

Genetic background of body reserves in laying hens through backfat thickness phenotyping

Nicolas Bédère^{1*}, Joëlle Dupont², Yannick Baumard³, Christophe Staub⁴, David Gourichon³, Frédéric Elleboudt⁵, Pascale Le Roy¹, Tatiana Zerjal⁶

¹ PEGASE, INRAE, Institut Agro, 35590, Saint Gilles, France

² CNRS, IFCE, INRAE, Université de Tours, PRC, 37380, Nouzilly, France

³ INRAE, PEAT, 37380, Nouzilly, France

⁴ INRAE, PAO, 37380, Nouzilly, France

⁵ INRAE, Université de Tours, CHU de Tours, Plate-forme PIXANIM, Nouzilly, France

⁶ Université Paris-Saclay, INRAE, AgroParisTech, GABI, 78350, Jouy-en-Josas, France

*Correspondence: nicolas.bedere@inrae.fr

ABSTRACT

In this study, we pursued three primary objectives: firstly to test and validate the phenotyping of backfat thickness as an indicator of the overall fatness of laying hens; secondly, to estimate genetic parameters for this trait; thirdly, to study the phenotypic and genetic relationships between this trait and other traits related to production and body composition. To address these questions, hens from two lines under divergent selection for residual feed intake, were phenotyped for body weight, body composition traits (backfat, total fat volume, and blood adipokines levels), and egg number. Linear mixed models enabled to estimate variance components and calculate genetic parameters. The two lines largely differed in body fatness: the efficient line had larger backfat and lower chemerin levels compared to the inefficient line. However, there were no significant differences between the two lines concerning body weight, total fat volume, other blood adipokines levels (adiponectin, ghrelin, and visfatin), and egg production. The genetic parameter estimation revealed moderate heritability (0.38 and 0.42) for backfat and body weight, high heritability (higher than 0.80) for blood adipokines levels and low heritability (0.24 and 0.27) for egg production and total fat volume. The Backfat and total fat volume were genetically highly and positively correlated (0.91). The body weight and total fat volume were also highly positively correlated (0.67). However, backfat and body weight were moderately positively correlated (0.39). The genetic correlation between backfat and egg number was moderate and negative. In conclusion, backfat could provide additional genetic information to that of the body weight as a selection criterion for body reserves. However, its correlation with laying performance should be taken into account to avoid undesired responses to selection.

Keywords: body composition, body reserves, backfat thickness, ultrasonography, CT-scan, adipokines, genetic correlations, heritability, laying hens

35 One of the major challenges of the egg production sector is to extend the egg production period of
36 laying hens, for ethical, environmental, and economical reasons (reviews: Bain *et al.* 2016; Preisinger 2018).
37 Laying hens have been selected for laying criteria for more than 60 years, resulting in animals able to
38 maintain profitable egg production from approximately 20 to 80 weeks of age. The priority of, at least
39 European stakeholders, is now to extend the laying period to 100 weeks of age, with the aim of producing
40 500 eggs per hen. This would further dilute the economic and environmental costs related to non-
41 productive life periods (such as growth and laying pauses), and reduce the number of hens by decreasing
42 the breeder stock.

43 The late-laying period, which goes beyond 80 weeks of age, remains relatively unexplored for what is our
44 understanding of its physiology, nutrition, and genetics. The existing literature on this laying period is
45 notably scarce, providing limited insights into these aspects, therefore, further research and investigation
46 are warranted to enhance our knowledge in these areas. Egg production is a major nutrient expenditure
47 for layers (energy, protein, calcium...) and about 25% of the gross energy intake goes to egg production
48 (Larbier and Leclercq, 1992; Luiting, 1990). Excessive investment in egg production may lead to different
49 metabolic diseases, and the longer the production cycle, the higher the risk. Risk factors mainly involve
50 genetics, physiology, nutrition and management (Bain *et al.*, 2016). For instance, extending the laying
51 period makes hens more likely to develop hepatic steatosis, a disease responsible for egg production drop
52 and obese conditions (Bain *et al.*, 2016). Therefore, we need to monitor both egg production and fattening
53 in laying hens, to select balanced hens that can ensure cost-effective egg production while maintaining
54 optimal fatness.

55 The monitoring of egg production and the pedigree of laying hens has been facilitated by cage-rearing
56 systems. In some regions of the world, cages are about to be banned and technical solutions are emerging
57 for individual recording systems and relevant selection criteria for egg production in alternative systems
58 (Bécot *et al.*, 2021). Regarding fatness in chicken, like in other species, the gold standard and most common
59 method to determine body composition are lethal and destructive because it is either a dissection with
60 adipose tissue weighing or a chemical analysis of the shredded body. This phenotyping method is
61 unsatisfactory because it requires the euthanasia of the animal, which raises ethical and practical
62 problems. Indeed, the animals can no longer be used for genetic selection, except as collateral information
63 when using allometric sequential slaughter designs to evaluate both states and dynamics of body reserves.

64 Alternative and non-invasive methods are now available to determine body composition in various species
65 (Lerch *et al.*, 2021; Staub *et al.*, 2019; Xavier *et al.*, 2022). In poultry, tomography has proven to be
66 sufficiently accurate to be considered as a reference method for body composition, with phenotypic
67 correlations above 0.80 in broilers (Cobo *et al.*, 2015; Mellouk *et al.*, 2018b). However, the routine use of
68 tomography is difficult to implement on a large number of animals as it cannot easily be performed on the

69 farm and because it requires sedation of the animal, which is time-consuming and costly and not without
70 risk for the animals. The methods relying on ultrasonography have been used effectively to assess body
71 fatness in chickens. A specific region was identified on top of the *synsacrum* where subcutaneous adipose
72 tissue thickness was highly correlated to chemical analyses of the shredded body ($r=0.92$; Mellouk *et al.*
73 2018), to the abdominal fat pad weight by dissection ($r=0.86$; Mellouk *et al.* 2018) and the body fat volume
74 estimated by tomography ($r>0.84$; Mellouk *et al.* 2018; Grandhaye *et al.* 2019). So far, body fatness traits
75 recorded by ultrasonography were all tested on broilers while no data are available on laying hens. Despite
76 belonging to the same species (*Gallus gallus domesticus*), broilers and layers have been subjected to
77 separate and intense genetic selection for over 60 years. As a result, they differ greatly in terms of growth
78 rate and energy metabolisms. In addition, selection and phenotype recording target different physiological
79 stages, focusing on young animals in broilers and adults in layers.
80 As they age, layers tend to become fatter, and breeders aim to achieve a balanced target fat level: neither
81 too thin nor too fat, to maintain sufficient body reserves in case of nutrient scarcity while avoiding
82 unnecessary energy storage.
83 Consequently, the present study aimed to achieve several objectives. Firstly, it sought to test and validate
84 the phenotyping by ultrasounds of the subcutaneous adipose tissue thickness on top of the *synsacrum* as
85 an accurate indicator of the overall fatness of the layer hen. Secondly, it aimed to estimate the heritability
86 of this new trait in laying hens. Finally, it aimed to study the phenotypic and genetic correlations between
87 this trait and other traits from the breeding goal of most of the lines of laying hens, in order to evaluate its
88 potential as a selection criterion.

