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Effects of oxygen concentration during incubation and broiler breeder age on embryonic heat production, chicken development, and 7-day performance

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ABSTRACT

Older breeder flocks produce eggs with a relatively larger yolk and thereby a higher nutrient availability than young breeder flocks. To optimise nutrient utilisation and embryonic development throughout incubation and posthatch period, embryos originating from older breeder flocks may require a higher oxygen availability. The current study investigated effects of broiler breeder flock age and incubational oxygen concentration on embryonic metabolism and chicken development until 7-day posthatch. Similar sized eggs of a young (28–32 week) or old (55–59 week) Cobb 500 breeder flock were incubated at one of three oxygen concentrations (17%, 21% or 25%) from day 7 of incubation until 6 h after emergence from the eggshell. Posthatch, chickens were reared until 7 days of age. Egg composition at the start of incubation, heat production during incubation, and embryo or chicken development at embryonic day (**ED**)14 and ED18 of incubation, 6 h after hatch and day 7 posthatch were evaluated. An interaction was found between breeder age and oxygen concentration for yolk-free body mass (**YFBM**) at ED18. A higher oxygen concentration increased YFBM in the old breeder flock, whereas no difference was found between 21 and 25% oxygen in the young breeder flock. Yolk size was larger in the old compared to the young flock from ED0 until 6 h after hatch. Breeder flock age did not affect YFBM at ED14 and 6 h after hatch nor daily embryonic heat production, but there were some effects on relative organ weights. Chickens of the old compared to the young breeder flock showed a higher weight gain at day 7, but at a similar feed conversion ratio (**FCR**). A higher oxygen concentration during incubation stimulated embryonic development, especially between 17% and 21% of oxygen, in both flock ages. Although this growth advantage disappeared at 7 days posthatch, a low oxygen concentration during incubation resulted in a higher FCR at 7 days posthatch. Results indicated that breeder flock age seemed to influence body development, with an advantage for the older breeder flock during the posthatch period. Oxygen concentrations during incubation affected body development during incubation and FCR in the first 7 days posthatch. Although an interaction was found between breeder flock age and oxygen concentration at ED18 of incubation, there was no strong evidence that nutrient availability at the start of incubation (represented by breeder flock ages) affected embryo and chicken development at a higher oxygen concentration.

Implications

In commercial hatcheries, incubation conditions are often equal for all eggs. However, egg size and composition vary amongst breeder flocks. Old compared to young breeder flocks produce larger eggs and have more nutrients available. To use these nutrients efficiently and optimise development, embryos of old compared to young flocks may require more oxygen during incubation. This study only found an indication at embryonic day 18 that eggs of older compared to young flocks reacted more on limitations in oxygen concentration. For incubation management, these results suggest that sufficient ventilation for oxygen is especially important for eggs of old breeder flocks.

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Introduction

Several abiotic and biotic factors can influence development of the chicken embryo during artificial incubation and thereby also posthatch survival and performance (Yalçin et al., 2005; Elibol and Brake, 2008). Two important factors affecting nutrient metabolism during development and growth of avian embryos are egg composition at oviposition and oxygen concentration during incubation (Wangensteen and Rahn, 1970; Wilson, 1997). The ratio between egg albumen and yolk is disproportionally changing with breeder flock age (Marion et al., 1964). Old compared to young breeder flocks produce larger eggs with a larger yolk, both absolutely and relatively (Nangsuay et al., 2013). Egg yolk has a high nutrient density and is mainly composed of lipids (Romanoff, 1967). Consequently, eggs from an old compared to young breeder flock have a higher energy content (Nangsuay et al., 2013 and 2016). Nutrient utilisation by the embryo is largely influenced by oxygen availability (Metcalfe et al., 1981). Until embryonic day (**ED**) 15, oxygen availability increases exponentially (Cirotto and Arangi, 1989), after which a plateau phase occurs (Dietz et al., 1998). Oxygen availability becomes restricted during this plateau phase because of limitations in diffusion rate through the eggshell and membranes in combination with a high metabolic rate of the growing embryo (Burton and Tullett, 1985). This process continues until hatching starts at approximately ED19.5. The limitation in oxygen availability for the embryo during the plateau phase has been shown by the finding that a higher oxygen concentration up to 25% during the second half of incubation increased the metabolic rate, shortened the plateau phase, and improved chicken development at hatch (Lourens et al., 2007; Molenaar et al., 2011).

Because of the higher nutrient availability in eggs originating from older compared to young breeder flocks, our hypothesis is that eggs from old breeder flocks need a higher oxygen availability to optimise nutrient utilisation to improve embryonic development and posthatch performance and this may be already needed after the first week of incubation. The aim of this study was therefore to investigate the interaction between breeder flock age (=variation of nutrient availability) and oxygen concentration after the first week of incubation on embryonic and chicken development up to 7 days posthatch. Preliminary results of this study have been presented by Molenaar et al. (2017) at the IFRG meeting, Wageningen, the Netherlands.

Material and methods

Experimental design

The experiment was designed as a 2 × 3 factorial arrangement with two breeder flock ages (young or old) and three oxygen concentrations (17%, 21% or 25%) applied from ED7 until hatch. Six consecutive batches of eggs were incubated with two treatments per batch. In each batch, eggs of both breeder flock ages were used, but only one oxygen concentration.

