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Article

Effects and Interactions of Incubation Time and Preplacement Holding Time on Mortality at Placement, Yolk Sac Utilization, Early Feeding Behavior and Broiler Live Performance

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Abstract: Effects and interactions of incubation time and chick preplacement holding time on mortality at placement, utilization of yolk sac, drive to eat and drink at placement and broiler live performance were investigated. Ross 308 broiler hatching eggs from a 39-week-old flock were set in two identical setters in a commercial hatchery,with setting time 12 hours earlier in one machine. At the end of incubation, chicks were removed from the hatchers at the same time. Thus, incubation times were either 504 h (normal incubation time (**NIT**) treatment) or 516 h (longer incubation time (**LIT**) treatment). After pull time, chicks from each incubation time group were subjected to either 6, 24, 48, 60 or 72 h preplacement holding times. At placement, chicks were given access to feed and water. In total, 19,200 chicks were randomly assigned to a total of 10 subtreatment groups (2 incubation times × 5 preplacement holding times). Therefore, a total of 1,920 chicks were used in each subtreatment group for the grow-out period in a commercial broiler house. An interaction was found between incubation time and preplacement holding time for residual yolk sac (**RYS**) weight (g, %) ($p < 0.001$). RYS weight was greater at pull time and at 6 and 24 h of preplacement holding time in the NIT treatment compared to the LIT treatment, while differences were no longer evident at 48–72 h. Chicks held for 6 hours before placement had fewer chicks with full crops and less eating activity 3 hours after placement than any of the other holding times. As expected, the initial BW at placement clearly decreased with increasing duration after the pull time ($p < 0.05$). Chicks held for 6 hours were the heaviest, and for 72 hours the lightest. Some of this BW trend was still evident at 35 d after placement with the lowest broiler weight recorded in the birds which had been held for 72 h after placement ($p < 0.001$). However, the growth of chicks held for 60 h caught up with those held for 6 hours by 35 d, and there was no signiifficant difference between them in BW. Mortality for the first 7 days after placement was increased only in the chicks held for 72 hours ($p = 0.031$). Grown to broiler weights (35 d post placement), chicks held for 72 h exhibited higher 0-35 day mortality compared to those held for 6 or 24 h ($p = 0.028$). However, neither BW nor mortality were affected by incubation time treatment ($p > 0.05$). In conclusion, there were no significant differences in average BW and mortality, where chicks were held in comfortable conditions for up to 60 h, but a 72 h preplacement holding time resulted in 35 d BW and mortality being negatively affected. In addition, LIT tended to have a beneficial effect on BW and mortality compared to NIT when the preplacement holding time was shorter (6–24 h) but had a negative effect for extended holding times (48–72 h).

Keywords: incubation time; preplacement holding time; residual yolk sac; mortality; body weight

1. Introduction

Under commercial conditions, individual chicks usually emerge from the egg shell over a 24 h to 36 h period, with the entire batch removed from the hatcher after 504 h [1]. Incubation time is known to be mainly influenced by strain, parental age, egg size, storage time and eggshell temperature before and during incubation [2–7]. Early collection of the chicks from hatcher tends to increase the

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percentage of second quality chicks with unhealed navels, while delaying the chick collection lead to a higher percentage of dehydrated chicks. After removal from the hatcher, access to feed and water will usually be delayed while chicks are selected, sexed, vaccinated and packed to be transported to the farm. The interval between take off and arrival on farm is usually 6 hours or less for broiler chicks. For breeding stock, often produced some distance from the farming base to maximize biosecurity and health status the holding time is usually longer and can be up to 60 hours [8]. Nevertheless, the energy content of fat in the residual yolk of the newly hatched chick is enough for the chicks needs for about 72 h under reasonable environmental conditions [9].

It has been reported that longer holding periods after hatching either in the hatcher or after pull time prior to placement in the broiler house, adversely affected early chick quality [10] increased early mortality [11–13] and reduced growth [3,13–16]. Several other studies, where chicks were similarly held in good conditions for a day or more post hatch, recorded no evidence of clinical dehydration of the chicks nor any effect on live broiler performance [1,17–22].

However, in all the above studies, pen sizes were small with relatively few replicates. As a result, mortality differences, in particular were unlikely to be statistically significant. On the other hand, in a recent study [23], a high number of chicks (19,200 chicks) with larger pens (160 chicks/pen) were used in a commercial broiler house. The study showed that for chicks held in comfortable conditions, holding times of up to 60 h had no effect on mortality, but a 72 h hold was associated with higher mortality to 7 days post placement. Although the increase was numerically small, it was statistically significant ($p = 0.02$).

It has been proposed in the EFSA (European Food Safety Authority) Panel on Animal Health and Welfare (AHAW) [24] that in order to improve bird welfare, the maximum time between hatching and first access to feed and water (including time spent in the hatchery, holding time, loading, transport and unloading time) should not exceed 48 h. However, the panel accepts that more research is needed to assess the maximum time before access to feed and water becomes detrimental for day-old chicks.

