Perinatal broiler physiology between hatching and chick collection in 2 hatching systems

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ABSTRACT Little is known about physiological responses of early- versus late-hatching chicks to early posthatch conditions in broiler practice. We investigated effects of hatching time on perinatal broiler physiology in 2 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 with 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 468 h (early), 483 h (midterm), and 498 h (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 515 h 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 triiodothyronine 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

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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 to 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,

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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 the 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, RH, 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., 1990; 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 2 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 wk. Eggs were stored for 2 to 3 d before being set in a Petersime (Zulte, Belgium) setter. A standard single-stage incubation program was used in the setter with a gradually decreasing machine temperature from 38.0°C at embryo (**E**) 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 ± 0.4 g, and was 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 the 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 d 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.

After decapitation, blood samples were collected in 4-mL fluoride tubes (BD Vacutainer, Becton Dickinson, Franklin Lakes, NJ) to which 10 μL of heparin was added, and put on ice immediately after collection. When 6 samples were collected, the tubes were centrifuged during 6 min at 2,300 × *g* at 10°C using a Rotofix 32 centrifuge (Hettich Zentrifugen, Tuttlingen, Germany). Plasma was collected and stored in 0.2 mL of Eppendorfs at −20°C until analysis.

The triiodothyronine ($\mathbf{T_3}$) and thyroxine ($\mathbf{T_4}$) concentrations were measured in plasma samples by RIA as described by Darras et al. (1992) using antisera and T_3 and T_4 standards (Byk-Belga, Brussels, Belgium). Corticosterone concentrations were analyzed as described previously (Decuypere et al., 1983; Meeuwis et al., 1989), using a double-antibody RIA-kit (IDS Ltd., Boldon, UK). Plasma glucose, lactate, and uric acid were analyzed by colorimetric diagnostics using a biochemical analyzer (VetTest 8008, Idexx Laboratories Inc., Westbrook, ME). 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 analyzed with the SAS 9.1 software package (SAS Institute Inc., 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 nonnormal 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 analyzed for 2 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:

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

where Y_{ij} is the dependent variable; SYS_i = system (hatcher, patio), HT_j = hatching time (early, midterm, late), and e_{ij} = residual error term.

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).

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. The 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 × HT interaction was observed: weights increased with increased hatching time in patio chicks, whereas 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, whereas 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 toward higher glycogen levels in patio compared with 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 × 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.

Physiological Changes Between Hatch and 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, whereas the YFBM of patio chicks, including possible feed and water residues in the intes-

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)

Item[1]	n	BW (g)	YFBM[2] (g)	Yolk (g)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Bursa (g)	Stomach (g)	Intestines (g)	Intestines (cm)
Treatment												
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 × HT												
Hatcher × 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 × 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 × 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 × 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 × 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 × 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 × HT		0.226	0.522	0.164	0.070	0.227	0.050	0.918	0.371	0.505	0.013	0.043

[a–c]Least squares means followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

[1]SYS = system (hatcher, patio); HT = hatching time (early, midterm, late).

[2]YFBM = yolk-free body mass.

tines 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

Table 2. Least squares means of total hepatic glycogen of chicks collected right after hatch or after 515 h of incubation (d E21.5 of incubation) from chicks hatched at 468 h (early), 483 h (midterm), or 498 h of incubation (late) in hatcher or in patio conditions

		Total hepatic glycogen (mg)		
Item[1]	n	At hatch	At d E21.5	Delta
Treatment				
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 × HT				
Hatcher × early	25	7.2 ± 1.5	1.3 ± 1.3^{c}	−5.9
Hatcher × midterm	25	8.3 ± 1.8	1.2 ± 0.3^{c}	−7.1
Hatcher × late	25	7.9 ± 1.7	3.6 ± 0.8^{c}	−4.3
Patio × early	25	9.5 ± 2.1	165.1 ± 35.8^{a}	+155.6
Patio × midterm	25	8.9 ± 1.9	69.0 ± 14.9^{a}	+60.0
Patio × late	25	9.6 ± 2.1	17.1 ± 3.7^{b}	+7.5
Source of variation				
HT		0.926	0.103	
SYS		0.071	<0.001	
SYS × HT		0.710	<0.001	

[a–c]Least squares means followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

[1]SYS = system (hatcher, patio); HT = hatching time (early, midterm, late).

