology and Immunopathology 105, 33–45.

Council of the European Union, 2007. Council directive 2007/43/EC of 28 June 2007 laying down minimum rules for the protection of chickens kept for meat production. Official Journal of the European Union L182, 19–29.

De Jong, I.C., van Riel, J., Bracke, M.B.M., van den Brand, H., 2017. A 'meta-analysis' of effects of post-hatch food and water deprivation on development, performance and welfare of chickens. PLoS One 12, 1–20.

Dibner, J.J., Knight, C.D., Kitchell, M.L., Atwell, C.A., 1998. Early feeding and development of the immune system in neonatal poultry. Journal of Applied Poultry Research 7, 425–436.

European Communities (EC), 1998. Commission directive 98/64/EC of 3 September 1998 establishing community methods of analysis for the determination of amino acids, crude oils and fats, and olaquindox in feeding stuffs and amending directive 71/393/EEC. Official Journal of European Communities L 257, 23.

Gracia, M.I., Araníbar, M.J., Lázaro, R., Medel, P., Mateos, G.C., 2003. α-Amylase supplementation of broiler diets based on corn. Poultry Science 82, 436–442.

Jennische, P., Larsson, K., 1990. Traditional Swedish methods to analyze feeds and feedstuffs (Report 60). Swedish Agrochemical Laboratory, Uppsala, Sweden.

Khalipina, D., Kaga, Y., Dacher, N., Chevalier, N.R., 2019. Smooth muscle contractility causes the gut to grow anisotropically. Journal of the Royal Society Interface 16, 1–10.

Lamot, D.M., van de Linde, I.B., Molenaar, R., van der Pol, C.W., Wijtten, P.J.A., Kemp, B., van den Brand, H., 2014. Effects of moment of hatch and feed access on chicken development. Poultry Science 93, 2604–2614.

Lindholm, C., Jönsson, J., Calais, A., Middelkoop, A., Yngwe, N., Berndtson, E., Hult, E., Altimiras, J., 2017. Growth heterogeneity in broiler breeder pullets is settled before onset of feed restriction but is not predicted by size at hatch. Journal of Animal Science 95, 182–193.

Marchaim, U., Kulka, R.G., 1967. The non-parallel increase of amylase, chymotrypsinogen and procarboxypeptidase in the developing chick pancreas. Biochimica et Biophysica Acta 146, 553–559.

Nielsen, B.L., Juul-Madsen, H.R., Steenfeldt, S., Kjaer, J.B., Sørensen, P., 2010. Feeding activity in groups of newly hatched broiler chicks: effects of strain and hatching time. Poultry Science 89, 1336–1344.

Nir, I., Nitsan, Z., Mahagna, M., 1993. Comparative growth and development of the digestive organs and of some enzymes in broiler and egg type chicks after hatching. British Poultry Science 34, 523–532.

Nitsan, Z., Dunnington, E.A., Siegel, P.B., 1991. Organ growth and digestive enzyme levels to fifteen days of age in lines of chickens differing in body weight. Poultry Science 70, 2040–2048.

Nordic Committee on Food Analysis, 2003. Determination in feeds and faeces according to Kjeldahl, 3rd edition. NMKL, Oslo, Norway.

Noy, Y., Sklan, D., 1995. Digestion and absorption in the young chick. Poultry Science 74, 366–373.

Noy, Y., Sklan, D., 1998. Metabolic responses to early nutrition. Journal of Applied Poultry Research 7, 437–451.

Noy, Y., Sklan, D., 1999. Energy utilization in newly hatched chicks. Poultry Science 78, 1750–1756.

Noy, Y., Sklan, D., 2001. Yolk and exogenous feed utilization in the posthatch chick. Poultry Science 80, 1490–1495.

Pinchasov, Y., 1994. Early transition of the digestive system to exogenous nutrition in domestic post-hatch birds. The British Journal of Nutrition 73, 471–478.

Powell, D.J., Velleman, S.G., Cowieson, A.J., Singh, M., Muir, W.I., 2016. Influence of chick hatch time and access to feed on broiler muscle development. Poultry Science 95, 1433–1448.

Ravindran, V., 2003. Development of digestive function in neonatal poultry: physiological limitations and potential. Proceedings of the Australian poultry science symposium, 10–12 February 2003, Sydney, Australia, pp. 1-7.

Sklan, D., Noy, Y., Hoyzman, A., Rozenboim, L., 2000. Decreasing weight loss in the hatchery by feeding chicks and poults in hatching trays. Journal of Applied Poultry Research 9, 142–148.

Sozcu, A., Ipek, A., 2015. Quality assessment chicks from different hatcher temperatures with different scoring methods and prediction of broiler growth performance. Journal of Applied Animal Research 43, 409–416.

Svihus, B., 2014. Starch digestion capacity of poultry. Poultry Science 93, 2394–2399.

Swedish Board of Agriculture, 2019. SJVFS 2019:23. Statens jordbruksverks föreskrifter och allmänna råd om fjäderfähållning inom lantbruket m.m. Retrieved on 9 July 2020 from. https://djur.jordbruksverket.se/download/18.7c1e1fce169bee5214fb0a6d/1553854723792/2019-023.pdf.

Tona, K., Bamelis, F., De Ketelaere, B., Bruggeman, V., Moraes, V.M.B., Buyse, J., Onagbesan, O., Decuypere, E., 2003. Effects of egg storage time on spread of hatch, chick quality, and chick juvenile growth. Poultry Science 82, 736–741.

Tong, Q., Romanini, C.E., Exadaktylos, V., Bahr, C., Berckmans, D., Bergoug, H., Etarradossi, N., Roulston, N., Verhelst, R., McGonnell, I.M., Demmers, T., 2013. Embryonic development and the physiological factors that coordinate hatching in domestic chickens. Poultry Science 92, 620–628.

Tullett, S., 2009. Investigating hatchery practice. Retrieved on 4 March 2020 from. http://en.aviagen.com/assets/Tech_Center/Ross_Tech_Articles/RossTechInvestigatingHatcheryPractice.pdf.

Uni, Z., Ganot, S., Sklan, D., 1998. Posthatch development of mucosal function in the broiler small intestine. Poultry Science 77, 75–82.

Van de Ven, L.J.F., van Wagenberg, A.V., Groot Koerkamp, P.W.G., Kemp, B., van den Brand, H., 2009. Effects of a combined hatching and brooding system on hatchability, chick weight, and mortality in broilers. Poultry Science 88, 2273–2279.

Van de Ven, L.J.F., van Wagenberg, A.V., Decuypere, E., Kemp, B., van den Brand, H., 2013. Perinatal broiler physiology between hatching and chick collection in 2 hatching systems. Poultry Science 92, 1050–1061.

Van der Pol, C.W., Maatjens, C.M., Aalbers, G., van Roovert-Reijrink, I., 2015. Effect of feed and water access on hatchling body weight changes between hatch and pull. Proceedings of the 20th European symposium on poultry nutrition (ESPN), 24–27 August 2015, Prague, Chech Republic, p. 255.

Willemsen, H., Debonne, M., Swennen, Q., Everaert, N., Careghi, C., Han, H., Bruggeman, V., Tona, K., Decuypere, E., 2010. Delay in feed access and spread of hatch: importance of early nutrition. World's Poultry Science Journal 66, 177–188.

Wilson, H.R., 1991. Interrelationships of egg size, chick size, posthatching growth and hatchability. World's Poultry Science Journal 47, 5–20.

Zelenka, J., Čerešňáková, Z., 2005. Effect of age on digestibility of starch in chickens with different growth rate. Czech Journal of Animal Science 50, 411–415.