

The length of the feed restriction period affects eating behavior, growth performance, and the development of the proximal part of the gastrointestinal tract of young broilers

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ABSTRACT We studied the effects of restricting the access to feed on the anticipatory eating behavior, growth performance, and the development of the proximal part of the gastrointestinal tract (**GIT**) in broilers. The experiment consisted in physical restriction of the access of broilers to feed for 0, 4, 6, or 8 h per day from 7 to 19 D of age. At 10, 13, 16, and 19 D of age, immediately before the start of the feed restriction (**FR**) period, 2 birds per cage were euthanized to evaluate crop and gizzard development. The experimental design was completely randomized, and the linear (**L**) and quadratic (**Q**) effects of fasting length on growth performance and GIT traits were determined. In addition, the effect of broiler age on GIT development was studied. From 7 to 19 D of age, ADFI (**L**, **Q**; $P \leq 0.05$) and BW gain (**L**; $P \leq 0.01$) decreased as the length of the FR period

increased, with most of the differences observed with 6 or more hours of fasting. However, feed conversion ratio was not affected by FR length. The relative weight of the crop (% BW) and its fresh content increased (**L**; $P \leq 0.001$) and the moisture of the digesta (%) decreased (**L**; $P \leq 0.001$) as the FR period increased. The DM content (g) of the crop increased with FR, with most of the differences observed with 6 or more hours of fasting (**L**, **Q**; $P \leq 0.001$). At 19 D of age, the *Lactobacillus* spp. count in the crop increased (**L**; $P \leq 0.05$) with increase in the FR period. Fasting did not affect any gizzard trait at any age. In summary, physical restriction of the access to feed for 6 h or more reduced BW gain but did not affect feed conversion ratio in broilers from 7 to 19 D of age. Feed restriction for 4 to 8 h stimulated the anticipatory feeding behavior and crop development in broilers.

Key words: broiler, crop trait, feed restriction, gizzard trait

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INTRODUCTION

Feed management practices, aiming to improve animal welfare without increasing production cost, are becoming more important for the poultry industry (Mateos et al., 2012). In broilers, *ad libitum* feeding of pellet feeds is associated with a reduction in starch digestibility (Svihus and Hetland, 2001; Abdollahi et al., 2011; Serrano et al., 2013) and an increase in the incidence of metabolic problems (e.g., ascites and lameness) and mortality (Leeson et al., 1999; Brickett et al., 2007; Serrano et al., 2012). Consequently, a reduction in growth early in life might improve welfare and flock productivity at slaughter in modern broiler strains.

Long fasting periods modify birds' behavior and cause anatomical and physiological changes in the gastrointestinal tract (**GIT**) of broilers (Buyse et al., 1993; Buys et al., 1998; Schwan-Lardner et al., 2013). In fact, feed restriction (**FR**) increases the size and the storage capacity of the crop (Svihus et al., 2013) and alters the feeding pattern of the birds, resulting in an increase in feed intake (**FI**) immediately before the start of the feed deprivation period (Shynkaruk et al., 2019). As per European Union guidelines, a minimum of 6-h darkness per day is mandatory after 7 D of age (Council Directive 2007/43/EC). It is assumed that FI is reduced during the dark period (Buyse et al., 1993), and therefore, intermittent light programs are associated with FR practices (Classen et al., 2016). Fasting procedures based on manipulating the light program are easier to implement and manage than physical restriction of the access to feed. Consequently, controlling light programs has been the preferred method to control FI in previous research studies (Buyse et al., 1996; Schwan-Lardner et al., 2013, 2016). Limited information is available on

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the effects of physical restriction to access to feed, while maintaining the light program unchanged, on the development of the proximal part of the GIT of birds. The hypothesis tested was that the response to fasting of broilers under a continuous 24-h lighting program could vary with the length of the FR period. The objective of this research was to evaluate the capacity of modern broilers to adapt GIT development to programs based on physical restriction of the access to feed and its effects on feeding behavior and growth performance from 7 to 19 D of age.

MATERIALS AND METHODS

Bird Husbandry and Experimental Design

The procedures used in this research were approved by the Animal Ethics Committee of the Universidad Politécnica de Madrid and were in compliance with the Spanish Guidelines for the Care and Use of Animals in Research ([Boletín Oficial del Estado, 2013](#)).

In total, 312 straight Ross 308 broilers, weighing 44.5 ± 0.75 g at the arrival to the experimental station, were randomly allotted to 24 cages ($145 \times 45 \times 40$ cm) in groups of 13. The cages (Alternative Design, Siloam Springs, AR) were equipped with 2 low-pressure nipple drinkers and a 65-cm-long open trough feeder and provided with an adjustable front door. The experimental design was completely randomized, with 4 treatments that consisted in the physical restriction of the access of the birds to feed, by manual closure of the feeders for 0, 4, 6, or 8 h per day from day 7 until the end of the experiment at 19 D of age. Each treatment was replicated 6 times, and the experimental unit for all measurements was the cage. All birds were kept under a 24-h continuous light program with *ad libitum* access to water throughout the experiment. Room temperature was maintained at 32°C during the first 3 D of life, and then, it was reduced gradually until reaching 23°C at 19 D of age. The ingredient composition and the calculated ([FEDNA, 2010](#)) and determined nutrient content of the crumble diet used during the whole experiment are shown in [Table 1](#).

