Phenotypic timeline of gastrointestinal tract development in broilers divergently selected for digestive efficiency

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ABSTRACT Sustainability of poultry farming relies on the development of more efficient and autonomous production systems in terms of feed supply. This implies a better integration of adaptive traits in breeding programs, including digestive efficiency, to favor the use of a wider variety of feedstuffs. The objective of the study was to better characterize the kinetics of development of the digestive tract in broilers, in relationship with digestive efficiency by measuring various digestive parameters as well as serum color. Absolute and relative growth of gastrointestinal tract organs were compared between 2 divergent chicken lines selected on digestive efficiency (AMEn) during 7 wk of development. We show that as early as 7 d of age, these 2 lines differs for several organs developments and that these differences remain visible later on. In addition, the allometry of the gizzard and intestine segments is different between the 2 lines, with efficient birds putting more effort in the upper part of the digestive tract during postnatal development and less-efficient birds putting more effort in the lower part of the gastrointestinal tract. Interestingly, we also showed that differences in serum pigmentation, which is a good biomarker for digestive capacity, could be a convenient diagnostic tool to discriminate between chickens with high or low digestive efficiency at early stages of development. In conclusion, this study showed that selection of chickens for AMEn had large impacts in gastrointestinal development including at early stages and is a valuable resource for further studies on the genetic and physiological control of the response of the animal to feed variations.

Key words: broiler, gastrointestinal tract, development, digestive efficiency

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INTRODUCTION

Feed represents the major proportion of production costs for meat-type chickens (Chenut, 2015). In addition, the increasing demand for poultry meat and consequently for crops required for poultry diets has accentuated the competition between animal and human consumption. Poultry breeding has until now favored highly performing animals, but this implies to feed them with high-quality resources and to rear them in an optimized production environment to enable the expression of their genetic potential. Currently, the evolution toward more sustainable livestock systems implies limiting inputs and the competition between animals and human for access to resources and thus using the adaptive capacity of the animals to variable and even unfavorable dietary conditions. This requires a better understanding of adaptation processes – especially those related to digestive efficiency – to improve poultry breeding schemes. Using high-quality feedstuffs, which are easily digested by all birds, does not make it possible to distinguish birds with a high or a low capacity for digestion. Feeding birds with wheat-based diets instead of corn-based diets is a way to challenge their digestive efficiency to characterize their ability to digest various types of feedstuffs (Carré et al., 2008). A divergent selection experiment on the digestive efficiency of the chicken (Mignon-Grasteau et al., 2004) using a wheat-based challenging diet led to marked differences in morphology and histology of the gizzard and small intestine at 23 d of age (Rideau et al., 2014). The transit time between the different sections of the digestive tract may also explain differences in digestive efficiency: for instance, particles, regardless of the size, spent 10 times less time in the gizzard of birds with low digestive capacity compared with birds with high digestive capacity (Rougière et al., 2012). The size and weight of the gizzard and the jejunum are highly different between birds as well (Rideau et al., 2014). These results suggest that several functions are expected to be involved in the control of digestive efficiency.

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Digestive tract development of broiler is a priority during general development at early stages (Lilja, 1983). At the opposite, digestive tract development is slow in layer (Nir et al., 1993). During early development, morphologic modifications of the digestive tract and maturation of its capacity are both observed when chicks are feeding externally on solid feed (Uni et al., 1998). Size of the intestine and side organs are increasing rapidly in the first d of development, and the allometric growth of the jejunum is high compared with other part of the intestine or other organs such as the liver (Nir et al., 1993; Uni et al., 1999). Despite some results showing that differences in digestive efficiency appeared as soon as 7 d of age between D+ and D− lines (de Verdal et al., 2013a), no data were available on the kinetics of development of the digestive tract on these lines.

The objective of the present project was thus to better characterize the kinetics of development of the digestive tract in broilers, in relationship with digestive efficiency.

MATERIALS AND METHODS

Ethics Approval and Consent to Participate

All animal care and experimental procedures reported in this publication were conducted at INRA UE1295 PEAT 37380 Nouzilly (license number D-37-175-1 for animal experimentation, DOI: 10.15454/1.5572326250887292E12) in accordance with French and European regulations concerning animal experimentation. The Ethics Committee for Animal Experimentation of Val de Loire (registered by the National Committee under the number C2EA-19) approved all procedures (procedure number #10100) including measures of digestive efficiency in individual cages, blood sampling procedures for serum analysis, and euthanasia procedures by injection of pentobarbital.

