Research in Veterinary Science 112 (2017) 201–207

Contents lists available at ScienceDirect

Research in Veterinary Science

journal homepage: www.elsevier.com/locate/rvsc

Effects of dust, formaldehyde and delayed feeding on early postnatal development of broiler chickens

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ARTICLE INFO

Keywords:
Hatching environment
Hatch window
Chicken quality
Perinatal development
Immunological development

ABSTRACT

We investigated effects of perinatal exposure to dust or formaldehyde and the moment of first feed intake after hatching on broiler chicken development during the first week of life. Four environmental treatments were used from 468 until 512 h of incubation: control (CONT), heat treated dust (HTD), untreated dust (UTD) or formaldehyde disinfection (FORM). After hatching, all chickens were assigned to 1 of 2 feeding treatments: early feeding (EF; feed and water available in the hatcher) or delayed feeding (DF). After 512 h of incubation (day 0), chickens were reared until day 7 of age. In DF chickens, body weight (BW), yolk free body mass (YFBM) and relative liver weight did not differ among environmental treatments at day 0. However, in EF chickens BW at day 0 was greater in HTD chickens than in UTD and FORM chickens. YFBM in EF chickens at day 0 was greater when chickens were exposed to HTD compared to the other environmental treatments. In EF chickens, relative liver weight was greater in HTD chickens than in FORM. In DF chickens, BW at day 0 was positively related with hatching time (HT). In EF chickens, YFBM was positively related to HT. Residual yolk weight at day 0 was positively related with HT, whereas relative liver weight and microbicidal capacity were negatively related with HT. This study demonstrated that formaldehyde and dust during the hatching phase affect broiler chicken development at pulling from the incubator, but not at day 7.

1. Introduction

Broiler chickens hatch over a period of 24–36 h, called the "hatch window" (Careghi et al., 2005). Chickens are normally pulled from the hatcher simultaneously at approximately 512 h of incubation, when most chickens have hatched. The period that chickens stay in a hatcher thus varies among individual chickens. In the period between hatching and pulling, chickens are exposed to various challenges: dust (Mitchell and Waltman, 2003), high pathogen loads (Mitchell and Waltman, 2003), formaldehyde treatment (Zulfifli et al., 1999) and feed and water deprivation (Careghi et al., 2005).

Dust, released during the hatching process, originates from fluff released from the hatchlings during drying (Mitchell and Waltman, 2003). Fluff breaks down into fine dust. Fine dust consists of particles ≤ 10 μm (called Particulate Matter 10 or PM10) and is able to settle in the trachea and lungs (Lai et al., 2012). In 3 weeks old layer chickens, it was found that dust particles of 10 μm are able to enter the body via the lungs and air sacs and settle in all parts of the body (Berghof et al., 2013). In preliminary experiments we observed high levels of PM10 in commercial hatchers (2.2 ± 1.2 mg/m^3; unpublished results). In broiler chicken houses an average of approximately 1.3 ± 1.0 mg/m^3 PM10 was found between 3 and 5 weeks of age (Lai et al., 2012). These findings suggest that newly hatched chickens are exposed to dust that might enter the body via the lungs and air sacs. Layer chickens of 3 weeks of age, exposed to dust or its components (e.g. lipopolysaccharides, NH_3, β-glucans, lipoteichoic acid; Lai et al., 2012) in combination with human serum albumin (HuSA), showed greater antibody titres against HuSA compared to unexposed chickens. Furthermore, exposure to heat inactivated fine dust resulted in greater growth rates 2 days after exposure in exposed chickens compared to non-exposed chickens (Lai et al., 2012), suggesting that dust might have a stimulatory effect on immune responses of chickens. Heat inactivated fine dust is used to mimic effects of dust, without the potential confounding effects of potential pathogens present in the dust. Consequences of exposure to fine dust during the hatching phase are, however, still unknown.

Apart from direct effects of dust, dust is also an important transport mechanism for (pathogenic) microorganisms, like bacteria, viruses and fungi (Mitchell and Waltman, 2003). To reduce the spread of (pathogenic) microorganisms during the hatching phase, formaldehyde is

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http://dx.doi.org/10.1016/j.rvsc.2017.04.021
Received 15 September 2016; Received in revised form 21 April 2017; Accepted 25 April 2017
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often evaporated in the hatcher (Mitchell and Baumgartner, 2007). Formaldehyde exposure at 18.5 days of incubation, however, causes lesions in the trachea of day-old chickens and increases the feed conversion ratio throughout the life of the broiler chicken (Zulfifli et al., 1999; Lourens et al., 2011).

