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1 GASTRO-INTESTINAL TRACT DEVELOPMENT IN BROILER

2 **Phenotypic timeline of gastro-intestinal tract development in broilers divergently**
3 **selected for digestive efficiency**

4

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17 **ABSTRACT**

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

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38 Key words: broiler, gastro-intestinal tract, development, digestive efficiency

INTRODUCTION

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Feed represents the major proportion of production costs for meat-type chickens (Chenut, 2015). Additionally, 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 towards 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 - in order 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 in order 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 days 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 to 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. Digestive tract development of broiler is a priority

64 during general development at early stages (Lilja, 1983). At the opposite, digestive tract
65 development is slow in layer (Nir, et al., 1993). During early development, morphological
66 modifications of the digestive tract and maturation of its capacity are both observed when
67 chicks are feeding externally on solid feed (Uni, et al., 1998). Size of the intestine and side
68 organs are increasing rapidly in the first days of development and the allometric growth of
69 jejunum is high compared to other part of the intestine or other organs such as liver (Nir, et
70 al., 1993; Uni, et al., 1999). Despite some results showing that differences in digestive
71 efficiency appeared as soon as 7 days of age between D+ and D- lines (de Verdal, et al.,
72 2013a), no data was available on the kinetics of development of the digestive tract on these
73 lines.

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

76 **MATERIALS AND METHODS**

77 *Ethics approval and consent to participate*

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

86 ***Animals and Sample Collection***

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

105 ***Recorded Traits***

106 ATOL nomenclature was used for the recorder traits (Golik, et al., 2012). Birds were weighed
107 at hatching, 7, 14, 20, 23, 35 and 50 days (Body Weight (BW). ATOL_0000351: weight_D0,
108 weight_D7, weight_D14, weight_D20, weight_D23, weight_D35 and weight_D50). Average
109 Daily Gain (ADG) was then calculated from 20 to 23 days of age. Twelve birds of each line
110 (D+ and D-) were sampled at hatching (D0), 7 days (D07), 14 days (D14), 23 days (D23), 35

111 days (D35) and 50 days (D50). Sex ratio for each line and each stage of sampling was close to
112 50/50. At each slaughter age, several sections of the gastro-intestinal tract (GIT) were
113 measured and/or weighed on 12 birds from the D+ line and on 12 birds from the D- line: crop
114 (weight_crop, ATOL_0001064), proventriculus (weight_proventriculus, ATOL_0001836),
115 gizzard (weight_gizzard, ATOL_0001067), duodenum (weight_duodenum, ATOL_0001848
116 and size_duodenum, ATOL_0001084), jejunum (weight_jejunum, ATOL_0001849 and
117 size_jejunum, ATOL_0001093), ileum (weight_ileum, ATOL_0001851 and size_ileum,
118 ATOL_0000571), caecum (weight_caecum, ATOL_0001843 and size_caecum,
119 ATOL_0001102), pancreas (weight_pancreas, ATOL_0000557) and liver (weight_liver,
120 ATOL_0001120). All weights were taken for empty organs/sections.
121 Feed consumption was also recorded between 20 and 23 days in order to calculate feed intake
122 (FI) and feed efficiency through Feed Conversion Ratio (FCR). Density for the intestine
123 segments were calculated as the ratio between the weight to the size of the organ. Allometric
124 growth was calculated for the different digestive traits as the ratio between the weight of a
125 specific organ over the body weight of the bird. The ratio of proventriculus and gizzard
126 weight to intestine weight was also calculated to estimate the balance between the upper and
127 lower parts of the digestive tract.

128 ***Apparent Metabolizable Energy***

129 AMEn (Apparent Metabolizable Energy corrected for zero nitrogen) was individually
130 measured during a balance trial between 20 and 23 days for 72 birds using a method based on
131 collection of total excreta, as described in (Bourdillon, et al., 1990; Mignon-Grasteau, et al.,
132 2004). AMEn was measured for all birds using Near Infrared (NIR) spectrophotometry
133 (Bastianelli, et al., 2010). The NIR spectra of all individual freeze-dried excreta were recorded
134 on Foss NIR system 6500 spin-cell equipment, between 400 and 2,500 nm. The NIR spectra
135 were measured in triplicate (with three different cup fillings) and averaged.