Laboratory Analysis and Measurements

A representative sample of the experimental diet was ground using a laboratory mill (Model Z-I, Retsch, Stuttgart, Germany) fitted with a 0.75-mm screen and analyzed for moisture by the oven-drying method (Method 930.15), ash by a muffle furnace (Method 942.05), and nitrogen content by combustion (Method 968.06) using a Leco equipment (FP-528, Leco Corporation, St. Joseph, MI), as indicated by [AOAC International \(2005\)](#). Gross energy of the feed was determined using an adiabatic bomb calorimeter (model 6400, Parr Instrument Company, Moline, IL). The neutral detergent fiber of the diet was determined as indicated by [Van Soest et al. \(1991\)](#) and expressed on ash-free basis. All the analyses were conducted in duplicate. The pH

Table 1. Ingredient composition and chemical analysis of the experimental diet (%).

Ingredient composition	
Wheat	37.16
Corn	20.00
Soybean meal, 47% CP	24.08
Extruded full-fat soybean, 37% CP	9.00
Barley	5.00
Calcium carbonate	1.37
Dicalcium phosphate	0.92
Lard	0.77
L-Lys, 78%	0.40
DL-Met, 99%	0.36
L-Thr, 99%	0.16
Sodium chloride	0.25
Sodium bicarbonate	0.13
Vitamin-mineral premix ¹	0.40
Determined analysis	
Moisture	9.6
Gross energy, kcal/kg	4,074
Crude protein	22.0
Total ash	6.4
Calcium	0.92
Phosphorus	0.57
Neutral detergent fiber	9.7
Calculated analysis	
AMEn, kcal/kg	3,000
Ether extract	4.2
Crude fiber	3.0
Digestible phosphorus	0.40
Sodium	0.15
Chloride	0.28
Digestible amino acids	
Lys	1.28
Met	0.65
Met + Cys	0.95
Thr	0.82
Trp	0.23
Ile	0.79

Abbreviation: AMEn, apparent metabolizable energy.

¹Included per kilogram of feed: vitamin A, 7,520 IU; vitamin D₃, 4,000 IU; vitamin E, 15 IU; vitamin K₃, 2 mg; vitamin B₂, 5.5 mg; vitamin B₆, 2 mg; vitamin B₁₂, 13 mcg; Zn, 52 mg; Cu, 6.2 mg; K, 1.2 mg; Se, 0.3 mg; Fe, 20 mg; niacin, 25 mg; Ca-pantothenate, 9 mg; pantothenic acid, 8 mg; folic acid, 0.5 mg; biotin, 100 mcg; choline chloride, 100 mg; betaine, 150 mg; butylated hydroxyanisole, 0.4 mg; butylated hydroxytoluene, 2 mg; 6-phytase (EC 3.1.3.26; DSM Nutritional Products Ltd., Madrid), 1,000 U; endo-1,4-beta-xylanase (EC 3.2.1.8; Adisseo France S.A.S., Montluçon, France), 1220 U; endo-1,3(4)-beta-glucanase (EC 3.2.1.6; Adisseo France S.A.S., Montluçon, France), 152 U.

of the drinking water and the diet (6.8 and 6.5, respectively) was measured using a portable pH meter (Model 507, Crison Instruments S.A., Barcelona, Spain), as described by [Giger-Reverdin et al. \(2002\)](#).

Feed disappearance and BW of the birds were recorded by cage at 7, 13, and 19 D of age to determine BW gain, ADFI, and feed conversion ratio (**FCR**) by period (7 to 13 D and 14 to 19 D of age) and cumulatively (7 to 19 D of age). At 10, 13, 16, and 19 D of age, immediately before the start of the FR period, 2 birds per cage were randomly selected, euthanized by CO₂ asphyxiation, and weighed individually. The crop and gizzard were carefully clamped to avoid any digesta mixing, excised, and weighed. The pH of the content of the 2 organs was measured in duplicate in situ using a digital pH meter equipped with a fine-tip glass electrode (model 507, Crison Instruments S. A., Barcelona, Spain), as indicated by [Jiménez-Moreno et al. \(2009\)](#). Then, the

Table 2. Effects of the length of the fasting period on growth performance of broilers from 7 to 19 D of age.¹