In the current study, following on from Özlü et al. [23], a large number of chicks (19,200) were placed and grown on to broiler weights to explore the effect of incubation time and its interaction with holding times before placement of between 6 and 72 h on yolk sac utilisation, the drive to eat and drink and mortality and growth to 7 and 35 d post placement.

2. Materials and Methods

All procedures in the current study were approved by the Animal Ethics Committee of Poultry Research Institute, Ankara.

2.1. Hatching Eggs and Incubation

Hatching eggs were obtained from a commercial broiler breeder flock of Ross 308 at 39 wk of age and stored for 3 d at 16 °C and 75% relative humidity (**RH**). After storage, a total of 38,400 hatching eggs were set equally and randomly in two identical setters (Petersime, Zulte, Belgium) in a commercial hatchery (Beypiliç Inc., Bolu, Türkiye). Half of the total eggs (19,200 eggs) were set 12 h earlier than the other half (19,200 eggs). Total setter capacity was 57,600 eggs, and the remaining space was used to incubate filler eggs, sourced from two other flocks of similar age and hatchability, that were not otherwise involved in the experiment. This was done to ensure that the incubation conditions were consistent with normal practice for the hatchery.

Eggs from both setters were prewarmed at 28 °C for 6 h before incubation. The setters were programmed to start incubation (E1) at 38.1 °C, falling to 37.0 °C at E19. The machines were minimally ventilated to E10, allowing RH to rise over 70%, after which they were ventilated and RH allowed to fall to 40%. Egg trays were turned hourly through 90°. On E19, the eggs were moved to hatcher baskets, and placed in hatchers, starting at 37.2 °C and gradually dropping to 36.4 °C by E21.

The experiment was designed to test the impact of incubation time on the livability and performance of chicks after various holding times between pull and placement on the farm. To ensure

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that chick holding and brooding conditions were identical, the eggs for the LIT treatment (516 h) were set in a separate setter 12 hours earlier than those for the NIT (504 h) treatment. Both groups spent 456 h in the setter, then on day 19 were transferred to two separate hatchers, where the LIT treatment eggs spent 12 hours longer than the NIT. The last 12 hours in the hatcher for the LIT treatment was programmed to deliver 36.4±0.4 °C and 53±2% RH, to achieve a chick vent temperature of 39.4–40.5 °C.

2.2. Chick Management and Experimental Design

During pull time, chicks were visually sorted according to commercial hatchery standards: weak chicks or those with physical abnormalities, unhealed navels, or red hocks considered unsaleable were culled. In total, 88.0% of the chicks hatched in the NIT treatment, and 88.8% of the chicks hatched in the LIT treatment. The percentage of second-grade chicks was 0.67% and 0.47% in the NIT and LIT treatments, respectively.

First quality chicks were counted, then spray vaccinated against Infectious Bronchitis and Newcastle disease. The boxes of chicks (80 chicks per box) were packed into an unlighted climate-controlled truck (H90, Heering, Vaassen, Holland). It took 2 hours to reach the broiler unit. Once at the unit, chicks were held in the truck which was maintained at 25.7±0.3 °C until they were placed in the broiler house.

For each incubation time treatment, boxes of chicks were randomly allocated into one of 5 different holding treatments, allowing holds of 6, 24, 48, 60 or 72 hours after the chicks were removed from the hatcher. Over the ten treatments (2 incubation times × 5 holding times) a total of 19,200 chicks were placed giving a total of 1,920 chicks in each subtreatment group for the grow-out period. For the first week of the experiment, 160 randomly selected as-hatched (not sexed) chicks belonging to one of the 10 subtreatment groups were placed in each of 12 replicate floor pens (120 total pens). From the second week of age onwards, chicks from two pens were combined into 6 replicate pens (60 total pens) each with 320 chicks per replicate.

2.3. Grow-out Housing and Management

The birds were grown from placement to 35 days in a commercial broiler house, which was preheated for 24 h before the first chicks were placed to deliver a uniform and steady litter temperature of 30 °C. From placement, the ambient temperature was gradually decreased from approximately 32 °C to 20 °C at the end of the experimental period (d 35). Chicks received 23 h of light (23L:1D) during the first 10 d. From Day 11 until the end of the study, the chicks were given 4 h of darkness between 23:00 h and 03:00 h.

