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Reducing the environmental impact of poultry breeding by genetic selection¹

H. de Verdal,* S. Mignon-Grasteau,† D. Bastianelli,‡ N. Mème,† E. Le Bihan-Duval,† and A. Narcy†²

*CIRAD, UMR 110 INTREPID, 34398 Montpellier Cedex 5, France; †INRA, UR83 Recherches Avicoles, F-37380 Nouzilly, France; and ‡CIRAD, UMR SELMET, 34398 Montpellier Cedex 5, France

ABSTRACT: Improving the sustainability of poultry production involves limiting its environmental impact and maintaining effectiveness. It has recently been shown that genetic selection on the ability of chickens to digest wheat at 23 d of age can decrease chicken excretion without decreasing BW at this age. The aim of this study was to check whether selection on digestibility modified excretion and growth performance over the whole production cycle. The 2 divergent lines selected for high (D+) and low (D-) apparent metabolizable energy corrected for 0 N balance (AMEn) values were compared with a reference line used at the beginning of the selection experiment (RL) to evaluate the potential excretion improvement that could be expected with such selection. These 3 lines were therefore compared for growth and excretion (raw and relative to feed intake, fresh and dry excreta weights, and moisture content of excreta) from 4 to 53 d. Between 4 and 7 d, 17 and 21 d, and 49 and 53 d, AMEn and N and P excretion rates were also compared between the 3 lines. Furthermore, body composition (breast meat and abdominal fat yields), bone breaking strength, and meat quality traits (lightness, redness, yellowness, and

ultimate pH) were compared between lines at 53 d. Over the whole rearing period, D+ birds excreted significantly less fresh and dry excreta (−56 and −61%) than D- and RL birds (−6 and −26%). Similarly, N and P excretion rates of D+ birds were 13% to 30% less than those of D- birds and 12% to 19% less than RL birds, depending on age. These excretion differences may be related to the differential development of the gastrointestinal tract. Differences between lines were already present at 7 d for relative gizzard weight and the weight of the upper to the lower part of the gastrointestinal tract ratio. Anatomic differences were maximum at 23 d for all traits except for relative weight of the duodenum. At slaughter age, BW, breast and fat yields, and meat color did not differ between D+ and RL birds, but D- birds were fatter than D+ and RL birds. Finally, ultimate meat pH was 1% to 2% greater in RL birds than in the D+ and D- lines. In conclusion, this study showed that selection of chickens for AMEn is a possible way to reduce the environmental impact of production over the whole rearing period without a negative impact on growth, body composition, or meat quality.

Key words: environment, poultry, selection

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INTRODUCTION

Environmental issues are of crucial importance for the poultry industry. Spreading chicken manure on fields causes environmental problems when undertaken

in excess in regions with a very dense production (Nahm, 2007; Bolan et al., 2010; Mignon-Grasteau et al., 2010a). Reducing poultry excretion and controlling its composition (especially in terms of N and P content) and physicochemical properties are therefore of major importance. It has long been accepted that digestibility is not controlled by genetics (Pym, 1990). Nevertheless, it has recently been shown that D+ birds (birds selected for high apparent ME corrected for 0 N retention; AMEn) excreted 48.4% and 36.7% less N and P, respectively, than the D- birds (birds selected for low AMEn) without modifying BW (Mignon-Grasteau et al., 2010b; de Verdal et al., 2011b). These differences in excretion rates

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²Corresponding author: agnes.narcy@tours.inra.fr

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could be explained by differential development of the gastrointestinal tract (de Verdal et al., 2011b). However, studies on these 2 divergent lines were performed on 3-wk-old chickens, which was the age at selection.

The aims of the present study were therefore to evaluate the impact of selection according to AMEn on excretion traits, especially N and P excretion, until birds reach a commercial BW and to estimate its indirect impact on growth, body composition, meat quality, and bone strength. The evolution of the gastrointestinal tract morphology with age was also studied to understand the mechanisms involved in differences between lines. The line used at the beginning of the selection experiment to create the D+ and D- lines was also included in the comparison to evaluate the potential practical improvement that can be expected from selection on digestibility. It is of note that this reference line (RL) has been selected for commercial purposes after the beginning of the selection experiment and is not properly a control line.

MATERIAL AND METHODS

The experiment was conducted under the guidelines of the French Ministry of Agriculture for Animal Research.

Birds and Housing

The experiment was performed on 144 birds (males and females), 48 birds from each line originating either from the ninth generation of the divergent selection experiment (8 generations of selection and 1 generation of maintenance) selected for high and low AMEn values or from the reference pure line used at the beginning of the selection experiment to create the D+ and D- lines and not selected according to AMEn in the meantime. The latter was therefore considered as a RL. At hatching, chicks were reared in cages of 4 or 5 birds. After 4 d, chicks were randomly allocated to individual cages in 2 rearing rooms. At 21 d, the remaining birds from each line were allocated to another rearing room, with cages appropriate to their BW. Environmental conditions were controlled for ventilation, lighting program (24 h light:0 h dark from d 1 to 6 and 18 h light:6 h dark from d 7 to 53), and temperature (from 33°C at d 1 to 20°C at d 53). Birds had free access to water and feed. Mortality was recorded daily. Birds were fed a starter and a grower wheat-based diet from hatching to 21 d and from 22 to 53 d, respectively (Table 1).