Hatching eggs

A total of 1 260 first grade hatching eggs of Cobb 500 breeder flocks, aged between 28 to 32 weeks (young) and 55 to 59 weeks (old), were obtained from commercial broiler breeder farms in the Netherlands, Germany and Belgium. Per batch, 105 hatching eggs per breeder flock age were selected at an egg weight range between 60.0 and 64.5 g.

Storage and incubation

Selected hatching eggs were stored for 2–3 days at 20 °C and 55–60% relative humidity at the research facility of Wageningen University and Research, the Netherlands. Per batch, 85 hatching eggs of each breeder flock age were placed evenly on two incubator trays with a capacity of 88 eggs. Incubator trays were set in the middle section in one HT-1408 setter (HatchTech B.V., Veenendaal, the Netherlands). Eggs were warmed in 24 h from the storage temperature of 20 °C to the required eggshell temperature (**EST**) of 37.8 °C. EST was measured by temperature sensors (NTC Thermistors, type DC95, Thermometrics, Somerset, UK) placed at the equator of four individual eggs. Temperature sensors were attached to the eggshell, using heat conducting paste (Dow Corning 340 Heat Sink Compound, Dow Corning GmbH, Wiesbaden, Germany) and a small piece of tape (approximately 2 × 2 cm). The incubator temperature was adjusted automatically to maintain an EST of 37.8 °C based on the median of the four temperature sensors. Until ED7, relative humidity was set at 55% and carbon dioxide concentration was maintained below 0.35%. Eggs were turned each hour over 90°.

After candling at ED7, fertile eggs of the young or old breeder flock were transferred to one of two identical small open-circuit climate respiration chambers (**CRCs**; Lourens et al., 2006). Each CRC contained eggs of one of the breeder flock ages and one out of three oxygen concentration treatments was applied. Until 6 h after emergence from the eggshell, oxygen concentration was maintained at either 17% (low), 21% (normal) or 25% (high).

For the 25% and 21% O_2 treatment, pure O_2 (>99.95%) was injected in a buffer (400 L), using a Mass Flow Controller (Bronkhorst, Veenendaal, the Netherlands, model F-201CV-500-RBD-33-Z, adjustment range 0–500 mL/min) at a rate to obtain the set point O_2 concentration average inside both CRCs. For the 17% O_2 treatment, pure N_2 (>99.95%) was injected in a buffer (400 L), using a Mass Flow Controller (Bronkhorst, Veenendaal, the Netherlands, model F-201CV-2K0-RBD-33-V, adjustment range 700–2 500 mL/min) at a rate to obtain the set point O_2 concentration average inside both CRCs. In this buffer, air was continuously mixed. From this buffer, air samples were collected each 9 min and analysed on O_2 and CO_2 concentration (Heetkamp et al., 2015). From this buffer, air was injected into both CRCs with its own gas volume meter (Itron G1.6). Air samples in the outgoing duct of both CRCs were also collected each 9 min and analysed on O_2 and CO_2 concentrations. Based on these concentrations, the injection volume of O_2 or N_2 into the buffer was automatically adjusted to maintain the set point O_2 in both chambers.

EST in the CRC was regulated in the CRCs as described by Lourens et al. (2007). EST was maintained at 37.8 °C, relative humidity was set at 55%, and carbon dioxide concentration ranged between 0.3 and 0.4% in all batches and treatments. Until ED18, eggs were turned each hour over 90°. Oxygen consumption and carbon dioxide production in the CRC were measured every 9 min from ED7 to ED18 as described by Lourens et al. (2006).

At ED18, all fertile eggs were transferred from the egg trays to hatching baskets and placed back in the same CRC. Temperature of the CRC was fixed at the temperature belonging to an EST of 37.8 °C, thereby allowing EST of the eggs to increase during the hatching phase (Molenaar et al., 2010). Relative humidity was set at 55%, and carbon dioxide concentration was maintained below 0.35% in all batches and treatments. From 467 h of incubation onward, complete emergence from the eggshell by the chicken was checked at a 6-h interval. 6 h after the emergence from the eggshell, 15 chickens of each treatment were sampled per batch to determine chicken development by measuring body and organ weights. These chickens were evenly distributed over the hatch window period. Another 26 chickens per treatment and batch,

which were evenly distributed over the hatch window period, were transferred to one of two medium-sized CRCs (Verstegen et al., 1987), where they were kept until day 7 of age.

Hatch until 7 days of age

Per treatment, chickens were kept in one pen with wood shavings within a medium-sized CRC (80 × 100 cm; Verstegen et al., 1987), where air temperature was maintained at 33 °C at day 0 and decreased gradually to 30 °C at day 7. Relative humidity was maintained between 40 and 60%. On the first day, 24 h of light was provided. From day 1 until day 4, lighting schedule was 23 h of light and 1 h of darkness. From day 5 until day 7, lighting schedule was 16 h of light and 8 h of darkness. A LED light source was used and the intensity was 40 lux on chicken level. After all chickens were transferred to the CRC (±520 h of incubation), all chickens were weighed (=day 0 measurement) and feed and water were provided *ad libitium*. Chickens were fed a commercially available crumbled starter diet (RDS, Wijk bij Duurstede, the Netherlands; 12.28 MJ/kg ME; 216.7 g/kg CP, 10.42 g/kg dLys). Water was provided by one bell drinker per CRC.