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)[1]

Item[2]	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
Treatment								
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 × HT								
Hatcher × early	25	173.8	42.39^{b}	5.16	26.25	1.07^{c}	5.50	0.22
Hatcher × midterm	25	190.4	57.49^{a}	5.73	21.61	1.68^{ab}	6.17	0.29
Hatcher × late	25	188.9	37.68^{b}	5.12	16.52	2.13^{a}	8.75	0.27
Patio × early	25	185.2	45.22^{b}	5.14	21.19	1.32^{bc}	5.19	0.28
Patio × midterm	25	190.2	42.67^{b}	5.31	20.15	1.53^{b}	5.09	0.33
Patio × 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 × HT		0.099	<0.001	0.551	0.077	0.012	0.298	0.574

[a–c]Least squares means followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

[1]T_3 = triiodothyronine; T_4 = thyroxine.

[2]SYS = system (hatcher, patio); HT = hatching time (early, midterm, late).

chicks. Because intestine and stomach weight included feed residues in patio chicks at d E21.5, weights of these organs were not analyzed.

Between hatch and 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 to 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 and d E21.5, corticosterone levels increased in hatcher chicks (+15.3%) and decreased in patio chicks (−36.5%). The 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 to d E21.5, the 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).

Physiological Parameters at d E21.5

Body and Organ Weights. The BW and absolute organ weights at d E21.5 are summarized in Table 4. For all variables, significant SYS × 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 ($P < 0.001$), whereas for late chicks, the difference was not significant. The SYS × 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, whereas 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 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 × HT interaction was found for hepatic glycogen. Glycogen was higher in early than in midterm and late chicks in patio, but it did not differ among hatching times in hatcher chicks.