Recorded Traits

Animals were weighed at 4, 7, 18, 21, 49, and 53 d (BW_x, where *x* is age). Parameters of the growth curve were estimated using Gompertz equation, according to Laird et al. (1965)

$$BW_x = BW_0 \times \exp\left\{\left[\frac{iGR}{K}\right] \times \left(1 - \exp(-Kx)\right)\right\}, \quad [1]$$

where BW_x is the recorded BW at age *x*, BW₀ = the BW at hatching, iGR = the initial specific growth rate $[(1/BW_x) \times (dBW_x/dx) \text{ when } x \rightarrow 0]$, and K = the maturation rate or the exponential factor of decay of the specific growth rate.

These parameters were estimated by nonlinear regression with the NLIN procedure (SAS Inst., Inc., Cary, NC), taking into account all available BW from hatching to slaughter. Age at inflection (Ti), at which growth rate is maximal, was calculated as follows:

$$Ti = (1/K) \ln (iGR/K). \quad [2]$$

Individual feed intake (FI) was recorded weekly, and the cumulated FI for the whole rearing period was calculated. The N (NI) and P (PI) intakes for each bird were calculated as the product of FI and N and P concentrations in the diet.

Droppings were individually collected twice a week for the whole rearing period (between 4 and 53 d of age) using the method of individual total collection of excreta (Bourdillon et al., 1990). Total excreta were weighed and dried to obtain both fresh excreta weight (FEW) and dry excreta weight (DEW) for the whole rearing period. The

Table 1. Diet composition (g/kg)

Item	Starter	Grower
Ingredients, as-fed basis		
Corn	60.4	60.2
Wheat (Rialto)	525.0	600.0
Soybean meal 48	284.0	224.5
Corn gluten 60	31.0	19.0
Soybean oil	60.0	60.0
DL methionine	1.2	0.8
L-Lysine 78	2.2	2.1
Calcium carbonate	13.4	13.0
Dicalcium phosphate	15.8	13.4
Sodium chloride	3.0	3.0
Mineral and vitamin mix ¹	3.5	3.5
Robenidine ²	0.5	0.5
Chemical composition ³ (as-fed basis)		
AMEn, kcal/kg ¹	2943	3002
CP, %	20.5	18.4
Ca, %	1.11	0.94
tP, %	0.66	0.60
NPP, %	0.42	0.37

¹Supplied per kilogram of diet: 0.5 mg Co, 16 mg Cu, 47 mg Fe, 1.6 mg I, 65 mg Mn, 0.2 mg Se, 72 mg Zn, 12,000 IU retinyl acetate, 3,440 IU cholecalciferol, 80 mg DL- α tocopheryl acetate, 4 mg thiamine, 6.4 mg riboflavin, 20 mg calcium pantothenate, 0.02 mg vitamin B12, 4 mg menadione, 5.6 mg pyridoxine hydrochloride, 0.4 mg folic acid, 0.24 mg biotin, 80 mg niacin, 440 mg choline, 40 mg antioxidant.

²Robenz, Alpharma Animal Health, Bridgewater, NJ.

³Calculated (Sauvant et al., 2004).

DEW:FI ratio was calculated for the whole rearing period. Freeze-drying was used as it has been found to minimize N conversion and NH_3 loss during processing (Mahimairaja et al., 1990). The water excreta content (**WEC**) was measured as the difference between FEW and DEW divided by FEW. Three balance trials were performed between 4 and 7 d (**B1**), 18 and 21 d (**B2**), and 49 and 53 d (**B3**) to measure digestibility and N and P excretion. Body weight gain (**WG**) and feed conversion ratio (**FCR**) were calculated for each balance trial. The AMEn and N excretion (**NEx**) were measured for all birds using near-infrared spectrophotometry (**NIRS**; Foss NIRSystems, Inc., Silver Spring, MD), using the method of Bastianelli et al. (2010). Phosphorus excretion (**PE**) was measured by colorimetric analysis. The ratios of NEx to PE, NEx to NI, PE to PI, NEx to WG, and PE to WG were calculated for each balance trial.

At 7, 21, and 53 d, 16 birds of each line were euthanized by intracardiac injection of 1.5 mL/kg BW of pentobarbital (Sanofi, Marne la Coquette, France). The proventriculus and gizzard were excised, cleaned, and weighed (**PRW** and **GZW**, respectively). The small intestine was cut into 3 parts: duodenum (from pylorus to pancreatic loop), jejunum (from the pancreatic loop to Meckel's diverticulum), and ileum (from Meckel's diverticulum to the ileocecal junction). Segment lengths were measured. Segments were then cleaned and weighed (**DW**, **JW**, and **IW**, for duodenum, jejunum, and ileum weights, respectively). The weight to length ratio of each segment (**DD**, **JD**, and **ID**, respectively) was also calculated as an indicator of intestine density (Taylor and Jones, 2004). The ratio of PRW and GZW to small intestine weight (**PVG/INT**) was calculated as an efficient parameter for predicting AMEn variations (Garcia et al., 2007). All the data regarding organ weight were expressed per kilogram of BW to avoid any confusion between the gastrointestinal tract (**GIT**) weight trait differences and the potential BW differences between lines. Boluses in the gizzard and ileum were collected, and their pH was measured with a portable pH meter (model 506, Crison Instruments, SA, Barcelona, Spain) equipped with a Xerolyte electrode. The right tibia was removed and cleaned of muscle and then stored at -20°C until measurement. After bone defrosting, a 3-point loading test using an Instron Universal Testing Instrument (INSTRON, Guyancourt, France) was employed to measure the bone breaking strength (**BBS**).