	Fasting period (h/D)				SEM	<i>P</i> -value ^{2,3}	Regression	
	0	4	6	8	(n = 6)		Linear	Quadratic
7 to 13 days								
ADFI, g	46.1	47.4	43.6	42.5	0.71	<0.001	<0.01	<0.05
BW gain, g/d	36.8	37.8	34.5	33.7	0.50	<0.001	<0.001	<0.05
FCR, g/g	1.25	1.26	1.27	1.26	0.008	0.625	0.260	0.917
14 to 19 days								
ADFI, g	72.7	71.6	71.3	66.5	1.96	0.147	0.056	0.237
BW gain, g/d	55.6	54.0	54.1	50.8	1.59	0.229	0.065	0.446
FCR, g/g	1.31	1.33	1.32	1.31	0.019	0.837	0.994	0.365
7 to 19 days								
ADFI, g	56.3	56.7	54.2	51.7	1.04	<0.01	<0.01	<0.05
BW gain, g/d	44.0	44.0	42.0	40.3	0.81	<0.05	<0.01	0.079
FCR, g/g	1.28	1.29	1.29	1.28	0.011	0.867	0.686	0.489

Abbreviations: FCR, feed conversion ratio; FR, feed restriction.

¹The average BW of the birds at 7 D of age (131 ± 6.0 g) was similar for all treatments.

²*P*-values from the analysis of variance.

³Age of the birds affected all the variables studied ($P < 0.001$). No interactions between FR length and age of the birds were detected for any of the variables studied.

crop and gizzard were emptied of any digesta content and weighed again. The weight and the fresh content of the 2 organs, expressed as absolute (g) and relative weight (% BW) terms, were determined. In addition, the crop contents was dried in an oven and weighed, and the moisture content was calculated (Method 930.15) as indicated by [AOAC International \(2005\)](#). The anticipatory feeding behavior of the birds, as a response to the length of the FR period, was measured indirectly by weighing the digesta content of the crop immediately before the start of the fasting period. At 19 D of age, representative samples of the crop contents were collected from each replicate, frozen, and stored at -20°C for the quantification of *Lactobacillus* sp. colonies ([Ahmed et al., 2014](#)). In brief, the samples (approximately 1 g) were serially diluted (1:10 dilution) with 9 mL of a 0.9% sterile saline solution, thoroughly mixed, and then plated on a specific selective growth medium (DeMan, Rogosa, Sharpe agar medium). The results were expressed as \log_{10} cfu/g of the sample. The average of the 2 individual birds chosen at random from each replicate was used for further statistical analysis for all measurements.

Statistical Analysis

The linear (L) and quadratic (Q) effects of the length of the FR period (0, 4, 6, or 8 h per day) on growth performance, proximal GIT traits, and *Lactobacillus* spp. count were determined by the regression procedure as indicated by [SAS Institute \(2004\)](#). The same type of analysis was conducted to determine the effects of the age of the birds on the development of the organ traits. In addition, the effects of age and the interaction of age with FR length, on growth performance traits and on the development of the proximal part of the GIT, were tested as indicated by [Littell et al. \(1998\)](#).

RESULTS

No interactions between FR length and age of the birds were detected for any of the growth performance or GIT traits studied, and therefore, only main effects are presented.

Growth Performance

No mortality occurred during the experiment. From 7 to 19 D of age, an increase in the length of the fasting period from 0 to 8 h per day decreased ADFI (L, $P < 0.01$; Q, $P < 0.05$) and BW gain (L, $P < 0.01$; Q, $P = 0.079$), with effects that were more evident when the FR period lasted 6 h or more ([Table 2](#)). In addition, the effects of FR on ADFI and BW gain were more evident from 7 to 13 D of age (L, $P < 0.01$; Q, $P < 0.05$) than from 14 to 19 D of age (L; $P = 0.056$ and $P = 0.065$ for ADFI and BW gain, respectively). Feed restriction, however, did not affect FCR in any of the periods considered.

Organ Traits

Effects of Age From 10 to 19 D of age, the absolute weight of the empty crop increased (L; $P < 0.001$) with age ([Figure 1A](#)). In relative terms, however, an opposite effect was observed, and the relative weight of the empty crop decreased (L; $P < 0.001$) with age ([Figure 1B](#)). The absolute weight of the fresh content of the crop digesta (L, Q; $P < 0.05$) and their moisture content (%) (L; $P < 0.001$) increased with age ([Figure 2](#)). As a result, the DM content (g) of the crop digesta was not affected by bird age. In relative terms, however, the weight of the fresh content of the digesta of the crop decreased (L; $P < 0.01$) with age. The weight of the empty gizzard and its fresh content increased (L; $P < 0.01$) with age in absolute terms ([Figure 3A](#)) but

