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EFFECTS OF POST HATCH CONDITIONS ON BROILER CHICKENS

METABOLISM AND NUTRITION

Effects of Early Nutrition and Transport of One-Day-Old Chickens on Production Performance and Fear Response

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ABSTRACT

The importance of optimal early life conditions of broilers to sustain efficient and healthy production of broiler meat is increasingly recognized. Therefore, novel husbandry systems are developed, in which immediate provision of nutrition post hatch is combined with on-farm hatching. In these novel systems, one-day-old-chick handling and transport are minimized. To study whether early nutrition and reduced transport are beneficial for broiler performance and behavior, the effects of early or delayed nutrition and post-hatch handling and transport were tested from hatch until 35 d of age, in a 2*2 factorial arrangement. In total, 960 eggs were hatched in 36 floor pens. After hatch, chicks were given immediate access to water and feed (early nutrition) or after 54 h (delayed nutrition). Eighteen hours after hatch, chicks remained in their pens (non-transported control), or were subjected to short-term handling and transport to simulate conventional procedures. Subsequently, chicks returned to their pens. Compared with delayed-fed chickens, early-fed chickens had greater body weight up to 21 d of age, but not at slaughter (35 d of age). No effects of transport or its interaction with moment of first nutrition were found on performance. At 3 d post hatch, transported, early-fed chicks had a greater latency to stand up in a tonic immobility test than transported, delayed-fed chicks, but only in chicks that were transported. At 30 days post hatch, however, latency was greater in transported, delayed-fed chickens than in transported, early-fed chicks. This may indicate long-term deleterious effects of delayed nutrition on fear response in transported chickens. It is concluded that early nutrition has mainly beneficial effects on performance during the first two weeks post hatch, but these beneficial effects are less evident in later life. The combination of transport and early nutrition may influence the chicken's strategies to cope with stressful events in early and later life.

Keywords: broiler chicken, early nutrition, transport, behavior, production performance.

INTRODUCTION

The majority of broiler chickens hatch in conventional hatcheries after 19 to 21 days of incubation, having a hatch window of approximately 24 to 48 hours (**h**) (Careghi et al., 2005; Jacobs et al., 2016). The length of the hatch window is mainly affected by parent stock and incubation conditions (Lourens et al., 2005). During hatch in conventional hatchers, chicks have no access to nutrition until placement at the farm, which is considered suboptimal for broiler development and health (Uni et al., 2003b; Bar-Shira et al., 2005; Van De Ven et al., 2011; Simon et al., 2015). At the end of the hatch window, all chicks are simultaneously pulled and processed (e.g. sorting, sexing, counting, vaccinating) following standard procedures, stored for approximately 1 – 4 h, and transported to broiler farms.

Immediate post hatch provision of nutrition (water and feed) has been suggested to improve intestinal (Lilburn and Loeffler, 2015) and immunological development (Panda et al., 2014). Previous studies (Gonzales et al., 2003; Van De Ven et al., 2011; Simon et al., 2014, 2015) showed that effects of early nutrition on performance parameters seem to vanish in later life, making the long-term benefits of early nutrition on performance unclear. Practical implementation of early nutrition is implemented by hatching eggs within a broiler house (on-farm hatching), or supplying water and feed in the hatcher. Both systems are meant to provide hatchlings with immediate access to nutrition.

Various studies suggest that one-day-old chick transport may have negative effects on production performance and the chickens' ability to cope with stress, depending on transport duration (Valros et al., 2008; Mitchell, 2009; Bergoug et al., 2013; Jacobs et al., 2016). A drawback from these studies is that the effects of moment of first nutrition and transport are confounded, as the chicks that were subjected to a longer transport duration also did not have access to nutrition. It is therefore not clear whether the observed effects were caused by transport or delayed access to nutrition. Furthermore, to our best knowledge, interactions between access to nutrition and transport have not been studied so far.

The aim of the current study was to examine the effects of early nutrition and one-day-old-chick handling and transport, as well as their interaction, on growth performance and fear response of chickens in early and later life. Because both nutrition and transport in early life may affect neural and cognitive development (Candland et al., 1963; Jones and Waddington, 1992), we hypothesize that the chickens' fear reactions in a stressful situation will be affected by early life nutrition and transport procedures. Therefore, a tonic immobility test was performed to gain preliminary insights in the fear response (Forkman et al., 2007) of the chickens in early and later life.

MATERIALS AND METHODS

Experimental Design

Effects of delayed (**DN**) or early nutrition (**EN**) and no transport (**NT**) or transport (**T**) of one-day-old chicks were tested in a 2*2 factorial arrangement. This resulted in 4 treatment groups (**DN|NT**; **DN|T**; **EN|NT** and **EN|T**). In **Figure 1** the start and duration of these interventions are presented. Chick ages are expressed as chronological age (Careghi et al., 2005), starting from the end of the hatch window (0 d) until slaughter (35 d), unless specified otherwise.