At 24 h postmortem, the breast meat (Pectoralis major and P. minor) and abdominal fat were excised and weighed for each bird. The breast meat (**BM**) and abdominal fat (**AF**) yields were calculated. The ultimate pH of the meat (**pHu**) was recorded by direct insertion of the Xerolyte electrode into the muscle. Breast meat color was measured at 24 h postslaughter using a

Miniscan spectrophotometer (Hunterlab, Reston, VA) CIE $L^*a^*b^*$ system, where L^* represents the lightness, a^* is the redness, and b^* is the yellowness of the meat; greater L^* , a^* , and b^* values correspond to paler, redder, and yellower meat, respectively.

Statistical Analyses

All data were analyzed according to the General Linear Models (**GLM**) procedure of SAS, including a line effect, with all other effects being nonsignificant. For BW, sex effect was significant and thus was included in the model. Homogeneity of variances was not respected between groups because of a greater variance of D- birds. Analyses of variance were thus weighted by inverse of variance of each group. Differences were considered significant when the *P*-value was less than 0.05.

RESULTS

The elementary statistics for growth curve parameters, cumulated feed consumption, feed efficiency, and cumulated excretion characteristics for the whole rearing period (from hatching to 53 d of age) for both D+ and D- and the RL are reported in Table 2. Weekly analyses are reported in Figs. 1 and 2 and Table 3.

The selection criterion (AMEn) was always 4.6% to 25.0% greater ($P < 0.05$) in D+ than in D- whatever the age considered, with the difference being greatest at 3 wk (i.e., age for the selection experiment). The RL was intermediate, closer to D+ than D-, with differences being significant ($P < 0.05$) at 3 wk but not at 1 and 7 wk ($P = 0.06$ and $P = 0.07$, respectively).

Whatever the age, D- birds consumed 20.6% to 55.9% more ($P < 0.05$) feed than D+ birds. When compared with the RL line, D- birds still had a 12.6% to 38.2% greater FI, but only from 21 to 53 d. Furthermore, FI was greater ($P < 0.05$) in RL than in D+ birds between 7 and 42 d, with the former consuming 9.2% to 30.4% more than the latter.

Body weight was greater ($P < 0.05$) in RL birds than in D+ and D- birds until 42 d, but the difference did not continue after this age, with all 3 lines reaching similar BW at 53 d. Moreover, the line effect was not significant for the growth curve parameters. As a result of FI and growth differences, the FCR for D- birds was 131.2% greater ($P < 0.05$) than D+ between 17 and 42 d of age. The FCR of D+ was also 14.3% to 22.3% less than ($P < 0.05$) in the RL line between 7 and 28 d. After 42 d, the FCR did not differ between the 3 lines. As a result, the FCR over the whole rearing period was less ($P < 0.05$) in D+ (2.00) than in D- (2.93) line.

With regard to manure characteristics, D+ birds excreted less ($P < 0.05$) fresh and dry excreta than

D- birds, whatever the age, from 36.2% to 83.8% for fresh and from 40.0% to 76.3% for dry excreta weight, with the difference being greater between 21 and 35 d. The specific increase in FI, FCR, FEW, FEW/FI, DEW, and DEW/FI observed at 3 wk of age in D- birds corresponded to the age of selection. At the same time, birds were transferred from their initial room to another, which can partly explain the greater difference.

The RL birds were intermediate but produced 13.2% to 66.2% less fresh and 19.7% to 63.2% less dry excreta than D- birds. For all measurements, the greatest differences were observed between 21 and 35 d (i.e., around the selection age). Furthermore, except between 42 and 49 d, D+ birds produced 17.2% to 41.6% less ($P < 0.05$) dry excreta than RL birds, and the differences decreased with the age. The differences between D+ and RL birds were not significant for fresh excreta weight.

For the whole rearing period, the cumulated fresh excreta weight was reduced ($P < 0.05$) by 56.2% in D+ and by 53.3% in RL birds compared with D- birds. The total production of dry excreta was, in turn, reduced ($P < 0.05$) in D+ birds by 26.3% compared with RL birds and by 61.2% compared with D- birds. This difference between results for fresh and dry excreta was related to a significantly ($P < 0.05$) different water content (WC) of excreta between lines. Water content was greater for D+ than D-, with the differences being significant around the selection age (i.e., between 14 and 28 d). The RL birds had the lowest WC and differed significantly from D+ birds from 14 to 49 d.

When excretion weights were corrected for FI to take into account the FI differences between lines, the results were similar, with D- birds excreting significantly more fresh excreta than RL and D+ and the dry excreta weights in relation to FI for the

3 genotypes being significantly ($P < 0.05$) different from each other. For excretion traits, NEx/NI was 15.1% less ($P < 0.05$) in D+ than in the RL line during B1, but RL did not differ from D-. Similarly, during B1, D+ birds had 15.7% and 18.4% less ($P < 0.05$) P excretion rates than D- and RL, respectively, with the last 2 being not different. During B2, NEx/NI and PE/PI were 17.5% to 30.0% less in D+ than in D- and RL birds. As for AMEn, the greatest differences between lines were observed at 3 wk. Finally, NEx/PE was 7.5% less ($P < 0.05$) in D- than in D+ birds during B1 but 16.2% greater during B2. The NEx/PE ratio for RL birds did not differ from D- and D+ during B1. During B2, RL birds differed significantly from D- birds, with the NEx/PE ratio being 8.2% less than in D- birds. During B3, the ranking was the same, but differences between lines were not significant. Excretion of N and P in relation to WG differed significantly ($P < 0.05$) between the 3 lines at selection age, with D+ excreting 28.6% less P and 33.0% less N per gram of WG than RL birds and 43.7% and 51.4% less P and N than D- birds. Differences in PE/WG between lines were already present at 1 wk, with D+ showing reduced excretion compared with the other 2 lines (−10.5% and −18.4% when compared with RL and D-, respectively). At this age, D+ also excreted 12.1% less ($P < 0.05$) N per gram of WG than D- birds. At 7 wk, there was no longer a significant difference between lines in NE/WG and PE/WG.

