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Evidence for a constitutive cost of host resistance on body fat growth in ewe lambs from lines selected for resistance or susceptibility to experimental infections with *Haemonchus contortus*

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ABSTRACT

Although benefits of selection for host resistance to gastro-intestinal nematodes have long been recognized, its costs on production traits remain unclear. A main difficulty when studying those costs is to disentangle genetic effects due to selection from plastic responses induced by infection. Putative costs of host resistance have been extensively investigated in growing sheep. However, while most of those studies have relied on live weight to assess body growth, more comprehensive assessments accounting for body composition are advocated to detect trade-offs. In this study we used 90 female lambs from lines divergently selected on resistance to *Haemonchus contortus* that we experimentally infected ($n = 60$) or not ($n = 30$) under controlled conditions. As those conditions were defined to enable uninfected lambs to fully express their growth potential, we sought to precisely identify the effects of selection for host resistance on health traits and on growth traits. We assessed muscular and fat growth based on repeated measurements with dorsal ultrasonography for all lambs on farm, and with whole-body computed tomography (CT) scans for a subgroup of 18 infected lambs. Lambs achieved a high growth rate, including infected ones despite their high worm burden (confirmed at necropsy in the subgroup). As expected, lambs from the resistant (R) line were less infected than those from the susceptible (S) line. However, the clear pathogenic effects observed on muscular growth and voluntary feed intake were similar between lines. In contrast, a line difference in body fat was supported both by dorsal and volumetric CT measurements. Specifically, lower fat in the R line compared with the S line was observed equally in infected and uninfected groups, thus providing evidence for a constitutive cost of host resistance. Although this cost is not necessarily disadvantageous in nutrient-rich environments exposing animals to excess fat deposition, its consequences in nutrient-scarce environments may be important to promote sustainable breeding strategies for host resistance.

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1. Introduction

Selection on host resistance to parasites has long been considered of interest to improve animal health, welfare and productivity in livestock grazing systems exposing ruminants to gastro-intestinal nema-

todes (GIN) (Albers and Gray, 1987; Bisset and Morris, 1996; Stear et al., 2001). This interest is growing with the ongoing emergence of anthelmintic resistance around the world (Rose Vineer et al., 2020). As the systematic use of anthelmintic drugs becomes increasingly ineffective, breeding resistant animals may become one of the key components of parasite control strategies in the medium to long term (Torres-Acosta and Hoste, 2008; Kemper et al., 2009).

Selection for host resistance has obvious benefits on the reduction of parasitological damage (Sykes, 1994; Mavrot et al., 2015)

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but it may also lower livestock productivity if trade-offs exist between host resistance and production traits (Colditz, 2007; Greer, 2008). Although many studies addressed this issue, there is no clear genetic relationship between resistance traits (e.g. fecal parasite egg count (FEC)) and production traits (e.g. host growth rate or body weight). Instead, genetic correlations between those two types of traits vary extensively, from moderately unfavorable to favorable (see recent meta-analyses in sheep (Hayward, 2022; Mucha et al., 2022)). It has been suggested that such variation may reflect different balances between the beneficial consequences of being resistant versus the costs of being resistant (Bishop and Stear, 2003). Accordingly, unfavorable correlations should be more likely when high costs of host resistance exist. This can happen for instance if host resistance requires high amounts of scarce nutrients that could otherwise be used to fuel productive functions (Coop and Kyriazakis, 2001; Rauw, 2012). In addition to resource allocation trade-offs, several other mechanisms underlying the costs of resistance may exist but their relative importance remains largely unknown so far (Colditz, 2007).

From a genetic perspective, two types of resistance costs can be distinguished: (i) those that are directly related to the genetic potential of resistance or of production and (ii), those induced by the immune system activation during an infection (Knap and Bishop, 2000). As only the former are expected in the absence of parasite exposure, determining which one of those two types of cost prevails has emerged as a key issue to understand the evolution of host defenses in natural populations (Schmid-Hempel, 2003; Hamilton et al., 2008; Boots and Best, 2018). However it may also have important implications for livestock breeding in different contexts of infection. In the case of GIN infections, sheep lines that have been selected for high production seem to be less resistant to parasites (McEwan et al., 1992); that is consistent with the undesirable side-effects observed in other highly productive livestock (Rauw et al., 1998). However the reciprocal effects of selection for host resistance on production traits are unclear, as well as their dependence on infection. While genetically resistant sheep may grow slower than others during GIN infection, some studies attribute this effect to the nutritional cost of mounting an immune response induced by infection (e.g. Liu et al. (2005) in Merino sheep) whereas other studies link it to an inherent reduction in growth potential with increasing resistance, even in the absence of infection (e.g. Greer et al. (2018) in Romney sheep).