Animals and Sample Collection

Chickens from the D+ and D− lines had been divergently selected for high or low digestive efficiency, assessed by AMEn, at 3 wk, respectively (Mignon-Grasteau et al., 2004; Tran et al., 2014). The initial population, on which the selection experiment was performed, is a pure line of broilers used in a commercial cross dedicated to medium-growing broiler production, reaching the 2-kg market weight at 7 wk of age. During the first 8 generations, the birds were fed a difficult-to-digest diet that included 55% of Rialto wheat (Mignon-Grasteau et al., 2004). Breeders were selected for digestive efficiency using the AMEn measured during a balance trial at 3 wk of age. The 2 lines were then reproduced without selection for AMEn during the 12 following generations. At generation 20, the selection process was restarted with the same criterion. A total of 40 sires and 67 dams from the 21st generation of selection were used to set up the crosses to produce animals for the experiment. From hatching to 11 d of age, all 144 birds (72 of each line) were reared in 1 group on floor. They were transferred to individual cages from 12 to 23 d of age, to measure digestive efficiency at the same age as during the selection process (72 birds, 36 of each line). From 24 to 50 d of age, birds were brought back in their initial rearing cell, on floor. Throughout the experiment, birds were fed a diet similar to the one used during the selection experiment. This diet included 51% of wheat and 5% of rye, which is especially difficult for chickens to digest (Table 1, Mignon-Grasteau et al., 2020).

Recorded Traits

Animal Trait Ontology for Livestock (ATOL) nomenclature was used for the recorder traits (Golik et al., 2012). Birds were weighed at hatching, 7, 14, 20, 23, 35, and 50 d (BW ATOL_0000351: weight_D0, weight_D7, weight_D14, weight_D20, weight_D23, weight_D35, and weight_D50, respectively). ADG was then calculated from 20 to 23 d of age. Twelve birds of each line (D+ and D−) were sampled at hatching (D0), 7 d (D07), 14 d (D14), 23 d (D23), 35 d (D35), and 50 d (D50). Sex ratio for each line and each stage of sampling was close to 50/50. At each slaughter age, several sections of the gastrointestinal tract (**GIT**) were measured and/or weighed on 12 birds from the D+ line and on 12 birds from the D− line: crop (weight_crop, ATOL_0001064), proventriculus (weight_proventriculus, ATOL_0001836), gizzard (weight_gizzard, ATOL_0001067), duodenum (weight_duodenum, ATOL_0001848 and size_duodenum, ATOL_0001084), jejunum (weight_jejunum, ATOL_0001849 and size_jejunum, ATOL_0001093), ileum (weight_ileum, ATOL_0001851 and size_ileum, ATOL_0000571), cecum (weight_caecum, ATOL_0001843 and size_caecum, ATOL_0001102), pancreas (weight_pancreas, ATOL_0000557), and liver (weight_liver, ATOL_0001120). All weights were taken for empty organs/sections.

Feed consumption was also recorded between 20 and 23 d to calculate feed intake and feed efficiency through feed conversion ratio. Density for the intestine segments was calculated as the ratio between the weight and the size of the organ. Allometric growth was calculated for the different digestive traits as the ratio between the

Table 1. Composition of diet.

Ingredients	Percentage
Corn	4.31
Wheat	51.40
Rye	5.00
Soybean Oil	3.00
Palm Oil	3.00
Soybean cake 48	28.87
Calcium carbonate	1.14
Bicalcic phosphate	1.99
Salt	0.30
Vitamins and mineral	0.40
DL Methionine	0.26
HCl lysine	0.21
Threonine	0.07
Anticoccidial	0.05

Table 2. Mean, SD, line effect, and sex effect for traits recorded during the balance trial between 20 and 23 d of age (N = 12 for each line).