Gastrointestinal tract morphology is shown in Table 4. The relative PRW was 13.9% and 71.2% greater ($P < 0.05$) in D+ birds than in RL birds at 7 and 21 d, respectively, but the difference did not persist at 53 d. D- birds presented intermediate values at all ages. Relative gizzard weight was greater in D+ birds than in RL at all ages. Relative gizzard weight was also

Table 2. Basic statistics (least squares means $\pm$ SE) for BW at 53 d, growth curve parameters, feed intake, feed conversion ratio, and excretion characteristics for the whole rearing period for each line (n ranging from 42 to 47)

Variable ¹	D+	D-	RL	SEM	Line effect ²		
					D+ vs. D-	D+ vs. RL	D- vs. RL
BW53, g	1,974	1,893	1,989	281	NS	NS	NS
iGR, L/d	0.180	0.175	0.187	0.014	NS	NS	NS
K, L/d	0.038	0.038	0.041	0.004	NS	NS	NS
Ti, d	41.6	40.9	39.2	5.99	NS	NS	NS
FI, g	4,544	6,150	5,032	1,509	*	*	*
FCR, g/g	2.00	2.93	2.26	0.80	*	NS	NS
FEW, g	3,103	7,085	3,309	4,191	*	NS	*
DEW, g	897	2309	1217	1169	*	*	*
FEW/FI, g/g	0.67	1.07	0.64	0.104	*	NS	*
DEW/FI g/g	0.24	0.43	0.29	3.08	*	*	*
WC, %	68.9	66.4	62.7	0.33	NS	*	*

¹BW53 = BW at 53 d; iGR = initial specific growth rate; K = maturation rate; Ti = age of inflection; FI = feed intake; FCR = feed conversion ratio; FEW and DEW = fresh and dry excreta weight; FEW/FI and DEW/FI = fresh and dry excreta weight relative to feed intake; WC = water excreta content.

²NS = $P > 0.05$; * = $P < 0.05$.

significantly greater in D+ than in D- at 7 and 21 d ($P = 0.07$ at 53 d) but lighter ($P < 0.05$) in RL compared with D- at 7 d of age. Relative intestinal segment weights were similar in D+ and RL birds at 7 and 53 d, except for DW at 7 d, but D+ had a 23.3% and 23.2% lighter ($P < 0.05$) jejunum and ileum, respectively, than RL birds at 21 d. Furthermore, D+ birds had less dense ($P < 0.05$) intestines than RL birds whatever the age, with differences ranging from 15.9% to 34.5%. D+ also had 12.0% to 30.8% lighter and 16.7% to 25.0% less dense intestines than D- birds at 21 and 53 d. Finally, intestinal weights of D- and RL birds did not differ from

each other at 7 and 21 d, and densities did not differ at any age, except for IW at 7 d and JD at 21 d. The weights of intestinal segments were heavier in D- than in RL birds at 53 d. The differences in the development of the upper and lower parts of the GIT resulted in greater PVG/INT ratio values in D+ birds than in RL and D- birds at all ages, with differences being not significant between D- and RL birds. Differences were greatest at 21 d, with values for D+ birds being twice as high as for RL and D- at this age. Finally, the pH of gizzard content was lower ($P < 0.05$) in D+ than in D- and RL birds at 21 d. The pH of ileum content was higher ($P < 0.05$) in D+ birds than in D- birds at 21 and 53 d, but lower than in RL birds at 7 d.

Body composition (BMV and AFY) and BBS at 7, 21, and 53 d are shown in Table 5. The ranking of lines for BMV and AFY changed with age, with D+ birds having 18.4% and 22.4% less ($P < 0.05$) BMV than D- and RL birds at 7 d, respectively, but 8.32% greater ($P < 0.05$) BMV than D- birds at 53 d. At 53 d, AFY was less in RL and D+ birds than in D- birds, but RL were fatter than D+ and D- birds at 21 d.

Meat color characteristics (L^* , a^* , and b^*) at 53 d are shown in Table 6. The pHu was 1.06% higher ($P < 0.05$) in RL than in D+. Meat color was not significantly different between the 3 lines.

The BBS was greater ($P < 0.05$) in RL than in D- birds at 7 and 21 d, but the difference was not found at 53 d.

DISCUSSION

As expected, D+ birds showed significantly greater AMEn values for the 3 balance trials than D- birds, which was consistent with previous studies at 23 d of age (Mignon-Grasteau et al., 2004; de Verdal et al., 2011b). They had also greater AMEn than RL birds at 7 and 21 d. Compared with the selection of the RL based on commercial traits, it turns out that the selection for AMEn deteriorated the digestibility in D- birds more strongly than it improved it in D+ birds. This observation can be easily explained by the fact that starch digestibility nearly reached the maximum value of 100% (i.e., 96.3% in D+ vs. 88.4% in D- birds; Mignon-Grasteau et al., 2004), reducing the improvement margin regarding feed digestibility in D+. In addition, it should be noticed that in contrary to the phenotypic values, the evolution of the genetic values between the divergent lines was symmetric.