In the first generations of another meat sheep breed (Romane) divergently selected for resistance to *Haemonchus contortus*, we previously found evidence for a cost of host resistance on post-weaning growth by comparing lines during experimental infections indoors (Sallé et al., 2021; Douhard et al., 2022). Using the most recent generation of selection, here we aimed to disentangle the potential effects on growth directly due to genetic selection (*i.e.* occurring constitutively, even in the absence of infection) from those induced upon infection. We also attempted to better characterize this potential cost of host resistance by assessing growth not only in terms of live weight but also in terms of body composition. Indeed, even though the consequences of GIN parasites are usually considered mainly in terms of reduced protein synthesis impacting skeletal growth, muscle deposition and thus live weight gain (Sykes, 1994; Coop and Kyriazakis, 1999), our earlier findings suggested that a reduction in body fat growth might be closely associated with host resistance (Douhard et al., 2022).

2. Materials and methods

All procedures were approved by the French Ministry for Higher Education and Research and the Centre Val de Loire, France, Ethics Committee under the experimental approvals #23681 and #23800.

2.1. Animals

We used 90 female sheep (45 from the resistant (R) line and 45 from the susceptible (S) line) from the fourth generation of divergent selection (G4) on resistance to *H. contortus*. The experiment took place in the same experimental sheep farm where divergent selection was carried out (Institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), UE P3R, Osmoy, France; <https://doi.org/10.15454/1.5483259352597417E12>). The protocol of divergent selection has been previously described (Sallé et al., 2021; Douhard et al., 2022). Briefly, the phenotype of parasite resistance was based on FECs following a protocol of artificial infection with *H. contortus* L3s from the strain 'Humeau' (Lacroux et al., 2006). For each animal, two FEC records were used: a first record after an infection of 4 weeks in naïve lambs, a second record after another infection of 5 weeks. Those two FEC records were assumed to reflect a primary and secondary immune response, respectively. The two successive infections were based on a single dose of 10,000 L3s, were ended by drenching (0.2 mg/kg of live weight of ivermectin; Oramec, Boehringer Ingelheim, Lyon, France) and were separated by a 2-week recovery period. Estimated breeding values (EBVs) of the two FECs were computed using a model including fixed effects (lamb age, group pen, body weight, litter size, and sex) and an individual random effect estimated from the pedigree relationship matrix. The two EBVs per animal were then combined in an index used to select in each line 2–5% of males with the most extreme EBVs as fathers for the next generation (Sallé et al., 2021). Mating was planned to limit inbreeding.

At G4, the divergence in FECs between R and S sheep was maintained compared with previous generations. The 90 females largely differed in respect to their breeding values predicted from their parents (average index difference between lines = 1.85 genetic S. D. observed in G0). Within line, index values were not different between treatment groups of this study (Supplementary Fig. S1). Lambs from both lines were reared by their mother until 2 months of age and were managed identically after weaning. At the beginning of the experiment, initial age (Age₀) was on average 76 days (S.D. = 1.9, minimum = 72, maximum = 82). Lambs were not previously exposed to *H. contortus*, and were housed indoors during the whole experiment to prevent natural infection by GIN. The 90 lambs were transferred into pens where they could access individual automatic feeders distributing concentrates ad libitum. This feed was low-energy, high-protein concentrated pellets (dry matter 89.6%, crude protein 16.7%, crude fibre 16.1%, ether extract 2.7%, acid detergent fiber 18.5%, digestibility of organic matter 76.64%, net energy content 6.77 MJ/kg of dry matter) usually provided during the testing period to evaluate post-weaning growth potential of lambs in the breeding scheme of the Romane breed (Tortereau et al., 2020). There were four separate pens with a single feeder in each one so lambs were assigned to pens according to their body weight in order to alleviate intra-pen competition for feed access. The gate giving access to each feeder was controlled through radio frequency identification of the ear-tag so that only one individual could eat at a time, away from the others.

2.2. Experimental design

Out of the 90 female lambs, 60 were artificially infected while 30 (15 R and 15 S) were kept as uninfected controls during the whole experiment (Fig. 1). Allocation to the two treatment groups was applied at the pen level by randomly choosing two-thirds of the individuals for infection (and leaving the remaining third uninfected throughout the experiment). The protocol of artificial infection was similar to that used to phenotype parasite resistance in the divergent selection, except that a different anthelmintic-susceptible strain of *H. contortus* was used ('Weybridge') due to

production issues with the ‘Humeau’ strain. As this study focused on responses to secondary infection, Day 0 corresponds to the day when infection 2 started (i.e. Day –42 is the day of primary infection). Larvae from the secondary infection were from the same batch of coproculture as those of the primary infection. They were maintained refrigerated at 4 °C until Day –1 and revived at room temperature 24 h before secondary infection.