89 **Methods**

90 **Laying hen population and rearing condition**

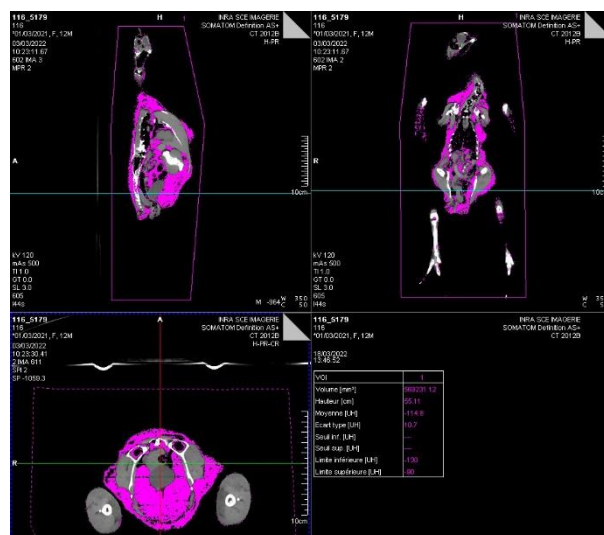
91 The laying hens used in this study belong to two experimental lines originating from the same Rhode
92 Island Red population, divergently selected since 1976 on the residual feed intake, a trait for feed efficiency
93 (Bordas *et al.*, 1992). These lines were chosen for this study because the selection process on RFI has also
94 led to marked differences in carcass adiposity with the efficient line (R-) being fatter than the inefficient
95 (R+) one, despite a reduced feed intake (El-Kazzi *et al.*, 1995). The RFI was estimated as defined in Byerly
96 *et al.* (1980) and represents the difference between the observed feed intake and the expected one
97 estimated based on known maintenance and production requirements.

98 In total, we used 394 animals, 215 from the R+ line and 179 from the R- line. There were 92 and 123 R+
99 phenotyped in 2019 and 2021 (from 9 sires and 38 dams in 2019, and 10 sires and 42 dams in 2021), and
100 75 and 104 R- in 2019 and 2021 (from 9 sires and 43 dams in 2019, and 9 sires and 41 dams in 2021). All
101 animals were hatched in two batches at the INRAE Pôle d'Expérimentation Avicole de Tours (UE PEAT,
102 Nouzilly, France ; <https://doi.org/10.15454/1.5572326250887292E12>). They were reared under standard
103 farming conditions in floor pens until 17 weeks of age when 46 birds were euthanized for body composition

104 recording (23 pullets per line), by neck cut and bleeding, immediately after head electrical stunning. The
105 remaining animals were transferred to individual cages with a lighting regime set at 14 h of light per day,
106 temperature was maintained between 19 and 21°C, and the hens were fed *ad libitum* a commercial diet
107 (15.5% CP and 2,650 kcal of ME/kg) automatically distributed at 8:00 a.m. (Appendix 1). Egg production
108 was recorded daily up to 53 weeks of age, when the hens were euthanized as described above. Because of
109 the adaptation of the experimental facility to both the sanitary situation and lockdown policy caused by
110 the COVID-19 pandemic, only the body weight (named BodyWeight) and last backfat thickness were
111 recorded in birds from the batch 2019.

112 Phenotypes

113 Tomography as the Gold Standard for body composition



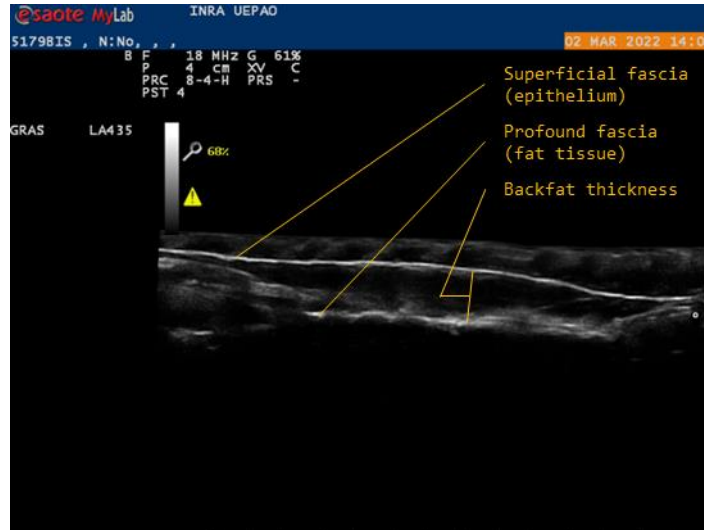
114

115 **Figure 1:** Example of CT-scan image visualizing the 3D axes (hen ID: PEA2021045179).
116 The pixels set between -130 and -90 HU and colored in pink to display the fatty components

117 The body composition of euthanized hens was determined immediately after euthanasia (within the hour
118 because *rigor mortis* occurs rapidly in chickens) with a CT scan (Siemens Somatom Definition AS, Siemens
119 Corp., Germany). During the scan, each hen was placed dorsally on the CT table. The X-ray source was set
120 at 120 kV and 500 mA/s. In humans, fat tissue typically displays Hounsfield Units (HU) ranging from -150 to
121 -50, although the exact limits do vary by individual and tissue type (Kim et al., 1999). To ensure these limits
122 align with laying hens, thresholds were estimated. Specifically, for all images, two discs of 10 mm² were
123 placed: one in the backfat tissue where the ultrasonography was done, and another in the abdominal fat
124 pad tissue. The minimum and maximum HU values were obtained for each disc. It is possible that non-fatty
125 components in the region, such as blood protein, were also captured. Therefore, the thresholds were set
126 at a 0.90 quantile for minimum values and a 0.10 quantile for maximum values. It resulted in a lower limit
127 of -130 HU and an upper limit of -90 HU, aligning with the updated range of -123 to -89 HU reported in a

128 recent study in humans (Pop and Mărușteri, 2023). The total volume of pixels within these bounds was
129 used as the total volume of fat in the animal (example in Figure 1; trait named TotFat).

130 *In-vivo indicators for body energy reserves*



131
132 **Figure 2:** Ultrasound scan panoramic image of the dorsal subcutaneous adipose tissue thickness
133 above the *synsacrum*, an example of the same hen as in Figure 1 (hen ID: PEA2021045179)

134 Dorsal subcutaneous adipose tissue thickness (trait named BackFat in this study) was recorded using an
135 ultrasound scanner (MyLab 30 Gold Vet, Hospimedi France, Saint-Crépin-Ibouwillers, France) equipped with
136 a high-frequency linear probe (18 MHz; L435, Esaote S.P.A., Genova, Italy). In previous studies in broilers,
137 a specific region was identified on top of the *synsacrum* as a good indicator of total fatness (Figure 2), based
138 on high correlations with TotFat (Mellouk *et al.* 2018; Grandhayé *et al.* 2019). The BackFat was recorded
139 according to the same protocol: the plumage was soaked with soapy water and then spread, ultrasound
140 gel was applied in contact with the epithelium and the probe was put in contact with the gel. The entire
141 recording process took about 1 min per hen and no feathers were plucked. BackFat was recorded 5 times
142 at 129, 192, 218, 289, and 371 days of age. The BodyWeight was recorded together with BackFat.

143 *Blood Adipokines levels*

144 A first blood sample was collected from the wing vein at 17 weeks of age and a second blood sample
145 was collected during the neck bleeding at the slaughter process, at 53 weeks of age. The difference in blood
146 sampling is not expected to bias the results, but it is a limitation of the experimental design. Plasma was
147 isolated from blood after centrifugation (5000 *g* for 10 min at 4°C) and then stored at -20°C. Consequently,
148 all hens had two blood samples available to determine adipokines concentrations. The concentrations of
149 four adipokines (visfatin, adiponectin, chemerin, and ghrelin) were determined in the plasma using
150 chicken-specific ELISA kits as previously described (Barbe *et al.*, 2020; Mellouk *et al.*, 2018b). Briefly,
151 MBS269004 (sensitivity 5 pg/mL), MBS016609 (sensitivity 0.1 µg/mL), MBS738819 (sensitivity 0.1 ng/mL),
152 and MBS2700427 (sensitivity 0.05 ng/mL) were used for visfatin, adiponectin, chemerin, and ghrelin,

153 respectively (My BioSource, San Diego, USA). The experiment was performed following the manufacturer's
 154 protocol with an intra-assay coefficient of variation $\leq 8\%$, $< 10\%$, $< 5.6\%$, and $< 12\%$, respectively. The
 155 absorbance was measured at 450 nm and then compared with reference values. The traits are named after
 156 the appropriated adipokines (visfatin, adiponectin, chemerin, and ghrelin).

157 *Egg production*

158 Egg production was recorded daily from the first egg laid until the end of the experiment (*i.e.* culling of the
 159 flock; trait named TotEggNum).