The differences were greatest at 3 wk for most traits, which could be expected as 3 wk corresponds to selection age. Differences could probably be increased further by also selecting for AMEn when older, although the additional progress that could be expected would

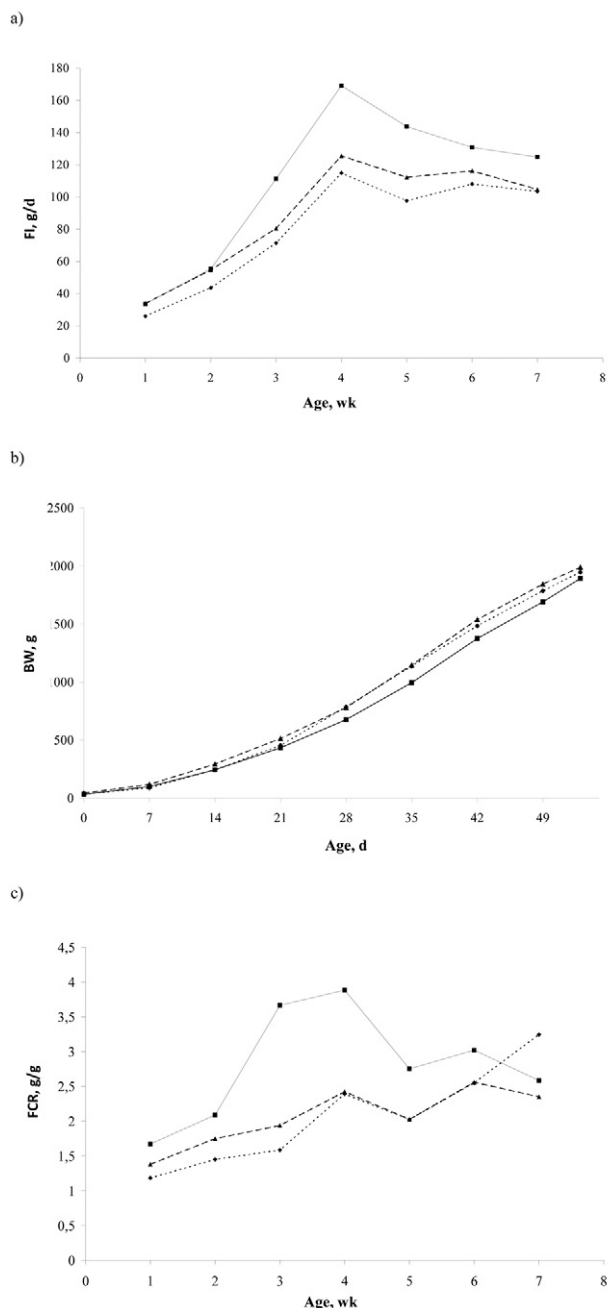


Figure 1. Effect of divergent line (D+, dotted line, and D-, solid line) and reference line (CL, dashed line) on (a) feed intake (FI), (b) BW, and (c) feed conversion ratio (FCR) for each rearing week. D+: line selected for high AMEn; D-: line selected for low AMEn; RL: reference line

probably be smaller. Indeed, AMEn is strongly linked to the development of the GIT, which is greatest during the first weeks of bird life (Palo et al., 1995). This assumption was consistent with the high genetic correlations between AMEn and gizzard and intestine weights (de Verdal et al., 2011a). The secreting and grinding activity of the proventriculus and gizzard increase with their weight (Svihus, 2011), which improves nutrient availability in the small intestine and thus digestibility (Wu and Ravindran, 2004). Our results indicated that D+ birds presented greater development of the upper part of the GIT at 7 d compared with D- and RL birds, which could

be explained by a greater sensitivity of the D+ to a wheat-based diet. In contrast, differences between lines in the weight of the lower part of the GIT appeared more marked at 21 and 53 d, which was consistent with the hypothesis that the development of the intestine occurs in response to the development of the gizzard and proventriculus (de Verdal et al., 2010). These differences resulted in a greater PVG/INT ratio in D+ than in D- and RL birds at all ages, which was consistent with previous results (Rougière et al., 2009) in which this ratio was considered as a relevant predictor of digestion efficiency in broilers. Until 42 d of age, D+ and D- birds presented a lighter BW than RL birds,