Trait	Age (d)	D^+ mean (±SD)	D^- mean (±SD)	D+/D− ratio (%)	Line effect	Sex effect
BW	20	385.7 ± 47.1	369.2 ± 47.3	4.5	0.14	0.072
BW	23	486.9 ± 60.7	461.8 ± 62.3	5.4	0.08	0.057
ADG	20–23	25.5 ± 4.4	22.8 ± 5.7	11.8	0.03	0.059
FI	20–23	171 ± 29	221 ± 80	−22.6	0.0006	0.11
FCR	20–23	1.69 ± 0.17	2.45 ± 0.75	−31.0	<0.0001	0.89
AMEn	20–23	3187.0 ± 87.4	3065.5 ± 134.2	4.0	<0.0001	0.67
FEW	20–23	145.6 ± 48.4	270.3 ± 188.2	−46.1	0.0002	0.095
DEW	20–23	42.6 ± 9.9	97.9 ± 68.6	−54.5	<0.0001	0.20
FEW/BW	20–23	0.29 ± 0.08	0.58 ± 0.39	−49.3	<0.0001	0.23
DEW/BW	20–23	0.09 ± 0.02	0.21 ± 0.14	−59.2	<0.0001	0.38
FEW/FI	20–23	0.84 ± 0.18	1.10 ± 0.39	−24.2	0.0004	0.13

Abbreviations: BW, animal weight (g); DEW, dry excreta weight (g); FCR, feed conversion ratio ($g.g^{-1}$); FEW, fresh excreta weight (g); FEW/BW, DEW/BW, fresh and dry excreta weight between 20 and 23 d of age relative to BW at 23 d ($g\ g^{-1}$); FEW/FI, fresh excreta weight relative to feed intake ($g.g^{-1}$); FI, feed intake (g).

weight of a specific organ and the BW of the bird. The ratio of proventriculus and gizzard weight to intestine weight was also calculated to estimate the balance between the upper and lower parts of the digestive tract.

AMEn

AMEn was individually measured during a balance trial between 20 and 23 d for 72 birds using a method based on collection of total excreta, as described in the studies by Bourdillon et al., 1990 and Mignon-Grasteau et al., 2004. AMEn was measured for all birds by near-infrared (**NIR**) spectrophotometry (Bastianelli et al., 2010). The NIR spectra of all individual freeze-dried excreta were recorded on Foss NIR system 6,500 spin-cell equipment, between 400 and 2,500 nm. The NIR spectra were measured in triplicate (with 3 different cup fillings) and averaged.

Serum Color

It has been previously shown on these lines that serum color could be a biomarker of digestive efficiency (Beauclercq et al., 2019). At each stage for all remaining birds, blood was thus sampled at the occipital sinus for serum color analyses. There were 60, 48, 36, 24 and 12 birds per line (D+ or D−) at 7, 14, 23, 35, and 50 d of age, respectively. Serum was prepared by keeping the blood at room temperature for 15 min until coagulation and centrifugation (3,000 × g for 10 min). Sera were aliquoted and stored at −20°C until further analysis. Samples of 200 μL from the serum of all birds were transferred to a transparent 96-well plate (Greiner Bio-One, Kremsmünster, Austria) and their absorption spectra were acquired between 300 and 600 nm (by 2 nm steps) in triplicate using an Infinite M200 spectrophotometer (Tecan, Männedorf, Switzerland).

Statistical Analyses

Each trait of interest was analyzed using ANOVA, with the following model:

$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

where y_{ijk} is the performance of animal k, μ the general mean, α_i the fixed effect of the line i (i = D+ or D−), β_j the fixed effect of the sex j (j = female or male), $(\alpha\beta)_{ij}$ the interaction between fixed effects line and sex, and ε_{ijk} the residual pertaining to animal k. Differences were considered significant when the P-value was lower than 0.05.

The equality of means between the D− and D+ serum absorption was tested for each wavelength from 300 to 600 nm by 2-nm step at each age with Welch's t test at the 95% confidence level. Linear correlations using Pearson correlation coefficient were also calculated between serum absorption for the same birds at 7, 14 and 23 d of age, for each wavelength from 300 to 600 nm by 2-nm step.

RESULTS AND DISCUSSION

Global Development Between Hatching and 50 d of Age

Animal weights at each age of interest are provided in Supplementary Data 1. Animals weighed in average 38.3 g, 118.9 g, 257.7 g, 474.3 g, 1008.8 g, and 1817.2 g at 0, 7, 14, 23, 35, and 50 d of age, respectively. These results are in accordance with previous observations and the expected weight of 2 kg at 7 wk in these lines (Mignon-Grasteau et al., 2004). No line effect was significant, no matter the stage (Supplementary Data 1). A sex effect was significant for animal weight only at 50 d of age. From 20 to 35 d of age, females are lighter, but the effect was not significant (Supplementary Data 1).