Measurements

Per batch, 20 eggs of each breeder flock age were randomly selected at onset of incubation to determine egg composition. Eggs were boiled for 10 min and albumen and yolk were separated and weighed after removal of the eggshell. The eggshell, including membranes, was dried for 24 h at room temperature and weighed. Individual weight of the other eggs was measured at ED0 and ED7 to calculate weight loss. Egg weight loss at ED7 was used to calculate eggshell conductance as described by Nangsuay et al. (2016). After correction for embryonic mortality up to ED18, heat production was calculated per egg containing a live embryo from average oxygen consumption and carbon dioxide production measurements per day, using the formula of Brouwer (1965). Heat production was expressed per day in mWatt per egg containing a live embryo. See also Lourens et al. (2006) for a detailed description. At ED14 and ED18, respectively, 12 and 15 eggs were randomly selected per treatment and batch for measurements. Embryos were killed by cervical dislocation and the embryo and residual yolk (**RSY**) were weighed. At ED18, heart, liver, stomach and intestines were weighed as well.

At 6 h after emergence from the eggshell, all chickens were weighed, and navel condition was scored as 1 (good; closed and clean navel), or 2 (moderate to poor; black string, black button exceeding 2 mm or open navel area; adapted from Molenaar et al., 2010). Per batch, 15 chickens of each treatment were killed by cervical dislocation and fresh weight of heart, liver, stomach, intestines, RSY, and bursa of Fabricius was determined. Yolk-free body mass (**YFBM**) was calculated as BW minus RSY weight, except at ED14 and ED18 when the YFBM was not retracted in the body yet and the YFBM represented the embryo weight. Incubation duration was calculated as time between setting the egg in the incubator and time that the chicken emerged from the eggshell, the latter was measured at 6-h intervals.

At day 7 posthatch, 14 chickens per treatment and batch were randomly selected and BW was measured. After cervical dislocation, fresh weight of heart, stomach (proventriculus and gizzard together), intestines, duodenum, jejunum, ileum, RSY, and bursa of Fabricius was determined. Per batch and treatment, total feed consumption was measured between day 0 and day 7. Feed conversion ratio (**FCR**) was calculated as total feed consumption divided by total weight gain per CRC.

Validation and quality assurance

To assure that measurements could be addressed to treatment and not to CRC, a repetition was included per treatment and CRC was alternated between treatments. Assessment of embryonic mortality and navel condition was performed by the same trained expert.

In the CRCs, a CO_2 recovery test was performed immediately prior to the start and after the study, according to procedures described by Heetkamp et al. (2015). In the four CRCs, on average 99.2, 99.3, 99.2 and 100.4% of the CO_2 released were recovered, respectively. There was no effect of the different O_2 concentrations used on recovery results. Sensors within the CRCs measuring temperature, relative humidity and gas flow were calibrated.

Statistical analysis of results

Egg or chicken was considered as experimental unit in the statistical analysis, except for heat production during incubation and feed consumption, growth and FCR between days 0 and 7 posthatch, where CRC was used as the experimental unit. Distributions of means and residuals were examined to verify model assumptions. Egg composition characteristics at onset of incubation were analysed using the MIXED procedure in SAS (Version 9.4; SAS institute, Cary, NC, USA), using the model:

$$Y_{ijk} = \mu + A_i + \varepsilon_{ijk} \quad (1)$$

where Y_{ijk} is the dependent variable, μ is the overall mean, A_i is the effect of breeder age (i = young or old) and ε_{ijk} is the error. Batch was added as a random factor. Yolk weight, albumen weight and eggshell weight were analysed with egg weight as a co-variable in the model.

All other data were analysed using the MIXED procedure in SAS (Version 9.4; SAS institute, Cary, NC, USA), using the model:

$$Y_{ijk} = \mu + A_i + B_j + AB_{ij} + \in_{ijk} \quad (2)$$

where Y_{ijk} is the dependent variable, μ is the overall mean, A_i is the effect of breeder age (i = young or old), B_j is the effect of oxygen level (j = 17%, 21%, or 25%), AB_{ij} is the interaction term, and ε_{ijk} is the error. Batch nested with oxygen level was added as a random factor. Egg weight at ED0 was included in model 2 as a co-variable for embryo measurements until ED18. Organ weights were expressed as percentages of either YFBM at 6 h after emergence from the eggshell or BW at day 7 of age.

Mean heat production per 24 h from ED8 to ED18 was analysed, using the MIXED procedure for repeated measurements. Model 2 was extended with the repeated factor day number (day_m; m = ED8 to ED18), and interactions of other factors with day number. An autoregressive covariance structure was used. Navel score was analysed using the GLIMMIX procedure for binary data, using model 2. Average growth from days 0 to 7 per batch and treatment was calculated based on individual BW measurements on days 0 and 7. Least square means were compared, using Bonferroni adjustments for multiple comparisons. Find input statements in Supplementary material S1. Data are presented as least square means ± SEM. In all cases, differences between treatments were considered significant at $P \leq 0.05$.