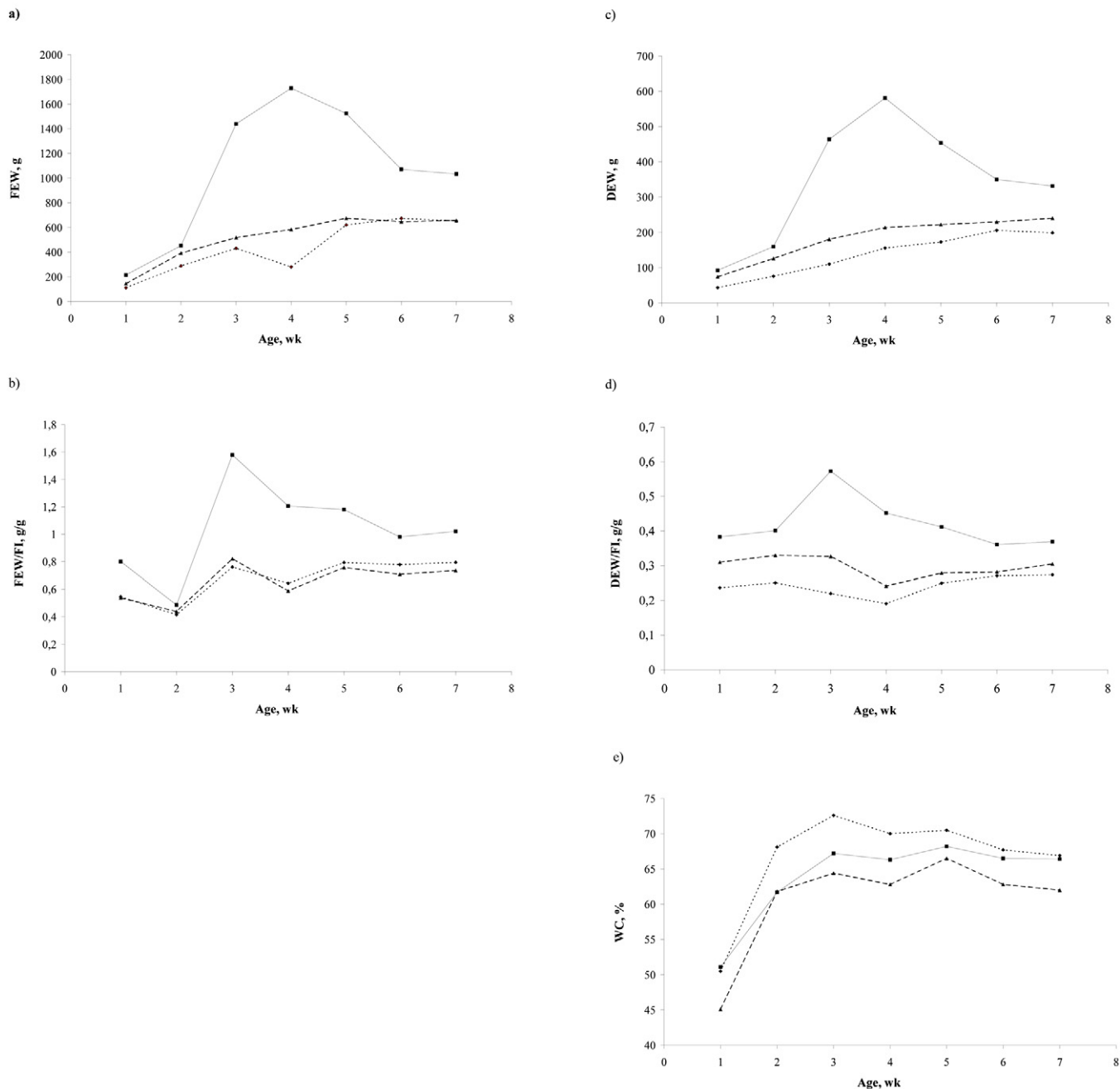


Figure 2. Effect of divergent line (D+, dotted line, and D-, solid line) and reference line (CL, dashed line) on (a) fresh excreta weight (FEW), (b) fresh excreta weight:feed intake (FEW/FI), (c) dry excreta weight (DEW), (d) dry excreta weight:feed intake (DEW/FI), and (e) water content (WC) for each rearing week. D+: line selected for high AMEn; D-: line selected for low AMEn; RL: reference line.

Table 3. Basic statistics (least squares means $\pm$ SE) for AMEn and excretion composition, NE/NI, NE/WG, PE/PI, PE/WG, and NE/PE, performed at each balance trial for each line¹

Age ²	Line	AMEn	NE/NI	NE/WG	PE/PI	PE/WG	NE/PE
B1	D+	3502	0.28	15.24	0.26 $\pm$ 0.01	2.19 $\pm$ 0.06	7.04 $\pm$ 0.15
	D-	3347	0.32	17.3	0.31 $\pm$ 0.01	2.69 $\pm$ 0.06	6.51 $\pm$ 0.15
	RL	3397	0.33	16.1	0.32 $\pm$ 0.01	2.45 $\pm$ 0.07	6.61 $\pm$ 0.15
	SEM	197	0.07	2.96	0.07	0.50	0.70
	D+ vs. D- ³	*	NS ($P = 0.08$)	*	*	*	*
	D+ vs. RL	NS ($P = 0.06$)	*	NS	*	*	NS
	D- vs. RL	NS	NS	NS	NS	*	NS
B2	D+	3229	0.42	19.7	0.47	3.60	5.49
	D-	2583	0.60	40.5	0.63	6.40	6.38
	RL	3038	0.51	29.4	0.57	5.04	5.86
	SEM	462	0.08	13.2	0.10	2.07	0.71
	D+ vs. D-	*	*	*	*	*	*
	D+ vs. RL	*	*	*	*	*	NS ($P = 0.06$)
	D- vs. RL	*	*	*	NS	*	*
B3	D+	3,073	0.58	59.6	0.59	9.24	5.03
	D-	2,730	0.73	58.1	0.68	10.6	5.54
	RL	2,966	0.66	38.4	0.66	7.75	5.12
	SEM	222	0.10	15.8	0.13	3.35	0.75
	D+ vs. D-	*	*	NS	NS	NS	NS
	D+ vs. RL	NS	NS	NS	NS	NS	NS
	D- vs. RL	*	NS	NS	NS	NS	NS

¹AMEn = apparent ME corrected for 0 N retention (kcal·kgMS⁻¹); NE/NI and PE/PI = N and P excreted relative to N and P intake (g/g); NE/WG and PE/WG = N and P excreted relative to BW gain (g/kg); NE/PE = N excreted relative to P excreted (g/g).

²B1: from 4 to 7 days of age, B2: from 18 to 21 days of age and B3: from 49 to 53 days of age.

³NS = $P > 0.05$; * = $P < 0.05$.