In most current broiler systems, chickens are deprived of feed and water until placement at the farm. This period of feed deprivation results in a lower body weight (BW) at placement (Careghi et al., 2005), lower organ weights (Bigot et al., 2003; Van de Ven et al., 2011; Van de Ven et al., 2013) and lower post-hatch growth, especially in early hatching chickens (Van de Ven, 2012; Wang et al., 2014). Early hatching chickens are withheld from feed and water and exposed to dust and formaldehyde for the longest duration before they are pulled from the hatcher. Taking together these environmental conditions, it can be suggested that early hatching chickens are more affected by conditions in the hatcher compared to later hatching chickens. It might be that delayed feeding post-hatching aggravates effects of environmental conditions in the hatcher. The potential interaction between exposure of broiler chickens to environmental conditions, like dust and formaldehyde and the moment of first access to feed and water has not been investigated until now.

The aim of this study was to investigate effects of perinatal exposure to dust or formaldehyde, combined with the moment of first feed and water access, on development of broiler chickens at hatch and during the first week of life.

2. Materials and methods

2.1. Experimental design

All procedures described in this study were approved by the Animal Use and Care Committee of Wageningen University and Research, the Netherlands. The experiment was set up as a 4 × 2 factorial arrangement, with 4 environmental and 2 feeding treatments. Hatching eggs and, later on, hatchlings were exposed to 1 of 4 environmental treatments from 468 h of incubation until the end of incubation (512 h). These 4 treatments were control (CONT), heat treated hatchery dust (HTD), untreated hatchery dust (UTD) or formaldehyde disinfection (FORM). Within 3 h after hatching, chickens within each environmental treatment were assigned to 1 of 2 feeding treatments: immediate access to feed and water (early feeding; EF) or deprivation of feed and water until 512 h of incubation (delayed feeding; DF). At 512 h of incubation (from now on referred to as day 0), chickens were pulled from the hatching cabinets, placed in grow-out pens and reared until day 7.

At 3 h after hatching and at day 0, 1 or 7 BW was determined. Thereafter, a blood sample of part of the chickens (for numbers see Table 1) was taken from the vena jugularis and used for a microbicidal capacity test and a monocyte activity test (see below; the latter only at day 7). After blood sampling at day 0 and 7, chickens were decapitated and weights of residual yolk (RY), bursa, spleen and liver were recorded.

Table 1
Number of broiler chickens sampled at day 0, 1 and 7 after pulling per environmental and feeding treatment.

	Day 0		Day 1		Day 7	
	DF	EF	DF	EF	DF	EF
Environmental treatment						
Control (CONT)	26	24	10	10	27	27
Formaldehyde (FORM)	25	25	10	10	26	26
Heat treated dust (HTD)	25	25	10	10	27	27
Untreated dust (UTD)	25	24	10	10	26	27

DF = delayed feeding; EF = early feeding.

2.2. Animals and housing

Ross 308 hatching eggs of one 45-weeks-old breeder flock were used in the experiment. Prior to incubation, all eggs were disinfected with Nontox© (Watter BV, Assen, the Netherlands) and thereafter incubated in one incubator (Petersime, Zulte, Belgium). At day 18 of incubation, eggs were candled and 504 fertile eggs within the range of 57 to 65 g were obtained from the hatchery (Van Hulst Belgabroed, Veldhoven, the Netherlands) and transported to the experimental facility of Wageningen University and Research (Wageningen, the Netherlands). Eggs were randomly divided across 4 environmental treatments. Each environmental treatment contained 126 eggs and was assigned to 1 of 4 identical climate respiration chambers (Heetkamp et al., 2015), in which 1 of 4 specially designed identical hatching cabinets of 0.9 m^3 was placed (Van de Ven et al., 2011). Within the climate respiration chambers and the hatching cabinets, the temperature and relative humidity can be maintained within very narrow limits (Heetkamp et al., 2015). The hatching cabinets consisted of two compartments: the bottom compartment was used to acclimatise and circulate the air through the hatching cabinet and contained a heater and a fan, whereas the upper compartment contained the eggs and later on the hatchlings. The walls and top of the upper compartment were made of transparent Plexiglas to allow visual inspection. Air was continuously recirculated within the hatching cabinet. Recirculation rates and air speeds were similar in all 4 hatching cabinets and eggs or chickens were continuously exposed to full-spectrum light.

Throughout the hatching phase, eggshell temperature (EST) was maintained at 37.8 °C until 500 h of incubation. At that moment the majority of the chickens had hatched. EST was measured by Pt-100 temperature sensors (Sensor Data BV, Rijswijk, the Netherlands) attached to 6 randomly selected eggs per hatching cabinet with the use of heat conducting paste (Shaffner Holding AG, Luterbach, Switzerland) and a small piece of tape (2 × 2 cm) as described by Lourens et al. (2006). After 500 h of incubation, the air temperature within each hatching cabinet was maintained at 35.5 °C and relative humidity at 52.5% for all 4 treatments until pulling at day 0.