160 **Statistical analyses**

161 *Models*

162 To calculate genetic parameters (correlations and heritabilities), variance components were estimated
 163 using bivariate animal model analyses (Henderson, 1975). Commonly in bivariate analyses, both traits have
 164 the same two variance strata, genetic and residual, or three strata, genetic, animal, and sampling. This
 165 common model with two strata can be described as:

166
$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix}$$

167 where \mathbf{y}_1 and \mathbf{y}_2 are vectors of the observed values for the first and second trait respectively, \mathbf{X}_1 and \mathbf{X}_2
 168 are design matrices for fixed effects and \mathbf{b}_1 and \mathbf{b}_2 are vectors of values for fixed effects (details at the end
 169 of the section), \mathbf{Z}_1 and \mathbf{Z}_2 are design matrices for the additive genetic random effects and \mathbf{u}_1 and \mathbf{u}_2 are
 170 vectors of breeding values, and \mathbf{e}_1 and \mathbf{e}_2 are vectors of residual values. The variance components are
 171 fitted as 2x2 matrices of variances-covariances for each stratum:

172
$$Var \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} = \mathbf{G} \times \mathbf{A} \quad \text{where } \mathbf{G} = \begin{bmatrix} \sigma_{u1}^2 & \sigma_{u1u2} \\ \sigma_{u1u2} & \sigma_{u2}^2 \end{bmatrix} \text{ and } \mathbf{A} \text{ is the additive genetic relationship matrix}$$

173
$$Var \begin{bmatrix} \mathbf{e}_1 \\ \mathbf{e}_2 \end{bmatrix} = \mathbf{R} \times \mathbf{I} \quad \text{where } \mathbf{R} = \begin{bmatrix} \sigma_{e1}^2 & \sigma_{e1e2} \\ \sigma_{e1e2} & \sigma_{e2}^2 \end{bmatrix} \text{ and } \mathbf{I} \text{ is the identity matrix}$$

174 For a bivariate analysis where both traits have three strata, the model can be described as:

175
$$\begin{bmatrix} \mathbf{y}_1 \\ \mathbf{y}_2 \end{bmatrix} = \begin{bmatrix} \mathbf{X}_1 & 0 \\ 0 & \mathbf{X}_2 \end{bmatrix} \begin{bmatrix} \mathbf{b}_1 \\ \mathbf{b}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{pe}_1 \\ \mathbf{pe}_2 \end{bmatrix} + \begin{bmatrix} \mathbf{Z}_1 & 0 \\ 0 & \mathbf{Z}_2 \end{bmatrix} \begin{bmatrix} \mathbf{samp}_1 \\ \mathbf{samp}_2 \end{bmatrix}$$

176 where fixed effects are the same as for the former model, variance components are fitted as 2x2 matrices
 177 for the genetic strata and the remaining variance is decomposed into an animal (non-genetic) stratum and
 178 a sampling stratum defined as:

179
$$Var \begin{bmatrix} \mathbf{u}_1 \\ \mathbf{u}_2 \end{bmatrix} = \mathbf{G} \times \mathbf{A} \quad \text{where } \mathbf{G} = \begin{bmatrix} \sigma_{u1}^2 & \sigma_{u1u2} \\ \sigma_{u1u2} & \sigma_{u2}^2 \end{bmatrix} \text{ and } \mathbf{A} \text{ is the additive genetic relationship matrix}$$

180 $Var \begin{bmatrix} \mathbf{pe}_1 \\ \mathbf{pe}_2 \end{bmatrix} = \mathbf{P} \times \mathbf{I}$ where $\mathbf{P} = \begin{bmatrix} \sigma_{pe1}^2 & \sigma_{pe1pe2} \\ \sigma_{pe1pe2} & \sigma_{pe2}^2 \end{bmatrix}$ and \mathbf{I} is the identity matrix

181 $Var \begin{bmatrix} \mathbf{samp}_1 \\ \mathbf{samp}_2 \end{bmatrix} = \mathbf{S} \times \mathbf{I}$ where $\mathbf{S} = \begin{bmatrix} \sigma_{samp1}^2 & \sigma_{samp1samp2} \\ \sigma_{samp1samp2} & \sigma_{samp2}^2 \end{bmatrix}$ and \mathbf{I} is the identity matrix

182 However, when one trait has two strata (\mathbf{y}_1 say) and the other has three strata (\mathbf{y}_2 say), the direct product
 183 variance structure breaks down; $\sigma_{e1}^2(\sigma_{e1e2})$ cannot be partitioned into $\sigma_{pe1}^2 + \sigma_{samp1}^2(\sigma_{pe1pe2} +$
 184 $\sigma_{samp1samp2})$. We can estimate four (three different) parameters:

185 $\sigma_{e1e2}^* = \sigma_{pe1pe2} + \sigma_{samp1samp2}$

186 $\sigma_{e1}^{2*} = \sigma_{e1}^2 - \sigma_{e1e2}^* = \sigma_{pe1}^2 + \sigma_{samp1}^2 - \sigma_{samp1samp2}$

187 $\sigma_{pe2}^{2*} = \sigma_{pe2}^2 - \sigma_{pe1pe2}$

188 $\sigma_{samp2}^{2*} = \sigma_{samp2}^2 - \sigma_{samp1samp2}$

189 The phenotypic variance components are then given by:

190 $\sigma_{total1}^2 = \sigma_{u1}^2 + \sigma_{e1}^{2*} + \sigma_{e1e2}^*$

191 $\sigma_{total1total2} = \sigma_{u1u2} + \sigma_{e1e2}^*$

192 $\sigma_{total2}^2 = \sigma_{u2}^2 + \sigma_{pe2}^{2*} + \sigma_{samp2}^{2*} + \sigma_{e1e2}^*$

193 The variance components were estimated using the average-information restricted maximum likelihood
 194 method (AI-REML algorithm; Gilmour et al., 1995). Reported heritability estimates are means calculated
 195 with all bivariate analyses. Genetic parameters were considered low between 0.00 and 0.25, moderate
 196 between 0.25 and 0.50, and high above 0.50. The fixed effects in the model include the genetic line to
 197 account for their mean differences (levels: R+ or R-), the effect of the batch (levels: 2019 or 2021) and the
 198 regression coefficient for the time of recording for the repeated trait. The genetic line was not used to
 199 stratify the random effects because preliminary analyses indicated that the variance components were
 200 similar in both lines. See the provided scripts "BEDERE_2023_ASREMLScript_bivariate_2x2strata.as",
 201 "[...]3x3strata.as", and "[...]3x2strata.as" for details.

202 *Bartlett's test*

203 Descriptive statistics of the data suggested a bimodal distribution of BackFat in both lines. This type of
 204 distribution may highlight the presence of a major gene controlling the trait. A simple test to detect a major
 205 gene effect on a trait is to test the homogeneity of the variances between families (Le Roy and Elsen, 1992).
 206 A Bartlett test was performed to test this hypothesis, using the sire as the family identifier (Bartlett, 1937).
 207 See the provided script "BEDERE_2023_RScript_BartlettTest.R" for details.

208 *Programs used*

209 Data handling, graphs, and the Bartlett test were performed in base R (R Core Team, 2023). Variance
 210 components and genetic parameters estimations were performed with ASReml 4.2 (Gilmour et al., 2021).

