



Effects of early nutrition and transport of 1-day-old chickens on production performance and fear response

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This is a "Post-Print" accepted manuscript, which has been published in "Poultry Science"

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Please cite this publication as follows:

Hollemans, M. S., de Vries, S., Lammers, A., & Clouard, C. M. (2018). Effects of early nutrition and transport of 1-day-old chickens on production performance and fear response. *Poultry Science*, [pey106]. DOI: 10.3382/ps/pey106

You can download the published version at:

<https://doi.org/10.3382/ps/pey106>

1           **EFFECTS OF POST HATCH CONDITIONS ON BROILER CHICKENS**

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**METABOLISM AND NUTRITION**

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**Effects of Early Nutrition and Transport of One-Day-Old Chickens**

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**on Production Performance and Fear Response**

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21 **ABSTRACT**

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

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

45

## INTRODUCTION

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

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

63 Various studies suggest that one-day-old chick transport may have negative effects on  
64 production performance and the chickens' ability to cope with stress, depending on transport  
65 duration (Valros et al., 2008; Mitchell, 2009; Bergoug et al., 2013; Jacobs et al., 2016). A  
66 drawback from these studies is that the effects of moment of first nutrition and transport are  
67 confounded, as the chicks that were subjected to a longer transport duration also did not have  
68 access to nutrition. It is therefore not clear whether the observed effects were caused by

69 transport or delayed access to nutrition. Furthermore, to our best knowledge, interactions  
70 between access to nutrition and transport have not been studied so far.

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

## 79 MATERIALS AND METHODS

### 80 *Experimental Design*

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

### 86 *Housing and Diets*

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

93 Services (Wijk bij Duurstede, The Netherlands). Floor temperature was 34 °C and ambient  
94 temperature was controlled at 36 °C. Average humidity ( $27.4 \pm 2.6$  %) and CO<sub>2</sub> ( $1100 \pm 156$   
95 ppm) levels were logged from placement until hatch. As a result of minimal ventilation, air  
96 speed was negligible. Embryonic temperature of 3 eggs per treatment was monitored indirectly  
97 by egg shell temperature (**EST**) and recorded every 5 min until hatch. EST sensors (NTC  
98 Thermistors: type DC 95, Thermometrics, Somerset, UK) were attached to the egg following  
99 procedures of Maatjens et al. (2016b). EST was maintained between 35.3 and 36.7 °C by  
100 manually adjusting floor heating and ventilation before and during hatch, based on  
101 recommendations of Maatjens et al. (2016a; b).

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

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

### 110 ***Animals and Treatments***

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

117 During their stay in the hatching baskets, water and feed were provided *ad libitum* to the EN  
118 groups, while DN groups did not receive any form of nutrition. To simulate post hatch holding  
119 and transport, all T groups were moved to an unconditioned room (20 °C, no air circulation,  
120 continuous lighting) and kept for 1.5 h in their original hatching baskets. Subsequently, the  
121 baskets with chicks were placed in a climate controlled chick transport van (33 °C; dark) and  
122 transported for 1 h. After transport, baskets were moved to their original pens and, after 0.5 h,  
123 all baskets were emptied allowing all chicks *ad libitum* access to water and feed. Thus, the  
124 period of handling and transport simulation was 3 h. NT groups remained in their hatching  
125 baskets within the barn according to conditions described in “housing and diets” and were  
126 placed in the pens simultaneously to the T groups. The experiment was performed according to  
127 the Guide For the Care and Use of Agricultural Animals in Agricultural Research and Teaching  
128 (2010).

## 129 ***Measurements***

### 130 ***Eggs and Chick Quality***

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

140 **Performance**

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

143 
$$Relative\ ADG = \frac{\left(\frac{BW_{end}}{BW_{start}} * 100\right)}{7}$$

144 Average daily feed intake (**ADFI**) and feed efficiency (**G:F**) were determined per pen at 3, 7,  
145 14, 28 and 35 d post placement.