136 ***Serum color***

137 It has been previously shown on these lines that serum color could be a biomarker of digestive
138 efficiency (Beauclercq, et al., 2019). At each stage for all remaining birds, blood was thus
139 sampled at the occipital sinus for serum color analyses. There was 60, 48, 36, 24 and 12 birds
140 per line (D+ or D-) at respectively 7, 14, 23, 35 and 50 days of age. Serum was prepared by
141 keeping the blood at room temperature for 15 min until coagulation and centrifugation
142 (3,000 g for 10 min). Sera were aliquoted and stored at –20°C until further analysis. Samples
143 of 200 µL from the serum of all birds were transferred to a transparent 96-well plate (Greiner
144 Bio-One, Kremsmünster, Austria) and their absorption spectra were acquired between 300 and
145 600 nm (by 2 nm steps) in triplicates using an Infinite M200 spectrophotometer (Tecan,
146 Männedorf, Switzerland).

147 ***Statistical Analyses***

148 Each trait of interest was analyzed using an analysis of variance (ANOVA), with the
149 following model:

150
$$y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + \varepsilon_{ijk}$$

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

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

160

161

RESULTS AND DISCUSSION

162 *Global development between hatching and 50 days of age*

163 Animal weights at each age of interest are provided in Supplementary data 1. Animals
164 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
165 and 50 days of age, respectively. These results are in accordance with previous observations
166 and the expected weight of 2 kg at 7 weeks in these lines (Mignon-Grasteau, et al., 2004). No
167 line effect was significant, no matter the stage (Supplementary data 1). A sex effect was
168 significant for animal weight only at 50 days of age. From 20 to 35 days of age, females are
169 lighter but the effect was not significant (Supplementary data 1).

170 *Digestive traits between 20 and 23 days of age*

171 Elementary statistics for digestive traits between 20 and 23 days for both lines are reported in
172 Table 2. A line effect was significant for all traits except for body weight at D20 and D23,
173 which is consistent with the fact that the lines have been selected for digestive efficiency at
174 constant body weight at 3 weeks. Indeed, the D+ birds had a 11.8% higher ADG, a 22.6%
175 lower FI, a 31.0% lower FCR and a 4.0% higher AMEn than D- birds. Whatever the trait, D+
176 birds excreted significantly less than D- birds (Table 2). Furthermore, D+ birds excreted 49.3
177 and 59.2% less fresh and dry excreta than D- birds for the same body weight at 23 days. This
178 is in accordance with what has been shown before (de Verdal, et al., 2011), with a lower
179 difference in AMEn between the two lines in the present study. Interestingly, sex had no effect
180 neither for body weight nor for digestive traits recorded between 20 and 23 days.

181 *GIT development between 7 and 50 days of age*

182 GIT sampling was performed at hatching, 7, 14, 23, 35 and 50 days of age. Measures at
183 hatching are presented in tables and supplementary files but due to the very small size of GIT

184 segments, they are hard to analyze, and observed variations between animals are mainly
185 within the range of the scale precision.

186 Gizzards were 17 to 25% heavier in D+ birds at 14, 23 and 50 days of age (Table 3), but not at
187 35 days of age, age at which the difference between lines was slightly lower than at other ages
188 (13%) and the coefficient of variation high (24%), especially for D- birds. While looking into
189 details of the gizzard development, significant differences between D+ and D- birds were
190 present only from 14 days on with D+ birds having both a higher absolute and relative gizzard
191 weight than D- birds. (Figure 1A), whereas de Verdal *et al.* (de Verdal, et al., 2010) found
192 differences as early at 7 days. The absence of difference at early stages in our study can
193 partially be explained by the large variability that is observed (Figure 1A). The 7 to 14 days
194 period is critical for the development of gizzard as at this age, the yolk sac and subdermal
195 reserves of the chick are exhausted and that gizzards have to take an active part in feed
196 grinding to provide nutrients to the organism (Chamblee, et al., 1992). Interestingly, yolk was
197 still present in D- birds at 7 days of age for most of the birds while none of the D+ birds
198 exhibits remaining yolk. It was visually observed that D+ and D- birds exhibited different
199 feeding behavior. D- birds went more to the feeder than D+ birds, including at very early
200 stages, consistently with previous observations on these lines (Mignon-Grasteau, et al., 2010).
201 This could explain the fact that D- birds do not use completely their yolk in the early days of
202 development, as they are keener to go to the feeders than D+ birds.