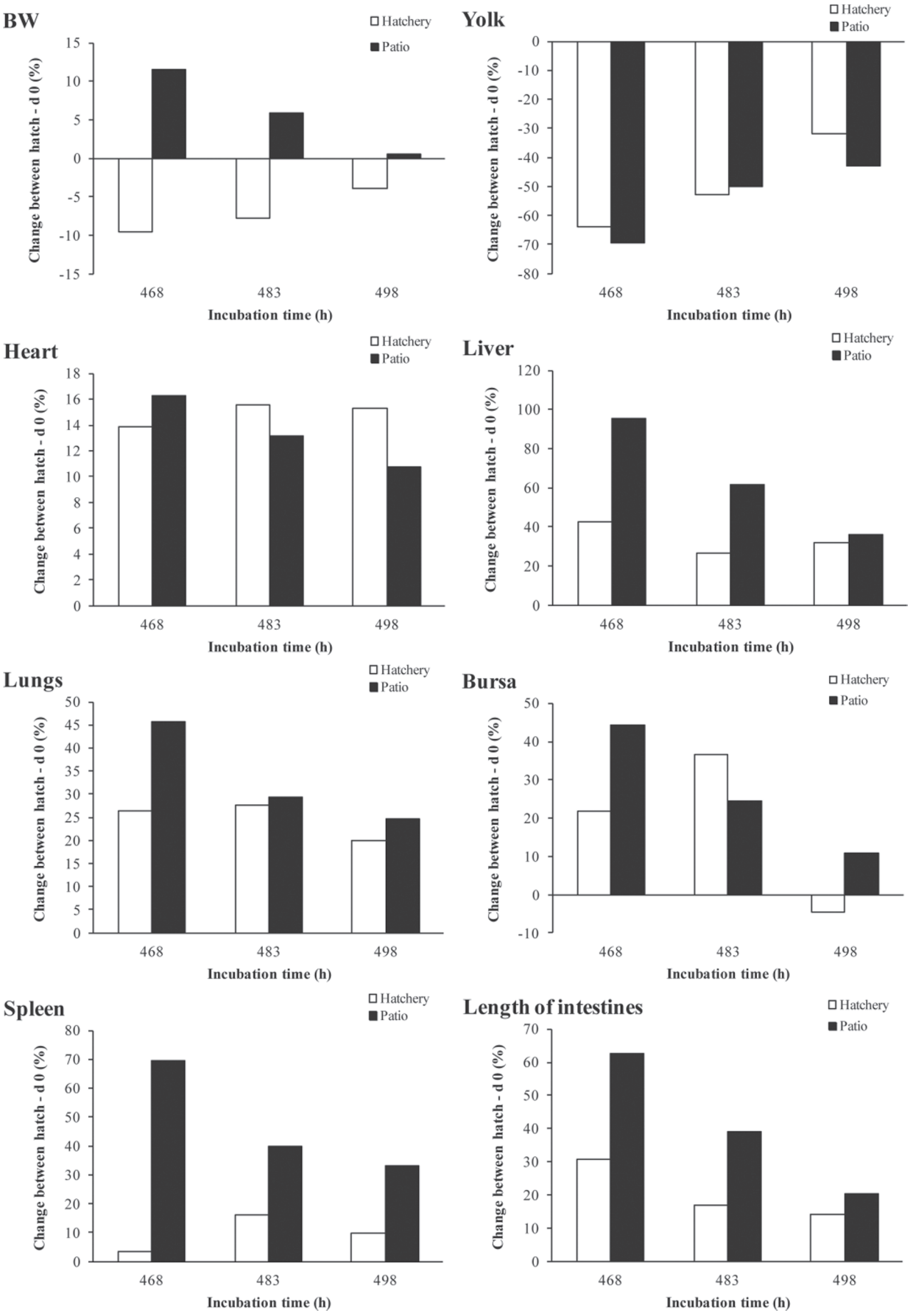

Figure 1. Least squares means of relative weight changes of the whole body, yolk, heart, liver, lung, bursa of Fabricius, spleen, and length of intestines of chicks hatched early, midterm, or late in the hatching process in a hatcher or a patio system, determined directly after hatch (468 h for early, 483 h for midterm, or 498 h for late chicks), or at the end of incubation (515 h for all 3 hatch groups).

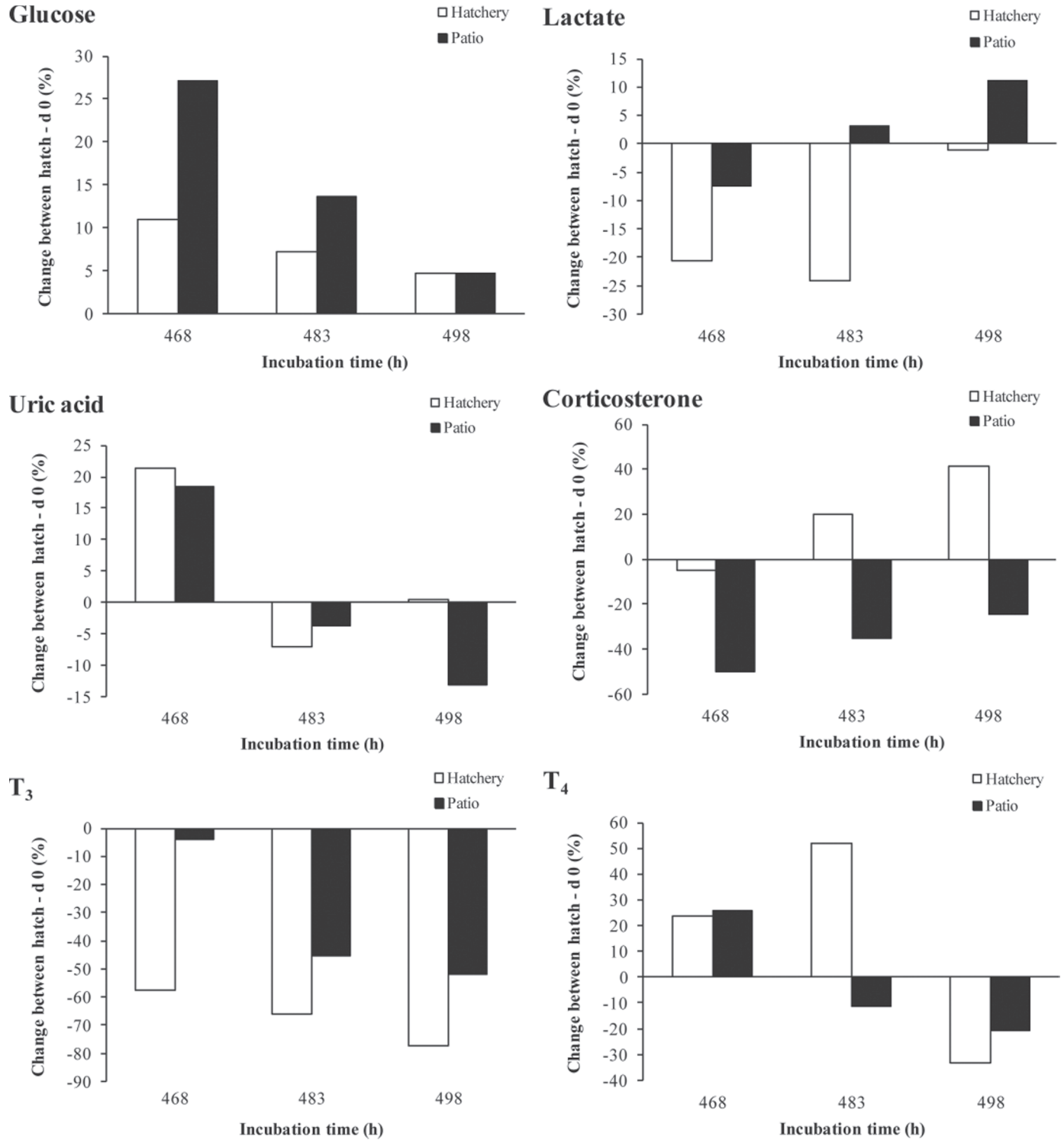

Figure 2. Least squares means of relative changes in plasma concentrations of glucose, lactate, uric acid, corticosterone, triiodothyronine (T_3), and thyroxine (T_4) in chicks hatched early, midterm, or late in the hatching process in a hatcher or a patio system, determined directly after hatch (468 h for early, 483 h for midterm, or 498 h for late chicks), or at the end of incubation (515 h for all 3 hatch groups).