146 **Tonic Immobility**

147 Tonic immobility tests were performed at 3 and 30 d post placement on 2 chickens per pen from  
148 7 randomly chosen pens per treatment. Different chickens were selected for the measurements  
149 at 3 and 30 d, to prevent habituation to the procedure (Jones, 1986). Results were averaged for  
150 each pen, resulting in 7 observations per pen. The procedure was adapted from Valros et al.  
151 (2008) with minor modifications. Briefly, one chicken was taken from the home pen and  
152 transferred in a bin to a quiet testing room, to ensure isolation from the flock. There, the chicken  
153 was restrained on the back for 10 s, using one hand to hold the chest and one to cover the neck  
154 and head. All tests were performed by the same experimenter and observer, who did not made  
155 direct eye contact with the chicken during both handling and testing. Experimental conditions  
156 were similar at both 3 and 30 d of age (*i.e.* same procedure of handling and transport to the test  
157 room (Jones and Waddington, 1992)). If the chicken stood up within 10 s after the end of  
158 restraining, the restraint was carried out again up to a maximum of 5 times. After 5 attempts,  
159 the test was stopped and the chicken was placed back in the home pen and recorded as missing  
160 value. The chicken was judged immobile when it stayed down for at least 10 s after removal of  
161 the hands. The latency (s) from immobility until standing was recorded. If the latency of  
162 immobility was  $\geq 300$  s, the test was stopped and the maximum latency of 300 s was noted.



163 ***Statistical Analyses***

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

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

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

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

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

187

## RESULTS

### 188 *Egg and Hatching Parameters*

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

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

### 200 *Performance*

201 No interactions between moment of access to nutrition and transport were found on  
202 performance. BW was significantly greater (46 g) for EN chicks until at least 28 d ( $F_{1, 32} = 4.38$ ,  
203  $P = 0.045$ ) compared with the DN chicks (**Table 2**). At slaughter (35 d), there was no significant  
204 difference between EN and DN chicks ( $F_{1, 32} = 2.13$ ,  $P = 0.152$ ). In **Table 3**, it is shown that  
205 moment of feeding affected ADG and ADFI, with a significant greater ADG at 0 – 3 and 3 – 7  
206 d (1.3 and 1.4 g/d, respectively) in EN chicks than in DN chicks. Furthermore, relative ADG  
207 was significantly ( $F_{1, 170} = 4.38$ ,  $P < 0.001$ ) higher in DN chicks compared with EN chicks, from  
208 0 until 14 d of age (**Figure 3**). G:F ratio was not affected by treatment. No effects of transport  
209 were found on BW (**Table 2**) or ADG, ADFI and G:F (**Table 4**).

## 210 *Tonic Immobility*

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

## 217 **DISCUSSION**

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

## 230 *Chick Quality and Progress of Grow-out Period*

231 Our results indicate that chick quality was identical in the different treatment groups. The  
232 increased cloacal temperature in EN chicks compared with DN chicks, is presumably due to  
233 heat generated by metabolism (Van den Brand et al., 2010). This increase in body temperature

234 in day-old chicks can be favorable, as these chicks might be less susceptible to temperature  
235 changes during transport and brooding.

236 ***Moment of First Nutrition \* Transport***

237 At 3 d of age, latency to stand up after tonic immobility was higher in EN|T chicks than in DN|T  
238 chicks. Although latency to stand up after tonic immobility is known to be a valid measure of  
239 fear levels in chickens (Jones and Mills, 1983; Forkman et al., 2007), no consensus has been  
240 reached concerning the validity of the TI test in very young chickens (Ratner and Thompson,  
241 1960; Salzen, 1963; Forkman et al., 2007). We, however, observed typical signs of immobility,  
242 such as no movement, and extended legs with tremor (Jones, 1986; Heiblum et al., 1998) at 3  
243 d of age. This seems to support the validity of the TI test to assess fear levels in very young  
244 chicks, too. The higher latency to stand up after tonic immobility in 3-day-old EN|T chicks  
245 compared with DN|T chicks may therefore indicate that EN|T chicks were more fearful than  
246 DN|T chicks in early life.