203 Despite a 14 to 49% heavier proventriculus in D+ than in D-, the line effect was not
204 significant at any age (Supplementary data 2). This can be explained by the high variability in
205 proventriculus weight in both D+ and D- birds (coefficient of variation of 40% and 20%
206 respectively, on average between 7 and 50 days of age), which might be due to the presence of
207 dilated proventriculus in some D+ animals, as already observed in these lines (de Verdal, et
208 al., 2010).

209 Crop development was significantly different between D+ and D- birds only at 23 and 35 days
210 of age (Supplementary data 2). Crop was 16.7 and 19.8 % heavier in D+ birds compared to D-
211 birds which is consistent with an increase in mass of the crop/proventriculus/gizzard section
212 in those birds.

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

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

226 Allometry reflects choices that are made during development by an organism to focus on the
227 development on whether one or another compartment of the body (Figure 1). Relative weight
228 of intestine was in the range of various types of broilers in the literature (Alshamy, et al.,
229 2018; Schmidt, Persia, Feierstein, Kingham and Saylor, 2009; Tickle, et al., 2014). As
230 expected from the literature on GIT development (Dror, et al., 1977; Lilburn and Loeffler,
231 2015), gizzard and intestine allometric growth are significantly decreasing over time of the
232 development (P -value $< 2.2 \cdot 10^{-16}$ for gizzard, duodenum, jejunum and ileum). Jejunum
233 allometric growth is higher than duodenum or ileum growth at early stages (Figure 1B, 1C

234 and 1D). It was previously shown that among intestine segments, jejunum is a priority for
235 birds, especially during early development (Nir, et al., 1993). Another hand, D+ and D- birds
236 do not allocate the same effort to the intestine development (Figure 1B, 1C and 1D).
237 Differences in allometric growth of the intestine with higher ratios in D- birds first appeared
238 in ileum (7 days), followed by jejunum (14 days) and at last duodenum (23 days). Jejunum
239 allometric growth is significantly higher in D- birds from 14 to 35 days of age. Ratio is also
240 higher in D- birds at 7 days of age but not significant, due to a very high variability in D-
241 birds. Selection based on digestive efficiency influenced the allometry of gizzard and intestine
242 development in the two studied lines as previously shown for selected or unselected breeds
243 (Alshamy, Richardson, Hunigen, Hafez, Plendl and Al Masri, 2018; Schmidt, Persia,
244 Feierstein, Kingham and Saylor, 2009; Tickle, Paxton, Rankin, Hutchinson and Codd, 2014).
245 The ratio of proventriculus and gizzard weight to intestine weight is a way to summarize
246 observed differences between lines for the upper and the lower part of the GIT (Table 5). This
247 was shown to be linked to digestibility (Carré, et al., 2010). Indeed, this ratio is significantly
248 different between D+ and D- birds from 14 to 50 days of age. The ratio is more than 25%
249 higher in D+ birds than in D- birds. A crosstalk between proventriculus/gizzard and intestine
250 seems to take place and molecular evidences are pointing toward this direction (Juanchich, et
251 al., 2019).
252 At early stages, it thus seems from our observations that gizzard and jejunum development are
253 driving digestibility phenotype.