Table 4. Basic statistics (least squares means $\pm$ SE) for all the measures of the gastrointestinal tract morphology for each line and age of slaughter¹

Age, d	Line	PRW	GZW	DW	JW	IW	PVG/INT	DD	JD	ID	pH gizzard	pH ileum
7	D+	11.5	46.3	18.8	23.1	16.0	1.00	0.11	0.07	0.05	3.55	7.46
	D-	10.7	40.3	14.8	25.1	17.8	0.89	0.12	0.09	0.07	3.46	7.39
	RL	10.1	36.0	15.9	24.2	15.2	0.85	0.13	0.10	0.07	3.34	7.69
	SEM	1.44	4.88	2.39	4.20	2.29	0.11	0.15	0.015	0.021	0.20	0.26
	D+ vs. D- ²	NS	*	*	NS	NS ($P = 0.10$)	*	NS	*	*	NS	NS
	D+ vs. RL	*	*	*	NS	NS	*	NS ($P = 0.06$)	*	*	NS	*
	D- vs. RL	NS	*	NS	NS	*	NS	NS ($P = 0.10$)	NS	NS	NS	*
21	D+	10.0	26.2	12.5	18.1	12.6	0.84	0.25	0.19	0.15	3.46	7.51
	D-	6.86	17.9	14.2	24.9	18.2	0.43	0.30	0.25	0.20	3.92	6.91
	RL	5.84	15.9	13.3	23.6	16.4	0.41	0.30	0.29	0.21	3.97	7.18
	SEM	1.77	4.47	1.67	2.91	2.85	0.08	0.03	0.03	0.03	0.51	0.35
	D+ vs. D-	*	*	*	*	*	*	*	*	*	*	*
	D+ vs. RL	*	*	NS	*	*	*	*	*	*	*	*
	D- vs. RL	NS	NS	NS	NS	NS	NS	NS	*	NS	NS	NS ($P = 0.10$)
53	D+	3.37	12.2	5.61	10.3	8.19	0.66	0.37	0.32	0.26	4.85	7.51
	D-	2.94	10.7	7.08	13.1	11.4	0.44	0.47	0.40	0.34	5.00	7.28
	RL	2.74	9.71	5.64	11.2	8.99	0.49	0.44	0.38	0.31	5.24	7.46
	SEM	0.79	1.36	1.18	1.99	1.71	0.08	0.08	0.08	0.06	0.65	0.22
	D+ vs. D-	NS	NS ($P = 0.09$)	*	*	*	*	*	*	*	NS	*
	D+ vs. RL	NS	*	NS	NS	NS	*	*	*	*	NS ($P = 0.07$)	NS
	D- vs. RL	NS	NS	*	*	*	NS	NS	NS	NS	NS	NS ($P = 0.07$)

¹PRW = proventriculus weight relative to BW (g/kg); GZW = gizzard weight relative to BW (g/kg); DW, JW, and IW = duodenum, jejunum, and ileum weights relative to BW (g/kg); PVG/INT = proventriculus and gizzard weight relative to small intestine weight (g/g); DD, JD, and ID = duodenum, jejunum, and ileum density (g/cm); pH gizzard and pH ileum = pH measurements of the content of the gizzard and ileum compartments.

²NS = $P > 0.05$; * = $P < 0.05$.

but the differences were not maintained at 53 d of age. However, the final BW of D- was achieved with 35.3% and 22.2% greater FI than D+ and RL birds, respectively. For the same BW at 53 d, D+ birds consumed 488 g less feed than RL birds. Consequently, for a poultry farm of 20,000 chickens, 9.76 tons of feed could be saved for each hatch if rearing D+ birds rather than RL birds, and that amount is not negligible in terms of saving natural resources.

Mignon-Grasteau et al. (2010b) and de Verdal et al. (2011b) showed that D- birds excreted 70.2% and 117.6% more FEW and DEW, respectively, at 3 wk than D+ birds. In the present study, D- birds excreted 73.4% and 98.7% more FEW and DEW than D+ birds during B2, which was consistent with previous results. These differences were intensified if we took into account the whole rearing period, with 128% and 157% greater FEW and DEW for D- than for D+ birds. These substantial differences between lines were probably at least partly linked to variations in FI between genotypes. Indeed, when correcting DEW for FI, excretion of the D+ birds was 44.2% and 17.4% less than D- and RL birds, respectively. The fact that differences between D+ and RL birds were significant for dry excreta but not for fresh excreta weights could be explained by the 9.9% greater water content in the excreta of D+ than in RL birds for the whole rearing period. This greater

WC of D+ birds had already been found at 3 wk (de Verdal et al., 2011b) and suggests that litter from D+ birds would be wetter than litter from D- or RL birds, thus increasing the risks of ammonia emissions (Miles et al., 2011). Excreta also differed in composition because N and P excretion rates were significantly different between the 3 lines. The D+ birds excreted significantly less N and P for the same BW gain during B1 and B2 than D- and RL birds, with greater N and P use efficiency. Over the whole rearing period, D+, D-, and RL birds excreted 53.7, 111.1, and 64.4 g of N and 9.77, 18.8, and 11.4 g of P, respectively. For a poultry house of 20,000 chickens, rearing D+ birds instead of RL birds would therefore result in a reduction of 215 and 31.9 kg of N and P excreted in litter, respectively (i.e., -968.4 and -143.6 kg of N and P per year at 4.5 hatches per year, which is the usual number of hatches for this type of chicken). Taking into account European regulations for spreading N and P (170 kg of N·ha⁻¹·yr⁻¹ and 80 kg P₂O₅·ha⁻¹·yr⁻¹) and the fact that around 50% of N is lost between excretion and spreading (ammonia volatilization), 14.2, 29.4, and 17.1 ha would be required to spread N and 25.2, 48.5, and 29.3 ha would be required to spread P for D+, D-, and RL chicken farms, respectively.