Digestive Traits Between 20 and 23 d of Age

Elementary statistics for digestive traits between 20 and 23 d for both lines are reported in Table 2. A line effect was significant for all traits except for body weight at D20 and D23, which is consistent with the fact that the lines have been selected for digestive efficiency at constant BW at 3 wk. Indeed, the D+ birds had a 11.8% higher ADG, a 22.6% lower feed intake, a 31.0% lower feed conversion ratio, and a 4.0% higher AMEn than D-birds. Whatever

Table 3. Mean, SD, and line effect for gizzard weight from hatching to 50 d of age.

Age (d)	D$^+$ mean (±SD)	D$^-$ mean (±SD)	D$^+$/D$^-$ ratio (%)	Line effect
0	1.9 ± 0.2	2.0 ± 0.3	−5.0	0.47
7	5.3 ± 0.6	4.9 ± 0.7	8.1	0.14
14	8.2 ± 1.2	6.5 ± 1.2	26.1	0.0024
23	9.5 ± 2.2	8.1 ± 1.3	17.3	0.041
35	15.8 ± 2.6	14.0 ± 3.4	12.9	0.14
50	27.9 ± 6.3	22.4 ± 7.2	24.6	0.048

the trait, D+ birds excreted significantly less than D-birds (Table 2). Furthermore, D+ birds excreted 49.3 and 59.2% less fresh and dry excreta than D-birds for the same BW at 23 d. This is in accordance with what has been reported before (de Verdal et al., 2011), with a lower difference in AMEn between the 2 lines in the present study. Interestingly, sex had no effect neither for BW nor for digestive traits recorded between 20 and 23 d.

Gastrointestinal Tract Development Between 7 and 50 d of Age

Gastrointestinal tract sampling was performed at hatching, 7, 14, 23, 35, and 50 d of age. Measures at hatching are presented in tables and supplementary files, but owing to the very small size of GIT segments, they are hard to analyze, and observed variations between animals are mainly within the range of the scale precision.

Gizzards were 17 to 25% heavier in D+ birds at 14, 23, and 50 d of age (Table 3) but not at 35 d of age, age at which the difference between lines was slightly lower than at other ages (13%) and high CV (24%), especially for D− birds. While looking into details of the gizzard development, significant differences between D+ and D− birds were present only from 14 d on with D+ birds having both a higher absolute and relative gizzard weight than D− birds. (Figure 1A), whereas de Verdal et al. (de Verdal et al., 2010) found differences as early