2.3. Treatments

Environmental treatments started after the first chicken had hatched (468 h of incubation). In the CONT treatment, air was continuously refreshed to reduce the amount of dust at egg and chicken level. Chickens in the UTD and HTD treatment were exposed to hatcher dust collected one week before the experiment was executed. Dust was obtained from a hatcher containing eggs from the same breeder flock as the eggs used in this experiment. After collection, the dust was divided into 2 portions: 1 portion was heat treated in a stove at 120 °C for 24 h (HTD) (Poole et al., 2008) and the other portion was not treated (UTD). Both portions were divided over 5 ml tubes, each containing approximately 0.25 g of dust and stored at 7 °C until using. From 468 h of incubation onward, every 3 h approximately 0.5 g of dust was sprayed into the hatching cabinets of the HTD and UTD treatment, with an adapted paint spraying mechanism to assure an adequate areolation of the dust particles in the hatching cabinet. A gradual increase in dust levels, mimicking dust patterns found in commercial hatcheries, was aimed for. PM10 levels were monitored and logged in all 4 hatching cabinets with a Dusttrak Aerosol Monitor Model 8520 (TSI Inc., 500 Cardigan road Shoreview, MN 55126-3996, USA) with 10 min intervals. Eggs and chickens in the FORM treatment were exposed to formaldehyde disinfectant from 468 h of incubation onward. A dilution of 30 ml of formaldehyde solution (37%) with 30 ml of water was put into an aluminium container with a surface of 132 cm^2, which was placed in the hatching cabinet. The formaldehyde was released by evaporation.

The concentration of formaldehyde was calculated with the formula of Kawamura and Mackay (1987):

$$E = A^{*}Km^{*}\left(\frac{Mw^{*}Pv}{R^{*}T}\right)$$

where E = evaporation rate, in kg/s, A = area of the evaporating area (0.0132 m^2), Km = mass transfer coefficient (0.00139 m/s), Mw = molecular weight of formaldehyde (30 kg/kmol), Pv = vapor pressure ($435.7 * 10^3$ Pa), R = the gas constant (8.314 J/(kmol * K)), and T = ambient temperature (308 K). This results in $1.78 * 10^{-6}$ kg formaldehyde per second (or $6.4 * 10^{-2}$ kg per hour) released. In a cabinet of 0.9 m^3 this equals a release of 7.13 ppm formaldehyde per hour. Every 3 h, the hatching cabinet was opened for chicken removal and a large part of the formaldehyde escaped. Concentrations would, subsequently, build up again. Thus, every 3 h a calculated formaldehyde concentration of approximately 21 ppm (3 * 7.13 ppm/h) was reached, which is in agreement with concentrations used in commercial hatchers and other experiments (Zulfifli et al., 1999; Steinlage et al., 2002).

Within 3 h after emergence from the shell, each chicken was assigned to 1 of 2 feeding treatments (delayed feeding (DF) or early feeding (EF)), but stayed in the hatching cabinet. The hatching cabinet was divided into two parts. The EF chickens obtained feed on an egg tray and water via a round drinker, whereas the DF chickens were not provided with feed and water. At 512 h of incubation, all environmental treatments were terminated and chickens within each environmental treatment were pulled from the hatching cabinets and placed in 1 of 2 littered floor pens, which were available within the same climate respiration chamber. One floor pen per climate respiration chamber contained chickens from the EF treatment, the other contained chickens from the DF treatment. From that moment onward, all chickens had ad libitum access to feed and water and were reared until day 7. During the rearing phase, environmental temperature was maintained at 34.5 °C at day 0 and was gradually decreased to 30.0 °C at day 7. Relative humidity (RH) was maintained at 52.5% throughout the rearing phase. Chickens were fed a commercially available, pelleted starter diet (12.58 MJ of ME/kg, 21.5% CP; 1.04% digestible lysine; 2.5 mm diameter) throughout the experiment.

2.4. Data collection

In Table 1 an overview of the number of chickens sampled per treatment and time point is given. At 3 h after emergence from the shell, all chickens were weighed, but not sampled. Chickens were sampled at 3 time points: at day 0, 1 or 7. Hatchability was calculated at day 0 by dividing the number of hatched chickens by the number of placed eggs.

2.4.1. Physiological development: BW and relative organ weight

During the hatching process (from 468 h of incubation onward), every 3 h, all newly hatched chickens were individually marked, using paw rings and BW was recorded. At day 0, 1 and 7, chickens to be sampled were weighed and thereafter sacrificed by decapitation. Subsequently, weights of bursa, spleen and liver were measured. At day 0, also weight of the RY was measured. Yolk free body mass (YFBM) was calculated by subtracting RY weight from BW. Relative organ weight was calculated by dividing organ weight by YFBM.