254 ***Differences in serum color between lines***

255 The color of the sera was noticeably different between the D+ and D- lines, which was
256 confirmed by spectrophotometry (Figure 2A). Sera were yellower in D+ than in D- birds as
257 already shown by Mignon-Grasteau *et al.* (Mignon-Grasteau, et al., 2020).. As presented in
258 Figure 2B, differences between D+ and D- birds in serum color were the most highly

259 significant at 23 days, age at which the absorption spectra of the two lines were different
260 between 300 and 600 nm. This maximal difference at 23 days of age could be expected as it is
261 the age at selection for these lines. In the present study, we show that this difference in serum
262 color can be observed as early as 7 days of age between 430 and 510 nm and remained until
263 23 days of age. The highest difference for the three stages is seen at 492 nm as previously
264 reported (Figure 2B) (Beauclercq, et al., 2019).

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

269 It has been previously shown that serum color at 23 days of age was highly genetically
270 correlated to digestive efficiency (Mignon-Grasteau, et al., 2020). As serum samples were
271 taken from the same birds at different ages, Pearson correlation coefficient between spectra
272 were thus calculated to evaluate whether sampling before 23 days of age could be a
273 precocious and reliable predictor of digestive efficiency (Figure 3). We observed a significant
274 correlation between spectra at 14 and 23 days of age, especially between 454 and 510 nm
275 (Figure 3A) for which the Pearson correlation coefficient is above 0.5 (P -value $< 2.3 \cdot 10^{-5}$,
276 Figure 3B). The maximal value of correlation is obtained at 492 nm between spectra at 14 and
277 23 days ($r = 0.58$). With the same wavelength window, spectra between 7 and 23 days are also
278 significantly correlated, but with a lower Pearson correlation coefficient (around 0.3).

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

283 In conclusion, we confirmed that selection of broilers on AMEn modified the morphology of
284 the digestive tract at three weeks of age and we showed that those modifications could be seen
285 as early as 7 days of age. Based on the allometric analyses, our results also suggest that
286 changes in the morphology of the gizzard and the small intestine are seen in order to
287 compensate functionality of one another. Furthermore, serum color can be used to predict
288 digestive capacity of the birds as early as 7 days of age. All together, we showed that
289 adaptation to feed is a process starts early in post-natal development and measurements could
290 be done at early stages to predict the digestive status of the bird.

291

292

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384 Uni, Z., Y. Noy, and D. Sklan. 1999. Posthatch development of small intestinal function in the
385 poul. *Poultry Sci* 78:215-222. doi DOI 10.1093/ps/78.2.215
386

387 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

388

389

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

Trait¹	Age (days)	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

392 ¹BW: animal weight (g), ADG: Average Daily Gain (g), FI: Feed Intake (g), FCR:
 393 Feed Conversion Ratio (g.g⁻¹), AMEn: Nitrogen corrected Apparent Metabolizable
 394 Energy (kcal.kg⁻¹ DM), FEW: fresh excreta weight (g), DEW: dry excreta weight (g),
 395 FEW/BW, DEW/BW: fresh and dry excreta weight between 20 and 23 days of age
 396 relative to BW at 23 d (g.g⁻¹), FEW/FI: fresh excreta weight relative to feed intake
 397 (g.g⁻¹)

398 Table 3. Mean, standard deviation (SD) and line effect for gizzard weight from
399 hatching to 50 days of age.

Age (days)	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

400

401 Table 4. Mean and standard deviation (SD) for jejunum and ileum length and density

402 from 7 to 50 days of age (N=12 for each line).

403

Trait	Age (days)	D⁺ mean (± SD)	D⁻ mean (± SD)	D⁺/D⁻ ratio (%)	Line effect
Jejunum density (g.cm⁻¹)	0	ND ¹	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

404 ¹ND: not determined

405

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

Trait¹	Age (days)	D⁺ mean (±sd)	D⁻ mean (±sd)	D⁺/D⁻ ratio (%)	Line effect
(Proventriculus + Gizzard) / Intestine weight (g.g⁻¹)	0	ND ¹	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

408

409 ¹ND: not determined

410

411 **Figure 1.** Allometric growth of the digestive tract during development

412 (A) Allometric growth of gizzard, i.e ratio of gizzard weight to body weight

413 (B) Allometric growth of duodenum, i.e ratio of duodenum weight to body weight

414 (C) Allometric growth of jejunum, i.e ratio of jejunum weight to body weight

415 (D) Allometric growth of ileum, i.e ratio of ileum weight to body weight

416 Each dot represents a single individual and box plot are showing median, first and

417 third quartile of each group. D- birds are in red, D+ birds are in light blue.