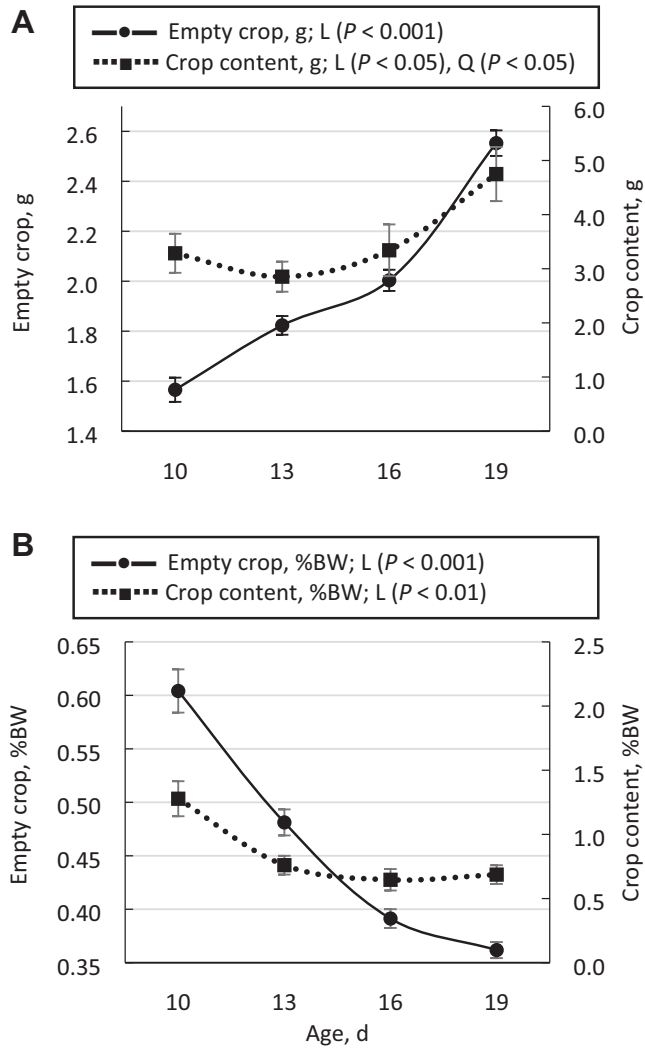


Figure 1. Influence of age (means \pm SEM) on the weight of the empty crop and its content in absolute (A) and relative (B) terms. Mean values per age correspond to 48 observations (4 treatments and 12 chicks per treatment). P -values in brackets correspond to data from the regression analysis (L = linear; Q = quadratic).

decreased (L; $P < 0.001$) in relative terms (Figure 3B). The pH of the crop decreased with age (L, $P < 0.001$; Q; $P < 0.05$), with the highest value observed at 13 D of age (Figure 4). Gizzard pH, however, was not affected by age.

Effects of the Length of the Fasting Period An increase in length of the FR period increased (L; $P < 0.001$) the weight of the empty crop in absolute and relative terms at all ages (Table 3). The weight of the fresh content of the crop, in absolute and relative terms, increased as the length of the FR period increased (L, Q; $P < 0.001$), with effects that were more evident with fasting periods of 6 h or more. The moisture content of the crop digesta (%) decreased (L; $P < 0.001$) as the length of the fasting period increased (Table 4). As a result, the content (g DM) of the crop digesta increased as the length of the fasting period increased (L, Q; $P < 0.001$), with effects that were more evident when the fasting period lasted 6 h or more. In general, fasting did not affect the weight of the gizzard at any age (Table 5).

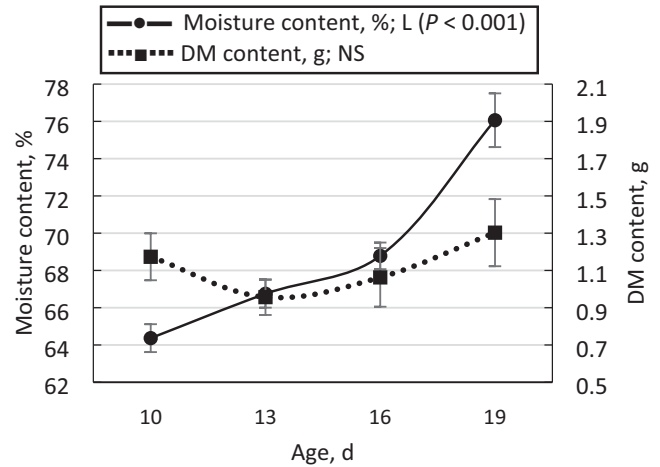


Figure 2. Influence of age (means \pm SEM) on the moisture (%) and DM (g) content of the crop. Mean values per age correspond to 48 observations (4 treatments and 12 chicks per treatment). P -values in brackets correspond to data from the regression analysis (L = linear; Q = quadratic).

On day 19, the pH of the crop increased (L; $P < 0.05$) as the length of the fasting period increased, but fasting did not affect gizzard pH at any age (Table 6). At this age, the number of *Lactobacillus* spp. colonies in the crop increased (L; $P < 0.05$) as the length of the fasting period increased (Table 7).