2.4.2. Immunological development: microbicidal capacity test

At day 0, 1 and 7, ten chickens per environmental and feeding treatment were randomly selected for a microbicidal capacity test. The microbicidal capacity test was performed in a whole blood sample. Prior to the test, a bacterial working solution with 20,000 CFU/ml was made with *E. coli* stock (ATCC#8739; Manassas, USA). The bacterial working solution was stored at 4 °C. For the microbicidal capacity test, the protocol of Millet et al. (2007) was slightly adapted. Blood samples were taken from the vena jugularis. Each blood sample was placed in a heparinized tube, closed off with a plastic cap and kept at room temperature. During the test, each blood sample was handled and diluted with pre-warmed (41 °C) CO_2-independent media (#18045; Gibco-Invitrogen, CA, California) plus 4 mM L-glutamine, as described by Millet et al. (2007). Each diluted blood sample was divided into 4 subsamples of 45 µl. To 3 of the 4 subsamples, 5 µl of bacterial working solution was added and thereafter subsamples were incubated at 40.5 °C for 1 h. The remaining subsample served as a negative control. After incubation, each subsample was inoculated on a MacConkey agar plate to check for bacteria concentration in the working solution (i.e. positive control). Subsequently, each MacConkey agar plate was incubated at 37 °C for 24 h. After incubation, the number of colonies per plate was counted and the average number of colonies of the three plates calculated. Microbicidal capacity was, subsequently, calculated as

$$1 - \frac{\text{average colonies per blood sample} - \text{average colonies in negative control}}{\text{average colonies in positive control}}$$

2.4.3. Immunological development: monocyte activity test

At day 7, a monocyte activity test was conducted in ten chickens per environmental and feeding treatment. Monocyte activity was determined by assessing nitrite production in isolated monocytes, either stimulated or not stimulated with lipopolysaccharides (LPS). To isolate monocytes, 0.5 ml of blood was diluted with 0.5 ml of RPMI-1640 medium. The dilution was added to 0.75 ml of Histopaque and centrifuged for 3 min at 13500 rpm. The monocytes were collected from the centrifuged solution and washed twice with RPMI-1640 medium. After each washing, the monocyte suspension was centrifuged at 14000 rpm for 10 s and the RPMI-1640 medium was removed from the monocytes. The monocytes were subsequently suspended in 1 ml of RPMI-1640 medium, supplemented with 2% penicillin-streptomycin and 2% foetal bovine serum. The monocyte suspension was then divided over 6 replicates of 100 µl: 3 replicates received 10 µl LPS solution (0.2 mg/ml PBS) and 3 replicates received 10 µl of PBS (negative control). The replicates were incubated at 41 °C and 5% CO_2 for 48 h. After incubation for 48 h, monocytes were precipitated and 50 µl of culture supernatant was taken from each replicate and added to 50 µl of Gries reagents (25 µl Sulphanilamide [1% H_3PO_4] and 25 µl N-(1-Naphthyl)Ethylenediamine dihydrochloride [0.1% H_3PO_4]) (Bailey et al., 1996). The replicates were gently shaken and kept at room temperature for 10 min. The nitric oxide concentration in each replicate was subsequently determined with an ELISA reader (manufacturer: Multiskan™ GO Microplate Spectrophotometer, Thermo Fisher Scientific Inc. Waltham, USA) at 550 nm according to the manufacturer's protocol. After calculations of nitric oxide concentrations, corrections were made to adjust for background noise. Background noise was calculated based on nitric oxide concentrations, produced by monocytes that were not stimulated by LPS. These concentrations were subtracted from the nitric oxide concentrations produced by the monocytes that were stimulated by LPS.

2.5. Statistical analysis

Individual chickens were considered as the experimental unit in all statistical analyses. Model assumptions were verified by examination of the distributions of the means and residuals. All data were analysed using SAS (version 9.2; SAS Institute, 2004).

Data on hatchability were analysed with a logistic procedure according to the model.

$$Y_i = \mu + ET_i + \varepsilon_i \quad (1)$$

Where: μ = overall mean, ET_i = environmental treatment and ε_{ij} = the residual error term.

BW at hatch was analysed with the GLM procedure, using the model:

$$Y_{ij} = \mu + ET_i + b_w{*}HT_j + (b_w{*}HT_j){*}ET_i + \varepsilon_{ij}, \quad (2)$$

where: μ = overall mean, ET_i = environmental treatment (i = control, formaldehyde, heat treated dust or untreated dust), b_w = the regression coefficient for hatching time, HT_j = hatching time (hours) after start of incubation, $(b_w * HT_j) * ETi$ = the interaction between hatching time and environmental treatment, ε_{ijk} = the residual error term.

For analysis of BW, YFBM, relative organ weights and microbicidal capacity at day 0 and day 1, the GLM procedure was used with the model.

$$Y_{ijk} = \mu + ET_i + FT_j + b_w{*}HT_k + ET_i{*}FT_j + (b_w{*}HT_k){*}ET_i + (b_w{*}HT_k) {*}FT_j + (b_w{*}HT_k){*}ET_i{*}FT_j + \varepsilon_{ijk}, \quad (3)$$

where: μ = overall mean, ET_i = environmental treatment (i = control, formaldehyde, heat treated dust or untreated dust), FT_j = feed treatment (j = early or delayed feeding), b_w = the regression coefficient for hatching time, HT_j = hatching time (hours) after start of incubation, $ET_i * FT_j$ = the interaction between environmental and feeding treatment, $(b_w * HT_k) * ET_i$ = the interaction between hatching time and environmental treatment, $(b_w * HT_k) * FT_j$ = the interaction between hatching time and feeding treatment, $(b_w * HT_k) * ET_i * FT_j$ = the interaction between hatching time, environmental treatment and feeding treatment, and ε_{ijk} = the residual error term. Preliminary analysis showed that the two-way interaction $(b_w * HT_k) * ET_i$ and the three-way interaction $(b_w * HT_k) * ET_i * FT_j$ were never significant for any of the parameters and were therefore removed from the models. For analysis of BW, relative organ weights, microbicidal capacity and monocyte activity at day 7, the GLM procedure with model 3 was used without $(b_w * HT_k)$ and its interactions.