418

419 **Figure 2.** Absorbance and significance of line effect [$-\log_{10}(P\text{-value})$] for sera
420 absorbance between 300 and 600 nm.
421 (A) Absorbance spectra of birds between 7 and 50 days in D+ (dashed line) and D-
422 (plain line). (B) Significance of line effect for spectra of birds between 7 and 50 days.
423 Green stands for measure at 7 days, brown line at 14 days, red line at 23 days, blue at
424 35 days and purple at 50 days. In (A), dashed lines are for D+ birds and plain line for
425 D- birds. In (B), the black line is the threshold for a P -value of 0.05 and the dashed
426 line indicates the peak of difference between lines at 492 nm.
427

428 **Figure 3.** Correlation between spectra at 7, 14 and 23 days. (A) Pearson correlations
429 between spectra, (B) *P*-value of Pearson correlations between spectra.
430 Orange, green and blue lines stand respectively for correlations between 7 and 14
431 days, between 7 and 23 days and between 14 and 23 days. The dashed line indicates
432 the peak of difference between lines at 492 nm and the black line indicates the
433 threshold for a *P*-value of 0.05.
434

435 **Abbreviations**

436 AMEn: Nitrogen corrected apparent metabolizable energy

437 QTL: Quantitative Trait Loci

438

439 **Declarations**

440 **Ethics approval and consent to participate**

441 All animal care and experimental procedures reported in this publication were
442 conducted at INRA UE1295 PEAT 37380 Nouzilly (license number D-37-175-1 for
443 animal experimentation, DOI: 10.15454/1.5572326250887292E12) in accordance
444 with French and European regulations concerning animal experimentation. The Ethics
445 Committee for Animal Experimentation of Val de Loire (registered by the National
446 Committee under the number C2EA-19) approved all procedures (procedure number
447 #10100) including measures of digestive efficiency in individual cages, blood
448 sampling procedures for serum analysis, and euthanasia procedures by injection of
449 pentobarbital.