For the first 7 days, 160 chicks were placed in each 1.50 x 2.75 m floor pen. The pens were spread with fresh wood shavings as litter material. From the second week of age onward, chicks from two pens were moved into one large pen in each subtreatment group, which measured 1.5 m × 12 m (18 m^2) and kept under uniform management conditions throughout the study. The initial chick density in each of the pens was approximately 0.026 m^2 per bird. From the second week onward, the chick density in each of the pens was 0.056 m^2 per bird. Water was provided via two or four nipple lines with 18 or 36 nipples per pen during the first and second week of age onward respectively throughout the remainder of the study. Feed was accessed from two (used for the first 7 d) or eight (used from 8–35 d) 33-cm diameter pan feeders running along the midline of each pen. For 4 days after placement, in line with commercial practice, feed was also placed on paper on all the pen floors.

The birds were fed starter (0-10 d), grower (11-24 d) and finisher (25-35 d) diets formulated and manufactured according to the recommendations of the breeding company [25]

2.4. Measurements

All on-farm measurements were performed a defined number of days after chicks were placed into the pens with free access to feed and water. The time elapsed since the chicks were removed from the hatcher thus varied by up to 3 d.

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2.4.1. Vent Temperature

Chick body temperature was determined by recording the vent temperature of 100 randomly selected chicks, sampling ten chicks from each box for each treatment. The measurements were taken using a Braun Thermoscan digital thermometer (IRT 4520, Braun GmbH, Kronburg, Germany) at pull time and at chick placement.

2.4.2. Mortality at Placement Time (**DOA**)

All chick boxes were opened, and dead chicks (dead on arrival, DOA) were recorded to determine the percentage of mortality relative to the total chicks at each placement time.

2.4.3. Residual Yolk Sac (**RYS**) Weight and Yolk-Free Body Mass (**YFBM**)

RYS and YFBM weights were measured at pull time and at the end of each of the 5 holding periods for both incubation time treatment groups. In addition, the RYS weight was also recorded to determine the yolk sac utilization of fasted and no-fasted chicks. Further information concerning the measurement of RYS and YFBM is provided by Özlü et al. [23].

2.4.4. Crop Filling and Feeding-Drinking Behavior

Crop filling was examined in a sample of chicks from each pen at 3 h after placement. Chick feeding and drinking behavior were determined at 1, 3 and 8 h after placement. The procedures for the crop filling and feeding-drinking behavior assessments were as previously described by Özlü et al. [23].

2.4.5. Live Broiler Performance (BW and Mortality)

Body weights (BWs) were recorded at placement and 7 d of age by bulk weighing in each pen. At 35 d from the day of placement, a random sample of 50 chickens (25 female and 25 male chickens) per pen was individually weighed in each group. Weighing times were organized so that each treatment had 35 full days of feed availability. Pens were checked and dead birds removed and recorded six times each day of the study.

2.5. Statistical Analysis

Five separate analyses consisting of Fisher's exact test (mortality at placement time), a one-way ANOVA (RYS weights between groups with differing access to feed and water [fasted/no-fasted] at each sampling time), and two or three factorial arrangement (2 × 5 or 2 × 5 × 2 factorial designs) were undertaken.

Effect of incubation time treatment (NIT of LIT), preplacement holding time (6, 24, 48, 60 or 72 h), gender, and access to feed and water (fasted or no-fasted) on collected data were analyzed using the General Linear Models procedure of SAS.

Residual yolk size (g or % of liveweight, YFBM, crop fill, the percentage of birds feeding or drinking at intervals after placement, BW and chick mortality were all analyzed appropriate for a 2 × 5 factorial arrangement of treatments. The two factors were incubation time treatment and preplacement holding time. Gender was included as a main factor for analysis of the BW at d 35, so the analysis was performed with a 2 × 5 × 2 factorial arrangement.

Additionally, data of RYS weights were analyzed appropriate for a 2 × 5 × 2 factorial arrangement of treatments. The three factors were incubation time, holding time, and whether or not the chicks had been able to eat and drink yet.

All data were expressed as Least Square Means and Duncan's multiple range tests was performed for the mean comparison.

All statistical analyses were performed with SAS version 9.1 (SAS Institute Inc., Cary, NC, USA).

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3. Results

3.1. Vent Temperature

Chick body temperature depends on surrounding environmental temperature and will affect the quality of day-old chicks and performance of chicks later in life [26,27]. For these reasons, cold and heat stress have been selected as the highly relevant welfare consequences for day-old chicks [24].

Chick body temperature is determined either with an infrared ear thermometer placed on the cloaca (vent) or by measuring deep body temperature by inserting a thermometer in the cloaca (rectal). Chick vent temperature is highly correlated with deep body (rectal) temperature [28], although it will normally measure 0.5 °C lower than true rectal temperature [29]. For a day-old chick to be comfortable, it is recommended that the vent temperature be in the range of 39.4–40.6 °C [8] or the rectal temperature be in the range of 40.0–41.0 °C [24].

In the present study, chick body temperature was easily and consistently determined by recording the vent temperature. No significant differences in vent temperature were found between the pull time and the preplacement holding times, and the average was maintained within the optimal range (39.7±0.65 °C), as shown in Table 1.

Table 1. Vent temperature of chicks.