Microbicidal capacity data were arcsine-transformed before analysis. Monocyte activity was log-transformed before analysis. For these parameters LSmeans before transformation are given, with P-values after transformation. Results are expressed as LSmeans ± SEM. Differences are considered significant at $P \leq 0.05$. Differences among treatments are indicated after correction for Tukey for multiple comparisons.

3. Results

Hatchability did not differ among treatments (average 97.6%). Average dust concentrations (n = 120; PM10) were 0.103 mg/m^3 in the control treatment (CONT), 0.504 mg/m^3 in the heat treated dust treatment (HTD), 0.550 mg/m^3 in the untreated dust treatment (UTD) and 0.184 mg/m^3 in the formaldehyde treatment (FORM).

3.1. Environmental and feeding treatments

BW at hatch did not differ among environmental treatments (Table 2). An interaction between environmental and feeding treatments for BW and YFBM was found at day 0 (Table 2). BW of chickens in the delayed feeding (DF) treatment did not differ among the environmental treatments. However, BW of chickens in the early feeding (EF) treatment was greater when exposed to heat treated dust (HTD) compared to the FORM and UTD treatments (average difference (Δ) = 3.0 g; P = 0.005), with the CONT treatment in between and not different from the other environmental treatments. Yolk free body mass (YFBM) of chickens in the DF treatment did not differ among the environmental treatments. However, YFBM of chickens in the EF treatment was greater when exposed to heat treated dust (HTD) compared to the other three environmental treatments (average Δ = 2.8 g; P = 0.006). At day 0, RY was not affected by environmental or feeding treatments.

An interaction was found for relative liver weight at day 0. Relative liver weight of chickens in the DF treatment did not differ among the environmental treatments. However, relative liver weight of chickens in the EF treatment was greater in the HTD treatment compared to the FORM treatment (Δ = 0.27%; P = 0.003), with CONT and UTD intermediate and not different from the other environmental treatments (Table 3). Regardless of feeding treatment, relative bursa weight was greater in the FORM treatment compared to the HTD treatment (Δ = 0.03%; P = 0.016), with both other treatments in between and not different from the other environmental treatments (Table 3). Relative spleen weight was greater in the HTD treatment compared to the CONT and UTD treatment (Δ = 0.014%; P < 0.001), with FORM in between and not different from the other environmental treatments.

BW of the EF treatment at day 7 was greater compared to the DF treatment (Δ = 10.5 g; P < 0.001; Table 2). No difference in BW at day 7 among environmental treatments was found. Relative liver weight at day 7 was greater in the EF treatment compared to the DF treatment (Δ = 0.15%; P = 0.048; Table 3), but was not affected by environmental treatment. No effects of environmental or feeding treatment were found for bursa and spleen weight at day 7 (Table 3).

No effects of environmental or feeding treatment were found for microbicidal capacity at day 0, 1 or 7 and for monocyte activity at day 7 (Table 4).

3.2. Hatch time effects

An interaction between feeding treatment and hatch time for BW and YFBM was found at day 0. In DF chickens, BW increased with hatching time (β = 0.11 g/h; P < 0.001), whereas in EF chickens, BW did not change with hatching time (Fig. 1A). In DF chickens, YFBM did not change with hatching time, whereas in EF chickens, YFBM decreased with hatching time (β = −0.16 g/h; P = 0.043) (Fig. 1B).

Regardless of treatments, RY weight at day 0 increased with hatching time (β = 0.11 g/h; P < 0.001). At day 0, relative liver weight (β = −0.011%/h; P ≤ 0.001) and microbicidal capacity (β = −0.02/h; P = 0.013) decreased with hatching time, regardless of treatments.

4. Discussion

In commercial hatchers, chickens are exposed to high levels of dust (Mitchell and Waltman, 2003), pathogens (Mitchell and Waltman, 2003) and formaldehyde (Zulfifli et al., 1999) and to feed and water deprivation (Careghi et al., 2005). This study investigated effects of perinatal exposure to dust or formaldehyde, combined with the moment of first feed and water provision on development of chickens during the first week of life.

