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# Effects of placement time on performance and gastrointestinal tract growth of male broiler chickens

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ABSTRACT - The main objective of this study was to evaluate the effect of placement time on the performance of broiler chickens and the development of their gastrointestinal tract. Two methodologies for measuring broiler performance were compared, one considering day of pulling as the first day, the other considering day of placement as the first day. A total of 1,056 one-day old male Cobb® 500 broiler chicks were subjected to treatments of different placement times after pulling from the hatchery: 3, 24, 48, and 72 h. The studied traits were: feed intake, body weight, feed conversion, viability, and gastrointestinal tract development. When day of pulling was considered the first day, feed intake and body weight at 39 days decreased as placement time increased. However, when day of placement was considered the first day, fasting up to 72 h did not have any negative effect on broiler performance at 39 days postplacement. Placement time did not affect yolk sac utilization or liver weight. At nine days post-placement, weights of gizzard + proventriculus, pancreas, and small intestine increased with increasing placement time. At seven days of age, there was no effect of placement time on villus height or crypt depth. It is possible to place broiler chicks up to 72 h post-hatching with no negative effects when day of placement is considered the first day for evaluating broiler performance.

Keywords: animal production, compensatory growth, newly hatched chick, villus

## Introduction

Several factors involved with hatching eggs and newly hatched chicks can influence performance, including chick holding conditions (e.g., duration of transportation) prior to placement on a broiler farm (Careghi et al., 2005). In addition, management during the initial phase (1 to 14 days) of broiler chick development is essential for good performance at the end of rearing period since significant development of the digestive system occurs during the first phase, followed by skeletal and muscular growth. Due to the shortness of the broiler rearing period, it is believed there is insufficient time for compensatory growth if failures occur (Halevy et al., 2000; Bigot et al., 2003; Kornasio et al., 2011; Attia et al., 2017). Thus, it is necessary to stimulate the initial growth of chicks to achieve maximum growth during the first days of life. Van de Ven et al. (2009) explained that this common practice does not provide chicks access to feed and water during the early post-hatch period until they are pulled, counted, transported to farms, and placed in a broiler house.

Obun and Osaguona (2013) defined the placement time as the time spent at the hatchery after pulling instead of the time spent during transportation to the farm, a period during which chicks are not fed.

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As this time increases, bird weight at the placement time decreases (Baião et al., 1998; Pedroso et al., 2006). During the first days post-hatching, there is a preferential growth of the gastrointestinal tract, as well as of other tissues. The objective of this major growth is to provide better nutrients to broiler chicks and support the development of other tissues (Noy et al., 2001; Bigot et al., 2003; Maiorka et al., 2003). Besides, according to Simon et al. (2019), 24-h fasting mainly affects the expression of structural proteins and proteins involved in lipid transport, general stress response, and intestinal defense.

Several studies have reported that the sooner chicks eat, the better their future performance will be (Noy and Sklan, 1999; Almeida et al., 2006a; Obun and Osaguona, 2013). On the other hand, other studies have shown that although the body weight (BW) of chicks fasted after hatching is lower than the BW of other broilers at the initial phase, the effect is not observed at the end of the growing period (Baião et al., 1998; Vieira and Moran Jr., 1999; Hooshmand, 2006; Bergoug et al., 2013). However, most studies considered day of pulling as the first day, and so conflicting results in the literature are mainly due to the methodology used to measure broiler age (Almeida et al., 2006b).

Thus, in this study, two methodologies for measuring the age of broiler chickens were compared: one considering day of pulling as the first day of age, the other considering day of placement as the first day. The objective of this study was to determine whether the methodology used to measure age interferes with broiler chicken performance, and to evaluate the effect of placement time on broiler chicken performance, gastrointestinal organ weight, and development of duodenum crypts and villi.

## **Material and Methods**

The experiment was conducted in Igarapé, Brazil (20°04'13" S and 44°18'06" W). The current experimental protocol was approved by the Ethical Principles in Animal Experimentation Committee (case no. 227/2012).

A total of 1,056 one-day-old male Cobb® 500 broiler chicks from 48-week old broiler breeders were obtained from a commercial hatchery, where the egg incubation period was average 504 h. On day 18 of incubation (432 h), *in ovo* vaccination against Marek's disease was performed.

