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

Nicolas Bédère, Joëlle Dupont, Yannick Baumard, Christophe Staub, David Gourichon, Elleboudt Frédéric, Pascale Le Roy, Tatiana Zerjal

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

Nicolas Bédère[1]*, Joëlle Dupont[2], Yannick Baumard[3], Christophe Staub[4], David Gourichon[3], Frédéric Elleboudt[5], Pascale Le Roy[1], Tatiana Zerjal[6]

[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

Peer-reviewed & recommended by PCI Animal Science

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 significantly 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

Introduction

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

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

The monitoring of egg production and the pedigree of laying hens has been facilitated by cage-rearing systems. In some regions of the world, cages are about to be banned and technical solutions are emerging for individual recording systems and relevant selection criteria for egg production in alternative systems (Bécot et al., 2021). Regarding fatness in chicken, like in other species, the gold standard and most common method to determine body composition are lethal and destructive because it is either a dissection with adipose tissue weighing or a chemical analysis of the shredded body. This phenotyping method is unsatisfactory because it requires the euthanasia of the animal, which raises ethical and practical problems. Indeed, the animals can no longer be used for genetic selection, except as collateral information when using allometric sequential slaughter designs to evaluate both states and dynamics of body reserves. Alternative and non-invasive methods are now available to determine body composition in various species (Lerch et al., 2021; Staub et al., 2019; Xavier et al., 2022). In poultry, tomography has proven to be sufficiently accurate to be considered as a reference method for body composition, with phenotypic correlations above 0.80 in broilers (Cobo et al., 2015; Mellouk et al., 2018b). However, the routine use of 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 2018b), to the abdominal fat pad weight by dissection (r=0.86; Mellouk et al., 2018b) and the body fat
74 volume estimated by tomography (r>0.84; Mellouk *et al.* 2018b; Grandhaye *et al.* 2019). So far, body
75 fatness traits recorded by ultrasonography were all tested on broilers while no data are available on laying
76 hens. Despite belonging to the same species (*Gallus gallus domesticus*), broilers and layers have been
77 subjected to separate and intense genetic selection for over 60 years. As a result, they differ greatly in
78 terms of growth rate and energy metabolisms. In addition, selection and phenotype recording target
79 different physiological 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

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

Phenotypes

Tomography as the Gold Standard for body composition

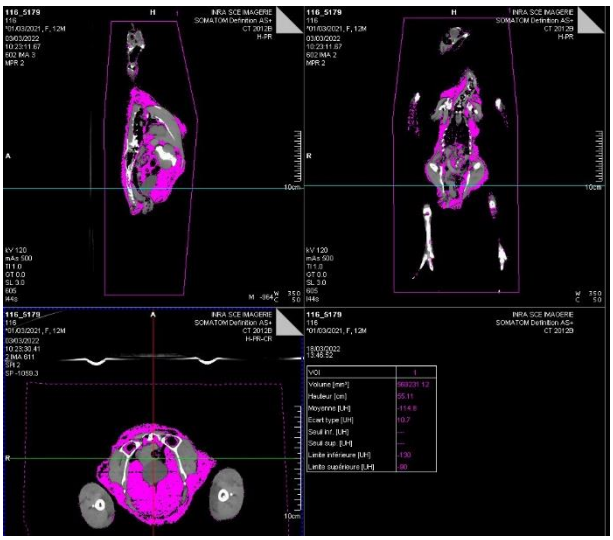

Figure 1: Example of CT-scan image visualizing the 3D axes (hen ID: PEA2021045179). The pixels set between -130 and -90 HU and colored in pink to display the fatty components

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

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

In-vivo indicators for body energy reserves

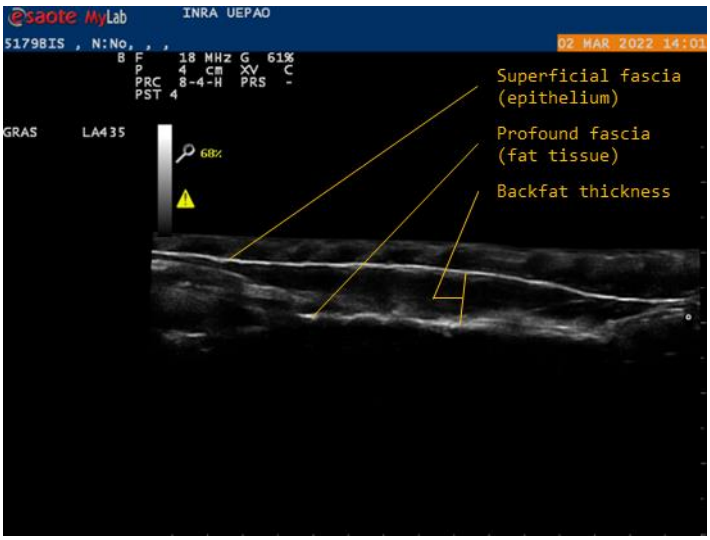

Figure 2: Ultrasound scan panoramic image of the dorsal subcutaneous adipose tissue thickness above the *synsacrum*, an example of the same hen as in Figure 1 (hen ID: PEA2021045179)