Treatment	Vent Temperature[1]
	(°C)
Incubation time (h)	
NIT (504)	39.73
LIT (516)	39.69
SEM	0.029
Preplacement holding time (h)	
0	39.61
6	39.72
24	39.67
48	39.78
60	39.73
72	39.74
SEM	0.060
P values	
Incubation time	0.326
Preplacement holding time	0.078
Incubation time × Preplacement holding time	0.121

[1]n=100 for all sub treatments

3.2. Mortality at Placement Time (DOA)

Cumulative preplacement mortality (DOA) was higher after the 72 h hold (0.107%) than it was when chicks were held for shorter periods of 6 h (0.005%), 24 h (0.024%) and 48 h (0.032%) holding times ($p < 0.001$, $p = 0.014$, and $p = 0.021$, respectively). Furthermore, the cumulative mortality at 60 h (0.057%) was also significantly higher than that at 6 h ($p = 0.005$). However, this was due to higher mortality for the LIT (0.075%) rather than the NIT (0.039%) treatment (Table 2).

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Table 2. Cumulative mortality during preplacement holding time.

Incubation time (h)	Preplacement holding time (h)				
	0-6	0-24	0-48	0-60	0-72
	(%)				
NIT (504)	0.010 ^{c}	0.022 ^{bc}	0.039 ^{bc}	0.039 ^{bc}	0.089 ^{a}
LIT (516)	0.000 ^{c}	0.025 ^{bc}	0.025 ^{bc}	0.075 ^{a}	0.125 ^{a}
Combined	0.005 ^{c}	0.024 ^{bc}	0.032 ^{bc}	0.057 ^{ab}	0.107 ^{a}

$^{a-c}$Mortality percentages followed by different letters differ significantly ($p < 0.05$).

DOA is also a relevant indicator for the assessment of chick welfare during preplacement holding time [30,31]. However, very little is known in the literature about the specific DOA of day-old chicks. In a field study from Yerpes et al. [32], the mean chick mortality during transport was 0.055%. The AHAW Panel concluded that DOA should not exceed 0.10% [24]. Recently, Özlü et al. [23] conducted an experiment similar to the current study and reported that mortality at placement was not affected up to 60 h of holding time. In that study, when the chicks were held another 12 hours, to 72 h, chick mortality to placement was higher (0.244%) significantly above that at 6 h (0.020%) or 24 h (0.039%) ($p < 0.05$). Similarly, in the current study, the highest mortality was observed for the 72 h holding time group, although it was very low (0.107%). In addition, LIT increased mortality at placement when the preplacement holding time was extended (60–72 h) (Table 2). Therefore, to reduce mortality at placement, chicks held for longer preplacement holding times should not also be exposed to long incubation times.

3.3. Yolk-Free Body Mass (YFBM) and Residual Yolk Weight (RYS)

In the current study, at placement, the chicks in the LIT group, an incubation time of 516 h, had a lower YFBM and less residual yolk ($p < 0.001$) than those in the NIT treatment (504 h). In addition, the YFBM was affected by the preplacement holding time and decreased with increasing preplacement holding time. In the time elapsed between chicks being pulled at the hatchery and placed after hold times of 24, 48, 60 or 72 h, YFBM dropped by 2.7%, 4.7%, 6.1% and 10.9%, respectively (Table 3). This finding was consistent with Özlü et al. [23], who found that the percentage of YFBM decreased more between 60 and 72 h.

As expected, the residual yolk weight, whether expressed in g or as a percentage of chick weight, decreased with each increase in holding time in both incubation time treatments. However, an interaction was found between incubation time and preplacement holding time for RYS weight (g, %) ($p < 0.001$). RYS weight was greater at pull time and at the 6 and 24 h holding times in the NIT treatment than in the LIT treatment, whereas the differences were no longer evident at 48 h and longer holding times.

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Table 3. Effects of incubation time and preplacement holding time on YFBM and RYS (g and percentage) of chicks.

Treatment		YFBM	Residual yolk sac (g and percentage)	
		g	g	%
Incubation time				
NIT (504)		39.83 [a]	2.86 [a]	6.38 [a]
LIT (516)		38.83 [b]	2.22 [b]	5.15 [b]
SEM		0.155	0.069	0.140
Preplacement holding time (h)				
0		41.09 [a]	4.73 [a]	10.20 [a]
6		40.57 [ab]	4.47 [a]	9.85 [a]
24		39.98 [b]	2.72 [b]	6.32 [b]
48		39.14 [c]	1.45 [c]	3.53 [c]
60		38.57 [c]	1.15 [c]	2.83 [c]
72		36.61 [d]	0.71 [d]	1.86 [d]
SEM		0.261	0.116	0.235
Incubation time × Preplacement holding time (h)				
NIT (504)	0	41.56	5.34 [a]	11.30 [a]
	6	41.03	5.13 [a]	11.10 [a]
	24	40.50	3.06 [c]	7.02 [c]
	48	39.48	1.59 [e]	3.83 [e]
	60	39.10	1.27 [ef]	3.10 [ef]
	72	37.27	0.76 [f]	1.94 [g]
LIT (516)	0	40.63	4.12 [b]	9.11 [b]
	6	40.10	3.81 [b]	8.60 [b]
	24	39.45	2.38 [d]	5.62 [d]
	48	38.79	1.31 [ef]	3.22 [ef]
	60	38.04	1.02 [ef]	2.56 [fg]
	72	35.94	0.66 [f]	1.78 [g]
SEM		0.369	0.183	0.367
P values				
Incubation time		<0.001	<0.001	<0.001
Preplacement holding time		<0.001	<0.001	<0.001
Incubation time × Preplacement holding time		0.978	<0.001	<0.001