Seven hundred and twenty of the one-day-old broiler chicks were used to evaluate broiler performance while the other 336 were used to evaluate organs. The two groups of chicks were kept in separate pens to prevent interference with stocking density and broiler performance. For the evaluation of broiler performance, chicks were placed, 30 per pen (12 birds/m²), in a poultry house with water and feed provided *ad libitum*. For the evaluation of organs, 84 chicks were placed per pen. A lighting program of 18 h of light + 6 h of darkness was adopted.

Treatments were defined according to time spent between pulling and placement as follows: chicks housed upon arrival at the poultry house (3 h after pulling); chicks housed 24 h after pulling; chicks housed 48 h after pulling; and chicks housed 72 h after pulling. Chicks were held inside transportation boxes without access to feed and water before placement. The transport truck maintained the temperature at 32 °C.

The studied traits were: weight loss from pulling to placement (WL), yolk-free body mass (YFBM) at placement time, residual yolk sac weight (YS), digestive organ weight, and villus height and crypt depth in the duodenum. In addition, broiler performance was evaluated using the following parameters: feed intake (FI), feed conversion ratio (FC), viability, and BW. Results of broiler performance were analyzed and compared, considering day of pulling as the first day (life period) and considering day of placement as the first day (rearing period), to determine whether the methodology used to measure age interferes with broiler chicken performance.

To analyze WL, the chicks were weighed in groups of 30 upon arrival on the farm, according to the treatments, and on the day of placement. Weight loss was calculated according to the following equation: (weight at pulling – weight at placement)/weight at pulling × 100. The results were expressed as percentages.

To determine yolk free body mass, 12 chicks of each treatment were weighed individually on the day of placement. The broiler chicks were then euthanized by cervical dislocation, and their respective residual yolk sacs were collected and weighed. Weight of chicks without residual yolk sacs was determined by the difference between BW and YS.

Residual yolk sac weight was determined for the following periods: just after hatching and at the ages of one, two, three, and four days post-pulling. On each day, 12 chicks from each treatment were euthanized by cervical dislocation and their respective yolk sacs removed and immediately weighed.

To determine digestive organ relative weight, eight chicks from each treatment were euthanized at the ages of three, six, and nine days post-pulling. Gizzard + proventriculus, liver, pancreas, and small intestine were removed and weighed. Weight was calculated as the percentage of chick BW using the equation:

## Organ relative weight = organ weight/body weight × 100

Intestine development was evaluated by determining villus height and crypt depth. For this, four chicks from each treatment were euthanized on the day of pulling and at one, four, and seven days post-pulling, and their small intestines removed. A 1.5-cm long section of the duodenum (washed with a 0.1% saline solution) was removed, fixed in 4% paraformaldehyde for 24 h, and placed in a 0.2 M phosphate buffer solution. Intestine segments were dehydrated in increasing concentrations of alcohol and xylene and embedded in paraffin. Sections (5-µm thick) were made, placed on slides, and stained with hematoxylin and eosin (Luna, 1968). The height of 15 villi and the depth of 15 crypts were measured per chick.

The parameters used to evaluate broiler performance were FI, FC, viability, and BW for the whole pen. All the broiler chicks were weighed at the age of 7, 21, and 39 days to determine performance. Analysis of broiler performance considered day of pulling as the first day (life period) and day of placement as the first day (rearing period) to compare aging methodologies and determine whether they interfere with broiler performance. All the broilers and diet were weighted twice, the first-time estimation considering days 7, 21, and 39 according to the pulling time and the second-time estimation of parameters was done according to the housing day.

Organ weight and intestine histology were evaluated using a completely randomized experimental design with four and eight, respectively, experimental units of one bird each per treatment. Evaluation of YS used a completely randomized design with 12 experimental units of one bird per treatment. Broiler performance was also evaluated using a completely randomized experimental design with six experimental units of 30 birds per treatment, while yolk-free body mass was evaluated using 12 experimental units of one bird each. All the data analyzed were firstly evaluated for normality using the Shapiro-Wilk test. Means were subjected to ANOVA at 0.05 probability. Variables were regressed on their linear and quadratic components to evaluate their pattern over time (3 to 72 h), with the regression model that adjusts the most being chosen. The choice of the best regression model was made by evaluating the regression model P-value and the greater R² value. Non-parametric ANOVA was used for data that did not meet normality and homoscedasticity, with means being compared by the Kruskal-Wallis test at 0.05 probability. Statistical procedures were performed using SAS (Statistical Analysis System, version 9.2).