On Days 7, 14 and 35, three infected lambs of each line were chosen and permanently removed from the experiment (i.e. $n = 18$ in total) to perform computed tomography (CT) and necropsy measurements in addition to the measurements on farm (Fig. 1). Lambs were chosen based on their body weight (BW) and back fat thickness (BFT) measured on the day of removal to limit the risk of sampling bias. For this, all infected lambs were first classified as ‘light’, ‘medium’ and ‘heavy’ based on BW distribution in each line. Then, as heavier lambs were likely fatter (e.g. at Day 7 Pearson’s correlation coefficient (r) between BFT and $BW = +0.53$), the BFT values used for the choice of lambs were adjusted through a simple linear regression of BFT on BW in each infected line. On each day of removal, we could thus choose three infected lambs per line representing each BW category that were close to the adjusted mean of BFT. In the process, we confirmed that a linear relationship was best supported after testing quadratic and cubic effects of BW on BFT.

2.3. Measurements on farm

On farm, traits related to parasite infection (FEC and blood haematocrit (HcT)) and to lamb growth (BW, BFT and muscle thickness (MT)) were measured by farm technicians. Fecal samples were collected to measure FEC within 48 h using the modified McMaster technique (Raynaud et al., 1970). Blood was also taken from the jugular vein to measure HcT by the microhaematocrit centrifugation technique. Lambs were sampled at the beginning and the end of each infection, and weekly during infection 2 (starting at Day 21 for FECs after the prepatent period).

BW was measured weekly (every day of blood and fecal sampling) and on additional days. During infection 2, dorsal ultrasound

scans were also performed with EasiScan™ (IMV Imaging, France) to measure BFT and MT on the left and the right at the 12th–13th lumbar vertebrae. Individual values of BFT and MT were defined as the average of the measures recorded on the left and right sides of the animals.

Finally, the voluntary consumption of concentrates (kg) was recorded individually with automatic feeders. Due to logistical constraints on the experimental farm, the phase of animal adaptation to automatic feeders took place at the beginning of the experiment (during infection 1) (Fig. 1). Adapted lambs were then only fed with hay ad libitum for approximately 2 weeks to prevent excessive fat gain before infection 2. At Day –3, lambs were given access to automatic feeders again and concentrate intake was continuously recorded afterwards until Day 56. This measure was considered to reflect total feed intake as the consumption of straw bedding was negligible. Measurements obtained at each lamb visit were summed daily to calculate the voluntary feed intake (VFI, in kg/day).

2.4. Volumetric traits based on CT imaging measurements

On Days 7, 14, and 35, the 18 selected lambs were transported to the experimental facilities where CT imaging and necropsy measurements were performed (INRAE Centre Val de Loire, Nouzilly). On the day of arrival, the six animals were placed together in a straw box and were provided hay and water ad libitum. On the day after (Days 8, 15, and 36), lambs received general anaesthesia to perform CT scanning and were subsequently placed supine and then prone to acquire the scans.

CT was performed with a Somatom Definition AS 128 (Siemens Germany). The scan parameters were set as follows: 120 kV, 250 mA/s and 1 mm thick slice. In order to visualize the images in three dimensions, the smooth medium safire I26 filter was chosen to facilitate image processing. The examinations were carried out with Syngo.Via software (Siemens Healthcare SAS, Saint Denis, France). Reconstructed images (e.g. Fig. 2A–C) were based on sequential CT of approximately 1,000 normal slices per animal (e.g. Fig. 2D). Tissue volumes were automatically calculated based