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

256 Unlike at 3 d of age, latency to stand up was shorter in the EN|T chicks compared with DN|T  
257 chicks at 30 d post placement, suggesting that EN|T chicks were less fearful than DN|T later in

258 life. Although it remains unclear why the impact of early nutrition in transported chicks was  
259 reversed from 3 d to 30 d, our results seem to indicate that early nutrition provided long-term  
260 advantages for the chicken's ability to cope with stress later in life.

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

### 272 *Moment of First Nutrition*

273 The lower BW of DN chicks until 28 d of age is consistent with previous research (Juul-Madsen  
274 et al., 2004; Van De Ven et al., 2011; Lamot et al., 2014), and might be explained by impaired  
275 organ and body development and dehydration during feed and water deprivation (Uni et al.,  
276 2003a; b; Smirnov et al., 2004; Lamot et al., 2014; Lilburn and Loeffler, 2015). The significant  
277 higher relative ADG in EN chicks compared with DN chicks from 0 to 14 d of age (**Figure 3**),  
278 might indicate compensatory growth of DN chicks (Zubair and Leeson, 1996).

### 279 *Transport*

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

282 (2013) transported broiler chicks from the hatchery under controlled climate conditions (0, 4,  
283 and 10 h transportation time) to an experimental facility and found that NT chicks had increased  
284 BW compared with T chicks until 21 d post hatch ADFI or G:F were not affected. Valros et al.  
285 (2008) found negative effects on fear-related behavior (e.g. latency to perch after transport, and  
286 latency to stand up after tonic immobility at 34 d post hatch) with increasing transport duration  
287 (4 and 10 h), but not on body weight. As no non-transported control was included in this study,  
288 effects of transport relative to no transport are unknown. As none of the above mentioned  
289 studies accounted for moment of access to nutrition after transport, the long-transported chicks  
290 were also deprived longer from nutrition than short-transported chicks. Therefore, the effects  
291 of transport reported in these studies could actually reflect the effect of DN instead of that of  
292 transport. This is in line for performance of the DN groups in the current study. We suggest that  
293 climate controlled transport of one-day-old chickens does not affects performance, as long as  
294 nutrition is provided. This is probably due to the fulfillment of the chicken's needs. Further  
295 investigation is required to explain why transport on itself does not result in differences in  
296 production performance.

297

## **ACKNOWLEDGEMENTS**

298 The authors are grateful to Conny Maatjens, Carla van der Pol, and Inge van Roover-Reijrink  
299 (HatchTech B.V., Veenendaal, The Netherlands) for critical review of the experimental setup  
300 and technical assistance during the experiment. Furthermore, Lotte Schakel is kindly  
301 acknowledged for her help during the experiment and data processing within her MSc thesis  
302 project. The accurate animal caretaking by Carin and Theo Nooijen is greatly appreciated.

303

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409

410

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

	Starter	Grower	Finisher
<b>Ingredients</b>			
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 <sup>1</sup>	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 <sup>2</sup>	0.02	0.02	0.02
Anti-coccidiostat <sup>3</sup>	0.06	0.06	-
Sodium butyrate coated	0.10	0.08	0.05
<b>Calculated nutrient composition<sup>4</sup></b>			
Moisture	11.7	11.9	11.8
Crude protein	22.5	20.0	19.5
Digestible lysine <sup>5</sup>	12.0	11.0	10.3
Digestible methionine + cysteine <sup>5</sup>	8.9	7.9	7.5
Digestible threonine <sup>5</sup>	8.0	7.2	6.9
Crude fat <sup>6</sup>	7.3	7.9	8.6
Crude fiber	2.5	2.6	2.6
Ash	5.8	4.9	4.8
Starch <sup>7</sup>	36	38.4	38.1
DE (kcal) <sup>5</sup>	3,000	3,040	3,080
Calcium	9.0	7.0	6.5
Available phosphorus	4.1	3.2	3.0

413

414 <sup>1</sup> Containing Vitamin A (2,500,000 IU); D3 (600,000 IU); E (3,350 IU); K3 (600 mg); B1 (600  
 415 mg); B2 (1,500 mg); B6 (800 mg) ; B12 (6,000 mg); niacin (9,000 mg); panthothenic acid

416 (2,000 mg); biotin (100,000 mg); choline chloride (100,000 mg); Mn (17,000 mg); Zn (18,000  
417 mg); Cu (3,000 mg); Fe (16,000 mg); I (400 mg); Se (50 mg).