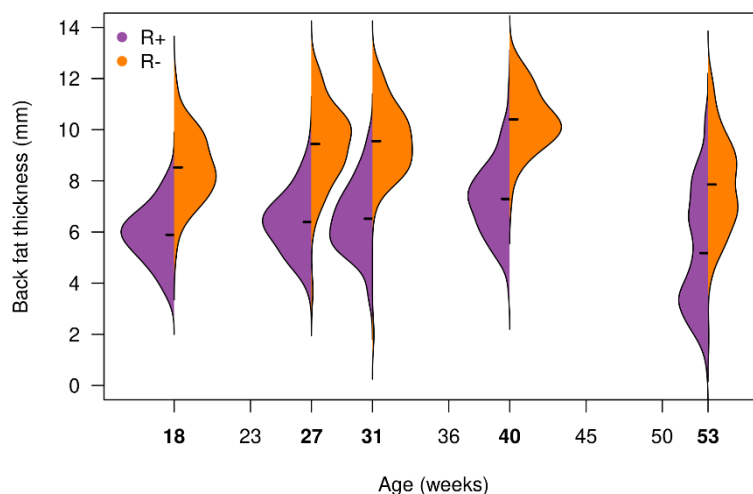
211 **Table 1:** Summary statistics for traits related to body composition (TotFat, BackFat, and BodyWeight), blood adipokines levels (Adiponectine, Chemerin,
 212 Ghrelin, and Visfatin), and egg production (TotEggNum) in two lines divergently selected for residual feed intake (efficient: R-, inefficient: R+).

Trait ¹	TotFat (mm ³)	BackFat (mm)	BodyWeight (g)	Adiponectin (µg/ml)	Chemerin (ng/ml)	Ghrelin (ng/ml)	Visfatin (ng/ml)	TotEggNum (count)
<i>Summary statistics of observed values</i>								
number of records	111	580	771	233	233	233	233	192
mean	540,367	6.1	2,339	2.4	39.5	57.0	58.0	158
R+ standard deviation	167,107	1.8	323	0.4	15.9	12.1	6.6	43
minimum	114,366	0.6	1,524	1.1	26.0	32.0	43.0	1
maximum	955,873	11.5	3,234	3.2	78.0	88.0	75.0	218
number of records	86	460	611	191	190	190	189	155
mean	633,050	8.9	2,304	2.0	27.5	60.3	59.2	150
R- standard deviation	141,608	1.8	351	0.4	11.9	14.3	6.2	56
minimum	380,231	1.9	1,375	1.0	3.0	34.0	43.0	0
maximum	1,087,374	12.7	3,465	3.2	68.0	112.0	74.0	194
<i>Fixed effect of the genetic line estimated by the mixed models (R+ compared to R-)</i>								
Estimated effect	-80,028	-3.1	23	0.3	12.1	-2.6	-1.8	-4
P-value	0.23	<.001	0.82	0.40	<.001	0.84	0.74	0.82

213 ¹The traits are named after their phenotypes: BackFat for dorsal subcutaneous adipose tissue thickness, BodyWeight for body weight, TotFat for the total volume of
 214 pixels of fat components, then the blood Adipokines levels named after the appropriate adipokine, and TotEggNum for the total number of eggs laid.
 215

217 **Phenotypic description of the divergent lines**

218 The two lines used, diverging for RFI, were very different regarding BackFat and chemerin blood
 219 concentration (Table 1, Figure 3): the R- line had a larger BackFat (+34%) and lower chemerin levels (-31%)
 220 than the R+ line. However, the lines were not significantly different regarding TotFat, BodyWeight,
 221 Adiponectin, Ghrelin, Visfatin, and TotEggNum. For the P-value for TotFat which is 0.23, given the
 222 difference in mean and the variance, there may be some lack of power in the analysis due to the fact that
 223 the tomography could be performed on one batch only. Previous studies about these lines reported that
 224 the R+ line (inefficient ones) has a higher feed intake, higher diet-induced thermogenesis and different
 225 endocrine responses, resulting in different lipid metabolism between the lines (Gabarrou et al., 2000, 1998,
 226 1997; Swennen et al., 2007) which could explain the observed differences in adiposity between lines.
 227 Interestingly, in pigs, the selection on residual feed intake was also associated with a difference in BackFat
 228 with the efficient line being fatter, although the magnitude of difference was smaller (Gilbert et al., 2017).
 229 The results on fat content observed in our study corroborate previous results about the R+ and R- lines,
 230 with the R+ being leaner than the R-. However, contrary to our expectation, no differences were observed
 231 in the blood level of adiponectin and ghrelin, which are hormones associated with feed intake acting as
 232 appetite-regulating signals (Mellouk et al., 2018a).



233

234 **Figure 3:** Distribution of the raw values for BackFat according to age in both lines. R+ are in purple on the
 235 left side of the beanplot, R- are in orange on the right side of the beanplot. The dash is the mean for each
 236 level.

237 **BackFat thickness is an indicator of body reserves**

238 BackFat and TotFat were genetically highly positively correlated, and phenotypically moderately
 239 positively correlated (Table 2). Previous studies reported a high phenotypic correlation between BackFat
 240 and TotFat in chicken ($r > 0.84$; Mellouk *et al.* 2018; Grandhaye *et al.* 2019) but neither heritability, nor

241 phenotypic and genetic correlations with other traits of interest were calculated. The lower phenotypic
 242 correlation reported in Table 2 takes into account the effects of the model, which can influence the
 243 correlation estimate (genetic line, the batch, the repetition of recording, and the genetic and permanent
 244 environment variances). When we repeated the estimation using the same approach (i.e. Person
 245 correlation using raw data; Mellouk *et al.* 2018; Grandhaye *et al.* 2019), we obtain a correlation value of
 246 0.71 (0.77 in R+ and 0.60 in R-), which is consistent with the findings previously published.

247 The BodyWeight and TotFat were also highly positively correlated. However, BackFat and BodyWeight
 248 were moderately positively correlated. The overall results show that BackFat is a good indicator of fatness
 249 in adult layers, consistent with previous findings in young broilers, where it exhibited a high phenotypic
 250 correlation with the fat fraction from chemical analyses of the shredded body ($r=0.92$) and the abdominal
 251 fat pad weight obtained by dissection ($r=0.86$; Mellouk *et al.* 2018). Given the genetic correlations between
 252 BackFat, TotFat, and BodyWeight, we can conclude that BackFat and BodyWeight hold different
 253 information related to fatness in chickens. Compared to TotFat, BackFat offers notable advantages as an
 254 easy-to-record trait: it is fast to record, does not require the animal to be asleep, and can be done with a
 255 portable machine. Our results combined with these technical aspects, make BackFat a very good indicator
 256 trait of fatness in chicken.

257 **Table 2:** Mean heritability estimates (in diagonal together with their associated mean standard errors),
 258 genetic correlation estimates (below the diagonal together with their associated standard errors), and
 259 phenotypic correlations (above the diagonal with their associated standard errors) for traits related to
 260 body composition (TotFat, BackFat, and BodyWeight), blood adipokine levels (Adiponectin,
 261 Chemerin, Ghrelin, and Visfatin), and egg production (TotEggNum) in two lines divergently selected
 262 for residual feed intake (efficient: R-, inefficient: R+).

	TotFat	BackFat	BodyWeight	Adiponectin	Ghrelin	Visfatin	TotEggNum
TotFat	0.27 (0.04)	0.39 (0.04)	0.54 (0.04)	-0.24 (0.06)	-0.32 (0.06)	-0.06 (0.07)	-0.01 (0.09)
BackFat	0.91 (0.13)	0.38 (0.06)	0.31 (0.04)	-0.18 (0.08)	-0.23 (0.07)	-0.07 (0.06)	-0.01 (0.06)
BodyWeight	0.67 (0.16)	0.39 (0.12)	0.42 (0.08)	-0.24 (0.07)	-0.28 (0.07)	-0.08 (0.07)	-0.17 (0.05)
Adiponectin	-0.37 (0.15)	-0.28 (0.22)	-0.42 (0.18)	0.92 (0.02)	0.39 (0.05)	-0.03 (0.06)	0.01 (0.08)
Ghrelin	-0.80 (0.16)	-0.44 (0.20)	-0.39 (0.18)	0.41 (0.06)	0.91 (0.02)	0.17 (0.06)	0.11 (0.08)
Visfatin	-0.32 (0.19)	-0.19 (0.13)	-0.26 (0.21)	-0.05 (0.07)	0.18 (0.07)	0.80 (0.03)	0.27 (0.06)
TotEggNum	-0.49 (0.34)	-0.34 (0.32)	-0.82 (0.19)	0.02 (0.16)	0.20 (0.20)	0.36 (0.15)	0.24 (0.05)

263 Genetic background of BackFat thickness

264 BackFat displayed a moderate heritability (Table 2). The distribution of the values for BackFat in both
 265 lines displayed a large variance, with apparently two modes, which seems to become exacerbated with
 266 time (Figure 3). The sire-family variances were heterogeneous according to the Bartlett test

267 (P-value=0.008). Both the multimodal distribution and the heterogeneity of sire-family variance are
268 evidence of a major gene effect (Le Roy and Elsen, 1992).