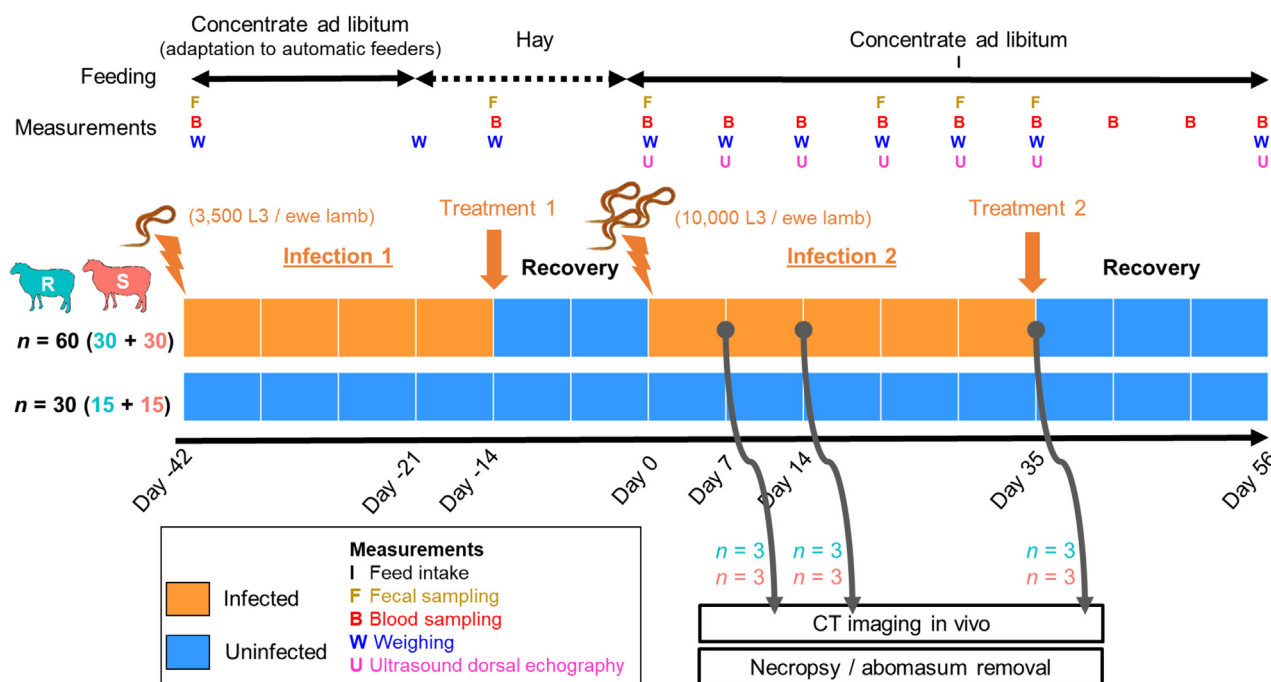


Fig. 1. Experimental design used in this study and applied to 90 female lambs from lines selected for parasite resistance (R) or susceptibility (S) to *Haemonchus contortus*.

on knowledge of the voxel resolution and its allocation to fat, muscle or 'other'. Volumes of fat (Vfat) and muscle (Vmuscle) and their sum (Vtotal) were calculated with two methods: first using the volume tool of the CT console (Somaris/7 syngo CT 2012B software), and second using the ITK-SNAP free software dedicated to image processing (Yushkevich et al., 2006). A calibration trial was previously run with sheep fat and muscle pieces that were scanned ex vivo to define Hounsfield Units (HU) threshold limits for the detection of fat (from -150 to -20 HU) and muscle (from 10 to 150 HU) in vivo. Those limits were close to that used elsewhere (e.g. from -194 to -23 HU; and from -22 to 146 HU for fat and muscle, respectively, in Kvame and Vangen (2007)). However, a lack of specificity in tissue detection was noticed, notably due to voxels wrongly allocated to fat while they actually correspond to other components in the digestive tract (e.g. food bolus, water) (Fig. 2B–D). To correct those errors, we re-analyzed images acquired between the neck and the hindquarters to discard volumes that clearly correspond to false positives in the digestive tract (Fig. 2E–G). Other potential sources of inaccuracy in the calculated volumes were not explicitly accounted for (e.g. false positives in other body parts, variation in animal position).

2.5. Necropsy measurements

Immediately after CT scanning, animals maintained under anaesthesia were humanely killed by exsanguination. The abomasum was ligated and removed. In the dissection room it was opened along the greater curvature to retrieve the contents and washings. Those were filtered through a 40 µm sieve to remove large particles and facilitate the visual detection of worms. In addition, the whole abomasum was digested in a pepsin-hydrochloric

acid solution (37 °C, 6 h) to collect the tissue-dwelling nematode stages. The volume of filtered materials was adjusted to 2 L with water and absolute alcohol (1/15 dilution), and conserved at 4 °C for later counting in the laboratory. Worms classified as L4s or adult L5s were counted in 1–3 aliquots, altogether representing at least 10% of the total volume. The total count of worms was the sum of L4s and L5s.

The mortality rate of the parasites within each lamb was calculated assuming an exponential decline (Anderson and Michel, 1977), so that for lamb *i* slaughtered at Day *k*, parasite mortality μ_{ik} was estimated as: $\mu_{ik} = -\log(\text{Total count}_{i,k}/\text{Dose}_0)/\text{Day}_k$, with Dose_0 the initial infection dose (i.e. 10,000 L3s per individual).

2.6. Statistical analysis

All statistical analyses were performed in R v4.2.2 (R Core Team, 2022. R: A language and environment for statistical computing). Linear mixed-effect models were fitted separately to each trait measured on farm (FEC, HcT, BW, BFT, MT, VFI) using the 'nlme' package (Pinheiro, J., Bates, D., 2023. nlme: Linear and Nonlinear Mixed Effects Models. <https://rdrr.io/cran/nlme/>) and following the protocol detailed by Zuur et al. (2009). Adjusted (least-square) means were calculated and compared pairwise using the package 'emmeans' (Lenth, R.V., 2023. emmeans: Estimated Marginal Means, aka Least-Squares Means. <https://cran.r-project.org/package=emmeans>). Detailed results of linear mixed-effect models including the summary of the selected model, testing of fixed effects, adjusted means and their contrasts are provided in [Supplementary Data S1](#).