[a-g] Means or percentages in a column followed by different letters differ significantly ($p \leq 0.05$).

It was assumed in the EFSA [33] that modern genetic lines may deplete their reserves more quickly due to the higher metabolic rates associated with faster growth. A higher metabolic rate during incubation will lead to a lower residual yolk weight and energy reserve for the hatchling that might affect posthatch development and performance. In contrast to EFSA [33], Aviagen [34] conducted a trial to compare the rate of yolk utilization in male line chicks from the 1972 genetic control line and the current equivalent line from 2017. The rate at which the residual yolk was depleted was very similar (83% of the residual yolk) in both the control lines and their modern counterparts at 72 h post chick takeoff. In the current study, similar to Aviagen [34], 85% of the residual yolk was utilized at the 72 h preplacement holding time. In addition, in a recent review on yolk sac utilization in poultry, Van der Wagt et al. [35] reported that genetic progress and improved management and incubation conditions have led to limited effects on yolk utilization efficiency and embryonic metabolic heat production.

In the current study, in which the maximum preplacement holding time was 72 h after pull time, the absorption of the yolk sac was not affected by fasting (Table 4). Other recent studies made similar observations, and did not find differences in yolk utilization or residual yolk weights between immediate and delayed posthatch feed intake up to 72 h [21,23,36–39].

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Table 4. Effects of access to feed and water on RYS (g and percentage) of chicks in the 504 and 516 h treatments.

Incubation time[1]	Feed and water access[2]	Age at sampling, h			
		24	48	60	72
		(g)			
NIT (504)	Fasted	3.06 ^{A}	1.59	1.27	0.76
	No-Fasted	3.20 ^{A}	1.51	1.06	0.74
	SEM	0.180	0.115	0.109	0.094
	P value [3]	0.598	0.611	0.173	0.863
LIT (516)	Fasted	2.38 ^{B}	1.31	1.02	0.66
	No-Fasted	2.41 ^{B}	1.28	0.98	0.70
	SEM	0.141	0.082	0.102	0.059
	P value [3]	0.861	0.852	0.758	0.631
		(%)			
NIT (504)	Fasted	7.02 ^{A,x}	3.83 ^{x}	3.10 ^{x}	1.94 ^{x}
	No-Fasted	5.88 ^{A,y}	2.27 ^{y}	1.55 ^{y}	0.95 ^{y}
	SEM	0.350	0.229	0.197	0.192
	P value [3]	0.024	<0.001	<0.001	0.001
LIT (516)	Fasted	5.62 ^{B,x}	3.22 ^{x}	2.56 ^{x}	1.78 ^{x}
	No-Fasted	4.46 ^{B,y}	1.99 ^{y}	1.39 ^{y}	0.89 ^{y}
	SEM	0.295	0.169	0.172	0.119
	P value [3]	0.007	<0.001	<0.001	<0.001

[1]Incubation time of chicks. [2]No-fasted group with access to feed and water at 6 h. [3]P value for comparison of the two groups (fasted/no-fasted; x,y: $p < 0.03$). $^{A-B}$Means in each cell followed by different letters differ significantly between incubation time treatments ($p < 0.05$).

3.4. Crop Fill Progression

Chicks which had been incubated for 504 h (NIT), then placed 6 hours after pull had a significantly higher percentage of empty crops three hours after placement ($p < 0.001$) and a significantly lower percentage of chicks with full rounded crops ($p < 0.01$). For all the longer holding periods, and all the LIT treatment, very few chicks had completely empty crops, and there was no significant difference between treatments. However, the percentage of full crops was significantly lower for the 6 hour holding time in both NIT and LIT treatments. Chicks held for 24 h were also slower to eat and drink than the 48h+ treatments, but all were over the target crop filling recommendations (75–80%) by Aviagen [40] at 3 h after placement in both incubation time treatments. Moreover, after a 6 h hold, the percentage of chicks with full crops in the NIT treatment (54.4%) was significantly lower than that in the LIT treatment (68.3%) at 3 h after placement ($p < 0.001$). Other recent studies have reported a similar trend, with chicks placed after six hours, slower to reach a high percentage with full crops than chicks held for longer before being given access to feed and water [23,41].