Housing and Diets

The facility consisted of 36 floor heated pens (1.55 * 0.95 m) covered with wood shavings. Before egg arrival, the bedding was covered with chick paper to prevent any litter uptake by the chicks. HatchCare baskets (HatchTech B.V., Veenendaal, The Netherlands) consisting of a chicken basket and an overlay egg tray were placed in each pen. Depending on the treatment, egg trays were filled with a commercial starter diet (EN) or left empty (DN), and 2 drinking nipples were attached to the basket (EN) or not (DN). Diets were produced by Research Diet

Services (Wijk bij Duurstede, The Netherlands). Floor temperature was 34 °C and ambient temperature was controlled at 36 °C. Average humidity (27.4 ± 2.6 %) and CO_2 (1100 ± 156 ppm) levels were logged from placement until hatch. As a result of minimal ventilation, air speed was negligible. Embryonic temperature of 3 eggs per treatment was monitored indirectly by egg shell temperature (**EST**) and recorded every 5 min until hatch. EST sensors (NTC Thermistors: type DC 95, Thermometrics, Somerset, UK) were attached to the egg following procedures of Maatjens et al. (2016b). EST was maintained between 35.3 and 36.7 °C by manually adjusting floor heating and ventilation before and during hatch, based on recommendations of Maatjens et al. (2016a; b).

After hatch, and before the chicks were taken out of the baskets and placed into the pen, each pen was provided with 2 trough feeders, and chick paper was removed.

Until 7 d post chick placement, 2 additional round feeding plates were placed in the pen to enhance feed uptake. A three-phase feeding schedule was applied including a starter, grower, and finisher diet (**Table 1)**. Water was provided *ad libitum* by 2 drinking nipples per pen. From egg placement until end of hatch, the experimental room was lighted continuously with a light intensity varying between 20 and 40 lux on the egg and animal level. After placement, a 16-h light : 8-h dark schedule was applied.

Animals and Treatments

In total, 960 incubated and candled eggs (embryonic age: 18 d) were obtained from a commercial hatchery (Probroed & Sloot, Langenboom, The Netherlands) and transported in a climate conditioned van (34 °C) to the research facility. Eggs were produced by a 50-week-old Ross 308 parent stock. All eggs were randomly assigned to one of the 4 treatments, with 27 eggs per pen, except for 4 pens (1 per treatment) in which 24 eggs were placed, resulting in 9 replicates per treatment group.

During their stay in the hatching baskets, water and feed were provided *ad libitum* to the EN groups, while DN groups did not receive any form of nutrition. To simulate post hatch holding and transport, all T groups were moved to an unconditioned room (20 °C, no air circulation, continuous lighting) and kept for 1.5 h in their original hatching baskets. Subsequently, the baskets with chicks were placed in a climate controlled chick transport van (33 °C; dark) and transported for 1 h. After transport, baskets were moved to their original pens and, after 0.5 h, all baskets were emptied allowing all chicks *ad libitum* access to water and feed. Thus, the period of handling and transport simulation was 3 h. NT groups remained in their hatching baskets within the barn according to conditions described in "housing and diets" and were placed in the pens simultaneously to the T groups. The experiment was performed according to the Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching (2010).

Measurements

Eggs and Chick Quality

After arrival at the research facility, eggs were weighed per pen. Sixty hours after placement of the eggs, i.e. just before transport simulation, the number of unhatched eggs were counted and collected for break-out, to determine the cause of not hatching. Chick quality of the hatched chicks was assessed before transport simulation, using chick length and navel score (n = 100 per treatment group), according to Maatjens et al. (2016b). Cloacal temperature was measured in 97 randomly selected chicks divided over 28 pens. Chicks with chick length lower than 17 cm or malformations (e.g. open navel) were classified second grade, and removed from the study (Tona et al., 2004). All non-hatched eggs (n = 19) were opened to determine the reason of not hatching.

Performance

Average body weight (**BW**) was evaluated per pen at 0, 3, 7, 14, 21, 28 and 35 d post placement to calculate average daily gain (**ADG**). Relative ADG of each week was calculated as follows:

$$Relative\ ADG = \frac{(\frac{BW_{end}}{BW_{start}} * 100)}{7}$$

Average daily feed intake (**ADFI**) and feed efficiency (**G:F**) were determined per pen at 3, 7, 14, 28 and 35 d post placement.