DISCUSSION

Growth Performance

As expected, ADFI and BW gain of the birds increased and feed efficiency decreased with age (7 to 14 D vs. 15 to 19 D of age), which is in agreement with previous research (Jiménez-Moreno et al., 2011; Kimiaetalab et al. 2017).

Feed restriction reduced the growth rate, especially during the first 6 D of the experiment, probably a consequence of the relative slow adaptation of the birds to the fasting conditions. Immediately after the implementation of a FR program to young birds, voluntary FI is reduced because the capacity of their GIT is limited, resulting in delayed growth (Rodrigues and Choct, 2018). In this respect, Zhan et al. (2007) reported that broilers fasted for 4 h per day from hatch to 21 D of age showed a 13% reduction in BW compared with broilers fed *ad libitum*. In addition, Farghly et al. (2019) observed that a 12-h/D fasting period, implemented immediately after hatch, reduced the BW of the birds at 21 D of age by 7.9%. The consistent disruption in growth performance observed for the first days after the start of the fasting period is often followed by a compensatory growth phase (Camacho et al., 2004) with a clear improvement in FCR (Rodrigues and Choct, 2018). Moreover, the compensatory growth is usually more pronounced when the FR period starts after the first 5 to 7 D of life (Plavnik and Hurwitz,

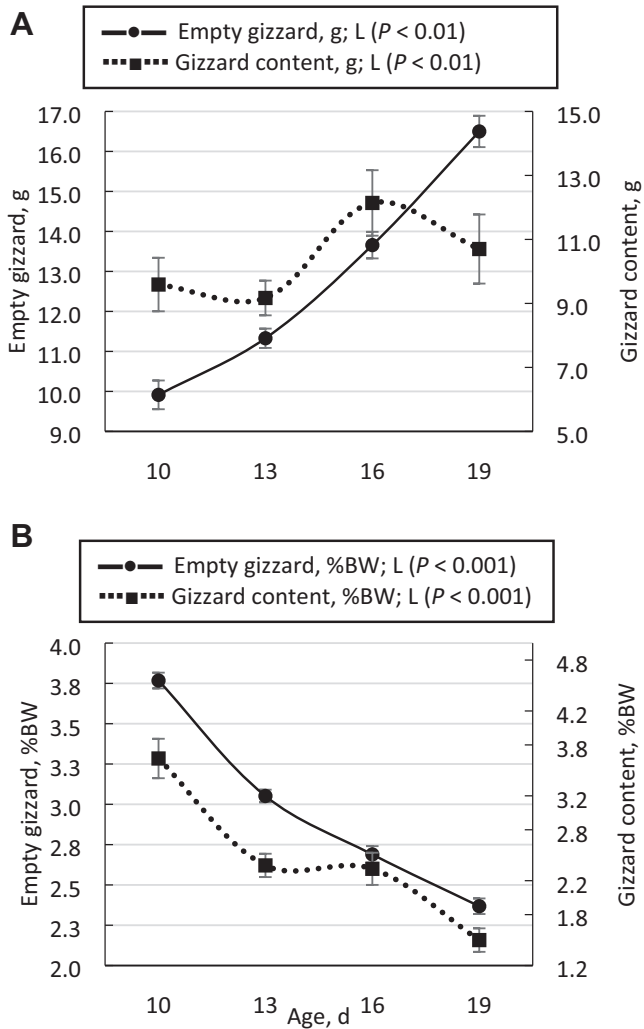


Figure 3. Influence of age (means \pm SEM) on the weight of the empty gizzard and its content in absolute (A) and relative (B) terms. Mean values per age correspond to 48 observations (4 treatments and 12 chicks per treatment). *P*-values in brackets correspond to data from the regression analysis (L = linear; Q = quadratic).

1985). In addition, the magnitude of the compensatory growth depends on the duration of the previous fasting conditions (Sahraei, 2012). In the present study, broilers that were fasted for less than 6 h/D from 7 to 19 D of age showed similar ADFI and BW gain as birds fed *ad libitum* in this period. Moreover, the negative effects of fasting on broiler growth disappeared after 14 D of age. Farghly et al. (2019) reported that the reduction in BW observed in birds that were fasted for 12 h/D from hatch to 21 D of age disappeared at 42 D of age. In the present study, the FR strategy started when the broilers were 7 D of age, and the experiment lasted only 12 D. Possibly, the reduction in BW observed at 19 D of age in birds that were fasted for 6 h/D or more could have been attenuated or even disappeared if the experiment could have lasted longer. This information suggests that birds adapted quickly to fasting conditions with age and that the initial reduction in FI could result in similar growth performance, if sufficient time is allowed for the adaptation of the birds to the fasting strategy.