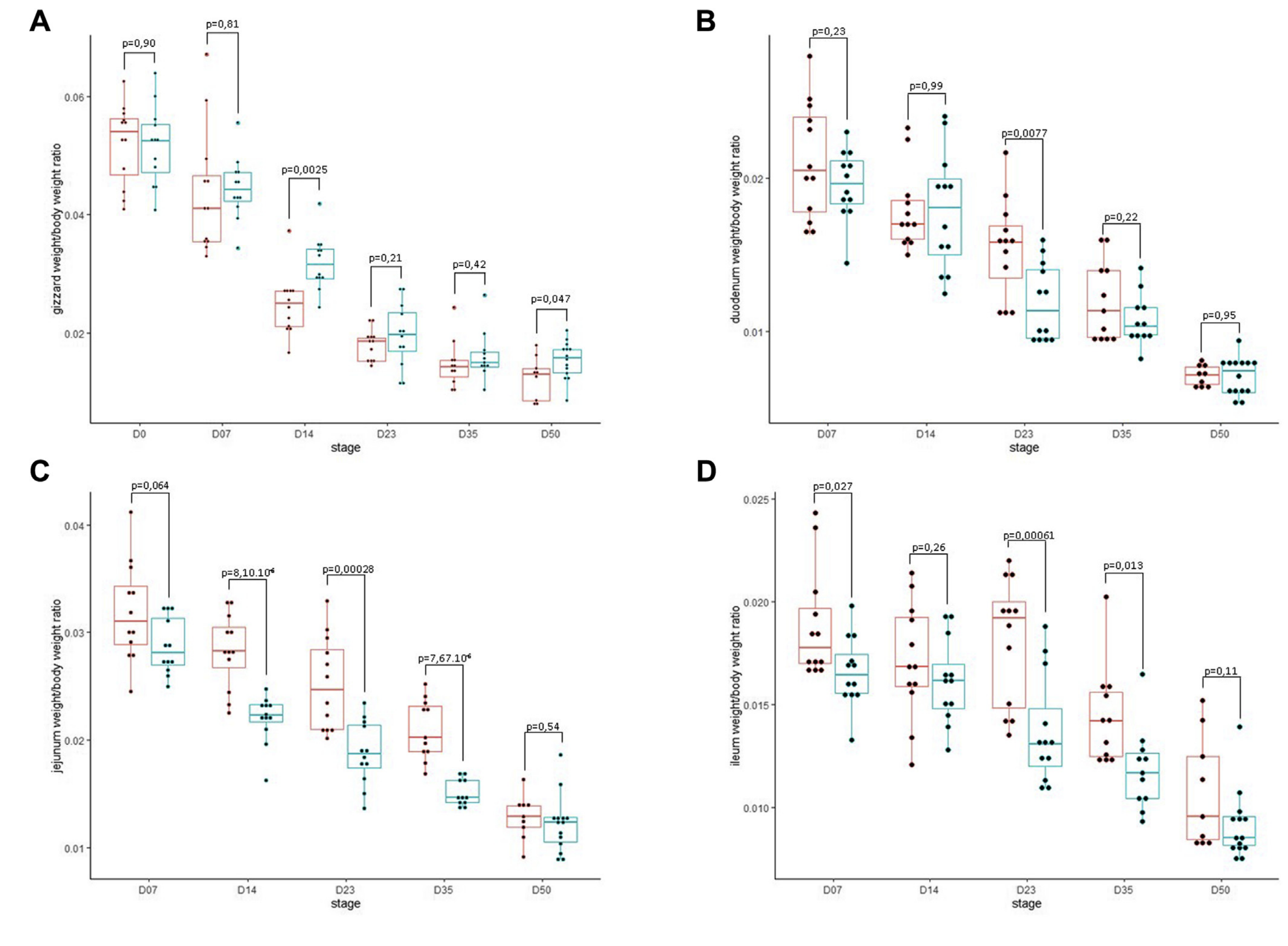

Figure 1. Allometric growth of the digestive tract during development. (A) Allometric growth of the gizzard, that is ratio of gizzard weight to BW. (B) Allometric growth of the duodenum, that is ratio of duodenum weight to BW. (C) Allometric growth of the jejunum, that is ratio of jejunum weight to BW. (D) Allometric growth of the ileum, that is ratio of ileum weight to BW. Each dot represents a single individual and box plot are showing median, first and third quartile of each group. D-birds are in red, D+ birds are in light blue.

Table 4. Mean and SD for jejunum and ileum length and density from 7 to 50 d of age (N = 12 for each line).

Trait	Age (d)	D^+ mean (±SD)	D^- mean (±SD)	D+/D− ratio (%)	Line effect
Jejunum density ($g.cm^{-1}$)	0	ND	ND	ND	ND
	7	0.11 ± 0.02	0.13 ± 0.03	−15.4	0.0093
	14	0.15 ± 0.02	0.21 ± 0.03	−28.6	<0.0001
	23	0.25 ± 0.05	0.29 ± 0.03	−13.8	0.0091
	35	0.32 ± 0.05	0.43 ± 0.08	−25.6	0.0020
	50	0.41 ± 0.10	0.49 ± 0.11	−16.3	0.050
Ileum density ($g.cm^{-1}$)	0	ND	ND	ND	ND
	7	0.07 ± 0.01	0.09 ± 0.02	−22.2	0.0051
	14	0.12 ± 0.02	0.14 ± 0.02	−14.3	0.020
	23	0.18 ± 0.03	0.21 ± 0.04	−14.3	0.024
	35	0.24 ± 0.04	0.29 ± 0.07	−17.2	0.049
	50	0.32 ± 0.06	0.37 ± 0.09	−13.5	0.13

Abbreviation: ND, not determined.

at 7 d. The absence of difference at early stages in our study can partially be explained by the large variability that is observed (Figure 1A). The 7- to 14-d period is critical for the development of the gizzard as at this age, the yolk sac and subdermal reserves of the chick are exhausted and that gizzards have to take an active part in feed grinding to provide nutrients to the organism (Chamblee et al., 1992). Interestingly, yolk was still present in D− birds at 7 d of age for most of the birds, whereas none of the D+ birds exhibits remaining yolk. It was visually observed that D+ and D− birds exhibited different feeding behavior. D− birds went more to the feeder than D+ birds, including at very early stages, consistently with previous observations on these lines (Mignon-Grasteau et al., 2010). This could explain the fact that D− birds do not use their yolk completely in the early days of development, as they are keener to go to the feeders than D+ birds.