418 <sup>2</sup> Commercial bacterial endo-1,3- $\beta$ -xylanase (Belfeed, Agrimex N.V., Lille, Belgium).

419 <sup>3</sup> Starter diet: Mixture of 45 mg narasin and 45 mg nicarbazin /kg feed (Maxiban, Elanco,  
420 Greenfield, USA); Grower diet: Salinomycin (72 mg/kg feed) (Sacox, Huvepharma, St. Louis,  
421 USA).

422 <sup>4</sup> Calculated based on feed table of Schothorst Feed Research (2015) and specified in g/kg  
423 unless specified otherwise.

424 <sup>5</sup> Apparent total tract digestibility.

425 <sup>6</sup> Ether extract with acid hydrolysis (ISO 6492).

426 <sup>7</sup> Amyloglucosidase method (ISO 15914)

427 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 =  
 428 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

429

430 <sup>1</sup> Model-established p-values for fixed effects of moment of first nutrition (water and feed), transport, and their interaction.

431

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

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

7 - 14	18	0.85 <sup>c</sup>	0.02	18	0.84 <sup>c</sup>	0.02	
14 - 28	18	0.65 <sup>d</sup>	0.02	18	0.65 <sup>d</sup>	0.02	
28 - 35	18	0.50 <sup>e</sup>	0.02	18	0.49 <sup>e</sup>	0.03	
0 - 35	18	0.63	0.01	18	0.63	0.01	0.337

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434

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



438 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  
 439 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 (g/d)										
	0 - 3	18	13.1 <sup>a</sup>	1.3	18	13.4 <sup>a</sup>	1.1	<.0001	0.402	0.501
	3 - 7	18	25.6 <sup>b</sup>	1.4	18	25.8 <sup>b</sup>	1.1			
	7 - 14	18	44.0 <sup>c</sup>	1.6	18	45.2 <sup>c</sup>	2.0			
	14 - 28	18	80.6 <sup>d</sup>	4.8	18	79.9 <sup>d</sup>	3.3			
	28 - 35	18	79.0 <sup>d</sup>	6.7	18	79.4 <sup>e</sup>	4.9			
	0 - 35	18	60.9	2.3	18	61.0	2.0			
Average daily feed intake (g/d)										
	0 - 3	17	14.2 <sup>a</sup>	1.5	18	15.0 <sup>a</sup>	2.4	<.0001	0.856	0.679
	3 - 7	18	35.1 <sup>b</sup>	4.4	18	34.3 <sup>b</sup>	2.3			
	7 - 14	18	52.4 <sup>c</sup>	1.8	18	53.1 <sup>c</sup>	1.9			
	14 - 28	18	123.4 <sup>d</sup>	4.3	18	123.3 <sup>d</sup>	3.6			
	28 - 35	18	159.6 <sup>e</sup>	7.0	18	160.2 <sup>e</sup>	8.1			
	0 - 35	18	97.0	3.0	18	97.2	3.1			
Gain to feed ratio										
	0 - 3	17	0.93 <sup>a</sup>	0.08	18	0.96 <sup>a</sup>	0.05	<.0001	0.502	0.136

3 - 7	18	0.75 <sup>b</sup>	0.05	18	0.76 <sup>b</sup>	0.05
7 - 14	18	0.84 <sup>c</sup>	0.01	18	0.85 <sup>c</sup>	0.02
14 - 28	18	0.65 <sup>d</sup>	0.02	18	0.65 <sup>d</sup>	0.02
28 - 35	18	0.49 <sup>e</sup>	0.03	18	0.50 <sup>e</sup>	0.02
0 - 35	18	0.63	0.010	18	0.63	0.008