4.1. Environmental and feeding treatments

Average dust level in commercial hatchers can rise up to 2.2 ± 1.2 mg/m^3 (PM10; unpublished results). In the current experiment, average dust levels in the UTD and HTD treatments were approximately 0.5 mg/m^3. Even though lower concentrations were reached in the current experiment than in practice, effects of exposure to dust on early postnatal development were found. Average formaldehyde levels between 20 and 80 ppm have been found in commercial hatchers (Sander et al., 1995). A concentration of 11 ppm has been shown to affect epithelial function and morphology as well as post hatch development (Zulfifli et al., 1999). In the current experiment, formaldehyde levels of approximately 21 ppm were used, which are based on calculated concentrations in Dutch hatcheries.

At day 0, exposure to heat treated dust (HTD) in combination with early feeding (EF) resulted in greater BW and YFBM compared to the FORM and UTD treatments. A comparable result was found by Lai et al. (2009) in 3 week old broiler chickens, where an intra-tracheal challenge with heat treated dust dissolved in PBS resulted in greater growth rates 2 days after the challenge (83.06 g; P < 0.001) compared to an intra-tracheal challenge with only PBS (63.00 g). It might be suggested

Table 2
Effects of environmental treatment (ET) and feeding treatment (FT) from 468 h of incubation until pulling on body weight (BW) at hatch, day 0 and 7, and on yolk free body mass (YFBM) and residual yolk (RY) at day 0 (LSmeans).

	Hatch	Day 0						Day 7	
	BW (g)	BW (g)		YFBM (g)		RY (g)		BW (g)	
Interaction: ET * FT	DF EF	DF	EF	DF	EF	DF	EF	DF	EF
Control		47.1^{bc}	48.5^{ab}	42.2^{bd}	44.0^{b}	5.0	4.6	179.9	196.9
Formaldehyde		46.2^{c}	47.3^{bc}	41.8^{bd}	43.0^{bc}	4.6	4.3	175.4	184.4
Heat treated dust		46.2^{c}	50.4^{a}	41.7^{cd}	46.2^{a}	4.5	4.2	179.8	185.8
Untreated dust		46.3^{bc}	47.6^{bc}	41.4^{cd}	43.3^{bc}	4.9	4.3	176.1	185.9
SEM		0.5		0.5		0.3		3.7	
Main effect: ET									
Control	50.8	47.8		43.1		4.7		188.4	
Formaldehyde	50.6	46.7		42.4		4.4		179.9	
Heat treated dust	50.4	48.3		43.9		4.3		182.8	
Untreated dust	50.4	47.0		42.4		4.6		181.0	
SEM	0.3	0.4		0.4		0.2		2.6	
Main effect: FT									
DF		46.5		41.8		4.7		177.8^{b}	
EF		48.4		44.1		4.3		188.3^{a}	
SEM		0.3		0.3		0.2		1.8	
ET	0.589	0.007		0.005		0.440		0.102	
FT	–	< 0.001		0.038		0.761		< 0.001	
HT	0.572	0.403		0.002		< 0.001		–	
ET * FT	–	0.005		0.006		0.904		0.497	
FT * HT	–	0.012		0.043		0.744		–	

DF = delayed feeding; EF = early feeding; HT = hatching time (hours).
[a–d]LSmeans lacking a common superscript within a column and factor differ (P ≤ 0.05).

Table 3
Effects of environmental treatment (ET) and feeding treatment (FT) from 468 h of incubation until pulling on relative liver, bursa and spleen weight at day 0 and 7 (LSmeans).

	Day 0						Day 7					
	Liver (% of YFBM)		Bursa (% of YFBM)		Spleen (% of YFBM)		Liver (% of YFBM)		Bursa (% of YFBM)		Spleen (% of YFBM)	
	DF	EF	DF	EF	DF	EF	DF	EF	DF	EF	DF	EF
Interaction: ET * FT												
Control	2.91^{bc}	3.10^{ab}	0.13	0.14	0.033	0.039	4.44	4.64	0.14	0.15	0.072	0.062
Formaldehyde	2.95^{bc}	2.90^{bc}	0.15	0.15	0.030	0.036	4.32	4.47	0.15	0.15	0.069	0.067
Heat treated dust	2.84^{c}	3.17^{a}	0.12	0.13	0.037	0.046	4.48	4.43	0.15	0.15	0.066	0.061
Untreated dust	2.94^{bc}	3.05^{ac}	0.14	0.13	0.028	0.027	4.12	4.40	0.15	0.14	0.064	0.073
SEM	0.05		0.01		0.003		0.10		0.01		0.005	
Main effect: ET												
Control	3.00		0.14 ^{ab}		0.036 ^{a}		4.54		0.14		0.033	
Formaldehyde	2.93		0.15 ^{a}		0.034 ^{ab}		4.40		0.15		0.030	
Heat treated dust	3.00		0.12 ^{b}		0.042 ^{a}		4.45		0.15		0.038	
Untreated dust	2.99		0.13 ^{ab}		0.028 ^{b}		4.26		0.14		0.025	
SEM	0.04		0.01		0.002		0.07		0.005		0.002	
Main effect: FT												
DF	2.91		0.14		0.033		4.34^{b}		0.15		0.029	
EF	3.05		0.14		0.037		4.49^{a}		0.14		0.034	
SEM	0.027		0.004		0.002		0.05		0.003		0.001	
ET	0.363		0.016		< 0.001		0.062		0.666		0.817	
FT	0.824		0.597		0.178		0.048		0.586		0.502	
HT	< 0.001		0.202		0.577		–		–		–	
ET * FT	0.003		0.736		0.569		0.463		0.499		0.202	
FT * HT	0.788		0.600		0.186		–		–		–	