450 **Consent for publication**

451 Not Applicable.

452 **Availability of data and material**

453 **Competing interests**

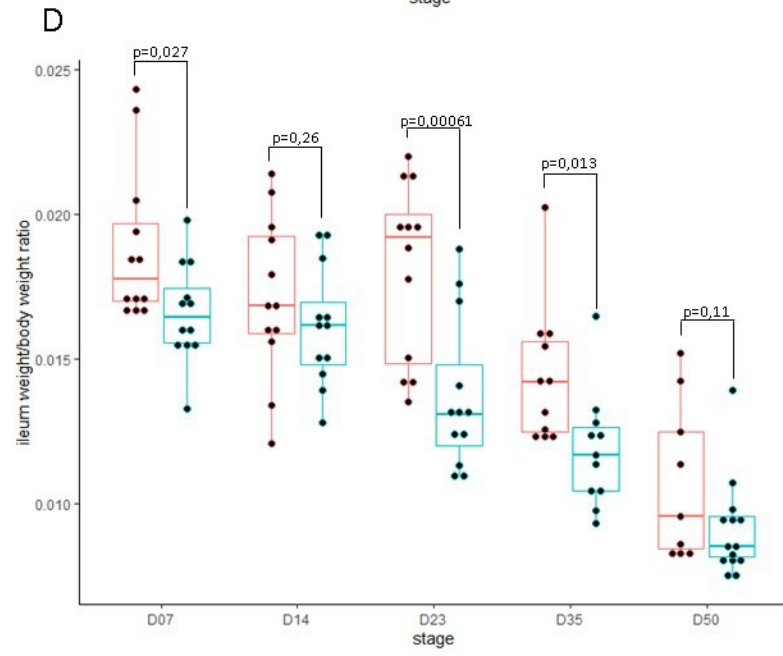
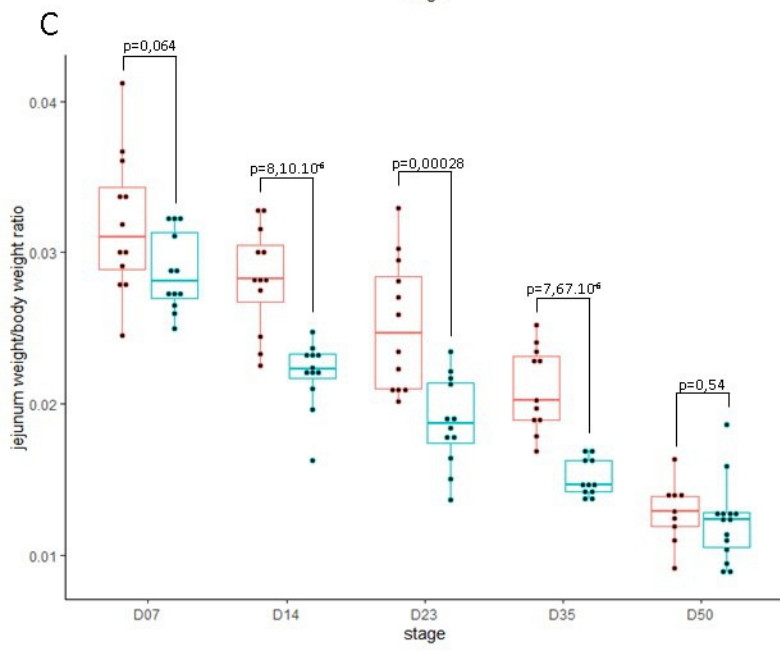
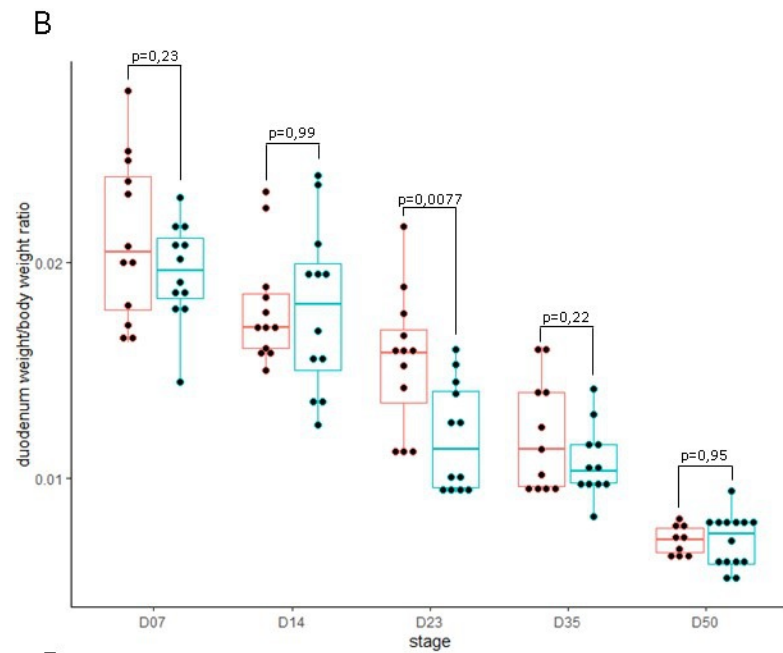
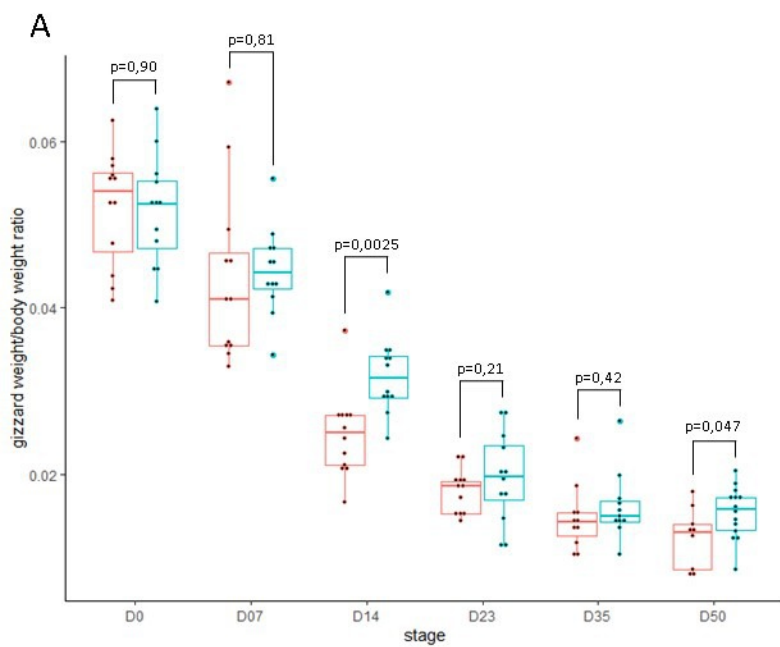
454 The authors declare that they have no competing interests.