The statistical model (quadratic or linear) used to test the effect of treatments was:

$$Y_{ij} = \beta_0 + \beta_1 X_i + \beta_2 X_i^2 + \varepsilon_{ij}$$
or
$$Y_{ij} = \beta_0 + \beta_1 X_i + \varepsilon_{ij}$$

in which  $Y_{ij}$  = dependent variables in i and replications j,  $\beta$ 's = regression coefficients,  $x_i$  = placement times (i = 3, 24, 48, and 72 h) and  $\epsilon_{ij}$  = residual random error.

## **Results**

Body weight of chicks at pulling was similar among treatments (P>0.05) while weight at placement decreased as placement time increased (WP, g = 43.2 - 0.07271 h;  $R^2 = 0.99$ ; P<0.05). As a result, WL increased as placement time increased (% = 0.20 + 0.18403 h;  $R^2 = 0.99$ ; P<0.05). The quadratic regression was also significant for WL but with a lower  $R^2$ , so the linear regression was chosen for this parameter. There was a significant quadratic regression for YFBM (g = 37.08 + 0.15927 h – 0.00255 h<sup>2</sup>;  $R^2 = 0.99$ ; P<0.05). On day 1, YS of chicks housed after 24 h was higher than that of those housed at 3 h (P<0.05). On day 2, YS was similar for all treatments (P>0.05). The regressions were not significant (P>0.05) on days 3 and 4 (Table 1).

At three, six, and nine days after pulling, the weight of the gizzard + proventriculus increased as placement time increased (P<0.05). Linear regression equations for weight of gizzard + proventriculus (WGP) as percentage of BW at three, six, and nine days are as follows: WGP3d, % = 6.85 + 0.0472 h (R<sup>2</sup> = 0.93); WGP6d, % = 5.30 + 0.02291 h (R<sup>2</sup> = 0.93); WGP9d, % = 7.79 + 0.01257 h (R<sup>2</sup> = 0.88). At three and six days after pulling, regressions were not significant for pancreas weight as percentage of BW (P>0.05), but at nine days after pulling, pancreas weight (WP) as percentage of BW increased as placement time increased (WP9d, % = 0.46 + 0.00138 h; R<sup>2</sup> = 0.79; P<0.05) (Table 2).

On day 3 after pulling, the small intestine weight (WSI) as percentage of BW decreased as placement time decreased (WSI3d, % = 7.54 - 0.04674 h;  $R^2 = 0.94$ ; P<0.05). On day 6 after pulling, regressions were not significant for small intestine weight as percentage of BW (P>0.05). On day 9 after pulling, small intestine weight as percentage of BW increased as placement time increased (WSI9d, % = 6.29 + 0.02296 h;  $R^2 = 0.65$ ; P<0.05) (Table 2). At three, six, and nine days after pulling, regressions for liver weight as percentage of BW (P>0.05) were not significant (data not shown).

At day 1, chicks that did not fast had greater villus height than those that fasted for 24 h (P<0.05). At four days of age, villus height (VH) decreased as placement time increased (VH4d,  $\mu$ m = 68.14 – 968.5 h; R<sup>2</sup> = 0.86; P<0.05). At seven days of age, regressions were not significant for villus height (P>0.05). At four days of age, crypt depth (CD) decreased as placement time increased (CD4d,  $\mu$ m = 97.47 – 0.38646 h; R<sup>2</sup> = 0.85; P<0.05); regressions were not significant for crypt depth (P>0.05) at seven days of age (Table 3).