In linear mixed-effect models, we included the fixed effects of the day of experiment ('Day *i*' with *i* ranging from -42 to 56; categorical),

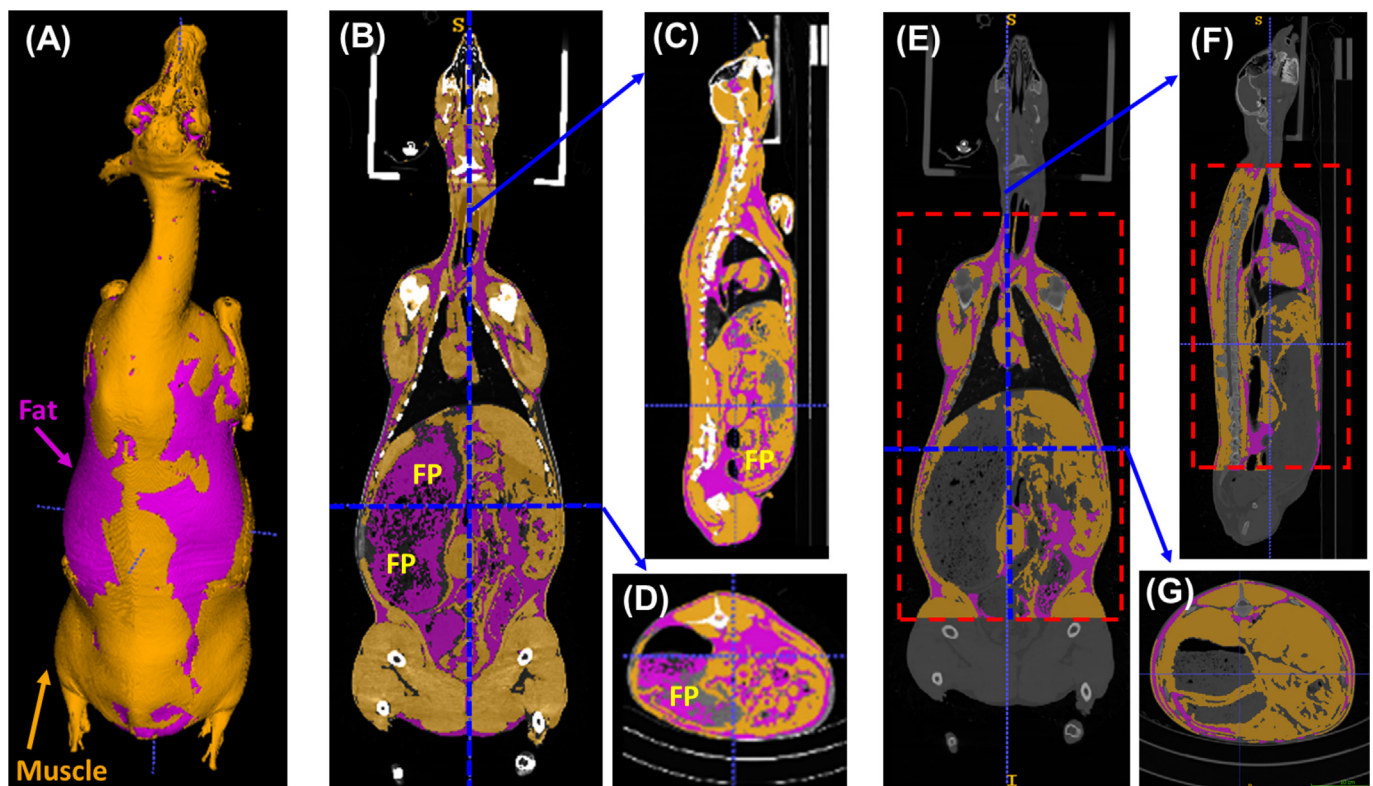


Fig. 2. Measurement of fat (in pink) and muscle (in ginger) tissues volumes based on computed tomography (CT) imaging and using ITK-SNAP free software dedicated to image processing (Yushkevich et al., 2006). Different views of the same sheep are presented, including a tridimensional reconstruction of the whole body (A) with coronal reconstructed images (B and E). The superimposed vertical and horizontal blue lines indicate planes of sections of sagittal reconstructed images (C and F) and axial normal CT images (D and G). In the whole-body analysis (B–D), volumes in the digestive tract were wrongly assigned to fat volumes (FP = false positive of fat volumes). A region from the neck to the hindquarters was re-analyzed (E–G; red dotted box) to correct fat volume. Here, muscle volume represents 13.66 dm³, and fat volume 4.56 dm³ (6.52 dm³ before correction). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