Results

Egg characteristics

Egg weight was 1.18 g lower in the young compared to the old breeder flock ($P < 0.001$; Table 1). Yolk weight was 3.61 g lower and

Table 1
Fresh egg composition of the young (28–32 weeks) or old (55–59 weeks) broiler breeder flock.[1]

Parameter	Young breeder flock	Old breeder flock	SEM	*P*-value
Egg weight (g)	61.58	62.76	0.11	<0.001
Yolk weight (g)	16.93	20.54	0.11	<0.001
Albumen weight (g)	39.50	35.85	0.13	<0.001
Yolk:Albumen ratio	0.43	0.57	0.004	<0.001
Eggshell weight (g)	5.74	5.77	0.04	0.57
Weight loss ED0[2] - ED7[3] (%)	4.20	4.19	0.03	0.69
Eggshell conductance ED0 - ED7 (mg/h/kPa)	5.66	5.76	0.29	0.82

Abbreviations: ED0 = Embryonic day 0; ED7 = Embryonic day 7.
[1] For all variables measured, n = 90 per breeder flock age group.

albumen weight was 3.65 g higher in eggs of the young compared to the old breeder flock ($P < 0.001$). Yolk to albumen ratio was lower in the young compared to the old breeder flock ($P < 0.001$). Eggshell weight, weight loss between ED0 and ED7, and eggshell conductance showed no significant difference between eggs of the young or old breeder flock ($P > 0.50$).

Embryonic development and chicken characteristics

No interaction between breeder age and O_2 concentration nor a breeder age effect was found for embryonic heat production from ED8 until ED18 ($P = 0.59$; Fig. 1). At ED14, ED15 and ED18, embryonic heat production was significantly higher in the 21 and 25% treatment compared to the 17% treatment ($P < 0.001$; Fig. 1). On ED16 and ED17, embryonic heat production increased with an increase in oxygen concentration ($P < 0.001$).

Effects of breeder age and oxygen concentration on embryo and chicken characteristics are shown in Tables 2 and 3. At ED18, an interaction was found between breeder flock age and oxygen concentration for YFBM ($P = 0.005$). In embryos of the young breeder flock, YFBM increased between the 17% and 21% oxygen concentration treatment, but not between the 21% and 25% oxygen concentration treatment. In embryos of the old breeder flock, YFBM increased with increasing oxygen concentrations. No other interactions were found between breeder flock age and oxygen concentration.

At ED14 and 6 h after emergence from the eggshell, YFBM was not affected by breeder flock age ($P > 0.50$). RSY weight was signif-