YFBM = yolk free body mass; DF = delayed feeding; EF = early feeding; HT = hatching time (hours).
[a–c]LSmeans lacking common superscripts within a column and factor differ (P ≤ 0.05).

that particles of the HTD are capable of entering the body (Lai et al., 2012), where they trigger an immunological response (Lai et al., 2009) which, in turn, triggers a metabolic response resulting in short-term increased growth (Henken and Brandsma, 1982). In 3-week old pullets, injection with sheep red blood cells increased energy retention by increased protein and fat deposition and decreased metabolizable energy for maintenance (Henken and Brandsma, 1982).

Relative liver weight was also increased in EF chickens in the HTD treatment compared to the FORM treatment, whereas relative spleen weight was greater in the HTD treatment compared to the CONT and UTD regardless of feeding treatment. It appears that dust particles stimulate cells in both liver and spleen related to the innate immune response (such as Kuppfer cells in the liver (Racanelli and Rehermann, 2006)), setting off a pathway that alters metabolism. Consequently, the presence of energy from the feed in the EF treatment in combination with the altered metabolism might have resulted in increased BW at day 0. The presence of live microorganisms in the UTD treatment and the absence of high dust levels in the CONT and FORM treatment possibly resulted in a different stimulation of cells related to the innate immune response and subsequently in another distribution of energy resources

Table 4
Effects of environmental treatment (ET) and feeding treatment (FT) from 468 h of incubation until pulling on microbicidal capacity at day 0, 1 and 7 and monocyte activity at day 7 (LSmeans).

Microbicidal capacity							Monocyte activity day 7	
	Day 0		Day 1		Day 7			
	DF	EF	DF	EF	DF	EF	DF	EF
Interaction: ET * FT								
Control	0.59	0.86	0.80	0.84	0.96	0.96	3.65	1.71
Formaldehyde	0.57	0.58	0.97	0.94	0.95	0.96	4.06	3.02
Heat treated dust	0.70	0.60	0.95	0.87	0.93	0.97	2.23	2.52
Untreated dust	0.67	0.67	0.92	0.96	0.97	0.94	3.35	3.60
SEM	0.12		0.06		0.02		0.65	
Main effect: ET								
Control	0.72		0.82		0.96		2.68	
Formaldehyde	0.57		0.96		0.95		3.54	
Heat treated dust	0.65		0.91		0.95		2.37	
Untreated dust	0.67		0.94		0.95		3.47	
SEM	0.09		0.04		0.02		0.47	
Main effect: FT								
DF	0.63		0.91		0.95		3.32	
EF	0.68		0.90		0.96		2.71	
SEM	0.06		0.03		0.01		0.30	
ET	0.501		0.158		0.965		0.966	
FT	0.563		0.301		0.855		0.856	
HT	0.013		0.100		–		–	
ET * FT	0.212		0.566		0.541		0.541	
FT * HT	0.563		0.303		–		–	

(Lochmiller and Deerenberg, 2000), which did not result in an increase of BW. This is supported by the lack of differences among environmental treatments in the DF chickens. The exact pathway, however, remains unclear. In accordance with previous research (Van de Ven et al., 2013), relative spleen weights tended to be greater in the EF treatment compared to DF treatment. The presence of energy from the feed enables the chickens to start their development early, which results in increased organ weights (Dibner et al., 1998; Bigot et al., 2003; Van de Ven et al., 2013).

At day 7, EF resulted in greater BW in accordance with earlier studies (Careghi et al., 2005; Van de Ven et al., 2011; Van de Ven et al., 2013). At this stage, effects of environmental treatments on BW were not significant (P = 0.102). However, BW of the CONT treatment was 3.1 to 4.7% greater than in the other environmental treatments, suggesting that exposure to dust or formaldehyde during the hatching phase might influence post hatch growth rate on the longer term.

Nitric oxide levels in chickens can reach levels up to 10.6 μM (Allen, 1997). The low nitric oxide production during the monocyte activity test in the current study might be caused by the young age of the chickens. It takes approximately 6 days for monocytes to differentiate after initial stimulation of the monoblast (Qureshi, 2003). Monocytes are, moreover, not observed in the liver before day 12 of age and in the spleen before day 16 of age (Qureshi et al., 2000). In 7 day old chickens, infected with a live smallpox virus, greater levels of nitric oxide (average 4.6 μM NO) at day 1 after inoculation were found compared to non-infected chickens (York et al., 1996). Because in the current experiment hardly any monocyte activity was found, it can suggested that the environmental treatments were either not able to affect the chickens to an extent that infection took place (and thus monocyte activity could be measured) or that a possible infection that had occurred during the hatching phase already disappeared by day 7 (and thus monocyte activity was already low again).