Blood Plasma Parameters. Plasma hormone and metabolite levels at d E21.5 are presented in Table 5. At d E21.5, a SYS × 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, whereas 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 × HT interaction was found, but when least squares means

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 (d E21.5 for all 3 hatch groups)

Item[1]	n	BW (g)	YFBM[2] (g)	Yolk (g)	Heart (g)	Lungs (g)	Liver (g)	Spleen (g)	Bursa (g)	Intestines (cm)
Treatment										
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 × HT										
Hatcher × 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 × 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 × 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 × 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 × 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 × 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 × HT		<0.001	<0.001	0.081	0.003	0.046	0.001	0.018	0.018	0.011

[a–d]Least squares means followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

[1]SYS = system (hatcher, patio); HT = hatching time (early, midterm, late).

[2]YFBM = yolk-free body mass.

were compared using post hoc Tukey's adjustment, no significant differences were observed. The T_3:T_4 ratios 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 because early chicks were collected

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 (d E21.5 for all 3 hatch groups)[1]

Item[2]	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
Treatment								
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 × HT								
Hatcher × early	25	192.81^{c}	33.67	6.27	25.01	0.45^{d}	6.80^{b}	0.07^{b}
Hatcher × midterm	25	204.20^{bc}	43.56	5.32	25.87	0.57^{cd}	9.37^{a}	0.06^{b}
Hatcher × late	25	197.58^{c}	37.24	5.14	23.34	0.48^{d}	5.81^{bc}	0.09^{b}
Patio × early	25	235.54^{a}	41.84	6.09	10.61	1.27^{a}	6.54^{b}	0.21^{a}
Patio × midterm	25	216.19^{b}	44.01	5.10	13.10	0.84^{b}	4.52^{c}	0.21^{a}
Patio × 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 × HT		<0.001	0.088	0.779	0.230	0.002	<0.001	0.038

[a–d]Least squares means followed by different superscripts within a column and factor are significantly different ($P \leq 0.05$).

[1]T_3 = triiodothyronine; T_4 = thyroxine.

[2]SYS = system (hatcher, patio); HT = hatching time (early, midterm, late).

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, conforming to commercial broiler practice in the 2 hatching systems. Although in the present experiment it was attempted to approach the situation as in broiler practice, it must be noted that presently 33% of the sampled chicks were collected as midterm chicks, whereas in broiler practice, most of the chicks would belong to this group.

Physiology at Hatch

Hatching Time × Hatching System. Plasma glucose levels were higher in chicks hatching late versus early in the hatch window, corresponding to previous findings in newly hatched chickens (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, 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 (2000) in poults. 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 (Molenaar et al., 2010b; Willemsen et al., 2010). 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 with earlier hatching chicks, because 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., 1990; 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 in the heart, liver, stomach, intestines, and lungs in patio chicks 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 the patio and 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 21 d 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 demanding hatching process (van de Ven et al., 2011). The background for these opposite results is not clear. A continuous lower incubation temperature (35 vs. 38°C) was shown to stimulate the expression of a metabolic regulator peroxisome proliferator-activated receptor-γ coactivator-1α in the liver of chicken embryos, which may activate gluconeogenesis (Walter and Seebacher, 2007). Hence, a lower temperature in patio chicks may have resulted in the increased glucose levels at hatch.

Early Posthatch Period

Hatch Time × Hatching System. The BW gain in fed patio chicks and BW loss in deprived hatcher chicks in the early posthatch period were comparable with 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 with 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 chickens is first converted to lactate before entering the circulation (Riesenfeld et al., 1982).

At d E21.5, T_3:T_4 ratios were lower than at hatch for all hatch groups, and 2 to 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 to 3 d posthatch found in fasted chicks (Noy and Sklan, 2001; Careghi et al., 2005). The 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 be related to the slightly lower yolk uptake in the hatcher chicks. In fed chicks, T_3 levels in the first 1 to 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, 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 analyzing the physiological status of hatchlings at chick pulling may result in different findings compared with analyzing 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 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 to 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 with 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 because 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 with 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 the 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. The present data indicate 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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