Tonic Immobility

Tonic immobility tests were performed at 3 and 30 d post placement on 2 chickens per pen from 7 randomly chosen pens per treatment. Different chickens were selected for the measurements at 3 and 30 d, to prevent habituation to the procedure (Jones, 1986). Results were averaged for each pen, resulting in 7 observations per pen. The procedure was adapted from Valros et al. (2008) with minor modifications. Briefly, one chicken was taken from the home pen and transferred in a bin to a quiet testing room, to ensure isolation from the flock. There, the chicken was restrained on the back for 10 s, using one hand to hold the chest and one to cover the neck and head. All tests were performed by the same experimenter and observer, who did not made direct eye contact with the chicken during both handling and testing. Experimental conditions were similar at both 3 and 30 d of age (*i.e.* same procedure of handling and transport to the test room (Jones and Waddington, 1992)). If the chicken stood up within 10 s after the end of restraining, the restraint was carried out again up to a maximum of 5 times. After 5 attempts, the test was stopped and the chicken was placed back in the home pen and recorded as missing value. The chicken was judged immobile when it stayed down for at least 10 s after removal of the hands. The latency (s) from immobility until standing was recorded. If the latency of immobility was ≥ 300 s, the test was stopped and the maximum latency of 300 s was noted.

Statistical Analyses

Data were processed and analyzed using SAS 9.3 software (SAS Institute Inc.,). Model residuals were inspected for outliers using histograms and QQ-plots. In total, 1 data point was removed because of erroneous recordings. Model residuals were tested to meet assumptions for homogeneity and normality. If needed, logarithmic or square root transformation was applied to normalize the data. Pen was the experimental unit, except for analyses of chick quality parameters, for which individual chicken was the experimental unit. All data are expressed as means and standard deviations.

Effects of treatments on ADG, relative ADG, ADFI and G:F were analyzed using a generalized linear mixed model (PROC GLIMMIX). Fixed factors were moment of feeding, transport, age, and the interaction between moment of feeding, transport, and age. Pen was included as random effect and age was modelled as R-side effect to account for repeated observations within pen. The covariance structure was selected based on assessing variograms, resulting in using a first order heterogeneous autoregressive structure (Wang and Goonewardene, 2004).

Effects of treatments on BW were analyzed per time point, due to heterogeneous variation between ages. Data were analyzed using a general linear model (PROC GLM) with moment of feeding, transport, and the interaction effect between moment of feeding and transport as fixed effects and pen as random effect.

Fixed effects of treatments (DN|NT; DN|T; EN|NT and EN|T) on the latency to stand up during the tonic immobility test were analyzed using a non-parametric Kruskal-Wallis H test, followed by two-by-two comparisons with a Mann-Whitney U test, when appropriate.

Data are presented as means and standard deviation, unless stated otherwise. Differences among means with $P < 0.05$ were considered statistically significant. Differences $P < 0.10$ were considered to represent statistical tendencies.

RESULTS

Egg and Hatching Parameters

The length of the hatch window (HW) of the chicks was approximately 33 h (latency in between first and last hatch), therefore, the time between end of HW and start of transport simulation was 18 h. As time of transport simulation was 3 h, we estimate the delay in nutrition to be between 54 for the first hatchers and 21 h for the last hatchers.

Chick quality after hatch (60 h after placement of the eggs of 18d), before transport, is presented in **(Table 1, supplementary material)**. Average cloaca temperature immediately after placement was 0.7 °C higher ($F_{1, 81} = 6.67$, $P < 0.001$) in the EN groups compared with the DN groups. Of the non-hatched embryos, 10.5% (n = 2) did not turn, 10.5 % (n = 2) died during external pipping, 63 % (n = 12) were underdeveloped or malformed, and 16 % (n = 3) were found to be slow hatchers or had a damaged egg shell. After hatch, 1 chick was removed as it was classified second grade. Each pen contained between 23 and 27 chicks after hatch.

Performance

No interactions between moment of access to nutrition and transport were found on performance. BW was significantly greater (46 g) for EN chicks until at least 28 d ($F_{1, 32} = 4.38$, $P = 0.045$) compared with the DN chicks (**Table 2**). At slaughter (35 d), there was no significant difference between EN and DN chicks ($F_{1, 32} = 2.13$, $P = 0.152$). In **Table 3**, it is shown that moment of feeding affected ADG and ADFI, with a significant greater ADG at 0 – 3 and 3 – 7 d (1.3 and 1.4 g/d, respectively) in EN chicks than in DN chicks. Furthermore, relative ADG was significantly ($F_{1, 170} = 4.38$, $P < 0.001$) higher in DN chicks compared with EN chicks, from 0 until 14 d of age (**Figure 3**). G:F ratio was not affected by treatment. No effects of transport were found on BW (**Table 2**) or ADG, ADFI and G:F (**Table 4**).

Tonic Immobility

Latencies to stand up after inducing tonic immobility are presented in **Figure 2**. Within transported chicks, at 3 d, latency to stand up was lower in the DN group compared with the EN group. At 30 d, DN|T chicks took more time to stand up than EN|T chicks. No differences of latency to stand up were found between EN and DN groups that were not subjected to transport. No significant correlations between body weight and latency to stand up were found (data not shown).