Poultry manure had an average N/P ratio of 2 (Gilbertson et al., 1979; Sharpley, 1999), whereas the N/P requirement of most crops is almost 8 (White and Collins, 1982; Moore et al., 1995). However, most fields have an excess of N and P in the soil, mainly because of repeated manure application over many years. According to the French and European regulations, the ideal N/P ratio when manure is spread on fields is 2.13, which is far below the crop requirements. Taking into account the litter and the water part of the manure spread (Institut Technique de l'Aviculture, 2001) and also the volatilization of NH₃, the N/P₂O₅ ratio found in the manure in the present study ranged from 1.04 to 1.50. The proportion of P₂O₅ compared with N was thus unbalanced. Nitrogen loss should be limited to improve the balance between N and P in manure. This

Table 5. Basic statistics (least squares means ± SE) for BMY, AFY, and BBS for each line and age of slaughter¹

Age, d	Line	BMY	AFY	BBS
7	D+	2.49	0.28	18.8
	D-	3.05	0.31	18.3
	RL	3.21	0.40	22.0
	SEM	0.46	0.12	3.10
	D+ vs. D- ²	*	NS	NS
	D+ vs. RL	*	*	NS (<i>P</i> = 0.06)
	D- vs. RL	NS	NS	*
21	D+	5.15	1.11	90.5
	D-	5.16	1.06	79.2
	RL	5.09	1.85	108
	SEM	0.53	0.52	24.4
	D+ vs. D-	NS	NS	NS
	D+ vs. RL	NS	*	NS (<i>P</i> = 0.08)
	D- vs. RL	NS	*	*
53	D+	6.25	3.94	227
	D-	5.77	4.61	206
	RL	6.38	3.87	222
	SEM	0.30	0.64	53.2
	D+ vs. D-	*	*	NS
	D+ vs. RL	NS	NS	NS
	D- vs. RL	*	*	NS

¹BMY = breast meat yield (%); AFY = abdominal fat yield (%); BBS = bone breaking strength (N).

²NS = *P* > 0.05; * = *P* < 0.05.

Table 6. Basic statistics (least squares means ± SE) for breast meat and bone quality traits for each line at 53 d of age

Trait ¹	D+	D-	RL	SEM	Line effect ²		
					D+ vs. D-	D+ vs. RL ²	D- vs. RL
L*	47.2	48.9	47.3	3.41	NS	NS	NS
a*	0.41	-0.30	0.06	1.60	NS	NS	NS
b*	12.9	11.4	11.5	2.65	NS	NS	NS
pHu	5.69	5.69	5.75	0.11	NS	*	NS

¹ L* = lightness; a* = redness; b* = yellowness; pHu = ultimate pH of the breast.

²NS = *P* > 0.05; * = *P* < 0.05.

could be achieved by decreasing manure pH or WC, which might limit the volatilization of N during rearing and storage (Lefcourt and Meisinger, 2001). As the greater WC of excreta of D+ birds should enhance N volatilization, N losses should be directly recorded to draw definitive conclusions.

The selection experiment based on AMEn modified nutrient retention rates and could thus impact body composition and perhaps also meat and bone quality. In the present study, BMY was less in D+ than in D- and RL at 7 d. This is consistent with more intense development of the GIT during the first days of life in D+ than in D- and RL birds, which implies that D+ birds have proportionally less energy to invest in muscle development. Differences in BMY were not significant at 3 wk of age, which is consistent with phenotypic results at 23 d of age and with the low genetic correlation estimated between AMEn and BMY ($r_g = 0.05$; H. de Verdal, unpublished data). The greater BMY and reduced AFY for D+ and RL birds compared with D- birds at 53 d could be explained by the differences in feed consumption and the metabolic consequences. Indeed, an increase in insulin secretion by the pancreas in response to greater feed consumption is known to increase fat deposition and, conversely, to decrease muscle protein deposition (Tomas et al., 1998; Buyse et al., 1999). Future studies should also examine the secretion concentrations of gastrointestinal hormones, which could explain the differences in body composition between D- and RL birds. Furthermore, Tomas et al. (1998) showed that the improvement in the efficiency of feed use is negatively linked to proteolysis activity and increases BMY and reduces AFY (Huyghebaert and Pack, 1996). Because D+ and RL birds retained more N than D- birds, the D+ line should show reduced proteolysis and increased breast meat mass. Thus, selection on high AMEn values improved BMY and AFY through a reduction in NE/WG as compared with D- birds and did not affect these traits compared with RL birds.

In the present study, RL birds showed 1.06% higher pHu than D+ birds, but the meat color traits (L^* , a^* , and b^*) were not significantly different between the 3 lines. The differences in pHu between lines seemed too small to cause noticeable changes in meat quality, and selection for AMEn should therefore not affect breast meat quality. However, according to Le Bihan-Duval et al. (2001) and Duclos et al. (2007), the breast meat of these 3 lines was rather acidic ($\text{pHu} < 5.7$), which could result in impairment of the technological processing ability.

Bone strength is important from an economic and welfare point of view. Although RL birds showed 17.0% to 36.4% greater BBS than D+ and D- at 7 and 21

d, chickens of the 3 lines did not show any differences in BBS at slaughter age. This was consistent with the very high genetic correlation between BBS and BW at 23 d (0.93; H. de Verdal, unpublished data) and with the fact that RL birds were heavier than D+ and D- at 7 and 21 d but not at 53 d. These results thus suggest that selection did not modify bone strength, a major risk of fracture at slaughter age.

Conclusion

This study clearly showed that selecting chickens for high AMEn values could improve DM use and retention of N and P, thus reducing the environmental impact of chicken excretion over the whole rearing period. Furthermore, such selection would not affect body composition and meat and bone quality traits at slaughter age compared with the reference genotype. This could be at least partly explained by the differential development of the GIT between the lines. Comparison of the hormone profiles of the 3 lines might help us to understand the mechanisms underlying the changes in terms of excretion, GIT morphology, and body composition observed in the present study.

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