selection line ('R' or 'S'), treatment group ('infected' versus 'control'), and the associated first-order and second-order interactions between those predictors. 'Age₀', that is the age of lambs at the start of the experiment, and 'Pen' were included as additional linear and categorical fixed effects, respectively. Individual identity was defined as a random effect to account for the non-independence between individual measurements. Prior to fixed effects testing, a residual variance structure was first selected among different possibilities including (i) heterogeneous variances according to the day of experiment, the treatment group or the selection line and, (ii) several temporal correlation structures to account for the potential dependence between residuals of values observed at close time points. The residual variance structure of each trait was selected based on the Akaike information criterion (AIC), where the AIC was calculated in models containing all fixed effects and interactions under consideration. For all traits, the first-order autoregressive correlation structure (AR1) yielded the best AIC whereas the choice of heterogeneous variance was trait-dependent (Supplementary Table S1). As FEC data was not normally distributed, we explored three different data transformations (logarithmic, square root, cube root). Generalized linear models are often more suitable than linear models for overdispersed parasite data but they are also more challenging to fit, especially for multivariate models with repeated measurements (Zuur et al., 2009). Here, we preferred to rely on a single and relatively simple linear framework for all traits after checking that data transformation provides acceptable results for the linear modelling of FECs. We chose the more suitable option (i.e. cube root) based on visual assessment of the residuals (Supplementary Fig. S2). Values predicted from the selected model were back-transformed for easier interpretation.

After selecting the residual variance structure, the fixed effects of each model were tested successively in a backward stepwise process using a likelihood ratio test. To account for the dependence between body size and body fat when interpreting BFT, we also tested the inclusion of a potential linear effect of mean-centered BW (and its interaction with experimental treatment selection line) as additional covariates. Finally, to assess the potential effects of different feeding periods on body growth, we also implemented a linear mixed model for BW (as previously described) but considering the day of experiment as a continuous covariate and all the potential interactions of this covariate with feeding period, treatment group, and selection line.

Traits derived from imaging measurements on the 18 animals were analyzed using a linear model including the fixed effects of line, day of experiment (*Day*; used as categorical), and *Age* (used as continuous). In addition, scaling relationships of volumetric traits with BW were tested based on the following model:

$$\log_{10}(V_{ijk}) = \beta_0 + \text{Line}_j + b_j \cdot \log_{10}(BW_{ijk}) + \beta_{\text{Age}_0} \cdot \text{Age}_{0ij} + \text{Day}_k + \varepsilon_{ijk}$$

where V_{ijk} is the volumetric trait of individual i from line j at Day k , β_0 is the intercept, β_{Age_0} the linear effect of Age_0 , and ε_{ijk} the residual term. In addition, the linear, possibly line-specific, effect b_j related to BW corresponded to the scaling exponent in the classic untransformed model with the scaling coefficient a_{ijk} :

$$V_{ijk} = a_{ijk} \cdot BW^{b_j} \text{ where } a_{ijk} = 10^{(\beta_0 + \text{Line}_j + \beta_{\text{Age}_0} \cdot \text{Age}_{0ij} + \text{Day}_k + \varepsilon_{ijk})}$$

3. Results

3.1. Responses to parasite infection

3.1.1. Traits measured on farm: FEC and HcT

FECs of the infected lambs were large during the first infection (median = 15,900 eggs/g with 6,580 and 28,380 for first and last decile, respectively, at Day -14) compared with the second infection (median = 4,825 eggs/g with 50 and 20,045 for first and last

decile, respectively, at Day 35). Although R lambs excreted less eggs than S lambs in general (Table 1, 'Line'), this was not statistically significant during the primary infection (Fig. 3A). Moreover, during this first infection both lines had a similarly large drop in HcT compared with their control group (Fig. 3B). At the day of second infection (Day 0), the HcT of infected animals was not fully recovered compared with control animals. Recovery was achieved 1 week later during the prepatent period. At the beginning of the patent period (c.a. Day 20), the HcT dropped again rapidly but started to recover before the peak of egg excretion at Day 35 in both lines, which indicates some degree of resilience. At Day 42, that is 1 week after treatment, the HcT was fully recovered in both lines. Although R lambs excreted less eggs than the S ones from Day 20 onwards, there was little evidence that the HcT responded differently to infection between lines (cf. Table 1, the lack of significance of terms including 'Line × Treatment'). In contrast, R lambs had higher levels of HcT independent of infection (adjusted means for HcT of controls during the whole experiment: $34.4 \pm 0.5\%$ in R versus $32.5 \pm 0.51\%$ in S). Effects related to animal batching (Table 1; 'Pen') were consistent with a higher susceptibility in the lighter batch.