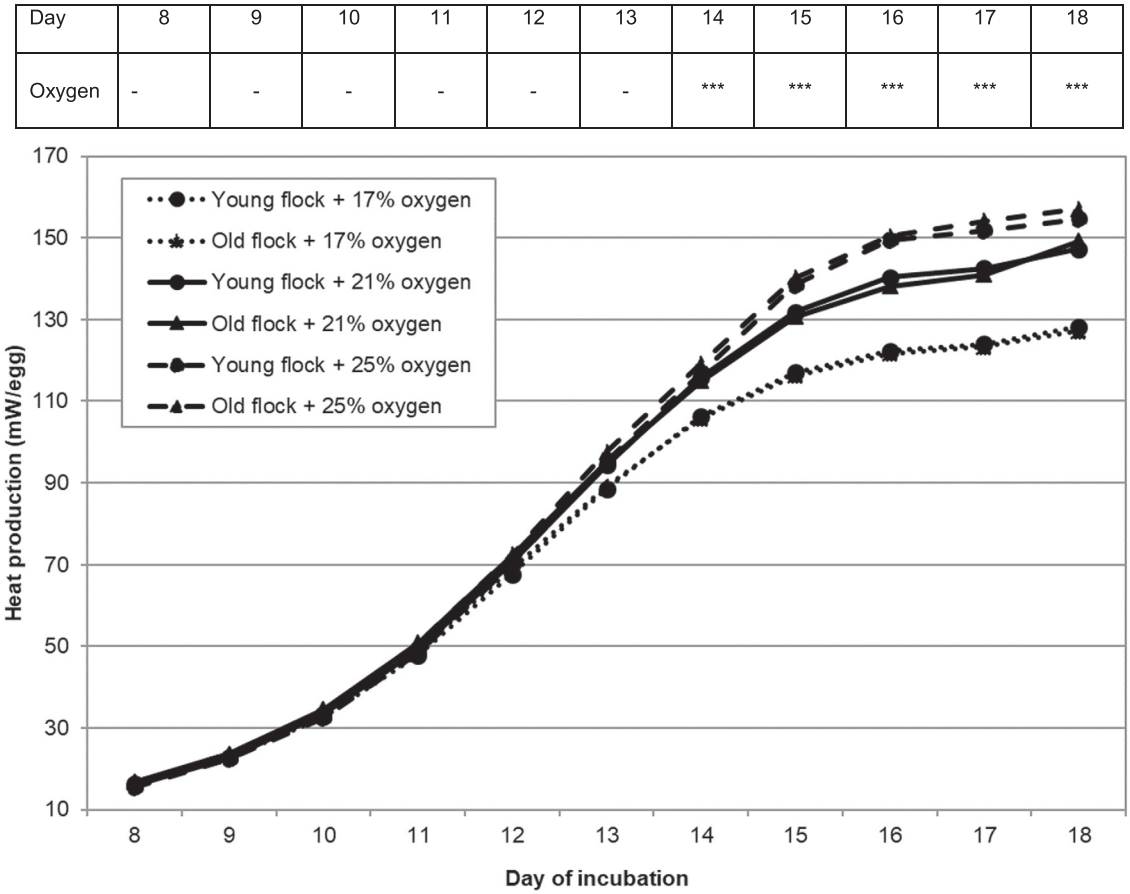

Fig. 1. Average daily heat production (mW/egg) of embryos of a young (28–32 weeks) or old (55–59 weeks) broiler breeder flock incubated at three oxygen concentrations (17%, 21%, or 25%) from day 7 of incubation until hatching. There was no significant difference in breeder age and interaction of breeder age × oxygen. The legend indicates the level of significance (*** = $P < 0.001$).

Table 2
Embryo and chicken characteristics at ED14, ED18 and 6 h after emerging from the eggshell of eggs originating of a young (28–32 weeks) or old (55–59 weeks) broiler breeder flock and incubated at 17, 21 or 25% oxygen from day 7 of incubation until hatching.[1]

	Interaction Breeder flock age × Oxygen concentration (O_2)						Main effect Breeder flock age (BF)			Main effect Oxygen concentration (O_2)				*P*-value		
Parameter	Young × 17%	Young×21%	Young × 25%	Old × 17%	Old × 21%	Old × 25%	SEM	Young	Old	SEM	17%	21%	25%	SEM	BF × O_2	BF	O_2
YFBM[2] (g)																	
ED14[3]	15.17	16.79	16.53	15.20	16.63	16.26	0.24	16.16	16.03	0.14	15.19[b]	16.71[a]	16.40[a]	0.17	0.80	0.52	0.02
ED18[3]	29.29[c]	32.89[a]	32.47[ab]	28.44[c]	31.31[b]	32.63[a]	0.32	31.55	30.79	0.19	28.87	32.10	32.55	0.25	0.005	0.002	0.004
6 h[4]	36.60	39.00	40.15	36.71	38.89	40.15	0.28	38.58	38.55	0.15	36.66[b]	38.94[a]	40.10[a]	0.18	0.90	0.90	0.002
RSY[5] (g)																	
ED14[3]	14.07	13.84	14.21	18.51	17.60	17.65	0.55	14.04[b]	17.92[a]	0.33	16.39	15.72	15.93	0.48	0.45	<0.001	0.72
ED18[3]	12.52	10.49	9.94	13.91	12.05	10.81	0.27	10.98[b]	12.26[a]	0.16	13.21[a]	11.27[b]	10.38[b]	0.19	0.40	<0.001	0.005
6 h[4]	8.06	5.83	5.47	8.40	7.18	6.01	0.23	6.45[b]	7.20[a]	0.13	8.23[a]	6.50[b]	5.74[b]	0.17	0.07	0.003	<0.001
Navel[6] (%)	55.4	72.6	80.5	57.3	68.2	70.9	-	69.5	65.6	-	56.4	70.4	75.6	-	0.46	0.26	0.22
Hatch time (h)	499	498	499	498	494	496	1.4	499[a]	496[b]	0.8	499	496	497	1.0	0.55	0.008	0.25

[1] For all variables, n = 24 per treatment.
[2] YFBM = Yolk-free body mass.
[3] ED14 or ED18 = Embryonic day 14 or 18, or 6 h after emergence of the eggshell.
[4] 6 h after emergence from the eggshell.
[5] RSY = Residual yolk.
[6] Average percentage of chickens per treatment group scored with a navel condition of 1 (=perfect) compared to 2 (=moderate to poor). SEM not applicable.
[a,b] Least square means within a row and factor lacking a common superscript differ ($P < 0.05$).

icantly lower in the young compared to the old breeder flock at ED14 ($\Delta = -3.88$ g), ED18 ($\Delta = -1.28$ g) and 6 h after emergence from the eggshell ($\Delta = -0.75$ g) (all $P < 0.004$). Navel condition was not affected by breeder flock age ($P = 0.88$). Incubation duration was affected by breeder flock age, where chickens of the young breeder flock hatched 3 h later than chickens of the old breeder flock ($P = 0.008$).

Embryos of the young breeder flock had a lower relative heart weight at ED18 than the old breeder flock ($\Delta = -0.03\%$, $P = 0.03$), but this difference disappeared at 6 h after emergence ($P = 0.07$). Relative liver weight was higher at ED18 ($\Delta = +0.07\%$, $P = 0.009$) and lower at 6 h after emergence from the eggshell ($\Delta = -0.08\%$, $P = 0.02$) in the young compared to the old breeder flock. Relative stomach weight was higher at ED18 ($\Delta = +0.37\%$) and at 6 h after emergence of the eggshell ($\Delta = +0.13\%$) in the young compared to the old breeder flock (both $P < 0.002$). Relative intestine weight was not affected by breeder flock age at ED18 or 6 h after emergence of the eggshell ($P > 0.40$; data not shown). Relative bursa of Fabricius weight was not affected by breeder flock age at 6 h after emergence from the eggshell ($P > 0.15$; data not shown).