**Table 1 -** Characteristics of chicks according to time between pulling and placement

Variable		Placem	ent time		– SEM	P-value	P-value
variable	3 h	24 h	48 h	72 h	SEIVI	$L^1$	$Q^2$
IW (g)	43.15	43.50	44.02	43.58	0.2150	0.3562	0.4417
WP (g)	43.15	41.42	39.97	37.80	0.4480	< 0.0001	0.5991
WL (%)	0.0	4.8	9.2	13.3	1.034	< 0.0001	< 0.0001
		Residual y	olk results				
YFBM (g)	37.09	39.41	38.88	35.35	0.6498	0.1754	0.0016
YSh (g)	4.19				-	-	-
$YSd1 (g)^3$	1.95b	3.58a			0.1997	< 0.00014	-
YSd2 (g) <sup>5</sup>	1.43	1.99	2.06		0.1465	$0.1581^{4}$	-
YSd3 (g) <sup>5</sup>	0.63	0.73	1.12	0.82	0.07263	0.1363	0.1281
YSd4 (g)	0.67	0.56	0.65	0.54	0.04016	0.4254	0.7279

IW - body weight at pulling; WP - body weight at placement; WL - weight loss between hatch and placement; YFBM - yolk free body weight; YS - residual yolk sac weight at puling (h) and at days one (d1), two (d2), three (d3), and four (d4) of male broiler chicks, according to time between hatch and placement.

<sup>&</sup>lt;sup>1</sup> P-value of linear regression.

<sup>&</sup>lt;sup>2</sup> P-value of quadratic regression.

<sup>&</sup>lt;sup>3</sup> Chick weight was used as a covariate.

<sup>&</sup>lt;sup>4</sup> P-value of F test.

<sup>&</sup>lt;sup>5</sup> Data transformed for analyses (square root).

a,b - Values within a row with different superscripts differ significantly at P≤0.05 by F test.

When day of pulling was considered the first day to evaluate performance, FI from 1 to 7 days after pulling and BW at seven days after pulling decreased as placement time increased (P<0.05). Linear regression equations for FI from 1 to 7 days after pulling and BW at seven days after pulling are as follows: FI7d, g = 144.29 - 1.17431 h ( $R^2 = 0.99$ ); BW7d, g = 185.73 - 1.03 h ( $R^2 = 0.98$ ). Quadratic regression was also significant for FI and BW, but  $R^2$  values were lower, so linear regression was used for these parameters. Feed conversion ratio from 1 to 7 days after pulling improved as placement time increased (FC7d = 1.023 - 0.00178 h;  $R^2 = 0.99$ ; P<0.05) (Table 4).

At 21 days after pulling, FI and BW decreased as placement time increased (P<0.05). Linear regression equations for FI from 1 to 21 days after hatching and BW at 21 days after pulling were as follows: FI21d, g = 1227.15 - 4.25122 h ( $R^2 = 0.97$ ); BW21d, g = 979.42 - 2.86565 h ( $R^2 = 0.93$ ). Furthermore, from 1 to 21 days after pulling, FC improved as placement time increased (FC21d = 1.311 – 0.00064167 h;  $R^2 = 0.83$ ; P<0.05) (Table 5).

**Table 2** - Relative weight of organs of male broiler chicks at three, six, and nine days after hatching, according to time between pulling and placement

Variable		Placem	ent time		CEM	P-value L <sup>1</sup>	P-value Q²
Variable	3 h	24 h	48 h	72 h	– SEM		
Gizzard + proventriculus (%)							
Three days	6.974	8.068	8.574	10.582	0.2940	< 0.0001	0.4548
Six days	5.345	5.917	6.125	7.108	0.1397	< 0.0001	0.3744
Nine days	4.815	5.175	5.194	5.812	0.1067	0.0007	0.7056
Pancreas (%)							
Three days	0.355	0.460	0.551	0.501	0.0162	0.0780	0.1513
Six days	0.489	0.443	0.539	0.565	0.0115	0.3151	0.3803
Nine days	0.481	0.484	0.533	0.497	0.0157	0.0160	0.9300
Small intestine (%)							
Three days	7.832	5.892	5.485	4.228	0.2906	< 0.0001	0.3713
Six days	7.179	7.974	7.438	7.604	0.1405	0.5659	0.4658
Nine days	6.719	6.460	6.884	8.414	0.2068	0.0016	0.1252

SEM - standard error of the mean.