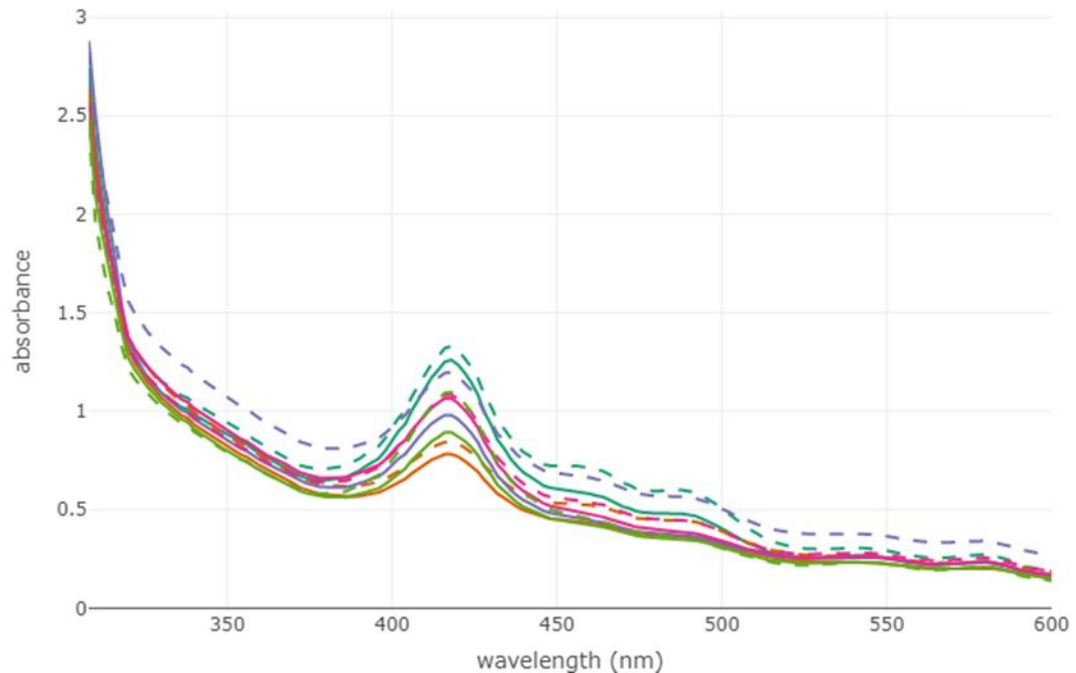
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458 **Authors' contributions**

459 AJ, CHA, AN and SMG designed the experiment. AJ, SU, AN and SMG sampled the
460 tissues. AJ, SU, SMG and CHA analyzed the results. AJ, SU, CHA, AN and SMG
461 discussed the results. AJ wrote the manuscript with significant input from the other
462 authors on their specific sections. All authors read and approved the final manuscript.



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