At ED14 and 6 h after emergence from the eggshell, YFBM was lower in the 17% oxygen concentration treatment compared to the 21% and 25% oxygen concentration treatment ($P < 0.003$). At ED18 and 6 h after emergence from the eggshell, RSY weight was higher in the 17% oxygen concentration treatment compared to the 21% and 25% oxygen concentration treatment ($P < 0.004$). RSY weight at ED14, navel condition, incubation duration and relative heart, liver and stomach weight at ED18 and 6 h after emergence from the eggshell were not affected by oxygen concentration (Tables 2 and 3; $P > 0.06$). At ED18 or 6 h after emergence from the eggshell, relative intestine weight was not affected by oxygen concentration ($P > 0.40$; data not shown). At 6 h after emergence from the eggshell, relative bursa of Fabricius weight was not affected by oxygen concentration ($P > 0.15$; data not shown).

Seven-day performance

Provision of feed and water was on average 22.9 h after emergence from the eggshell (=day 0) and weight loss during this period was on average 2.5 g and was similar between treatments ($P > 0.18$; data not shown). RSY weight at day 7 was on average 0.14 g and was similar between treatments ($P > 0.30$; data not shown).

All other posthatch performance data are shown in Table 4. At 6 h after emergence from the eggshell, chicken weight was 0.58 g lower in the young compared to the old breeder flock ($P < 0.001$). At day 0, this difference was 0.33 g ($P = 0.04$). At day 7, chicken weight was 4.8 g lower in the young compared to the old breeder flock ($P = 0.01$). Growth between days 0 and 7 was 4.3 g lower in the young compared to the old breeder flock ($P = 0.04$). At 6 h after emergence from the eggshell, and days 0 and 7 posthatch, chicken weight did not differ between oxygen concentration treatments ($P > 0.11$; Table 4). No effect of breeder age was found for feed consumption (144.9 g on average) or FCR between days 0 and 7 (1.08 on average) (both $P > 0.20$). No effect of oxygen concentration during incubation was found for growth or feed consumption between days 0 and 7 ($P > 0.20$). FCR between days 0 and 7 was highest in the 17% oxygen and lowest in the 21% oxygen concentration treatment ($P = 0.04$), with the 25% oxygen concentration treatment in between.

At day 7 of age, relative heart, liver, stomach, total intestines, duodenum, jejunum, and bursa of Fabricius weight did not differ between the two breeder flock ages ($P > 0.05$; data not shown). At day 7, relative ileum weight was 0.03% higher for the old flock compared to the young flock ($P = 0.02$; data not shown). At day 7, no difference was found between oxygen concentration

Table 3
Relative heart, liver and stomach weight at ED18 and 6 h after emerging from the eggshell of eggs originating of a young (28–32 weeks) or old (55–59 weeks) broiler breeder flock and incubated at 17, 21 or 25% oxygen from day 7 of incubation until hatching.[1]

	Interaction Breeder flock age (BF) × Oxygen concentration (O_2)							Main effect Breeder flock age (BF)			Main effect Oxygen concentration (O_2)				*P*-value		
Parameter	Young × 17%	Young ×21%	Young × 25%	Old ×17%	Old ×21%	Old ×25%	SEM	Young	Old	SEM	17%	21%	25%	SEM	BF × O_2	BF	O_2
Heart[2]																	
ED18[3]	0.68	0.69	0.76	0.73	0.75	0.75	0.03	0.71[b]	0.74[a]	0.02	0.70	0.72	0.75	0.03	0.10	0.03	0.61
6 h[4]	0.81	0.84	0.90	0.84	0.85	0.93	0.02	0.85	0.87	0.01	0.82	0.85	0.91	0.02	0.72	0.07	0.07
Liver[2]																	
ED18[3]	2.00	1.93	2.09	1.87	1.92	1.99	0.05	2.00[a]	1.93[b]	0.03	1.93	1.93	2.04	0.05	0.16	0.009	0.37
6 h[4]	2.47	2.47	2.48	2.57	2.55	2.52	0.04	2.47[b]	2.55[a]	0.02	2.52	2.51	2.50	0.04	0.68	0.02	0.96
Stomach[2]																	
ED18[3]	5.29	4.85	5.01	4.74	4.55	4.75	0.18	5.05[a]	4.68[b]	0.10	5.02	4.70	4.88	0.16	0.35	<0.001	0.47
6 h[4]	2.42	2.52	2.43	2.20	2.37	2.39	0.05	2.45[a]	2.32[b]	0.03	2.31	2.45	2.41	0.04	0.22	0.002	0.15

[1] For all variables, n = 24 per treatment.
[2] Expressed as percentage of yolk-free body mass.
[3] ED18 = Embryonic day 18.
[4] 6 h after emergence from the eggshell.
[a,b] Least square means within a column and factor lacking a common superscript differ ($P < 0.05$).