4.2. Hatching time effects

At day 0, YFBM decreased with hatching time in EF chickens and RY weight increased with hatching time regardless of feeding treatment in accordance with (Van de Ven et al., 2013). Thus, earlier hatching EF chickens had a greater YFBM and less RY at day 0 than later hatching EF chickens, which suggests further physiological development. This effect is probably caused by the fact that early hatched chickens had longer time to ingest feed and water than later hatched chickens, allowing them to start their development earlier (Careghi et al., 2005; Van de Ven et al., 2011). Earlier hatching DF chickens also showed a lower RY weight at day 0 than later hatching DF chickens. YFBM, however, was not affected by hatching time in DF chickens. It appears that RY in DF chickens was not used for development, but only for maintenance and survival.

Microbicidal activity at day 0 was negatively related to hatching time. This relationship might be explained by the difference in stress and corticosterone (CORT) levels at the moment of sampling. Hatching is a stressful event for the chicken and results in releases of high CORT levels (Everaert et al., 2008; Van de Ven et al., 2013). Both stress (Mitchell and Baumgartner, 2007; Tieleman et al., 2010) and CORT (Stier et al., 2009) affect microbicidal capacity. The time between hatching and sampling was shorter for the late hatching chickens, which suggests that the late hatching chickens may not yet have recovered from the stressful hatching process at the moment of sampling at day 0. Research of Van de Ven et al. (2013) showed that early hatching chickens seem to have lower, though not significant, CORT levels at day 0 compared to later hatching chicks. It could therefore be suggested that the decreased microbicidal capacity in later hatching chickens was still a result of stress from the hatching process. A lower microbicidal capacity in later hatching chickens, consequently, might suggest that late hatching chickens are more vulnerable to infections at the moment of transfer from the hatcher to the broiler house. During transfer from the hatcher to the broiler house, chickens are exposed to hatchery automation, transport, temperature variation and a new environment. This process might result in stress again (Donofre et al., 2014) and cross contamination among the chickens (Cason et al., 1994; Boonprasert et al., 2014) and, thus increase the risk for infection, especially in the more vulnerable late hatching chicks.

To conclude, BW at day 0 was affected by both hatching environ-

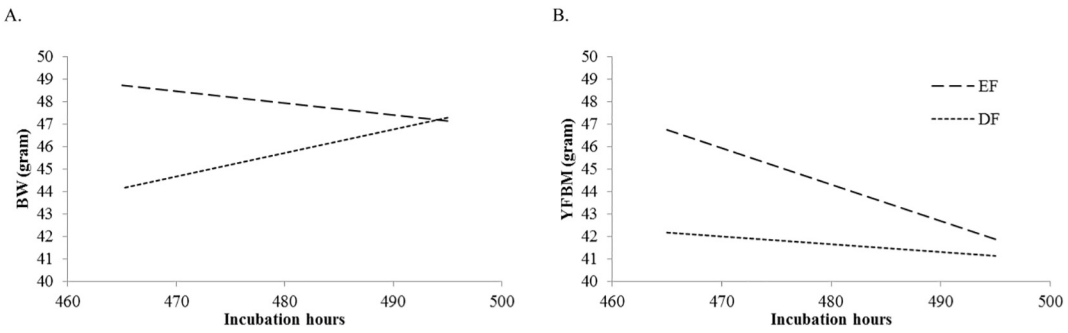

Fig. 1. Predicted means of BW (A) and yolk free body mass (YFBM; B) at day 0 in relationship to the duration of incubation. Chickens were subjected to 2 feeding treatments; Early feeding (EF) and Delayed feeding (DF).

ment and early feed and water provision. Effects of hatching environment disappeared at day 7. It can be speculated that hatching environment will affect later life performance when chickens are under a greater metabolic and immunological pressure. Hatching time affected residual yolk weight, YFBM and microbicidal capacity at day 0, which indicates a further physiological and immunological development of early hatching chickens compared to later hatching chickens at day 0, especially in early fed chickens.

Conflict of interest

P. de Gouw and L.J.F. van de Ven are employed at Vencomatic B.V., Eersel, the Netherlands, but do not have any conflict of interest regarding the topic described in this article. The other authors do not have any conflict of interest.

Acknowledgements

This study was financially supported by Vencomatic B.V., Eersel, the Netherlands. The funding agency had no role in study design, data collection and analysis, but was involved in the preparation of this article. The authors thank the personnel of hatchery Van Hulst (Belgabroed NV) for their technical assistance. The help of colleagues from the Adaptation Physiology Group (Wageningen UR), Agnes de Wit (Wageningen Livestock Research) and colleagues of Vencomatic BV during data collection and analysis is highly appreciated.

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