269 In quails, a study reported a low heritability of 0.17 for fat skin percentage (recorded as the fat content
270 of the shredded skin) as an indicator similar to BackFat (Lotfi et al., 2011). In pigs, BackFat displayed a high
271 heritability (from 0.63 to 0.72; Cai et al., 2008; Gilbert et al., 2007; Suzuki et al., 2005) while in cattle,
272 BackFat presented moderate ones (from 0.36 to 0.59; Arnold et al., 1991; Nkrumah et al., 2007; Schenkel
273 et al., 2004). Many quantitative trait loci (QTL) associated with fatness in chickens are reported: there are
274 129 QTL listed in chickenQTLdb (<https://www.animalgenome.org/QTLdb/chicken/>) from 69 scientific
275 articles. Some genes are known to be involved in lipogenesis and differently expressed in lean and fat
276 broilers (Bourneuf et al., 2006; Resnyk et al., 2017). Yet, major genes for BackFat were not explicitly
277 identified, further analyses including segregation analyses and genome-wide association studies
278 accounting for dominant effects would help to identify them.

279 **Genetic background of other traits related to fatness**

280 Moderate heritabilities were observed for TotFat and BodyWeight (Table 2), with the latter **aligning**
281 with previous studies reporting estimates ranging from 0.32 to 0.53 (Rowland et al., 2019; Wolc et al., 2011,
282 2009). Heritability in the R+ and R- lines may have changed a little because estimates for BodyWeight were
283 reported to be 0.56 and 0.61 in females and males respectively in the 15 first generations (Tixier-boichard
284 et al., 1995). Carcass percentage of fat displayed a moderate heritability in other studies using other
285 chicken lines (0.43 to 0.55; Moreira et al., 2018; Nunes et al., 2011).

286 The chosen adipokines in this study are known to be indicators of body reserve status and dynamics
287 (review: Mellouk et al., 2018a). Adiponectin is used as an indicator of energy deficit: the leaner the bird the
288 higher the level of adiponectin. Chemerin is used as an indicator of body lipid mobilization: the lower the
289 abdominal fat pad, the higher the level of chemerin. Ghrelin is used as an indicator of general body reserves
290 accretion: it is known to stimulate intake and growth hormone release. Visfatin is acting like a myokine in
291 birds (Krzysik-Walker et al., 2008) and it is used as an indicator of lean body reserve status compared to
292 body lipid reserves. The genetic background, particularly the genes coding for these proteins are well
293 described. All adipokines except chemerin displayed very high heritability (Table 2). This indicates that
294 genetics is the primary source of phenotypic variation, and that environmental fluctuations have minimal
295 **influence** in our setup, where hens are housed in individual cages and fed *ad libitum*. **We observed a**
296 **significant increase in blood levels of adiponectin (P < 0.001) and visfatin (P = 0.007), a significant decrease**
297 **in chemerin (P < 0.001), and no significant change in ghrelin (P = 0.14) between 17 and 53 weeks of age. It**
298 **has been reported in turkeys that plasma levels of adiponectin, chemerin, and visfatin decrease during the**
299 **laying period (Diot et al., 2015). A kinetic experimental design would be required to further investigate the**
300 **effect of physiological stage on blood levels of adipokines. Blood adipokine levels are also known to vary**
301 **with dietary intake and composition in broilers (Mellouk et al., 2018a, 2018b), but these were similar**

302 between hens in our experimental setup. Genetic parameters for chemerin could not be estimated because
303 the estimated additive genetic variance was too close to the zero boundary. This means that almost none
304 of the observed variance is due to genetics, despite a phenotypic coefficient of variation close to 40%. We
305 hypothesize that there may be a single haplotype per line in the population, explaining why there is no
306 genetic variance observed despite a significant difference in mean between the lines. Consequently, no
307 genetic correlation with other traits could be estimated (explaining why chemerin is not in Table 2).
308 Adiponectin displayed a moderate and positive genetic correlation with Ghrelin, no correlation with
309 Visfatin and TotEggNum, and moderate and negative genetic correlations with TotFat, BackFat, and
310 BodyWeight. This is consistent with its role in chicken: increased blood level of adiponectine is associated
311 with decreased lipid deposition, decreased body weight and increased feed intake (Mellouk et al., 2018a).
312 Ghrelin displayed low and positive genetic correlations with Visfatin and TotEggNum, moderate and
313 negative genetic correlations with BackFat and BodyWeight, and a high and negative genetic correlation
314 with TotFat. This is consistent with its role in chicken: increased blood level of ghrelin is associated with
315 decreased feed intake and increased lipolysis (Murugesan and Nidamanuri, 2022). These correlations
316 further support BackFat as a good indicator trait for fatness and energy reserves in chickens. Chemerin
317 levels were significantly higher in the R+ line, which is consistent with the fact that it is associated with
318 lower body fatness (Mellouk et al., 2018a). Visfatin displayed a low and positive genetic correlation with
319 TotEggNum, and low-to-moderate and negative genetic correlations with TotFat, BackFat, and
320 BodyWeight. We were expecting a lower genetic correlation between visfatin and fat-related traits given
321 its biological function: visfatin is acting like a myokine in chicken (Krzysik-Walker et al., 2008). Increased
322 blood levels of visfatin are associated with increased feed intake and body weight (lean part; Mellouk et
323 al., 2018a). It is important to note the high standard errors reported for genetic correlations between
324 adipokines and other traits, pinpointing they could gain from additional data.

325 Tradeoff between body reserves and egg production

326 The TotEggNum displayed a moderate heritability (Table 2). This phenotype is capturing two distinct
327 biological processes: puberty (age at first laying) and laying rate. Total egg number displayed a low
328 heritability in other studies (from 0.01 to 0.20; Bedere et al., 2022; Liu et al., 2019; Wolc et al., 2011a), but
329 in most papers the early period (before 25 weeks of age) is skipped to start recording after the laying peak.
330 Again, the same trait in the first 15 generations was reported to be more heritable ($h^2 = 0.48$; Tixier-
331 boichard et al., 1995).

332 The genetic correlation of TotEggNum was moderate and negative with BackFat, and high and negative
333 with BodyWeight (Table 2). These correlations suggest a tradeoff between body reserves and egg
334 production in some populations. The genetic correlation between TotEggNum and BodyWeight was higher
335 (-0.82) than that with TotFat (-0.49) or BackFat(-0.34). This means that the genetic share between
336 TotEggNum and BodyWeight is stronger than with TotFat or BackFat. We hypothesize that this could be

337 explained by a larger tradeoff, possibly including energy, minerals and protein, whereas the tradeoff
338 between egg production and fatness would be limited to energy resources. The BodyWeight is partly
339 composed of fat, consistent with the share of their genetic architecture, as indicated by the moderate-to-
340 high and positive genetic correlations between BackFat or TotFat and BodyWeight. The few studies
341 mentioning genetic correlations between egg production and body weight reported moderate and
342 negative correlations (-0.29 to -0.42; Yoo et al., 1988) or no correlation (Wolc et al., 2011b). The very high
343 value estimated in our study may be a specificity of the R+ and R- lines, which is an unusual population for
344 the egg industry. Both the size and fatness are optimum-based breeding goals: a targeted neither too big
345 nor too small size and fatness are desired, whereas egg production is mostly maximized. This means that
346 the selection index must consider these genetic correlations to combine selection criteria such as
347 TotEggNum, BackFat, and BodyWeight to breed multi-performing laying hens. In fact, if similar genetic
348 correlations were found in commercial lines, including BackFat in the selection index would allow avoiding
349 the indirect response of fatness to selection on egg production. Breeding companies may be interested in
350 stabilizing fatness in chickens to avoid health, welfare and performance problems due to metabolic
351 disorders associated with extreme conditions: leanness and obesity (Baéza and Le Bihan-Duval, 2013; Bain
352 et al., 2016).