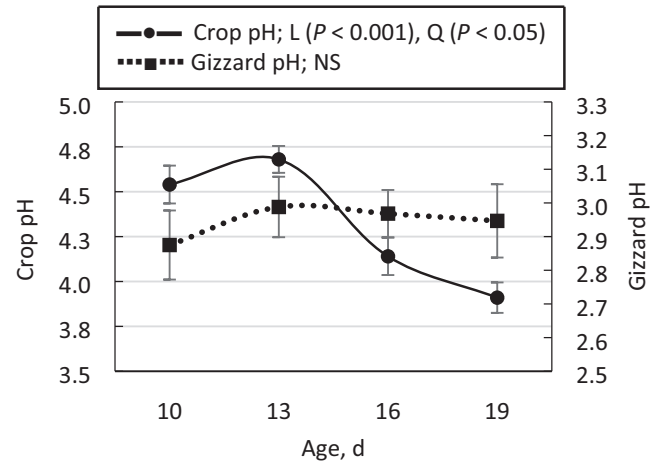


Figure 4. Influence of age (means \pm SEM) on crop and gizzard pH. Mean values per age correspond to 48 observations (4 treatments and 12 chicks per treatment). *P*-values in brackets correspond to data from the regression analysis (L = linear; Q = quadratic).

Organ Traits and Feeding Behavior

Effects of Age In absolute terms, the weight of the crop increased with age, whereas an opposite effect was observed in relative terms. Similar results were observed for the weight and the fresh content of the gizzard, which is in agreement with data of Ravindran et al. (2006) and González-Alvarado et al. (2008). The differences observed between absolute and relative terms, on the weight of the 2 organs of the proximal part of the GIT, are probably related to differences in the rate of growth of organs and tissues with age (Jiménez-Moreno et al., 2019). The weight of the fresh digesta of the crop, as well as their moisture content, increased with age independent of the length of the fasting period. The authors have not found any study on the effects of age on the weight of the crop content during the early adaptation period to FR to compare with the results reported herein. However, the results suggest that birds adapted their eating and drinking behavior to the fasting conditions imposed with age, improving feed moisturization in the crop.

Crop digesta pH depends, at least in part, on the fermentation capability of the microbial population and the concomitant increase in organic fatty acid production in this organ. The fermentation activity of the crop is expected to increase with age (Classen et al., 2016), and consequently, the pH of the crop might decrease with age, which is in agreement with the results reported herein.

In the present study, gizzard pH was not affected by bird age. The pH of the gizzard is expected to decrease during the first week of life because of the increase in the secretion of hydrochloric acid in the proventriculus as the GIT matures (Mahagna and Nir, 1996). After this age, the pH of the gizzard varies widely, depending on factors such as the retention time of the digesta in this organ and the amount and chemical characteristics of the original feed (Svihus, 2011).

Table 3. Effects of the length of the fasting period on selected crop traits.

	Age, days	Fasting period, h/D				SEM	P-value ^{1,2}	Regression	
		0	4	6	8	(n = 6)		Linear	Quadratic
Absolute weight, g									
Content	10	0.75	1.63	4.26	6.50	0.721	<0.001	<0.001	<0.05
	13	0.75	1.96	2.57	6.14	0.555	<0.001	<0.001	<0.01
	16	1.00	1.68	3.37	7.31	0.956	<0.001	<0.001	<0.05
	19	2.07	3.19	4.83	8.92	1.000	<0.001	<0.001	<0.05
	Average	1.14	2.11	3.76	7.22	0.380	<0.001	<0.001	<0.001
Empty organ	10	1.38	1.53	1.57	1.78	0.097	<0.05	<0.01	0.513
	13	1.47	1.85	1.88	2.10	0.075	<0.001	<0.05	0.632
	16	1.61	1.92	2.14	2.34	0.085	<0.001	<0.01	0.811
	19	2.42	2.33	2.56	2.90	0.102	<0.001	<0.001	0.653
	Average	1.72	1.91	2.04	2.28	0.042	<0.001	<0.001	0.327
Relative weight, % BW									
Content	10	0.32	0.60	1.61	2.58	0.273	<0.001	<0.001	<0.05
	13	0.20	0.49	0.68	1.67	0.146	<0.001	<0.01	<0.01
	16	0.19	0.31	0.64	1.44	0.167	<0.001	<0.001	<0.01
	19	0.28	0.44	0.67	1.35	0.146	<0.001	<0.001	<0.05
	Average	0.25	0.46	0.90	1.76	0.089	<0.001	<0.001	<0.001
Empty organ	10	0.55	0.57	0.60	0.70	0.040	<0.05	<0.05	0.184
	13	0.38	0.46	0.51	0.57	0.024	<0.001	<0.01	0.976
	16	0.31	0.36	0.41	0.48	0.018	<0.001	<0.001	0.650
	19	0.33	0.32	0.36	0.44	0.015	<0.001	<0.001	0.317
	Average	0.39	0.43	0.47	0.55	0.014	<0.001	<0.001	0.336

Abbreviation: FR, feed restriction.