Table 3 - Villus height ( $\mu$ m) and crypt depth ( $\mu$ m) of male broiler chicks on hatching day, at one, four, and seven days after hatching, according to time between pulling and placement

Variable		Placem	ent time		- SEM	P-value L¹	P-value
variable	3 h	24 h	48 h	72 h	SEM		$Q^2$
Villus height (μm)							
Hatching	582.3				-	-	-
Day 1	669.2a	582.7b			19.038	$0.00642^{3}$	-
Day 4	1112.0	983.4	906.3	888.0	26.816	0.0003	0.0610
Day 7	1380.0	1371.7	1489.0	1258.8	33.499	0.4109	0.1334
Crypt depth (µm)							
Hatching	18.9				-	-	-
Day 1	55.9a	54.2a			2.318	$0.9000^{3}$	-
Day 4	100.1	80.6	83.8	68.6	3.207	< 0.0001	0.6877
Day 7	95.8	82.0	96.8	93.1	3.506	0.8965	0.8620

SEM - standard error of the mean.

P-value of linear regression.
 P-value of quadratic regression.

<sup>&</sup>lt;sup>1</sup> P-value of linear regression.

<sup>&</sup>lt;sup>2</sup> P-value of quadratic regression.

<sup>&</sup>lt;sup>3</sup> P-value of F test.

a,b - Values within a row with different letters differ significantly at P $\leq$ 0.05 by F test.

At 39 days after pulling, FI and BW decreased as placement time increased (P<0.05). Linear regression equations for FI from 1 to 39 days after pulling and BW at 39 days after pulling were as follows: FI39d, g = 4251.63 - 6.62864 h ( $R^2 = 0.99$ ); BW39d, g = 2736.12 - 3.9342 h ( $R^2 = 0.98$ ). Regressions were not significant for FC from 1 to 39 days after pulling (P>0.05) (Table 6).

Evaluation of broiler performance considering day of placement as the first day showed a significant quadratic regression for FI from 1 to 7 days after placement and for BW at seven days (P<0.05). Quadratic regression equations for FI from 1 to 7 days after placement and BW at seven days after placement were as follows: FI7d,  $g = 141.36 + 0.83691 h - 0.00943 h^2$  ( $R^2 = 0.93$ ); BW7d,  $g = 182.04 + 0.78573 h - 0.0097 h^2$  ( $R^2 = 0.98$ ). Linear regression was also significant for FI from 1 to 7 days after placement, but the  $R^2$  value was lower, so quadratic regression was used for this parameter. Regressions were not significant for FC from 1 to 7 days after placement (P>0.05). Treatments had no effect on viability from 1 to 7 days after placement (P>0.05) (Table 4).

At 21 days after placement, BW decreased as placement time increased (BW21d, g = 960.85 - 0.69058 h;  $R^2 = 0.91$ ; P<0.05). From 1 to 21 days after placement, there was a significant quadratic regression for FC (FC21d = 1.32102 + 0.00316 h = 0.00004065 h<sup>2</sup>;  $R^2 = 0.98$ ; P<0.05). Regressions were not significant

**Table 4 -** Performance of male broiler chickens in the period from 1 to 7 days, according to time between pulling and placement

Variable		Placem	ent time		CEM	P-value L¹	P-value Q²
	3 h	24 h	48 h	72 h	- SEM		
		1 to 7	days after pullir	ng			
Feed intake (g)	142.2	117.8	90.9	57.2	6.62	< 0.0001	< 0.0001
Body weight (g)	182.4	163.6	141.0	107.5	5.92	< 0.0001	< 0.0001
Feed conversion (g/g)	1.022	0.981	0.938	0.894	0.01	< 0.0001	0.9063
		1 to 7 da	ys after placem	ient			
Feed intake (g)	142.2	153.6	162.2	152.0	1.74	0.0111	0.0008
Body weight (g)	182.4	194.2	198.6	188.0	1.79	0.1992	< 0.0001
Feed conversion (g/g)	1.022	1.006	1.022	1.013	0.005	0.7938	0.9275
Viability (%)	100	100	99.4	99.4	-	$0.5823^{3}$	-

SEM - standard error of the mean.