3.1.2. Traits at necropsy: worm counts

Worm were found in the 18 necropsied animals (Table 2). Worm counts were consistent with a progressive decline of the population and its differentiation into adult L5s at the peak of egg excretion (Day 35). In the R line, the heavy lamb had less worms than the two others at each sampling day whereas no clear pattern appeared in the S line. Numerically, total worm count and the percentage of L4s also suggested that parasite development and survival was reduced in the R line. Estimated mortality rates μ were 0.07 ± 0.040 and 0.13 ± 0.034 in lines R and S, respectively. However no difference in μ was statistically detected between lines (paired t -test $t_{(8)} = 0.90$, $P = 0.39$).

3.2. Growth and feed intake responses

3.2.1. Traits measured on farm: BW, BFT, MT and VFI

Within control groups, lines had similar BW patterns. Control lambs gained on average 178 ± 3.6 g/day from Day -45 to Day 56. However variations were observed between the different feeding periods (Supplementary Fig. S3): during the first (Day -56 to Day -21) and second (Day 0 to Day 56) ad libitum periods with concentrate, lambs gained 211 and 233 g/day, respectively (Tukey-adjusted $P = 0.27$) whereas during the intermediary period with hay (Day -21 to Day 0), BW gain of control lambs was much reduced ($+102 \pm 45$ g/day). Compared with control lambs, infected lambs had similar BW during the first ad libitum and during the intermediary period with hay. In general, infection did not strongly affect BW gain in general despite the high worm burden (Table 1). However, during the second ad libitum period infected S lambs became lighter than control S lambs from Day 28 onwards whereas infection had no effect on BW in R lambs (Supplementary Fig. S3). Of note, this BW deficit of approximately 6% in the S line was not caught up 3 weeks after the end of the second infection (Day 56), whereas some resilience was observed in the HcT even 1 week before drenching. In contrast, R lambs demonstrated a high resilience in HcT and in their body growth throughout the experiment. As the initial batching was based on BW, 'Pen' had a significant effect on BW and on most other traits dependent on BW (Table 1).

In comparison with BW, the negative effect of infection on lamb condition was more clearly and earlier reflected on MT in both lines (Table 1; Fig. 4A and B). Within each treatment group, lines had similar MT levels in general, although MT of infected R lambs dropped later than S lambs and recovered faster, almost com-

Table 1

Significance of fixed effects for the linear mixed models applied to each trait measured on farm including fecal parasite egg count (FEC) measured in the 60 female lambs infected with *Haemonchus contortus* in this study, and other traits measured in all female lambs (n = 90): blood hematocrit (HcT); body weight (BW), muscle thickness (MT), back fat thickness BFT) and voluntary feed intake (VFI). Detailed results are available in [Supplementary Data S1](#).

Model term	Trait					
	FEC ^a	HcT	BW	MT	BFT	VFI
Day (categorical)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Line (R vs. S)	<0.001	<0.001	0.108	0.482	<0.001	0.001
Treatment (infected vs. control)		0.167	0.552	<0.001	0.666	0.049
Day × Line	0.062	<0.001	<0.001	0.013	0.309	<0.001
Day × Treatment		<0.001	0.020	0.005	0.972	0.004
Line × Treatment		0.657	0.260	0.285	0.500	0.067
Day × Line × Treatment		0.049	0.182	0.866	0.740	0.951
Age ₀ (continuous)	0.118	0.290	0.178	0.456	0.156	0.009
Pen (categorical)	0.057	0.080	<0.001	<0.001	<0.001	<0.001

R, resistant; S, susceptible.

^a Using cube root transformation. Treatment effect and its interactions with Day and Line not tested as FEC was only analyzed in the infected groups (FEC = 0 in control groups).