Despite a 14 to 49% heavier proventriculus in D+ than in D−, the line effect was not significant at any age (Supplementary Data 2). This can be explained by the high variability in proventriculus weight in both D+ and D− birds (CV of 40 and 20%, respectively, on average between 7 and 50 d of age), which might be owing to the presence of dilated proventriculus in some D+ animals, as already observed in these lines (de Verdal et al., 2010).

Crop development was significantly different between D+ and D− birds only at 23 and 35 d of age (Supplementary Data 2). The crop was 16.7 and 19.8% heavier in D+ birds compared with D− birds, which is consistent with an increase in mass of the crop/proventriculus/gizzard section in those birds.

Overall, intestinal development was different between D+ and D− birds (Supplementary Data 2), but differences vary from age to age. For example, some segments are different in weight but not in size, such as the duodenum at 7 d of age or the ileum at 23 d of age. In more details, the jejunum and ileum (Table 4) were lighter but longer for D+ birds than for D− birds. The jejunum and ileum were therefore less dense for D+ birds than for D− birds at all ages, except for ileum density at 50 d of age, which is not significantly different between lines. Differences at 23 d were in the same range than those previously found by de Verdal et al. (de Verdal et al., 2011).

It can be stated that most significant differences between lines for length of segments appeared from 0 to 14 d, whereas weight differences were mostly significant from 14 to 50 d. This is consistent with the different phases of intestine development in chicks, which starts with elongation of the intestine, followed by a hypertrophy of enterocytes to increase the surface of absorption of nutrients (Schmidt et al., 2009).

Allometry reflects choices that are made during development by an organism to focus on the development on whether one or another compartment of the body (Figure 1). Relative weight of the intestine was in the range of various types of broilers in the literature (Schmidt et al., 2009; Tickle et al., 2014; Alshamy et al., 2018). As expected from the literature on GIT development (Dror et al., 1977; Lilburn and Loeffler, 2015), gizzard and intestine allometric growth are significantly decreasing over time of the development (P-value $< 2.2.10^{-16}$ for the gizzard, duodenum, jejunum, and ileum). Jejunum allometric growth is higher than

Table 5. Mean, SD, and line effect of the ratio of proventriculus and gizzard weight to intestine weight from 7 to 50 d of age (N = 12 for each line).

Trait	Age (d)	D^+ mean (±SD)	D^- mean (±SD)	D+/D− ratio (%)	Line effect
(Proventriculus + Gizzard)/Intestine weight ($g.g^{-1}$)	0	ND	ND	ND	ND
	7	0.79 ± 0.09	0.70 ± 0.13	12.9	0.082
	14	0.66 ± 0.12	0.48 ± 0.093	37.5	0.00050
	23	0.52 ± 0.18	0.37 ± 0.068	40.7	0.00098
	35	0.51 ± 0.15	0.37 ± 0.065	37.8	0.016
	50	0.62 ± 0.19	0.46 ± 0.15	34.8	0.050

Abbreviation: ND, not determined.