¹P-values from the analysis of variance.

²No interactions between FR length and age of the birds were detected for any of the traits studied.

Effects of the Length of the Fasting Period Typically, the feeding behavior of broilers fed *ad libitum* consists in short and frequent approaches to the feed with little utilization of the crop as a storage organ. In contrast, birds subjected to fasting conditions learn to modify their eating behavior with a voluntary increase in feed consumption immediately before the initiation of the FR period. This information suggests that broilers are able to predict the subsequent lack of feed availability (Svihus et al., 2010; Schwan-Lardner et al., 2014; Shynkaruk et al., 2019). A relevant consequence of this strategy is the development and utilization of the crop as a place for feed storage (May and Lott, 1994; Svihus et al., 2013). In the present study, compared with the birds that were fed *ad libitum*, the weight of the crop and its content of the restricted birds, measured immediately before the start of the fasting period, increased with prolonged FR periods, consistent with

data of Duve et al. (2011) and Schwan-Lardner et al. (2013). Similarly, Shynkaruk et al. (2019) observed that the crop content of broilers exposed to dark for 4–10 h/D reached a peak immediately before the start of the dark period, an effect that was not observed when the dark period lasted only 1 h. The results of the present study suggest that the birds anticipated their behavior to the expected fasting conditions, by increasing FI at the end of the feeding period, an effect that was more evident when the subsequent FR period lasted 6 h or more.

Longer residence of the feed in the crop is associated with an improvement in the moisturization of the feed, which in turn might facilitate feed utilization (Svihus et al., 2013). In experiments conducted altering the length of the lighting period, both feed and water restrictions start at the same time (initiation of the dark period), and thus, the availability of feed and water is

Table 4. Effects of the length of the fasting period on selected crop traits.

	Age, days	Fasting period, h/day				SEM	P-value ^{1,2}	Regression	
		0	4	6	8	(n = 6)		Linear	Quadratic
Crop content, g DM									
	10	0.24	0.52	1.51	2.43	0.252	<0.001	<0.001	<0.05
	13	0.14	0.55	0.87	2.26	0.192	<0.001	<0.001	<0.01
	16	0.27	0.49	1.00	2.50	0.315	<0.001	<0.001	<0.05
	19	0.31	0.43	1.24	3.23	0.360	<0.001	<0.001	<0.01
	Average	0.24	0.50	1.15	2.60	0.122	<0.001	<0.001	<0.001
Moisture content, %									
	10	67.7	66.6	64.1	62.4	1.50	0.146	<0.05	0.856
	13	83.0	71.8	65.3	63.1	1.49	<0.001	<0.001	0.206
	16	73.2	70.7	69.8	65.8	1.43	<0.05	<0.05	0.415
	19	86.1	86.7	77.7	63.8	2.89	<0.001	<0.001	0.498
	Average	77.2	74.0	69.3	63.8	0.77	<0.001	<0.001	0.764

Abbreviation: FR, feed restriction.

¹P-values from the analysis of variance.

²No interactions between FR length and age of the birds were detected for any of the traits studied.

Table 5. Effects of the length of the fasting period on selected gizzard traits.

	Age, days	Fasting period, h/day				SEM	P-value ^{2,3}	Regression ¹
		0	4	6	8	(n = 6)		Quadratic
Absolute weight, g								
Content	10	9.16	9.36	9.34	10.38	1.673	0.946	0.893
	13	7.88	10.42	9.18	9.10	1.086	0.444	0.166
	16	11.54	10.81	14.97	11.25	2.047	0.460	0.206
	19	10.17	10.77	11.40	10.36	2.160	0.980	0.589
	Average	9.69	10.34	11.17	10.30	1.151	0.832	0.541
Empty organ	10	9.74	10.34	9.66	9.92	0.719	0.911	0.711
	13	11.02	11.88	11.22	11.20	0.483	0.614	0.165
	16	13.76	13.79	14.23	12.85	0.664	0.519	0.330
	19	16.33	17.03	17.00	15.64	0.784	0.554	0.352
	Average	12.71	13.26	13.03	12.40	0.383	0.422	0.673
Relative weight, g								
Content	10	3.56	3.44	3.51	4.03	0.489	0.823	0.245
	13	2.03	2.59	2.46	2.45	0.268	0.489	0.326
	16	2.23	2.00	2.87	2.29	0.386	0.436	0.866
	19	1.35	1.47	1.60	1.56	0.305	0.946	0.738
	Average	2.29	2.38	2.61	2.58	0.240	0.743	0.655
Empty organ	10	3.81	3.59	3.77	3.90	0.092	0.137	<0.05
	13	2.98	3.12	3.01	3.10	0.080	0.533	0.086
	16	2.53	2.81	2.73	2.69	0.101	0.261	0.946
	19	2.47	2.35	2.21	2.44	0.093	0.195	0.068
	Average	2.95	2.97	2.93	3.03	0.046	0.405	0.169

Abbreviation: FR, feed restriction.