353

Conclusion

354 To conclude, this study showed, on two Rhode Island lines diverging for feed efficiency differing also in
355 fat content, that backfat thickness is a potentially accurate indicator of the overall fatness of laying hens.
356 Backfat thickness can be recorded repeatedly during the production cycle, creating opportunities to better
357 understand body reserve dynamics in chickens. In addition, backfat thickness displayed a moderate
358 heritability, implying that there is room for genetic improvement, probably canalization around an
359 optimum to be defined. Both the bimodal distribution of the trait and the heterogeneity of the variances
360 between families are signs of the presence of a major gene segregating backfat thickness in the population.
361 The genetic correlation with body weight was moderate, implying that backfat holds complementary
362 genetic information about fatness that is currently not considered in breeding programs including body
363 weight in their breeding goal. Finally, the genetic correlation with egg production was moderate and
364 unfavorable. This correlation should be taken into account to avoid undesired responses to selection. It is
365 important to keep in mind that all the reported results are based on particular genetic lines, divergently
366 selected since 1976 on the residual feed intake. They need to be confirmed on regular commercial genetic
367 lines to consider backfat thickness in the breeding goal.
368

CRediT (Contributor Roles Taxonomy, <https://credit.niso.org/>)

Initials ¹	NB	JD	YB	CS	DG	FE	PLR	TZ	BR	FL	CR	MCi	MD	MCh	LG	AG
Conceptualization	✓	✓		✓	✓		✓	✓		✓						
Data curation	✓															
Formal Analysis	✓															
Funding acquisition	✓															
Investigation	✓	✓	✓	✓	✓	✓			✓	✓	✓	✓	✓	✓	✓	
Methodology	✓															
Project administration	✓															
Resources		✓	✓	✓	✓	✓										
Software																✓
Supervision	✓	✓		✓	✓		✓	✓								
Validation		✓	✓	✓	✓	✓	✓	✓								✓
Visualization	✓			✓		✓										
Writing – original draft	✓															
Writing – review & editing	✓	✓		✓			✓	✓								✓

370 ¹NB: Nicolas Bédère, JD: Joëlle Dupont, YB: Yannick Baumard, CS: Christophe Staub, DG: David Gourichon, FE: Frédéric Elleboudt, PLR: Pascale Le Roy, TZ: Tatiana
371 Zerjal, BR: Baudouin Rivet, FL: François Lecompte, CR: Christelle Ramé, MCi: Marine Cirot, MD: Mathilde Daudon, MCh: Marine Chahnamian, LG: Lola Guerche, AG:
372 Arthur Gilmour
373

374

Acknowledgements

375 The authors are grateful to:

- 376 - the staff of the different research units that helped and lot: Baudouin Rivet, François Lecompte,
- 377 Christelle Ramé, Marine Cirot, Mathilde Daudon, Marine Chahnamian, and Lola Guerche
- 378 - the Animal Genetic Division of INRAE for supporting this study
- 379 - Arthur Gilmour for his guidance in the bivariate analyses parametrization

380

Ethics statement regarding animals

381 All data coming from living animals were recorded as part of the breeding program of INRAE Poultry
382 experimental facility (UE PEAT, Nouzilly, France ; <https://doi.org/10.15454/1.5572326250887292E12>)
383 conducted in compliance the French Ministry of higher education, research and innovation authorization
384 (number agreement 02414.01). The traits involved are egg number, body weight, and backfat thickness.
385 The other traits were recorded *post-mortem*, after the animals were euthanized in compliance with
386 national regulations pertaining to livestock production and according to procedures approved by the
387 French Veterinary Services. The traits involved are body composition by tomography, blood adipokines
388 concentrations, and carcass traits (*e.g.* abdominal fat pad weight).

389

Data, scripts, code, and supplementary information availability

390 Data are available online: [link forthcoming upon acceptance](#)

391 Scripts and code are available online: [link forthcoming upon acceptance](#)

392

Conflict of interest disclosure

393 The authors declare that they comply with the PCI rule of having no financial conflicts of interest
394 concerning the content of the article.

395

Funding

396 This research was funded by the Animal Genetic Division of INRAE.

397

- 399 Arnold, J.W., Bertrand, J.K., Benyshek, L.L., Ludwig, C., 1991. Estimates of genetic parameters for live
400 animal ultrasound, actual carcass data, and growth traits in beef cattle. *Journal of Animal*
401 *Science* 69, 985–992. <https://doi.org/10.2527/1991.693985x>
- 402 Baéza, E., Le Bihan-Duval, E., 2013. Chicken lines divergent for low or high abdominal fat deposition:
403 a relevant model to study the regulation of energy metabolism. *Animal* 7, 965–973.
404 <https://doi.org/10.1017/S1751731113000153>
- 405 Bain, M.M., Nys, Y., Dunn, I.C., 2016. Increasing persistency in lay and stabilising egg quality in longer
406 laying cycles. What are the challenges? *British Poultry Science* 57, 330–338.
407 <https://doi.org/10.1080/00071668.2016.1161727>
- 408 Barbe, A., Mellouk, N., Ramé, C., Grandhaye, J., Staub, C., Venturi, E., Cirot, M., Petit, A., Anger, K.,
409 Chahnamian, M., Ganier, P., Callut, O., Cailleau-Audouin, E., Metayer-Coustard, S., Riva, A.,
410 Froment, P., Dupont, J., 2020. A grape seed extract maternal dietary supplementation in
411 reproductive hens reduces oxidative stress associated to modulation of plasma and tissue
412 adipokines expression and improves viability of offsprings. *PLOS ONE* 15, e0231131.
413 <https://doi.org/10.1371/journal.pone.0231131>
- 414 Bartlett, M.S., 1937. Some Examples of Statistical Methods of Research in Agriculture and Applied
415 Biology. *Supplement to the Journal of the Royal Statistical Society* 4, 137–183.
416 <https://doi.org/10.2307/2983644>
- 417 Bécot, L., Bédère, N., Roy, P.L., 2021. Sélection sur la ponte des poules en systèmes alternatifs à la
418 cage: (Full text available in English). *INRAE Productions Animales* 34, 1–14.
419 <https://doi.org/10.20870/productions-animales.2021.34.1.4680>
- 420 Bedere, N., Berghof, T.V.L., Peeters, K., Pinard-van der Laan, M.-H., Visscher, J., David, I., Mulder,
421 H.A., 2022. Using egg production longitudinal recording to study the genetic background of
422 resilience in purebred and crossbred laying hens. *Genetics Selection Evolution* 54, 26.
423 <https://doi.org/10.1186/s12711-022-00716-8>
- 424 Bordas, A., Tixier-Boichard, M., Merat, P., 1992. Direct and correlated responses to divergent
425 selection for residual food intake in Rhode island red laying hens. *British Poultry Science* 33,
426 741–754. <https://doi.org/10.1080/00071669208417515>
- 427 Bourneuf, E., Héroult, F., Chicault, C., Carré, W., Assaf, S., Monnier, A., Mottier, S., Lagarrigue, S.,
428 Douaire, M., Mosser, J., Diot, C., 2006. Microarray analysis of differential gene expression in
429 the liver of lean and fat chickens. *Gene* 372, 162–170.
430 <https://doi.org/10.1016/j.gene.2005.12.028>
- 431 Byerly, T.C., Kessler, J.W., Gous, R.M., Thomas, O.P., 1980. Feed Requirements for Egg Production.
432 *Poultry Science* 59, 2500–2507. <https://doi.org/10.3382/ps.0592500>
- 433 Cai, W., Casey, D.S., Dekkers, J.C.M., 2008. Selection response and genetic parameters for residual
434 feed intake in Yorkshire swine1. *Journal of Animal Science* 86, 287–298.
435 <https://doi.org/10.2527/jas.2007-0396>
- 436 Cobo, E., Molette, C., Touze, J.-L., Venturi, E., Bernadet, M.-D., Staub, C., 2015. Mise en place d'une
437 méthodologie en imagerie pour prédire des caractères de production chez les palmipèdes à
438 foie gras. Presented at the Onzièmes Journées de la Recherche Avicole et Palmipèdes à Foie
439 Gras, Tours, France.
- 440 Diot, M., Reverchon, M., Rame, C., Froment, P., Brillard, J.-P., Brière, S., Levêque, G., Guillaume, D.,
441 Dupont, J., 2015. Expression of adiponectin, chemerin and visfatin in plasma and different
442 tissues during a laying season in turkeys. *Reproductive Biology and Endocrinology* 13, 81.
443 <https://doi.org/10.1186/s12958-015-0081-5>
- 444 El-Kazzi, M., Bordas, A., Gandemer, G., Minvielle, F., 1995. Divergent selection for residual food
445 intake in Rhode Island Red egg-laying lines: Gross carcass composition, carcass adiposity
446 and lipid contents of tissues. *British Poultry Science* 36, 719–728.
447 <https://doi.org/10.1080/00071669508417816>