Table 4
Chicken weight at 6 h after emergence from the eggshell and days 0 and 7 posthatch of eggs originating of a young (28–32 weeks) or old (55–59 weeks) broiler breeder flock and incubated at 17, 21 or 25% oxygen from 7 days of incubation until hatching.[1]

	Interaction Breeder flock age (BF) × Oxygen concentration (O_2)							Main effect Breeder flock age (BF)			Main effect Oxygen concentration (O_2)				*P*-value		
Parameter	Young x17%	Young x21%	Young x25%	Old x17%	Old x21%	Old x25%	SEM	Young	Old	SEM	17%	21%	25%	SEM	BF*O_2	BF	O_2
Chick weight (g)																	
6 h	44.69	45.20	45.32	45.22	46.00	45.72	0.19	45.07[b]	45.65[a]	0.11	44.95	45.6	45.52	0.16	0.36	<0.0001	0.12
Day 0	42.41	42.83	42.71	42.84	43.96	42.86	0.29	42.65[b]	42.98[a]	0.17	42.63	42.96	42.86	0.25	0.90	0.04	0.66
Day 7	172.6	177.1	174.9	178.7	178.4	181.9	3.08	174.9[b]	179.7[a]	1.78	175.6	177.8	178.4	2.63	0.40	0.01	0.75
Growth (g)	130.5	134.4	132.2	136	135.3	138.9	2.64	132.4[b]	136.7[a]	1.52	133.2	134.8	135.6	2.41	0.27	0.04	0.79
Feed (g)	142.6	142.2	144.4	153.3	140.2	146.5	3.11	143.1	146.7	1.80	148	141.2	145.5	2.20	0.26	0.25	0.24
FCR[2]	1.09	1.06	1.09	1.13	1.04	1.05	0.01	1.08	1.07	0.008	1.11[a]	1.05[b]	1.07[ab]	0.009	0.14	0.48	0.04

[1] For chicken weight 6 h after emergence and day 0, n = 76 per treatment; for chicken weight at day 7, n = 24 per treatment.
[2] FCR = Feed Conversion Ratio (total feed consumption in g/total BW gain in g).
[a,b] Least square means within a column and factor lacking a common superscript differ ($P < 0.05$).

treatments for relative heart, liver, stomach, intestines, duodenum, jejunum, ileum or bursa of Fabricius weight ($P > 0.07$; data not shown).

Discussion

Although egg weight was slightly higher in the current study, egg yolk weight was considerable higher in the old compared to young breeder flock and this is consistent with other studies (Nangsuay et al., 2013 and 2016). The increase in ovulation intervals and a higher availability of lipids in hens of old compared to young breeder flocks explain the higher deposition of yolk in these eggs (Nasri et al., 2020). The higher yolk deposition in eggs of old compared to young breeder flock resulted in a higher total nutrient availability at the onset of incubation, as confirmed by Nangsuay et al. (2013 and 2017). Based on our hypothesis, the embryos of an old compared to a young flock would increase their nutrient utilisation with an increase in oxygen concentration, resulting in a better embryonic development, chicken quality and posthatch performance.

At ED14, there was no interaction found between breeder age (and thus initial total nutrient availability in the egg) and oxygen concentration for any of the developmental parameters. Metabolic heat production showed no plateau phase yet at ED14 and oxygen may therefore not have been limited at that point in time. An interaction was found between breeder age and oxygen concentration for YFBM at ED18, showing no difference in YFBM between 21% and 25% of oxygen in the young breeder flock, but YFBM in the old breeder flock increased with an increase in oxygen concentration. The metabolic heat production did not seem to follow the same trend as the YFBM, because no interaction between breeder age and oxygen concentration was found there. These results suggest that a higher oxygen concentration increased embryonic development during the plateau phase of incubation when oxygen becomes limited, especially for the embryos of older flocks that have more nutrients initially available. Our hypothesis could be strongly confirmed if an increase in oxygen concentration resulted in a higher YFBM at ED18 for the embryo of an old breeder flock compared to a young breeder flock, but the results were opposite. The absolute values of the YFBM at ED18 showed that the YFBM in the old breeder flock incubated at 21% oxygen was 1.58 g lower than the YFBM of the young breeder flock incubated at 21% oxygen and there was no difference between breeder flock ages at 25% of oxygen. The seemingly opposite result of our expectation might be related to nutrient concentrations within the egg. Yolk size was larger in the old compared to young breeder flock but this is mainly composed of lipids that are used for energy during incubation. Especially proteins seem to be important for body development, as shown by studies on in ovo injection of amino acids or albumen removal (Ohta et al., 1999; Willems et al., 2014; Peebles, 2018). Nangsuay et al. (2013) showed that albumen of an old breeder flock contained relatively less proteins compared to albumen of a young breeder flock and this may have further contributed to the lack in a higher embryonic growth in the old compared to young breeder flock when more oxygen was provided. At hatch, no interaction between breeder flock age and oxygen concentration was found anymore for YFBM or residual yolk weight and this may indicate that more oxygen will not benefit the embryos anymore during and after the hatching process when there are no physical limitations anymore to access oxygen. The lack of a strong interaction between initial total nutrient availability (represented by different breeder flock ages) and oxygen concentration during incubation may suggest that limitation of embryonic development is not only caused by total nutrient availability and oxygen concentration but also by other factors that influence the oxygen availability and metabolism at tissue level. This hypothesis needs to be further investigated in future studies.