DISCUSSION

This study shows that EN affects production performance in early life, but not in later life, which is consistent with prior research (Gonzales et al., 2003; Juul-Madsen et al., 2004; Van De Ven et al., 2011; Simon et al., 2014, 2015). It should be noted, however, that, in our study and those of others, chickens were kept at relatively non-challenging, experimental conditions. Effects of EN on later life production performance in more challenging, i.e. field conditions, can therefore not be excluded, which can be suggested from Simon et al. (2015). Transport, and its interactions with moment of first nutrition did not affect production performance. The analysis of the latencies to stand up after tonic immobility suggests that EN and DN chicks express a different fear response after transport at different ages. To the best of our knowledge, this study is the first to investigate effects of early nutrition and transport separately. This is in contrast to prior research on post-hatch transport, where effects of transport were confounded with nutritional effects (Valros et al., 2008; Bergoug et al., 2013).

Chick Quality and Progress of Grow-out Period

Our results indicate that chick quality was identical in the different treatment groups. The increased cloacal temperature in EN chicks compared with DN chicks, is presumably due to heat generated by metabolism (Van den Brand et al., 2010). This increase in body temperature in day-old chicks can be favorable, as these chicks might be less susceptible to temperature changes during transport and brooding.

Moment of First Nutrition * Transport

At 3 d of age, latency to stand up after tonic immobility was higher in EN|T chicks than in DN|T chicks. Although latency to stand up after tonic immobility is known to be a valid measure of fear levels in chickens (Jones and Mills, 1983; Forkman et al., 2007), no consensus has been reached concerning the validity of the TI test in very young chickens (Ratner and Thompson, 1960; Salzen, 1963; Forkman et al., 2007). We, however, observed typical signs of immobility, such as no movement, and extended legs with tremor (Jones, 1986; Heiblum et al., 1998) at 3 d of age. This seems to support the validity of the TI test to assess fear levels in very young chicks, too. The higher latency to stand up after tonic immobility in 3-day-old EN|T chicks compared with DN|T chicks may therefore indicate that EN|T chicks were more fearful than DN|T chicks in early life.

That EN|T chicks expressed higher fear responses than DN|T chicks at 3 d might result directly from the impact of early nutrition on brain and cognitive development and, thus, on the ability for chicks to express fear responses at such a young age. Various studies (Candland et al., 1963; Andrew and Brennan, 1983; Cashman et al., 1989) have shown that fear responses develop parallel to body development. It is possible that a delay in access to nutrition might have led not only to impaired body and organ (brain) development, but also to a delayed development of fear-related behavior in DN chicks. Alternatively, early access to water and feed might have acted as an early life environmental enrichment, thus stimulating brain development and the early ability to express early fear responses in EN chicks (Jones and Waddington, 1992).

Unlike at 3 d of age, latency to stand up was shorter in the EN|T chicks compared with DN|T chicks at 30 d post placement, suggesting that EN|T chicks were less fearful than DN|T later in life. Although it remains unclear why the impact of early nutrition in transported chicks was reversed from 3 d to 30 d, our results seem to indicate that early nutrition provided long-term advantages for the chicken's ability to cope with stress later in life.

It is worth noting that differences in fear responses between EN and DN chicks were only found in chicks that have been transported in early life. This implies that handling and transport at very young ages may accentuate the impact of early or delayed nutrition on the chickens' fear responses in both early and later life. Accordingly, research has shown that stressful early life events (*e.g.* transport) can alter TI responses in chickens in later life (Al-Aqil et al., 2009) and brain development in rodents and humans (Teicher et al., 2003; Hoeijmakers et al., 2014). Although additional research using alternative fear tests would be needed to confirm the short- and long-term impact of early nutrition on fear responses of transported chicks, the reported findings could have important implication for hatcheries, chick transporters or slaughterhouses. For instance, our findings indicate that EN|T chicks may be able to cope better with stressful events in later life, such as thinning and pre-slaughter procedures (Jacobs et al., 2017).

Moment of First Nutrition

The lower BW of DN chicks until 28 d of age is consistent with previous research (Juul-Madsen et al., 2004; Van De Ven et al., 2011; Lamot et al., 2014), and might be explained by impaired organ and body development and dehydration during feed and water deprivation (Uni et al., 2003a; b; Smirnov et al., 2004; Lamot et al., 2014; Lilburn and Loeffler, 2015). The significant higher relative ADG in EN chicks compared with DN chicks from 0 to 14 d of age (**Figure 3**), might indicate compensatory growth of DN chicks (Zubair and Leeson, 1996).

Transport

Our results suggest that short-term holding time and transport simulation (3 h) do not affect early and later-life performance. This seems to be in contrast with other studies. Bergoug et al.