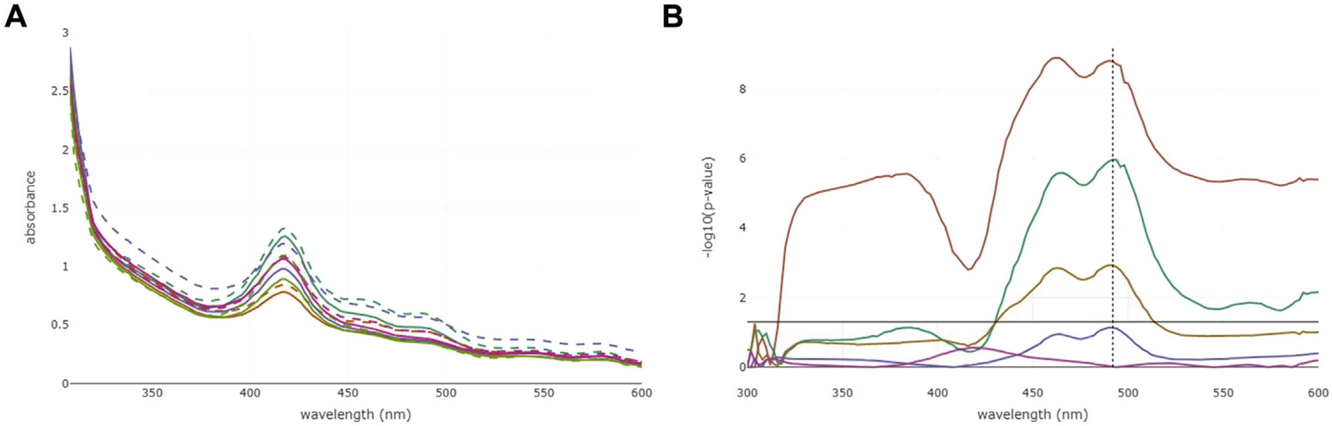

Figure 2. Absorbance and significance of line effect [-log10(P-value)] for sera absorbance between 300 and 600 nm. (A) Absorbance spectra of birds between 7 and 50 d in D+ (dashed line) and D- (plain line). (B) Significance of line effect for spectra of birds between 7 and 50 d. Green stands for measure at 7 d, brown line at 14 d, red line at 23 d, blue at 35 d, and purple at 50 d. In (A), dashed lines are for D+ birds and plain line for D-birds. In (B), the black line is the threshold for a P-value of 0.05, and the dashed line indicates the peak of difference between lines at 492 nm.

duodenum or ileum growth at early stages (Figure 1B–D). It was previously shown that among intestine segments, the jejunum is a priority for birds, especially during early development (Nir et al., 1993). On the other hand, D+ and D− birds do not allocate the same effort to the intestine development (Figure 1B–D). Differences in allometric growth of the intestine with higher ratios in D− birds first appeared in the ileum (7 d), followed by the jejunum (14 d), and at last the duodenum (23 d). Jejunum allometric growth is significantly higher in D− birds from 14 to 35 d of age. Ratio is also higher in D− birds at 7 d of age but not significant, owing to a very high variability in D− birds. Selection based on digestive efficiency influenced the allometry of gizzard and intestine development in the 2 studied lines as previously shown for selected or unselected breeds (Schmidt et al., 2009; Tickle et al., 2014; Alshamy et al., 2018).

The ratio of proventriculus and gizzard weight to intestine weight is a way to summarize observed differences between lines for the upper and the lower part of the GIT (Table 5). This was shown to be linked to digestibility (Carré et al., 2010). Indeed, this ratio is significantly different between D+ and D− birds from 14 to 50 d of age. The ratio is more than 25% higher in D+ birds than in D− birds. A cross talk between the proventriculus/gizzard and intestine seems to take place and molecular evidences are pointing toward this direction (Juanchich et al., 2019).

At early stages, it thus seems from our observations that gizzard and jejunum development are driving digestibility phenotype.

Differences in Serum Color Between Lines

The color of the sera was noticeably different between the D+ and D− lines, which was confirmed by spectrophotometry (Figure 2A). Sera were yellower in D+ than in D− birds as already shown by Mignon-Grasteau et al. (Mignon-Grasteau et al., 2020). As presented in Figure 2B, differences between serum colors of D+ and D− birds were most highly significant at 23 d, age at which the absorption spectra of the 2 lines were different between 300 and 600 nm. This maximal difference at 23 d of age could be expected as it is the age at selection