- 448 Gabarrou, J.-F., Geraert, P.A., Francois, N., Guillaumin, S., Picard, M., Bordas, A., 1998. Energy
449 balance of laying hens selected on residual food consumption. *British Poultry Science* 39,
450 79–89. <https://doi.org/10.1080/00071669889439>
- 451 Gabarrou, J.-F., Géraert, P.-A., Picard, M., Bordas, A., 1997. Diet-Induced Thermogenesis in Cockerels
452 Is Modulated by Genetic Selection for High or Low Residual Feed Intake. *The Journal of*
453 *Nutrition* 127, 2371–2376. <https://doi.org/10.1093/jn/127.12.2371>
- 454 Gabarrou, J.-F., Geraert, P.A., Williams, J., Ruffier, L., Rideau, N., 2000. Glucose–insulin relationships
455 and thyroid status of cockerels selected for high or low residual food consumption. *British*
456 *Journal of Nutrition* 83, 645–651. <https://doi.org/10.1017/S0007114500000829>
- 457 Gilbert, H., Bidanel, J.-P., Gruand, J., Caritez, J.-C., Billon, Y., Guillouet, P., Lagant, H., Noblet, J.,
458 Sellier, P., 2007. Genetic parameters for residual feed intake in growing pigs, with emphasis
459 on genetic relationships with carcass and meat quality traits. *Journal of Animal Science* 85,
460 3182–3188. <https://doi.org/10.2527/jas.2006-590>
- 461 Gilbert, H., Billon, Y., Brossard, L., Faure, J., Gatellier, P., Gondret, F., Labussière, E., Leuret, B.,
462 Lefaucheur, L., Le Floch, N., Louveau, I., Merlot, E., Meunier-Salaün, M.-C., Montagne, L.,
463 Mormede, P., Renaudeau, D., Riquet, J., Rogel-Gaillard, C., Van Milgen, J., Vincent, A.,
464 Noblet, J., 2017. Review: divergent selection for residual feed intake in the growing pig.
465 *Animal* 11, 1427–1439. <https://doi.org/10.1017/S175173111600286X>
- 466 Gilmour, A.R., Gogel, B.J., Cullis, B.R., Welham, S.J., Thompson, R., 2021. ASReml User Guide Release
467 4.2 Functional Specification.
- 468 Gilmour, A.R., Thompson, R., Cullis, B.R., 1995. Average Information REML: An Efficient Algorithm for
469 Variance Parameter Estimation in Linear Mixed Models. *Biometrics* 51, 1440–1450.
470 <https://doi.org/10.2307/2533274>
- 471 Grandhayé, J., Lecompte, F., Staub, C., Venturi, E., Plotton, I., Cailleau-Audouin, E., Ganier, P., Ramé,
472 C., Brière, S., Dupont, J., Froment, P., 2019. Assessment of the body development kinetic of
473 broiler breeders by non-invasive imaging tools. *Poultry Science*.
474 <https://doi.org/10.3382/ps/pez112>
- 475 Henderson, C.R., 1975. Best linear unbiased estimation and prediction under a selection model.
476 *Biometrics* 31, 423–447.
- 477 Kim, S., Lee, G.H., Lee, S., Park, S.H., Pyo, H.B., Cho, J.S., 1999. Body fat measurement in computed
478 tomography image. *Biomed Sci Instrum* 35, 303–308.
- 479 Krzysik-Walker, S.M., Ocón-Grove, O.M., Maddineni, S.R., Hendricks, G.L., Ramachandran, R., 2008.
480 Is visfatin an adipokine or myokine? Evidence for greater visfatin expression in skeletal
481 muscle than visceral fat in chickens. *Endocrinology* 149, 1543–1550.
482 <https://doi.org/10.1210/en.2007-1301>
- 483 Larbier, M., Leclercq, B., 1992. L'œuf et l'alimentation des poules pondeuses, in: *Nutrition et*
484 *alimentation des volailles, Du labo au terrain*. INRA, Paris, pp. 195–226.
- 485 Le Roy, P., Elsen, J.M., 1992. Simple test statistics for major gene detection: a numerical comparison.
486 *Theoret. Appl. Genetics* 83, 635–644. <https://doi.org/10.1007/BF00226909>
- 487 Lerch, S., De La Torre, A., Huau, C., Monziols, M., Xavier, C., Louis, L., Le Cozler, Y., Faverdin, P.,
488 Lambertson, P., Chery, I., Heimo, D., Loncke, C., Schmidely, P., Pires, J.A.A., 2021. Estimation
489 of dairy goat body composition: A direct calibration and comparison of eight methods.
490 *Methods* 186, 68–78. <https://doi.org/10.1016/j.ymeth.2020.06.014>
- 491 Liu, Z., Yang, N., Yan, Y., Li, G., Liu, A., Wu, G., Sun, C., 2019. Genome-wide association analysis of egg
492 production performance in chickens across the whole laying period. *BMC Genet* 20, 67.
493 <https://doi.org/10.1186/s12863-019-0771-7>
- 494 Lotfi, E., Zerehdaran, S., Ahani Azari, M., 2011. Genetic evaluation of carcass composition and fat
495 deposition in Japanese quail. *Poultry Science* 90, 2202–2208.
496 <https://doi.org/10.3382/ps.2011-01570>

497 Luiting, P., 1990. Genetic variation of energy partitioning in laying hens: causes of variation in
498 residual feed consumption. *Worlds Poult. Sci. J.* 46, 133–152.
499 <https://doi.org/10.1079/WPS19900017>

500 Mellouk, N., Ramé, C., Barbe, A., Grandhay, J., Froment, P., Dupont, J., 2018a. Chicken Is a Useful
501 Model to Investigate the Role of Adipokines in Metabolic and Reproductive Diseases.
502 *International Journal of Endocrinology* 2018, 1–19. <https://doi.org/10.1155/2018/4579734>

503 Mellouk, N., Ramé, C., Marchand, M., Staub, C., Touzé, J.-L., Venturi, É., Mercierand, F., Travel, A.,
504 Chartrin, P., Lecompte, F., Ma, L., Froment, P., Dupont, J., 2018b. Effect of different levels of
505 feed restriction and fish oil fatty acid supplementation on fat deposition by using different
506 techniques, plasma levels and mRNA expression of several adipokines in broiler breeder
507 hens. *PLoS ONE* 13, e0191121. <https://doi.org/10.1371/journal.pone.0191121>

508 Moreira, G.C.M., Boschiero, C., Cesar, A.S.M., Reecy, J.M., Godoy, T.F., Pértille, F., Ledur, M.C.,
509 Moura, A.S.A.M.T., Garrick, D.J., Coutinho, L.L., 2018. Integration of genome wide
510 association studies and whole genome sequencing provides novel insights into fat
511 deposition in chicken. *Sci Rep* 8, 16222. <https://doi.org/10.1038/s41598-018-34364-0>