0.982

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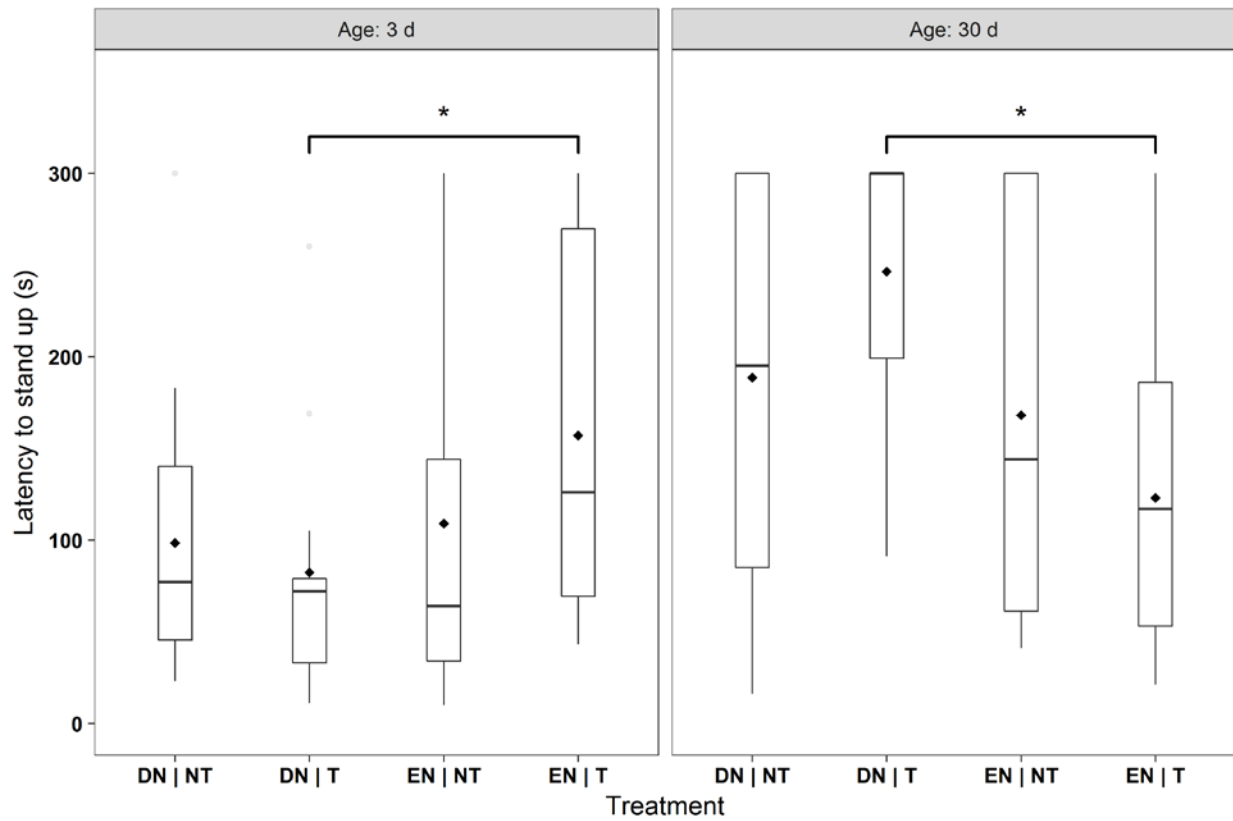
<sup>1</sup> Model-established p-values for fixed effects of transport, age, and their interaction. Superscripts within columns (a, b, c, d, e) indicate differences between age-intervals. No differences between transport groups were

442

observed.