**Table 5 -** Performance of male broiler chickens from 1 to 21 days after pulling or after placement and viability in the period from 1 to 21 days, according to time between pulling and placement

V		Placeme	ent time		CEM	P-value L¹	P-value Q²
Variable	3 h	24 h	48 h	72 h	- SEM		
		1 to 21 d	lays after pullir	ıg			
Feed intake (g)	1207.0	1147.0	1060.7	902.7	25.52	< 0.0001	0.1212
Body weight (g)	957.7	937.1	874.0	756.2	17.98	< 0.0001	0.6553
Feed conversion ratio (g/g)	1.319	1.284	1.278	1.268	0.006	0.0081	0.4190
		1 to 21 day	ys after placem	ient			
Feed intake (g)	1207.0	1241.8	1246.5	1181.1	11.04	0.1759	0.0783
Body weight (g)	957.7	944.6	955.1	905.2	7.52	0.0103	0.8898
Feed conversion ratio (g/g)	1.319	1.378	1.368	1.359	0.008	0.4633	0.0078
Viability (%)	99.4	98.9	99.4	98.9	-	$0.8560^{3}$	-

SEM - standard error of the mean.

<sup>&</sup>lt;sup>1</sup> P-value of linear regression.

P-value of quadratic regression.
 P-value of Kruskal-Wallis test.

<sup>&</sup>lt;sup>1</sup> P-value of linear regression.

<sup>&</sup>lt;sup>2</sup> P-value of quadratic regression.

<sup>&</sup>lt;sup>3</sup> P-value of Kruskal-Wallis test.

the period from 1	to 39 days, a	iccording to t	ime between	pulling and p	nacement		
Vaniabla	Placement time					P-value	P-value
Variable	3 h	24 h	48 h	72 h	- SEM	$L^1$	$\mathbb{Q}^2$
		1 to 39 d	ays after pullir	ng			
Feed intake (g)	4239.8	4111.4	3930.6	3768.7	43.56	< 0.0001	0.6718
Body weight (g)	2728.1	2658.7	2535.4	2454.0	26.12	< 0.0001	0.7713
Feed conversion ratio (g/g)	1.579	1.572	1.578	1.564	0.004	0.3502	0.6246
		1 to 39 day	ys after placem	ient			
Feed intake (g)	4239.8	4328.8	4343.0	4253.2	26.48	0.7749	0.2433
Body weight (g)	2728.1	2782.9	2746.4	2728.1	13.12	0.8085	0.3470
Feed conversion ratio (g/g)	1.579	1.580	1.607	1.585	0.007	0.4952	0.6200

96.7

96.1

 $0.9000^{3}$ 

**Table 6 -** Performance of male broiler chickens from 1 to 39 days after pulling or after placement and viability in the period from 1 to 39 days, according to time between pulling and placement

97.3

96.1

Viability (%)

days after placement (P>0.05) (Table 5).

for FI from 1 to 21 days after placement (P>0.05). Treatments had no effect on viability from 1 to 21

At 39 days after placement, regressions were not significant for FI and FC from 1 to 39 days after placement, and for BW at 39 days after placement (P>0.05). Treatments had no effect on viability from 1 to 39 days after placement (P>0.05) (Table 6).

## **Discussion**

As placement time increased, chick weight at placement decreased and, consequently, weight loss increased (Table 1). Delayed access to water and feed after hatching causes weight loss during the holding of chickens. Each 1-h increase in placement time decreased weight at placement by 0.07271 g and increased WL by 0.18403%. This weight loss from hatching to placement may be due to water loss and/or use of yolk sac and muscle protein for gluconeogenesis (Pedroso et al., 2005; Careghi et al., 2005).

The results for YFBM (Table 1) indicated that the heaviest YFBM at pulling was when chicks were placed 31.2 h after hatching. This result indicated that until 31.2 h after pulling, the main cause of weight loss was residual yolk sac utilization, after which, until 72 h after pulling, chicks may be experiencing effects from dehydration and muscle utilization, in addition to residual yolk sac utilization. Chicks housed 31.2 h or more after pulling still had an amount of yolk sac, but possibly not enough to provide nutrients for bird activity. The similar YS on day 2 and no significant linear and quadratic regressions on days 3 and 4 indicated that time of fasting had no negative effect on residual yolk sac utilization.