¹The linear effects were not significant for any trait.

²P-values from the analysis of variance.

³No interactions between FR length and age of the birds were detected for any of the traits studied.

limited at the same time (Shynkaruk et al., 2019). However, when the access to feed is restricted by physical impediment, as in the present study, birds have no access to feed, but water is freely available during the whole FR period. Consequently, at the beginning of the FR period, feeding activity is more important for these birds than drinking activity, with broilers giving preference to FI over water intake. As a result, the moisture content of the crop digesta at the start of the fasting period in the physically restricted birds is reduced because birds postpone drinking compared with eating as water will be available afterward. This suggestion is consistent with the results of the present study, in which the DM content of the crop increased at the start of the FR period.

Table 6. Effects of the length of the fasting period on crop and gizzard pH.¹

	Age, days	Fasting period, h/day				SEM	P-value ^{3,4}	Regression ²
		0	4	6	8	(n = 6)		Linear
Crop	10	4.77	4.46	4.56	4.43	0.211	0.681	0.877
	13	4.53	4.67	4.85	4.72	0.150	0.543	0.503
	16	4.17	3.99	4.18	4.28	0.208	0.815	0.146
	19	3.87	3.61	4.00	4.16	0.170	0.131	<0.05
	Average	4.17	4.08	4.18	4.14	0.170	0.131	<0.05
Gizzard	10	2.95	2.75	2.86	2.95	0.205	0.894	0.926
	13	2.96	3.12	2.94	2.93	0.180	0.869	0.233
	16	2.84	2.94	3.08	3.01	0.140	0.663	0.872
	19	3.03	2.90	2.93	2.92	0.218	0.976	0.940
	Average	2.95	2.97	2.93	2.93	0.140	0.663	0.872

¹The pH of the original feed and of the drinking water was 6.80 and 6.53, respectively.

²The quadratic effects were not significant for any trait.

³P-values from the analysis of variance.

⁴No interaction between FR length and age of the birds were detected for any of the traits studied.

The effects of fasting on the utilization of the crop as a feed storage organ were evident at 10 D of age, only 3 D after the start of the fasting period. At this age, the crop digesta content increased as the length of the FR period increased, which is consistent with data of Svihus et al. (2013), who reported a quick adaptation of the birds to the fasting conditions imposed. Furthermore, the adaptation to FR was maintained during the whole experiment, proving the consistent effects of fasting on crop development and bird feeding behavior.

Longer retention times of the digesta in the crop are associated with more nutrients available for fermentation and with an increase in the activity of the microbiota present in this organ (Svihus et al., 2013; Classen et al., 2016). Consequently, a reduction in crop pH was expected as the length of the fasting period increased. In the present study, however, the increase in FI observed immediately before the start of the fasting period resulted in an increase in the pH of the crop digesta, probably because of the higher FI and the pH of the feed (pH = 6.5). Fasting increased linearly the number of *Lactobacillus* spp. colonies in the crop, which is in agreement with the results of Dalal (2016) who reported that increasing the dark period from 1 to 11 h per D tended to increase the number of *Lactobacillus* spp. colonies in the crop of broilers at 33 D of age. The data of the present study suggest that fasting promotes the colonization of the crop by *Lactobacilli*, a consequence of the increased amount of the substrate available for fermentation.

The implementation of the FR strategy increased the weight of the crop and its content but had no effects on gizzard weight or gizzard pH. Similar lack of effects of

Table 7. Effects of the length of the fasting period on *Lactobacillus* spp. count (log cfu/g of the sample) in the crop content at 19 d of age.

Fasting period, h/day				SEM (n = 6)	P-value ¹	Regression ²
0	4	6	8			Linear
5.28	5.97	6.36	6.35	0.318	0.086	<0.05

¹P-values from the analysis of variance.

²The quadratic effect was not significant.

fasting on gizzard development and size has been reported by Hoopaw and Goodman (1976) and Svihus et al. (2013).

In conclusion, FR for 4, 6, and 8 h from 7 to 19 D of age decreased feed consumption in broilers for the first days after the implementation of the fasting strategy, but did not affect FCR or mortality. Fasting conditions changed broiler feeding behavior quickly with an increase in FI, in anticipation of the lack of feed available, as soon as 3 D after the start of the feed restriction strategy on day 10. Moderate increases in the length of the FR period (from 4 to 8 h/D) stimulated the storage capacity of the crop without showing any effect on gizzard traits. The data confirm that broilers are able to anticipate their feeding behavior to the fasting conditions by increasing voluntary FI rapidly. Fasting broilers for moderate periods of time (6-h darkness per day after 7 D of age), as proposed by the European Union, had limited influence on the growth performance of broilers at 21 D of age, with the potential negative effects decreasing quickly as the bird aged.

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