Hatching time was 3 h earlier in the old flock compared to the young flock and this could be related to an advanced embryonic stage at the start of incubation. Eggs of old compared to young breeder flocks contain often embryos in a more advanced morphological stage (Reijrink et al., 2009; Pokhrel et al., 2017), which is probably a result of a shorter clutch length and a higher number of first clutch eggs that have a longer uterine period (Scott and Warren, 1936; Fasenko et al., 1992).

At 6 h after emergence from the eggshell, RSY weight of chickens from the old flock was higher than from the young flock. Nangsuay et al. (2011 and 2013) also showed that RSY weight was higher in the old compared to the young flock at ED18, but this difference disappeared at hatch (Nangsuay et al., 2011 and 2013). This discrepancy between studies might be related to strain differences (Nangsuay et al., 2017) or the moment that the RSY was measured. Nangsuay et al. (2011 and 2013) evaluated chickens at pull time (±517 h), which corresponds to their chronological age. In the current study, we evaluated chickens 6 h after their emergence from the eggshell (±504 h), which corresponds to their biological age.

The RSY weight at hatch is a result of the initial yolk size, the influx of other egg components and the utilisation of yolk throughout incubation (Yadgary et al., 2010). In the current study, we observed that the larger yolk in the initial egg of the old compared to young breeder flock resulted in a larger residual yolk at hatch. When subtracting the initial yolk size from the residual yolk at 6 h after emergence, total yolk utilisation throughout incubation seemed to be larger in the old (Δ = 13.34 g) compared to the young breeder flock (Δ = 10.48 g). This finding is in agreement with results of O'Sullivan et al. (1991) and Nangsuay et al. (2011). Metabolic pathways may have been different in old compared to young breeder flocks, explaining the higher relative organ weights of especially heart and liver that were found in the current study and higher relative amounts of DM, fat and protein of the YFBM found in chickens of an old compared to young breeder flock in a study of Nangsuay et al. (2013). These results indicate a better development of embryos during incubation, which may have contributed to the higher BW and growth up to 7 days of age in the old compared to young breeder flock.

Results of the current study showed that especially the decrease in oxygen concentration from 21% to 17% affected the development of the embryo and chicken, expressed by a lower metabolic heat production and decreased YFBM during incubation and at hatch. Studies of Lourens et al. (2007) and Molenaar et al. (2010) with a similar experimental set-up (oxygen concentration treatments were only applied shorter, until ED19 instead of hatch) showed comparable results. The limited advantages in development related to an increase in oxygen concentration from 21% to 25% might be related to factors that influence embryonic oxygen availability at tissue level or the abilities of tissue to utilise extra oxygen.

Eggshell characteristics and the functionality of the yolk sac membrane and/or CAM may limit the diffusion rate of oxygen through the eggshell and membranes or may impair a higher transportation of oxygen towards embryonic tissue (Wangensteen and Rahn, 1970; Ar et al., 1987). This may be particularly occurring until the embryo has internally pipped and pulmonary ventilation starts. An oxygen concentration of 25% compared to 21% might facilitate body development more after pulmonary ventilation started and this might be indicated in the current study by a relatively higher YFBM increase from ED18 until 6 h after emergence from the eggshell in the 25% compared to 21% oxygen treatment (+2.8% vs +1.3%, respectively, numerical difference).

No effect of oxygen concentrations within the range of 17 to 25% was found on relative organ weights at hatch or in the

posthatch period, which was found in previous studies as well (Chan and Burggren, 2005; Lourens et al., 2007; Molenaar et al., 2010). Development of organs seems not to be affected by oxygen concentrations ranging between 17 and 25% during incubation. Although there was on average a difference found in YFBM of 2.86 g (=7.2%) at hatch between the 17%, and the 21% and 25% oxygen treatment, BW at hatch and at 7 days of age was comparable between treatments. Although BW at 7 days was similar, the feed efficiency (as expressed by FCR) was negatively influenced by oxygen level at 17% during incubation. This may suggest that functionality of the intestinal tract was impaired, which could occur already during incubation as indicated by less yolk utilisation (large RSY at hatch) in the low oxygen treatment group.

Conclusion

It can be concluded from the current study that although an interaction was found between breeder flock age and oxygen concentration at ED18 of incubation, there was no strong evidence that breeder flock age (reflecting differences in nutrient availability at the start of incubation) affects embryo and chicken development with a higher oxygen concentration. Breeder flock age seemed to affect body development, with an advantage for the older breeder flock during the posthatch period. Oxygen concentrations during incubation exhibited effects on body development during incubation and feed efficiency in the first 7 days posthatch.

Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.animal.2021.100323.

Ethics approval

The experimental protocol was approved by the Institutional Animal Care and Use Committee of Wageningen University, Wageningen, the Netherlands, protocol approval number 2014092c.

Data and model availability statement

None of the data were deposited in an official repository but data are available upon request.

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Declaration of interest

No conflict of interest declared for any of the authors.

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