During the first 48 h after pulling, newly-hatched chicks must make the transition from using energy in the form of lipid from yolk to using exogenous carbohydrate-rich food (Noy and Sklan, 1999). It has been suggested that with post-hatch food and water deprivation, yolk is mainly resorbed directly into the blood via the circulatory system. On the other hand, the presence of food in the gastrointestinal tract stimulates the release of yolk of the yolk sac, thereby enhancing yolk secretion in the intestine (Noy and Sklan, 2001). Thus, early post-hatch feeding may stimulate residual yolk sac resorption in the intestine (Bhanja et al., 2009), although studies also found that early post-hatch feeding does not stimulate residual yolk sac resorption (Gonzales et al., 2008; Gaglo-Disse et al., 2010).

In the present study, post-hatch feed and water deprivation for 24 h reduced yolk absorption when compared that of chicks housed immediately after pulling. Araújo et al. (2018) suggested that better resorption of the residual yolk sac is considered positive for chicken gut development and performance, yet, it has been suggested to stimulate the transport of immunoglobulins from the yolk to the chicken.

SEM - standard error of the mean.

<sup>&</sup>lt;sup>1</sup> P-value of linear regression.

P-value of quadratic regression.
 P-value of Kruskal-Wallis test.

Moreover, a decrease of residual yolk sac over time was observed. On day 4, chicks had depleted about 85% of the yolk sac. According to Jong et al. (2016), chickens are able to survive on yolk sac reserves (only) for 72 h after hatching. In the present experiment, it was observed that the deprivation of water and feed after collection, also called pulling, for 72 h, impaired the performance of broiler chicks. Thus, transport of chicks to a farm for periods longer than 48 h could be damaging to chicks.

Increased weight of gizzard + proventriculus of chicks as placement time increased (Table 2) may be explained by the decrease in BW. Body weight of chicks at seven days after pulling decreased as placement time increased (Table 4). This fact may explain the heavy weight of gizzard + proventriculus as percentage of BW. Increased pancreas weight at nine days after hatching as placement time increased (Table 2) can also be explained by BW. According to Jong et al. (2016), gizzard and proventriculus size can be expected to increase more rapidly in fed birds than in post-hatch food-deprived chicks.

The period of fasting had a negative effect on small intestine development during the first days (Table 2). Each hour increase in placement time decreased small intestine weight three days after hatching by 0.04674%. Although fasting had negative effect on the development of the small intestine during the first three days, compensatory growth occurred after feeding and, at nine days after hatching, small intestine weight increased as placement time increased (Table 2). Each hour increase in placement time increased small intestine weight as percentage of BW nine days after hatch by 0.02296%. These results also confirmed that faster growth of the small intestine during the first days of life does not depend on placement time (Noy et al., 2001).

According to the results, the lack of nutrients after hatching did not have a negative effect on liver development, which indicates that the development of this organ is independent from placement time. These results are not in accordance with Maiorka et al. (2003), who demonstrated that liver development is associated with substrate from intestinal absorption. Still, Van de Ven et al. (2013) demonstrated high liver weight when chicks are not deprived of feed and water.

It was observed that, in general, the effect of water and feed deprivation appears to be transient due to a late onset of FI that is crucial for early organ development, with weights becoming matched at the end of the first week of life. This can also be observed in the lack of effects on chick performance when considering the day of placement.

Similarly to the results found in our study, Ribeiro et al. (2018) found that a post-hatching fasting of up to 24 h did not interfere with the weights of digestive organs of 10-day-old female broiler chickens. However, 48- and 72-h post-hatch fasting adversely affected the weight and growth of digestive organs in the birds.

The presence of feed in the intestinal lumen was important for stimulating villus development during the first 24 h (Maiorka et al., 2003). This occurred until four days after hatching, with each hour increase in placement time causing a decrease of 968.5  $\mu$ m in villus height (Table 3). Negative effects of fasting disappeared at seven days after hatching. Periods of fasting just after hatching resulted in slower development of villi in the duodenum; however, development after feeding was sufficient for the chicks to reach complete intestine growth at seven days post-hatching.