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

Blood Adipokines levels

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

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

Egg production

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

Statistical analyses

Models

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

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

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 $\mathbf{X}_2$ 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 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 vectors of breeding values, and $\mathbf{e}_1$ and $\mathbf{e}_2$ are vectors of residual values. The variance components are fitted as 2x2 matrices of variances-covariances for each stratum:

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

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

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

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

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

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

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

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

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

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

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

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

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

The phenotypic variance components are then given by:

$$\sigma^2_{total1} = \sigma^2_{u1} + \sigma^{2*}_{e1} + \sigma^*_{e1e2}$$

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

$$\sigma^2_{total2} = \sigma^2_{u2} + \sigma^{2*}_{pe2} + \sigma^{2*}_{samp2} + \sigma^*_{e1e2}$$

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

Bartlett's test

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

Programs used

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

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

Trait[1]		CT-TotFat (mm^3)	BackFat (mm)	BodyWeight (g)	Adiponectin (µg/ml)	Chemerin (ng/ml)	Ghrelin (ng/ml)	Visfatin (ng/ml)	TotEggNum (count)
Summary statistics of observed values									
R+	**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**
	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
R-	**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**
	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
Fixed effect of the genetic line estimated by the mixed models (R+ compared to R-)									
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

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

Results & Discussion

Phenotypic description of the divergent lines

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

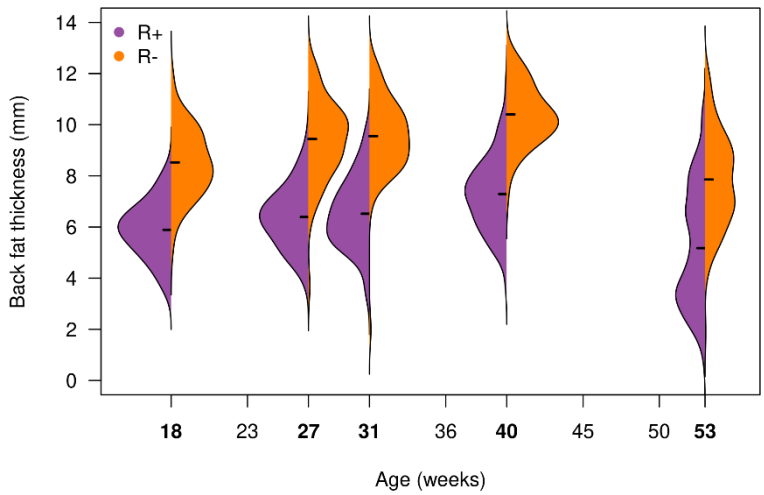

Figure 3: Distribution of the raw values for BackFat according to age in both lines. R+ are in purple on the left side of the beanplot, R- are in orange on the right side of the beanplot. The dash is the mean for each level.

BackFat thickness is an indicator of body reserves

BackFat and CT-TotFat were genetically highly positively correlated, and phenotypically moderately positively correlated (Table 2). Previous studies reported a high phenotypic correlation between BackFat and CT-TotFat in chicken ($r > 0.84$; Mellouk *et al.* 2018b; Grandhaye *et al.* 2019) but neither heritability,

nor phenotypic and genetic correlations with other traits of interest were calculated. The lower phenotypic correlation reported in Table 2 takes into account the effects of the model, which can influence the correlation estimate (genetic line, the batch, the repetition of recording, and the genetic and permanent environment variances). When we repeated the estimation using the same approach (i.e. Pearson correlation using raw data; Mellouk et al., 2018b; Grandhaye et al., 2019), we obtain a correlation value of 0.71 (0.77 in R+ and 0.60 in R-), which is consistent with the findings previoulsy published.