(2013) transported broiler chicks from the hatchery under controlled climate conditions (0, 4, and 10 h transportation time) to an experimental facility and found that NT chicks had increased BW compared with T chicks until 21 d post hatch ADFI or G:F were not affected. Valros et al. (2008) found negative effects on fear-related behavior (e.g. latency to perch after transport, and latency to stand up after tonic immobility at 34 d post hatch) with increasing transport duration (4 and 10 h), but not on body weight. As no non-transported control was included in this study, effects of transport relative to no transport are unknown. As none of the above mentioned studies accounted for moment of access to nutrition after transport, the long-transported chicks were also deprived longer from nutrition than short-transported chicks. Therefore, the effects of transport reported in these studies could actually reflect the effect of DN instead of that of transport. This is in line for performance of the DN groups in the current study. We suggest that climate controlled transport of one-day-old chickens does not affects performance, as long as nutrition is provided. This is probably due to the fulfillment of the chicken’s needs. Further investigation is required to explain why transport on itself does not result in differences in production performance.

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Table 1: Composition of starter (0 – 14 d) grower (14 – 28 d) and finisher (28 – 35 d) diets (%, as-fed basis, unless indicated otherwise).

	Starter	Grower	Finisher
Ingredients			
Wheat	41.39	50.59	56.52
Soybean meal	23.66	23.19	22.70
Maize	20.00	15.00	10.00
Soybean oil	4.26	5.22	5.82
Soy protein concentrate (CP: 55%)	1.50	1.00	1.50
Fishmeal	2.50	-	-
Potato protein	2.50	1.00	-
Mineral and vitamin premix[1]	0.50	0.50	0.50
L-Lysine	0.17	0.31	0.27
DL-Methionine	0.28	0.31	0.29
L-Threonine	0.08	0.14	0.13
Limestone	1.34	1.20	1.01
Monocalcium phosphate	1.29	0.98	0.79
Sodium bicarbonate	0.27	0.33	0.31
Sodium chloride	0.07	0.07	0.08
Xylanase[2]	0.02	0.02	0.02
Anti-coccidiostat[3]	0.06	0.06	-
Sodium butyrate coated	0.10	0.08	0.05
Calculated nutrient composition[4]			
Moisture	11.7	11.9	11.8
Crude protein	22.5	20.0	19.5
Digestible lysine [5]	12.0	11.0	10.3
Digestible methionine + cysteine [5]	8.9	7.9	7.5
Digestible threonine [5]	8.0	7.2	6.9
Crude fat[6]	7.3	7.9	8.6
Crude fiber	2.5	2.6	2.6
Ash	5.8	4.9	4.8
Starch[7]	36	38.4	38.1
DE (kcal)[5]	3,000	3,040	3,080
Calcium	9.0	7.0	6.5
Available phosphorus	4.1	3.2	3.0

[1] Containing Vitamin A (2,500,000 IU); D3 (600,000 IU); E (3,350 IU); K3 (600 mg); B1 (600 mg); B2 (1,500 mg); B6 (800 mg) ; B12 (6,000 mg); niacin (9,000 mg); panthothenic acid

(2,000 mg); biotin (100,000 mg); choline chloride (100,000 mg); Mn (17,000 mg); Zn (18,000 mg); Cu (3,000 mg); Fe (16,000 mg); I (400 mg); Se (50 mg).

[2] Commercial bacterial endo-1,3-β-xylanase (Belfeed, Agrimex N.V., Lille, Belgium).

[3]Starter diet: Mixture of 45 mg narasin and 45 mg nicarbazin /kg feed (Maxiban, Elanco, Greenfield, USA); Grower diet: Salinomycin (72 mg/kg feed) (Sacox, Huvepharma, St. Louis, USA).

[4] Calculated based on feed table of Schothorst Feed Research (2015) and specified in g/kg unless specified otherwise.

[5] Apparent total tract digestibility.

[6] Ether extract with acid hydrolysis (ISO 6492).

[7] Amyloglucosidase method (ISO 15914)

Table 2: Body weight of chickens that received one of 4 treatments groups (DN | NT, EN | NT, DN | T or EN | T). (DN = delayed nutrition; EN = early nutrition; NT = no transport; T = transport; n = 9 pens per treatment).

Age (d)	Treatment								Effects		
	DN | NT		DN | T		EN | NT		EN | T		Feeding * Transport	Feeding	Transport
	Mean	SD	Mean	SD	Mean	SD	Mean	SD			
0	4	1	43	1	49	2	49	2	0.539	< .0001	0.995
3	80	2	81	2	90	6	91	4	0.850	< .0001	0.439
7	179	5	182	4	195	10	197	8	0.897	< .0001	0.384
14	484	15	498	20	506	16	514	13	0.571	< .0001	0.052
21	1023	38	1015	22	1047	32	1053	29	0.537	0.005	0.930
28	1596	66	1608	53	1652	87	1643	52	0.923	0.045	0.637
35	2163	79	2158	75	2192	88	2204	61	0.747	0.154	0.874

[1] Model-established p-values for fixed effects of moment of first nutrition (water and feed), transport, and their interaction.

Table 3: Average daily gain, average daily feed intake and gain to feed ratio of chickens that received delayed nutrition (54 h) or immediate nutrition after hatch.