These results are in accordance with Geyra et al. (2001), who documented decreased villus area in the duodenum during fasting after hatching, followed by increased villus area after feeding and, at six days of age, no negative effect of fasting on intestine development. Fasting until 72 h after hatching was harmful to crypt development at four days of age, with each hour increase in placement time causing a decrease of  $0.38646~\mu m$  in crypt depth. Crypt development was affected by fasting during the first days after hatching but, at seven days after hatching, the duodenum achieved maturity with no effect of fasting on duodenum development.

The results for broiler performance at seven days after pulling (Table 4) were as expected, because chicks housed for 24, 48, and 72 h had one, two, and three days less to eat compared with those that were placed immediately after pulling. Therefore, each hour increase in placement time decreased FI by 1.17431 g from hatching to seven days post-hatching, and a decrease of 1.0 3 g in BW seven days after pulling.

Feed intake 21 days after pulling (Table 5) decreased as placement time increased (P<0.05), also as expected because the chickens were fed during 21, 20, 19, and 18 days, respectively. Each hour increase in placement time decreased FI by 4.25122 g from hatching to 21 days after pulling. In addition, each hour increase in placement time decreased BW 21 days after placement by 2.86265 g. This was expected because chickens that had eaten for less time could not recover their weight as fast as other chickens and, consequently, were lighter. The results for broiler performance at 39 days after pulling (Table 6) were as expected because broiler chickens housed for 24, 48, and 72 h were given fewer days to eat. Each hour increase in placement time decreased FI by 6.62864 g from hatching to 39 days after pulling and 3.9342 g in BW at 39 days after pulling.

Between 3 and 72 h, the greatest FI from 1 to 7 days after placement was for chicks housed 44.3 h after hatching (FI7d, g =  $141.36 + 0.83691 \, h - 0.00943 \, h^2$ ). In addition, chicks were heavier at the age of seven days after pulling when they were placed 40.5 h after pulling (BW7d, g =  $182.04 + 0.78573 \, h - 0.0097 \, h^2$ ). Body weight at seven days after pulling increased when chicks were housed from 0 to 40.5 h after pulling, and decreased when they were housed after 40.5 until 72 h. The reason for the lighter BW for chicks housed immediately after pulling may be the smaller amount of FI of those chicks. Chicks housed immediately after pulling have an endogenous source of nutrients (residual yolk sac), which inhibits feed intake (Pedroso et al., 2005). Moreover, fasted chicks show a voracious appetite due to fasting and yolk sac absorption. These results may also explain the increase in weight gain of fasted chicks after they are fed.

Cançado and Baião (2002) and Bigot et al. (2003) observed that chicks housed for 24 and 48 h after pulling had increased BW gain. At 21 days after pulling, each hour increase in placement time decreased BW by 0.69058 g. At 21 days after pulling, chicks that were housed immediately recovered BW. The 72 h of fasting had negative effects on BW at this age. From 1 to 21 days after pulling, FC worsened for chicks housed from 0 to 38.8 h, but FC improved when they were housed after 38.8 until 72 h after pulling (FC21d = 1.32102 + 0.00316 h = 0.00004065 h<sup>2</sup>).

The result of broiler performance at 39 days after pulling (Table 6) indicated that housing chicks until 72 h after pulling had no negative effect on broiler performance when day of placement is considered the first day. These results support hatcheries that have to transport broiler chicks to distant farms. Hatcheries may place chicks up to 72 h with no negative effect on broiler performance, as long as they are transported in good conditions. These results confirmed what was suggested by Almeida et al. (2006b), insofar as that the conflicting results in the literature are mainly due to the methodology used to measure broiler chicken age.

# **Conclusions**

Pre-placement fasting has a negative effect for up to 72 h; however, chicks can recover from this adverse effect after the first week of life with no influence on performance results at 39 days of age. It is also possible to house male broiler chicks for up to 72 h after pulling with no negative effect on broiler performance or digestive organ development, when day of placement is considered first day for performance analysis. In these conditions, the rearing period will be the same for all chicks and they would be able to eat during the same period. Good transport conditions are also important to avoid more stressful situations.

## **Conflict of Interest**

The authors declare no conflict of interest.

## **Author Contributions**

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