443





451

452 Figure 2: Latency to stand up in seconds after induced tonic immobility in the 4 treatment

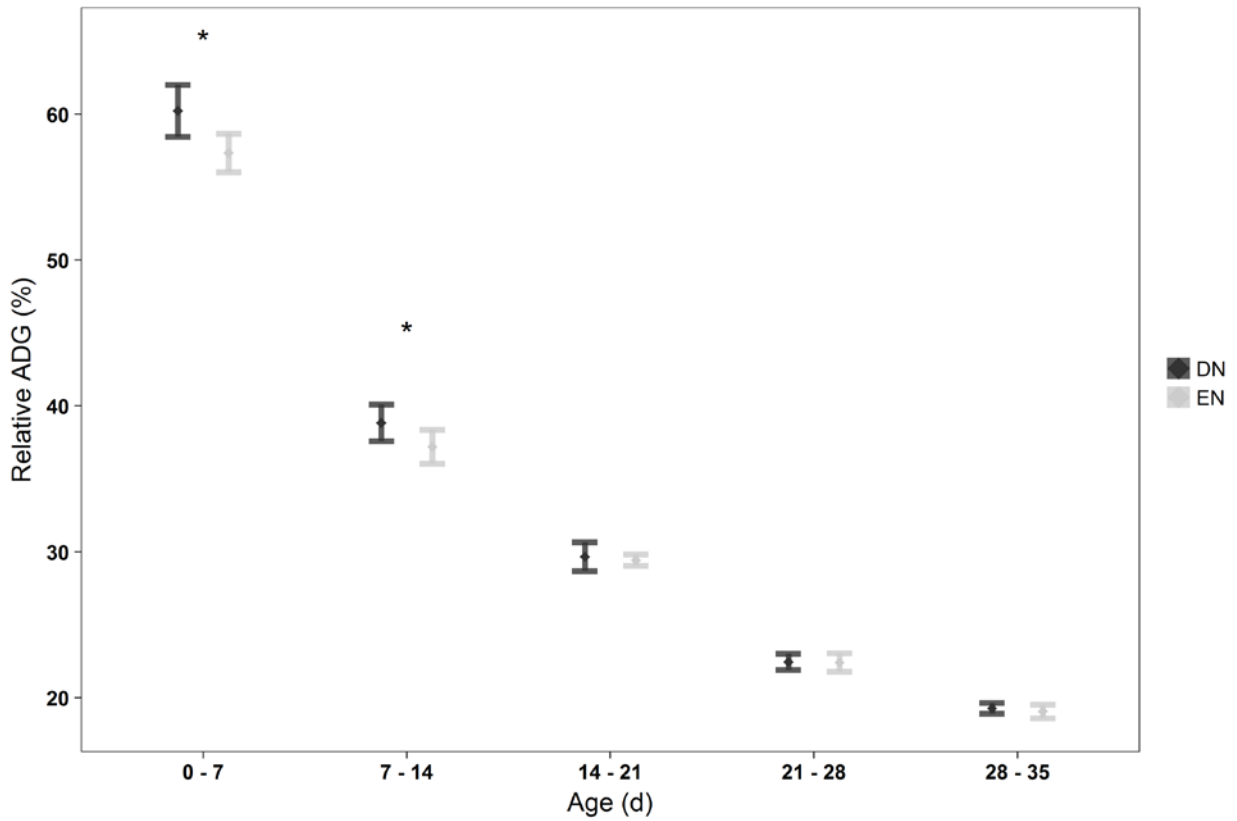
453 groups (DN|NT; DN|T; EN|NT and EN|T) at 2 ages (3 and 30 d). (DN = delayed nutrition; EN

454 = early nutrition; NT = no transport; T = transport). Asterisks represent significant ( $P \leq 0.05$ )

455 differences between treatments and diamonds represent means.

456

457



458

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

462

**SUPPLEMENTARY MATERIAL**

463

464

465 Table 5: Egg weight, hatchability, and chick quality (chick length, cloaca temperature, and navel quality) of chicks that received delayed nutrition  
 466 (54 h) or immediate nutrition after hatch and prior to transport.

	Egg weight (g) <sup>1</sup>			Hatchability (%) <sup>1</sup>			Chick length (cm)			Navel quality (%) <sup>2</sup>			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 <sup>b</sup>	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 <sup>a</sup>	0.1
P-value	.661			.128			.085			.539	.597	.814	< .001		

467

468 <sup>1</sup> Analyzed at the pen level.

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

470