	Age (d)	Treatment						Fixed effects[1]		
		Delayed feeding			Early feeding			Age	Feeding	Age * Feeding
		n	Mean	SD	n	Mean	SD			
Average daily gain								<.0001	0.041	0.091
(g/d)	0 - 3	18	12.6 [a, x]	0.7	18	13.9 [a, y]	1.2			
	3 - 7	18	25.0 [b, x]	0.9	18	26.4 [b, y]	1.2			
	7 - 14	18	44.3 [c]	2.3	18	44.8 [c]	1.4			
	14 - 28	18	79.3 [d]	3.7	18	81.3 [d]	4.4			
	28 - 35	18	79.8 [d]	5.4	18	78.6 [d]	6.2			
	0 - 35	18	60.5	2.1	18	61.4	2.1		0.524	
Average daily feed intake								<.0001	0.044	0.269
(g/d)	0 - 3	17	13.5 [a]	1.5	18	15.3 [a]	2.1			
	3 - 7	18	34.8 [b]	4.6	18	34.5 [b]	1.9			
	7 - 14	18	51.9 [c]	1.9	18	53.6 [c]	1.4			
	14 - 28	18	122.0 [d]	3.6	18	124.7 [d]	3.9			
	28 - 35	18	159.0 [e]	7.7	18	160.6 [e]	7.3			
	0 - 35	18	96.1	3.1	18	98.0	2.7		0.069	
Gain to feed ratio								<.0001	0.686	0.136
	0 - 3	17	0.95 [a]	0.07	18	0.93 [a]	0.06			
	3 - 7	18	0.74 [b]	0.05	18	0.77 [b]	0.05			

7 - 14	18	0.85^{c}	0.02	18	0.84^{c}	0.02	
14 - 28	18	0.65^{d}	0.02	18	0.65^{d}	0.02	
28 - 35	18	0.50^{e}	0.02	18	0.49^{e}	0.03	
0 - 35	18	0.63	0.01	18	0.63	0.01	0.337

[1] Model-established p-values for fixed effects of moment of first nutrition (water and feed), age, and their interaction. Superscripts within columns (a, b, c, d, e) indicate differences between age-intervals. Superscripts within rows (x, y) indicate differences between treatment groups within age interval.

Table 4: Average daily gain, average daily feed intake and gain to feed ratio of chickens that were not transported after hatch and chicks that were transported after hatch.

	Age (d)	Treatment						Fixed effects		
		No transport			Transport			Age	Transport	Age * Transport
		n	Mean	SD	n	Mean	SD			
Average daily gain								<.0001	0.402	0.501
(g/d)	0 - 3	18	13.1 [a]	1.3	18	13.4 [a]	1.1			
	3 - 7	18	25.6 [b]	1.4	18	25.8 [b]	1.1			
	7 - 14	18	44.0 [c]	1.6	18	45.2 [c]	2.0			
	14 - 28	18	80.6 [d]	4.8	18	79.9 [d]	3.3			
	28 - 35	18	79.0 [d]	6.7	18	79.4 [e]	4.9			
	0 - 35	18	60.9	2.3	18	61.0	2.0		0.877	
Average daily feed intake								<.0001	0.856	0.679
(g/d)	0 - 3	17	14.2 [a]	1.5	18	15.0 [a]	2.4			
	3 - 7	18	35.1 [b]	4.4	18	34.3 [b]	2.3			
	7 - 14	18	52.4 [c]	1.8	18	53.1 [c]	1.9			
	14 - 28	18	123.4 [d]	4.3	18	123.3 [d]	3.6			
	28 - 35	18	159.6 [e]	7.0	18	160.2 [e]	8.1			
	0 - 35	18	97.0	3.0	18	97.2	3.1		0.845	
Gain to feed ratio								<.0001	0.502	0.136
	0 - 3	17	0.93 [a]	0.08	18	0.96 [a]	0.05			

3 - 7	18	0.75 [b]	0.05	18	0.76 [b]	0.05	
7 - 14	18	0.84 [c]	0.01	18	0.85 [c]	0.02	
14 - 28	18	0.65 [d]	0.02	18	0.65 [d]	0.02	
28 - 35	18	0.49 [e]	0.03	18	0.50 [e]	0.02	
0 - 35	18	0.63	0.010	18	0.63	0.008	0.982

[1] Model-established p-values for fixed effects of transport, age, and their interaction. Superscripts within columns (a, b, c, d, e) indicate differences between age-intervals. No differences between transport groups were observed.