3.5. Behavior Observations

In the current study, feeding and drinking behaviors were observed in each treatment at 1, 3 and 8 h after placement. There were no interactions between incubation time and holding time for feeding or drinking behavior at any of the three observation times, nor were there any differences between the two incubation times (Table 5). However, the chicks held for 6 h showed least interest in feeding at all three observation times ($p < 0.001$), which was consistent with the differences in crop fill reported above (Table 6). However, these changes in crop fill and early eating and drinking activity did not lead to comparable improvements in 7- and 35-d BW and mortality. Therefore, crop fill and eating activity at the beginning of production might not be good indicators of animal well-being and health, which was also confirmed by Özlü et al. [23].

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Table 5. Behavior observations of chicks at 1, 3 and 8 h after placement.

Treatment	Observation at 1 h[1]		Observation at 3 h		Observation at 8 h	
	Eating	Drinking	Eating	Drinking	Eating	Drinking
Incubation time (h)	(%)					
NIT (504)	23.65	10.06	22.52	10.10	14.02	6.48
LIT (516)	23.79	10.71	23.40	10.90	15.56	7.52
SEM	0.883	0.515	0.662	0.547	0.808	0.580
Preplacement holding time (h)						
6	12.76 ^{b}	8.70 ^{b}	14.90 ^{b}	7.45 ^{c}	7.81 ^{b}	6.56 ^{b}
24	26.15 ^{a}	9.70 ^{b}	26.20 ^{a}	15.47 ^{a}	15.63 ^{a}	9.90 ^{a}
48	25.73 ^{a}	10.94 ^{ab}	24.27 ^{a}	11.56 ^{b}	16.35 ^{a}	6.46 ^{b}
60	28.80 ^{a}	12.81 ^{a}	26.09 ^{a}	9.27 ^{bc}	16.15 ^{a}	5.37 ^{b}
72	25.16 ^{a}	9.79 ^{b}	23.33 ^{a}	8.75 ^{c}	18.02 ^{a}	6.72 ^{b}
SEM	1.396	0.493	1.047	0.865	1.277	0.917
P values						
Incubation time	0.907	0.161	0.091	0.225	0.183	0.210
Preplacement holding time	<0.001	0.010	<0.001	<0.001	<0.001	0.014
Incubation time × Preplacement holding time	0.142	0.166	0.481	0.510	0.776	0.348

[1]Percentage of the behaviors of eating from the feeder and chick paper or drinking water from the nipples at 1 h, 3 h or 8 h after placement. [a-c]Percentages of chicks in a column followed by different letters differ significantly ($p \leq 0.05$).

Table 6. Crop fill progression at 3 h after placement of chicks.

Treatment		Crop fill progression at 3 h after placement of chicks[1]				
		Empty	Water	Feed	Full, rounded	Total
Incubation time (h)		(%)				
NIT (504)		3.33 ^{a}	7.57	6.19	82.90 ^{b}	100.0
LIT (516)		0.56 ^{b}	6.78	5.22	87.44 ^{a}	100.0
SEM		0.343	1.048	0.774	2.231	
Preplacement holding time (h)						
6		8.61 ^{a}	15.28 ^{a}	14.72 ^{a}	61.39 ^{c}	100.0
24		0.28 ^{b}	8.33 ^{b}	5.28 ^{b}	86.61 ^{b}	100.0
48		0.56 ^{b}	3.89 ^{b}	5.56 ^{b}	90.00 ^{ab}	100.0
60		0.28 ^{b}	5.04 ^{b}	1.59 ^{c}	93.10 ^{a}	100.0
72		0.00 ^{b}	3.33 ^{b}	1.39 ^{c}	95.28 ^{a}	100.0
SEM		0.542	1.658	1.223	1.864	
Incubation time × Preplacement holding time						
NIT (504)	6	15.56^{a}	13.33	16.67	54.44 ^{e}	100.0
	24	0.00 ^{b}	7.78	5.00	87.22 ^{abc}	100.0
	48	1.11 ^{b}	6.11	6.11	86.67 ^{bc}	100.0
	60	0.00 ^{b}	6.75	2.06	91.19 ^{abc}	100.0
	72	0.00 ^{b}	3.89	1.11	95.00 ^{ab}	100.0
LIT (516)	6	1.67 ^{b}	17.22	12.78	68.33 ^{d}	100.0
	24	0.56 ^{b}	8.39	5.06	86.00 ^{bc}	100.0
	48	0.00 ^{b}	1.67	5.00	93.33 ^{ab}	100.0
	60	0.56 ^{b}	3.33	1.11	95.00 ^{ab}	100.0
	72	0.00 ^{b}	2.78	1.67	95.56 ^{a}	100.0
SEM		0.766	2.244	1.730	2.636	
P values						
Incubation time		<0.001	0.595	0.380	0.009	
Preplacement holding time		<0.001	<0.001	<0.001	<0.001	
Incubation time × Preplacement holding time		<0.001	0.395	0.699	0.037	

[1]Crop fill progression divided into the following categories: empty, water only, feed only, and full, soft, and rounded (water and feed). [a-d]Percentages of chicks in a column followed by different letters differ significantly ($p \leq 0.05$).