512 Murugesan, S., Nidamanuri, A.L., 2022. Role of leptin and ghrelin in regulation of physiological
513 functions of chicken. *World's Poultry Science Journal* 78, 1021–1036.
514 <https://doi.org/10.1080/00439339.2022.2119917>

515 Nkrumah, J.D., Basarab, J.A., Wang, Z., Li, C., Price, M.A., Okine, E.K., Crews, D.H., Jr., Moore, S.S.,
516 2007. Genetic and phenotypic relationships of feed intake and measures of efficiency with
517 growth and carcass merit of beef cattle1. *Journal of Animal Science* 85, 2711–2720.
518 <https://doi.org/10.2527/jas.2006-767>

519 Nunes, B. do N., Ramos, S.B., Savegnago, R.P., Ledur, M.C., Nones, K., Klein, C.H., Munari, D.P., 2011.
520 Genetic parameters for body weight, carcass chemical composition and yield in a broiler-
521 layer cross developed for QTL mapping. *Genet. Mol. Biol.* 34, 429–434.
522 <https://doi.org/10.1590/S1415-47572011005000019>

523 **Pop, M., Mărușteri, M., 2023. Fat Hounsfield Unit Reference Interval Derived through an Indirect**
524 **Method. *Diagnostics (Basel)* 13, 1913. <https://doi.org/10.3390/diagnostics13111913>**

525 Preisinger, R., 2018. Innovative layer genetics to handle global challenges in egg production. *British*
526 *Poultry Science* 59, 1–6. <https://doi.org/10.1080/00071668.2018.1401828>

527 R Core Team, 2023. R: A Language and Environment for Statistical Computing.

528 Resnyk, C.W., Carré, W., Wang, X., Porter, T.E., Simon, J., Le Bihan-Duval, E., Duclos, M.J., Aggrey,
529 S.E., Cogburn, L.A., 2017. Transcriptional analysis of abdominal fat in chickens divergently
530 selected on bodyweight at two ages reveals novel mechanisms controlling adiposity:
531 validating visceral adipose tissue as a dynamic endocrine and metabolic organ. *BMC*
532 *Genomics* 18, 626. <https://doi.org/10.1186/s12864-017-4035-5>

533 Rowland, K., Ashwell, C.M., Persia, M.E., Rothschild, M.F., Schmidt, C., Lamont, S.J., 2019. Genetic
534 analysis of production, physiological, and egg quality traits in heat-challenged commercial
535 white egg-laying hens using 600k SNP array data. *Genet Sel Evol* 51, 31.
536 <https://doi.org/10.1186/s12711-019-0474-6>

537 Schenkel, F.S., Miller, S.P., Wilton, J.W., 2004. Genetic parameters and breed differences for feed
538 efficiency, growth, and body composition traits of young beef bulls. *Can. J. Anim. Sci.* 84,
539 177–185. <https://doi.org/10.4141/A03-085>

540 Staub, C., Venturi, E., Cirot, M., Léonard, L., Barrière, P., Blard, T., Gaudé, Y., Gascogne, T., Yvon, J.M.,
541 Lecompte, F., Ramé, C., Reigner, F., Dupont, J., 2019. Ultrasonographic measures of body
542 fatness and their relationship with plasma levels and adipose tissue expression of four
543 adipokines in Welsh pony mares. *Domestic Animal Endocrinology* 69, 75–83.
544 <https://doi.org/10.1016/j.domaniend.2019.02.002>

545 Suzuki, K., Irie, M., Kadowaki, H., Shibata, T., Kumagai, M., Nishida, A., 2005. Genetic parameter
546 estimates of meat quality traits in Duroc pigs selected for average daily gain, longissimus

547 muscle area, backfat thickness, and intramuscular fat content. *Journal of Animal Science* 83,
548 2058–2065. <https://doi.org/10.2527/2005.8392058x>

549 Swennen, Q., Verhulst, P.-J., Collin, A., Bordas, A., Verbeke, K., Vansant, G., Decuyper, E., Buyse, J.,
550 2007. Further Investigations on the Role of Diet-Induced Thermogenesis in the Regulation of
551 Feed Intake in Chickens: Comparison of Adult Cockerels of Lines Selected for High or Low
552 Residual Feed Intake. *Poultry Science* 86, 1960–1971. <https://doi.org/10.1093/ps/86.9.1960>

553 Tixier-boichard, M., Boichard, D., Groeneveld, E., Bordas, A., 1995. Restricted Maximum Likelihood
554 Estimates of Genetic Parameters of Adult Male and Female Rhode Island Red Chickens
555 Divergently Selected for Residual Feed Consumption. *Poultry Science* 74, 1245–1252.
556 <https://doi.org/10.3382/ps.0741245>

557 Wolc, A., Arango, J., Settar, P., O’Sullivan, N.P., Dekkers, J.C.M., 2011a. Evaluation of egg production
558 in layers using random regression models. *Poult. Sci.* 90, 30–34.
559 <https://doi.org/10.3382/ps.2010-01118>

560 Wolc, A., Lisowski, M., Hill, W.G., White, I.M.S., 2011b. Genetic heterogeneity of variance in
561 production traits of laying hens. *British Poultry Science* 52, 537–540.
562 <https://doi.org/10.1080/00071668.2011.625493>

563 Wolc, A., White, I.M.S., Avendano, S., Hill, W.G., 2009. Genetic variability in residual variation of
564 body weight and conformation scores in broiler chickens. *Poult. Sci.* 88, 1156–1161.
565 <https://doi.org/10.3382/ps.2008-00547>

566 Xavier, C., Le Cozler, Y., Depuille, L., Caillot, A., Lebreton, A., Allain, C., Delouard, J.M., Delattre, L.,
567 Luginbuhl, T., Faverdin, P., Fischer, A., 2022. The use of 3-dimensional imaging of Holstein
568 cows to estimate body weight and monitor the composition of body weight change
569 throughout lactation. *Journal of Dairy Science* 105, 4508–4519.
570 <https://doi.org/10.3168/jds.2021-21337>

571 Yoo, B.H., Sheldon, B.L., Podger, R.N., 1988. Genetic parameters for Oviposition time and interval in
572 a white leghorn population of recent commercial origin. *British Poultry Science* 29, 627–637.
573 <https://doi.org/10.1080/00071668808417089>

574

575

577 **Appendix 1: Diet composition (AVRIL NUTRITION ANIMALE, Bruz, France)**

578 **Ingredients:** wheat, soybean meal, corn, sodium carbonate, dried and soluble corn distillers grains, barley,
579 monocalcium phosphate, sodium chloride, soybean oil, soybeans, wheat bran, rapeseed meal.

580

581 **Additional feedstuff:** vitamins (A: 10 000 UI/kg, D3: 3 000 UI/kg, E: 21 UI/kg), oligoelements (iron sulfate:
582 50.3 mg/kg, anhydrous calcium iodate: 1.5 mg/kg, copper sulfate: 10 mg/kg, manganese oxide II: 50 mg/kg,
583 hydrated glycine manganese chelate 30 mg/kg, zinc oxide: 50 mg/kg, hydrated glycine zinc chelate
584 30 mg/kg, sodium selenite: 0.3 mg/kg), amino-acids (L-lysine sulfate: 545 mg/kg), digestibility enhancer
585 (endo-1.4-beta-xylanase: 560 TXU/kg, endo-1.4-beta-glucanase: 250 TGU/kg, 3-phytase: 5000 FTU/kg)
586 other (lutein extract: 6.0 mg/kg, carotenoids: 4.6 mg/kg, canthaxanthine: 2.0 mg/kg), grappeseed dried
587 extract, organic acids).

588

589 **Proximate analyses:** 17.3% protein, 3.2% cellulose, 2.3% fat, 13.0% ashes, 0.9% Lysine, 0.4% Methionine,
590 3.9% calcium, 0.1% sodium, 0.4% phosphorus.