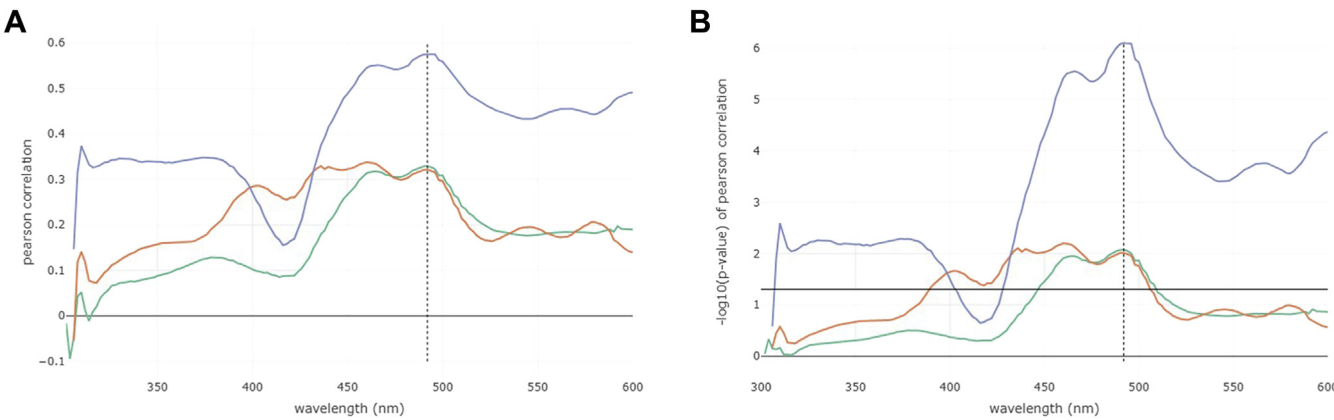

Figure 3. Correlation between spectra at 7, 14, and 23 d. (A) Pearson correlations between spectra, (B) P-value of Pearson correlations between spectra. Orange, green, and blue lines stand for correlations between 7 and 14 d, between 7 and 23 d, and between 14 and 23 d, respectively. The dashed line indicates the peak of difference between lines at 492 nm and the black line indicates the threshold for a P-value of 0.05.

for these lines. In the present study, we show that this difference in serum color can be observed as early as 7 d of age between 430 and 510 nm and remained until 23 d of age. The highest difference for the 3 stages is seen at 492 nm as previously reported (Figure 2B) (Beauclercq et al., 2019).

These differences in serum color were not observed anymore at 35 and 50 d of age. This is consistent with the fact that at these ages, GIT morphologic differences (Supplementary Data 2) and digestive efficiency differences are also less important between the 2 lines (de Verdal et al., 2013b).

It has been previously shown that serum color at 23 d of age was highly genetically correlated to digestive efficiency (Mignon-Grasteau et al., 2020). As serum samples were taken from the same birds at different ages, Pearson correlation coefficient between spectra was thus calculated to evaluate whether sampling before 23 d of age could be a precocious and reliable predictor of digestive efficiency (Figure 3). We observed a significant correlation between spectra at 14 and 23 d of age, especially between 454 and 510 nm (Figure 3A) for which the Pearson correlation coefficient is higher than 0.5 (P-value $< 2.3.10^{-5}$, Figure 3B). The maximal value of correlation is obtained at 492 nm between spectra at 14 and 23 d (r = 0.58). With the same wavelength window, spectra between 7 and 23 d are also significantly correlated but with a lower Pearson correlation coefficient (around 0.3).

These differences in serum pigmentation could be a convenient diagnostic tool to discriminate between chickens with high or low digestive efficiency at early stages (around 14 d of age instead of 23 d of age) with a simple blood sampling and without the use of cages to determine their ability to digest.

In conclusion, we confirmed that selection of broilers on AMEn modified the morphology of the digestive tract at 3 wk of age, and we showed that those modifications could be seen as early as 7 d of age. Based on the allometric analyses, our results also suggest that changes in the morphology of the gizzard and the small intestine are seen to compensate functionality of one another. Furthermore, serum color can been used to predict digestive capacity of the birds as early as 7 d of age. All together, we showed that adaptation to feed is a process that starts early in postnatal development, and measurements could be carried out at early stages to predict the digestive status of the bird.

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Ethics approval and consent to participate

All animal care and experimental procedures reported in this publication were conducted at INRA UE1295 PEAT 37380 Nouzilly (license number D-37-175-1 for animal experimentation, DOI: 10.15454/1.557232625 0887292E12) in accordance with French and European regulations concerning animal experimentation. The Ethics Committee for Animal Experimentation of Val de Loire (registered by the National Committee under the number C2EA-19) approved all procedures (procedure number #10100) including measures of digestive efficiency in individual cages, blood sampling procedures for serum analysis, and euthanasia procedures by injection of pentobarbital.

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DISCLOSURES

The authors declare no conflicts of interest.

SUPPLEMENTARY DATA

Supplementary data associated with this article can be found in the online version at https://doi.org/10.1016/j.psj.2020.11.013.

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