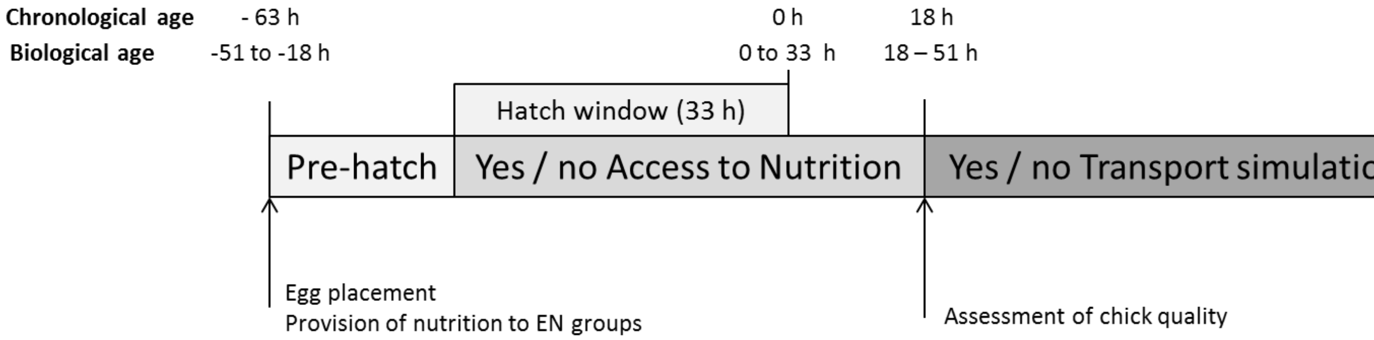

Figure 1: Experimental procedures and start of treatments (DN = delayed nutrition; EN = early nutrition) in time. Chicks were pulled at 63 h post placement, resulting in a biological age (defined by Careghi et al. 2005) of 0 – 33 h at pulling (chronological age = 0 h). Treatments were applied from 3 h chronological age (corresponding with 3 – 36 h biological age).

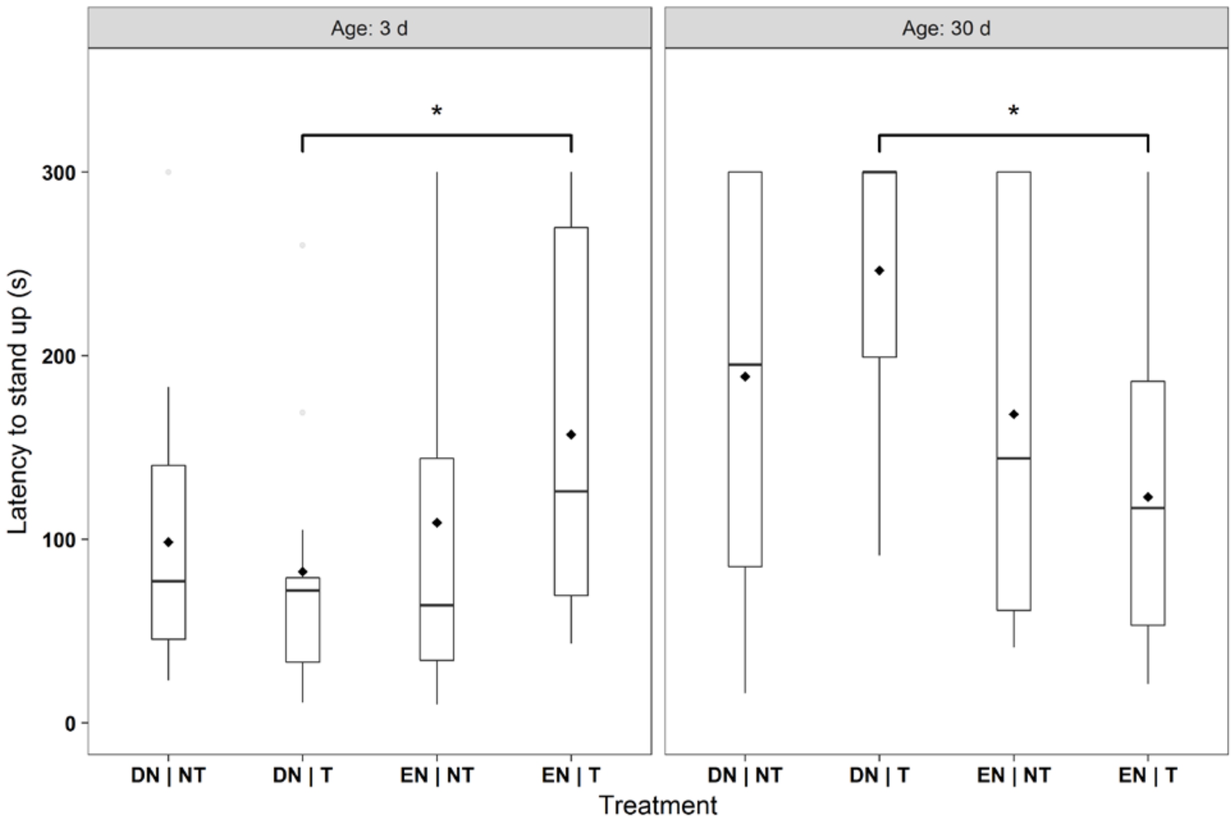

Figure 2: Latency to stand up in seconds after induced tonic immobility in the 4 treatment groups (DN|NT; DN|T; EN|NT and EN|T) at 2 ages (3 and 30 d). (DN = delayed nutrition; EN = early nutrition; NT = no transport; T = transport). Asterisks represent significant ($P \leq 0.05$) differences between treatments and diamonds represent means.