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3.6. Body Weight

At placement, chicks from the longer incubation treatment (LIT) were 4.06% lighter than those from the normal incubation time treatment (NIT). However, after 35 d with ad libutum access to feed and water the weights of the birds on the two treatments showed no difference (Table 7).

In this study, the length of time chicks were held after pull significantly reduced chick weight at placement (p <0.05). The chicks held for 6 h were the heaviest (44.8 g) and those held for 72 h the lightest (38.0 g) (p <0.001). There was no interaction between hold time and incubation time for BW at placement. However, an interaction was found between the two treatments for BW at 7 d after placement. The BW differences between the NIT and LIT treatments were -3.2, -1.1, +3.4, +5.8 and +7.9 g at 6, 24, 48, 60 and 72 h, respectively, at 7 d after placement, which was an advantage in the LIT treatment for short holding times (6–24 h) but a negative effect for prolonged holding times (48–72 h).

The longest holding time (72 h) resulted in a lower 35 d BW (p < 0.01). In contrast to placement time, no significant differences occurred among the 6 to 60 h preplacement holding times, and the highest BW was found in the 24 h holding time at the end of the trial. Numerous studies have shown that BW was negatively affected by longer posthatch holding period in the early period of growing, but no differences were observed at the end of growout period [17,42–48].

Previous studies have shown that it is possible for broilers to catch up after 36-54 h of fasting between pull and placement, provided that they are allowed access to feed for the same amount of the end of the rearing period [20,21,49,50]. In a similar study by Cardeal et al. [51], chicks were subjected to 3, 24, 48, and 72 h preplacement holding times after pulling from the hatchery. When day of placement was considered the first day, fasting up to 72 h did not have any negative effect on BW, FCR and mortality at 39 d. In the current study, BW at 35 d was similar in the 6 h and 60 h preplacement holding time groups (2179 vs. 2148 g), whereas 72 h holding time (2098 g) without feed and water access after pull time could not compensate for the loss in growth compared with the other holding time groups at 35 d after placement. Although there was no interaction between the preplacement holding time and incubation time for BW at 35 d after placement, with a similar trend at 7 d, the LIT treatment that did positively affect BW for the short holding times was not favorable for longer holding times. Furthermore, at 7 d after placement, the BW difference between 6 and 72 h in the NIT treatment was 10.3 g, whereas this difference was 21.4 g in the LIT treatment. Similarly, BW differences were 2 times higher in the LIT than in the NIT treatment (111 g vs. 50 g) between 6 and 72 h holding times at 35 d after placement (Table 7).

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Table 7. Body weight of chickens from 0 to 7 and 35 d of placement.

Treatment		Body weight		
		0 d	7 d	35 d
Incubation time			(g)	
NIT (504)		42.16 [a]	171.6 [a]	2163
LIT (516)		40.45 [b]	169.0 [b]	2162
SEM		0.077	0.47	8.6
Preplacement holding time (h)				
6		44.82 [a]	176.2 [a]	2179 [ab]
24		43.08 [b]	177.1 [a]	2202 [a]
48		41.04 [c]	170.7 [b]	2177 [ab]
60		39.64 [d]	167.1 [c]	2148 [b]
72		37.95 [e]	160.3 [d]	2098 [c]
SEM		0.122	0.74	13.5
Incubation time × Preplacement holding time (h)				
NIT (504)	6	45.68	174.6 [bc]	2158
	24	43.96	176.6 [ab]	2181
	48	41.85	172.4 [cd]	2190
	60	40.61	170.2 [de]	2168
	72	38.70	164.3 [f]	2108
LIT (516)	6	43.95	177.8 [a]	2199
	24	42.19	177.7 [a]	2222
	48	40.23	169.0 [e]	2164
	60	38.68	164.0 [f]	2129
	72	37.19	156.4 [g]	2088
SEM		0.172	1.04	19.1
P values				
Incubation time		<0.001	<0.001	0.887
Preplacement holding time		<0.001	<0.001	<0.001
Sex[1]				<0.001
Incubation time × Preplacement holding time		0.797	<0.001	0.120

[1]As expected, males exhibited greater BW than females at 35 d ($p < 0.001$), and no interaction effects were observed between sex and other factors ($p > 0.05$). [a-e]Means in a column followed by different letters differ significantly ($p \leq 0.05$).