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

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

	CT-TotFat	**BackFat**	**BodyWeight**	**Adiponectin**	**Ghrelin**	**Visfatin**	**TotEggNum**
CT-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)**

Genetic background of BackFat thickness

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

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

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

Genetic background of other traits related to fatness

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

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

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

Tradeoff between body reserves and egg production

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

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

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

Conclusion

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

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	✔	✔		✔			✔	✔								✔

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

Acknowledgements

The authors are grateful to:

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

Ethics statement regarding animals

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

Data, scripts, code, and supplementary information availability

Data are available online: https://doi.org/10.57745/HUQOXW

Scripts and code are available online: https://doi.org/10.57745/HUQOXW

Conflict of interest disclosure

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

Funding

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

References

Arnold, J.W., Bertrand, J.K., Benyshek, L.L., Ludwig, C., 1991. Estimates of genetic parameters for live animal ultrasound, actual carcass data, and growth traits in beef cattle. Journal of Animal Science 69, 985–992. https://doi.org/10.2527/1991.693985x

Baéza, E., Le Bihan-Duval, E., 2013. Chicken lines divergent for low or high abdominal fat deposition: a relevant model to study the regulation of energy metabolism. Animal 7, 965–973. https://doi.org/10.1017/S1751731113000153

Bain, M.M., Nys, Y., Dunn, I.C., 2016. Increasing persistency in lay and stabilising egg quality in longer laying cycles. What are the challenges? British Poultry Science 57, 330–338. https://doi.org/10.1080/00071668.2016.1161727

Barbe, A., Mellouk, N., Ramé, C., Grandhaye, J., Staub, C., Venturi, E., Cirot, M., Petit, A., Anger, K., Chahnamian, M., Ganier, P., Callut, O., Cailleau-Audouin, E., Metayer-Coustard, S., Riva, A., Froment, P., Dupont, J., 2020. A grape seed extract maternal dietary supplementation in reproductive hens reduces oxidative stress associated to modulation of plasma and tissue adipokines expression and improves viability of offsprings. PLOS ONE 15, e0231131. https://doi.org/10.1371/journal.pone.0231131

Bartlett, M.S., 1937. Some Examples of Statistical Methods of Research in Agriculture and Applied Biology. Supplement to the Journal of the Royal Statistical Society 4, 137–183. https://doi.org/10.2307/2983644

Bécot, L., Bédère, N., Roy, P.L., 2021. Sélection sur la ponte des poules en systèmes alternatifs à la cage: (Full text available in English). INRAE Productions Animales 34, 1–14. https://doi.org/10.20870/productions-animales.2021.34.1.4680

Bedere, N., Berghof, T.V.L., Peeters, K., Pinard-van der Laan, M.-H., Visscher, J., David, I., Mulder, H.A., 2022. Using egg production longitudinal recording to study the genetic background of resilience in purebred and crossbred laying hens. Genetics Selection Evolution 54, 26. https://doi.org/10.1186/s12711-022-00716-8

Bordas, A., Tixier-Boichard, M., Merat, P., 1992. Direct and correlated responses to divergent selection for residual food intake in Rhode island red laying hens. British Poultry Science 33, 741–754. https://doi.org/10.1080/00071669208417515

Bourneuf, E., Hérault, F., Chicault, C., Carré, W., Assaf, S., Monnier, A., Mottier, S., Lagarrigue, S., Douaire, M., Mosser, J., Diot, C., 2006. Microarray analysis of differential gene expression in the liver of lean and fat chickens. Gene 372, 162–170. https://doi.org/10.1016/j.gene.2005.12.028

Byerly, T.C., Kessler, J.W., Gous, R.M., Thomas, O.P., 1980. Feed Requirements for Egg Production. Poultry Science 59, 2500–2507. https://doi.org/10.3382/ps.0592500

Cai, W., Casey, D.S., Dekkers, J.C.M., 2008. Selection response and genetic parameters for residual feed intake in Yorkshire swine1. Journal of Animal Science 86, 287–298. https://doi.org/10.2527/jas.2007-0396

Cobo, E., Molette, C., Touze, J.-L., Venturi, E., Bernadet, M.-D., Staub, C., 2015. Mise en place d'une méthodologie en imagerie pour prédire des caractères de production chez les palmipèdes à foie gras. Presented at the Onzièmes Journées de la Recherche Avicole et Palmipèdes à Foie Gras, Tours, France.

Diot, M., Reverchon, M., Rame, C., Froment, P., Brillard, J.-P., Brière, S., Levêque, G., Guillaume, D., Dupont, J., 2015. Expression of adiponectin, chemerin and visfatin in plasma and different tissues during a laying season in turkeys. Reproductive Biology and Endocrinology 13, 81. https://doi.org/10.1186/s12958-015-0081-5

El-Kazzi, M., Bordas, A., Gandemer, G., Minvielle, F., 1995. Divergent selection for residual food intake in Rhode Island Red egg-laying lines: Gross carcase composition, carcase adiposity and lipid contents of tissues. British Poultry Science 36, 719–728. 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., Lebret, 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 Grandhaye, 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 Lamberton, 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

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

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

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

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

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

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

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

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

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

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

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

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

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

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

Suzuki, K., Irie, M., Kadowaki, H., Shibata, T., Kumagai, M., Nishida, A., 2005. Genetic parameter 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., Decuypere, 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

Appendix

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

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

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

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