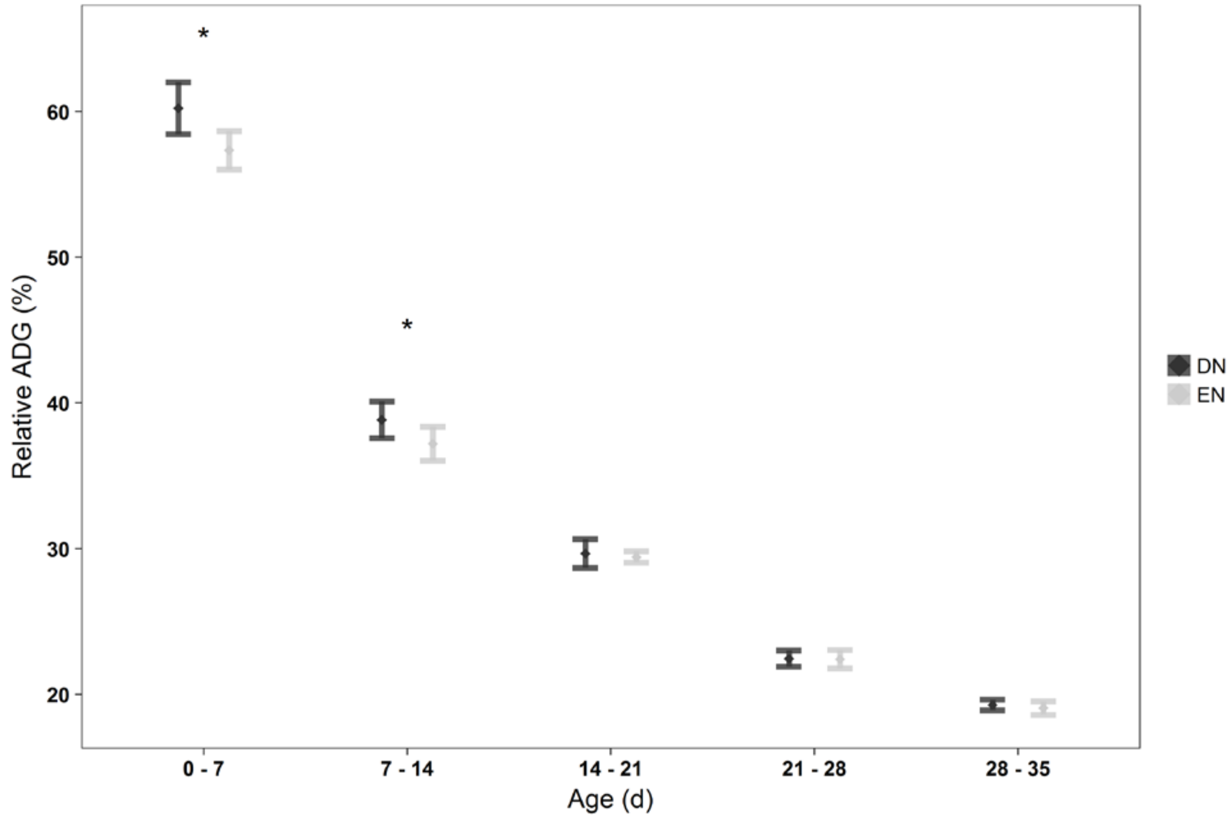

Figure 3: Relative average daily gain of chicks that received delayed nutrition (DN) or immediate nutrition (EN) after hatch. Asterisks represent significant ($P < 0.001$) differences between treatments and error bars represent standard deviation.

SUPPLEMENTARY MATERIAL

Table 5: Egg weight, hatchability, and chick quality (chick length, cloaca temperature, and navel quality) of chicks that received delayed nutrition (54 h) or immediate nutrition after hatch and prior to transport.

	Egg weight (g) [1]			Hatchability (%) [1]			Chick length (cm)			Navel quality (%)[2]			Cloaca temperature (° C)		
	n	mean	SD	n	mean	SD	n	mean	SD	Score 1	Score 2	Score 3	n	mean	SD
Feed access															
Delayed	18	56.7	1.9	18	98.1	2.3	206	20.1	0.5	62.1	33.0	4.9	49	38.7^{b}	0.1
Early	18	56.1	1.4	18	96.9	2.3	206	20.2	0.5	65.0	30.6	4.4	48	39.4^{a}	0.1
P-value		.661			.128			.085		.539	.597	.814		< .001	

[1] Analyzed at the pen level.

[2]Expressed as percentage of chicks within each score. Navel quality was assessed and each chick was scored from 1-3 (Maatjens et al., 2016).