3.7. Mortality

In the current study, incubation time had no impact on mortality at 7 and 35 d after placement. However, mortality was affected by preplacement holding time and reached 1.82% in the 72 h group at 7 d, which was significantly higher than that in the other preplacement holding time groups ($p = 0.031$). Özlü et al. [23] also reported similar findings that holding chicks for 72 h increased 7-d mortality more than those held for 6–60 h preplacement. Similarly, a large number of chicks (19,200) were reared in the current study, and mortality, a direct indicator of flock health, was not affected by holding times up to and including 60 h at 35 d. However, similar to 7 d, holding for a further 12 to 72 h increased mortality (6.15%) compared to the 6 h (4.85%) and 24 h (4.22%) preplacement holding time groups ($p = 0.028$; Table 8).

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Table 8. Mortality of chickens from placement to 7 and 35 d of placement.

Treatment		Mortality	
		0-7 d	0-35 d
Incubation time		——— (%) ———	
NIT (504)		0.94	5.13
LIT (516)		1.04	5.27
SEM		0.197	0.246
Preplacement holding time (h)			
6		0.57 [b]	4.85 [b]
24		0.52 [b]	4.22 [b]
48		0.89 [b]	5.37 [ab]
60		1.15 [b]	5.42 [ab]
72		1.82 [a]	6.15 [a]
SEM		0.311	0.389
Incubation time × Pre-Placement time (h)			
NIT (504)	6	0.73	5.31
	24	0.52	4.38
	48	0.94	5.10
	60	1.25	5.21
	72	1.25	5.63
LIT (516)	6	0.42	4.38
	24	0.52	4.06
	48	0.83	5.63
	60	1.04	5.63
	72	2.40	6.67
SEM		0.440	0.550
P values			
Incubation time		0.710	0.680
Preplacement holding time		0.031	0.028
Incubation time × Preplacement holding time		0.465	0.435

[a-b]Percentages in a column with different superscripts differ significantly ($p \leq 0.05$).

De Jong et al. [52] carried out a detailed meta-analysis of data from multiple peer-reviewed published experiments examining the impact of delayed feeding on broiler performance and welfare traits. While a total of 84 experiments were initially identified as possible candidates for meta-analysis, in categorizing the data prior to analysis, they found that while some of the experiments started timing in the hatcher, soon after individual chicks emerged from the shell (Category 1), others started when the hatcher was opened to remove all the chicks (Category 2), while for a final group the experiment only started when the chicks were placed in pens at the experimental farm, an unknown number of hours after both emergence and pull (Category 3). Of the 84 data sets originally identified, 42 supplied body weight data suitable for analysis, whereas only 12 of them reported usable mortality data, either to 7 days, 42 days or both. The meta-analysis suggested that a post hatch delay of 48 hours or longer in offering feed increased total mortality to 42 days. However, the 12 data sets included had equal numbers falling into each of the start time categories, with half of them also having either small pen sizes or low numbers of replicates. In addition, some of the delayed-fed chicks were held in boxes in the chicken house. Given that optimal house brooding temperatures are some 6-8 °C warmer than those normally used in chick delivery vehicles, these birds almost certainly overheated, which could be expected to increase early mortality [27]. On the other hand, in much larger scale experiments, Dişa et al. [50] examined the interaction effect of hatching time and pull time on broiler live performance. Chicks were held in the hatcher for 7, 17, 26, 31, 41, or 50 h after hatch and the highest mortality at 41 d was found in chicks that were held in the hatcher for the shortest time (7 h) ($p < 0.001$). In the current study, although there was no interaction ($p > 0.05$) between incubation time and preplacement holding time on mortality at 35 d (Table 8), mortality differences between NIT and LIT treatments were -0.93, -0.32, +0.53, +0.42 and +1.04% at 6, 24, 48, 60 and 72 h, respectively. This was

apparently beneficial for mortality in the LIT treatment, as indicated by BW, at short holding times (6–24 h) but detrimental at long holding times (48–72 h). In addition, the mortality difference between 6 and 72 h in the NIT treatment was 0.5%, whereas this difference was 2.0% in the LIT treatment at 7 d after placement. Similarly, mortality differences were 0.3 and 2.3% in the NIT and LIT treatments, respectively, at 35 d (Table 8), which was consistent with the percentage of mortality at placement (Table 2).

4. Conclusions

In the present study, using a total of 19,200 chicks, preplacement holding times up to and including 60 h after pull time under optimum thermal conditions had no effect on BW and mortality at 35 d of age but reduced BW and increased the mortality when the preplacement holding time was extended to 72 h. In addition, the longer incubation time (LIT-516 h) tended to have a beneficial effect on BW and mortality when the preplacement holding time was shorter (6–24 h), but these parameters were negatively affected with extended holding times (48–72 h) compared to normal incubation time (NIT-504 h).

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