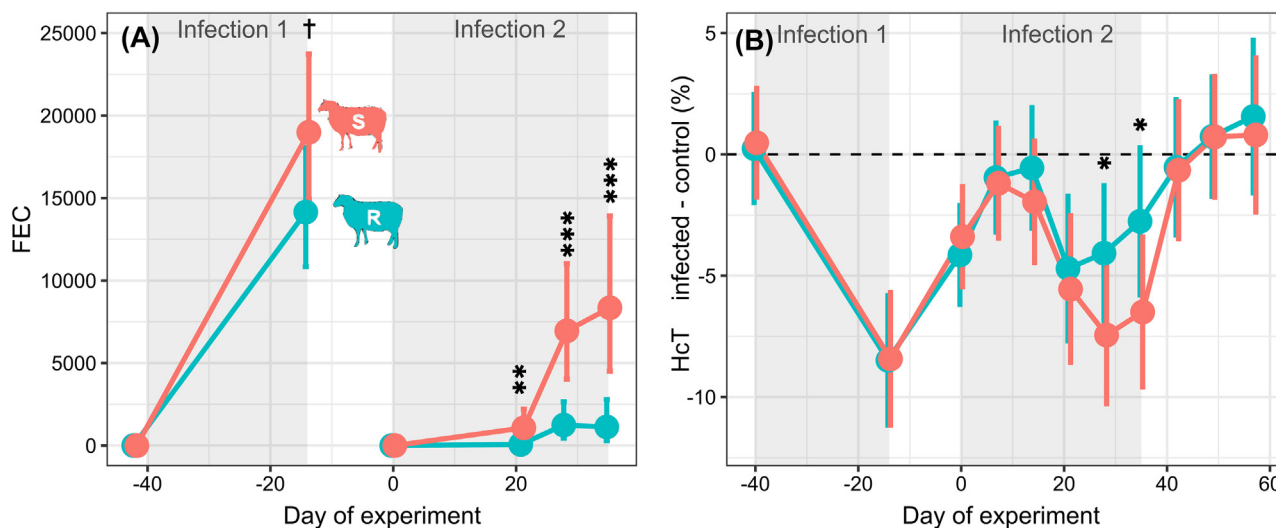


Fig. 3. Responses to two successive infections in lambs from lines selected for parasite resistance (R) or susceptibility (S) to *Haemonchus contortus*. (A) Fecal parasite egg count (FEC) of infected lambs (back-transformed after cube root transformation for data analysis). (B) Blood haematocrit (HcT) difference between infected and control lambs. Grey areas indicate the time period of primary infection (3,500 L3s of *H. contortus* per lamb at Day -41; drenching at Day -14), and of second infection (10,000 L3s of *H. contortus* per lamb at Day 0; drenching at Day 35). Circles are adjusted means in (A) and contrasts of adjusted means in (B), both obtained from linear mixed models, with error bars representing 95% confidence intervals. Statistical differences between lines: [†]P < 0.1; **P < 0.01; ***P < 0.001).

Table 2

Individual worm count data obtained for the 18 lambs slaughtered at Day 8, 15 and 35. A secondary infection took place at Day 0 with 10,000 *Haemonchus contortus* L3s per lamb. Data includes the total number of worms counted in the abomasum contents and their classification into L4s and L5s, expressed as a percentage of the total count.

Day of experiment	Stage	Total count ^a		L4 (%)		L5 (%)	
		R	S	R	S	R	S
		BW group					
8	Light	7,540	6,540	23	3	77	97
	Medium	8,200	4,160	2	0	98	100
	Heavy	440	9,030	0	0	100	100
15	Light	1,156	6,879	9	0	91	100
	Medium	6,323	65	0	0	100	100
	Heavy	363	2,805	49	20	51	80
35	Light	2,781	5,616	0	0	100	100
	Medium	113	4,602	0	0	100	100
	Heavy	29	6,880	0	0	100	100

BW, body weight; R, resistant; S, susceptible.

^a Sum of L4s and L5s sampled in the abomasum contents.

pletely, 3 weeks p.i. (Fig. 4A) contrary to S lambs that maintained their deficit (Fig. 4B).

In contrast to MT, BFT was independent of treatment group in both lines but clearly differed between lines (Table 1; Fig. 4C and

D). On average BFT of R lambs was 10% lower (3.55 ± 0.074 mm) than S lambs (3.98 ± 0.075 mm) and this effect of the line did not significantly depend on the day of secondary infection or on post-infection recovery. As expected, BFT was also strongly influ-

enced by BW (+0.071 mm/kg of BW, S.E. = 0.013, $P < 0.001$). This additional effect did not depend on the selection line nor on the treatment group. Once BW has been included as a covariate of BFT, differences between lines were confirmed ($P < 0.001$). With the model including BW, differences between lines remained independent of treatment group (as with the model excluding BW) but were dependent on the day of experiment ($P = 0.006$ for term 'Line × Day').

The difference in BFT between lines was not associated with differences in feed intake as the VFI was similar between control R lambs and control S lambs most of the time (Fig. 5). Most differences in VFI were observed within the S line between control and infected lambs. The reduction in VFI in heavily infected S lambs after the prepatent period was consistent with parasite-induced anorexia as levels progressively recovered in the control S lambs after drenching.

3.2.2. Volumetric traits based on image analysis

As expected, volumetric traits were strongly correlated between the two methods ($r = 0.97, 0.98$ and 0.99 for Vfat, Vmuscle and Vtot, respectively). However volumes calculated with ITK-SNAP tended to be lower than those obtained with the CT console (differences of 13.8%, 4.7% and 7.1% for Vfat, Vmuscle and Vtot, respectively). Moreover, Vtot obtained with the CT console scaled almost isometrically with BW as expected ($b = 1.05$; Fig. 6A), whereas Vtot calculated with ITK-SNAP tended to increase faster than BW (i.e. scaling exponent $b > 1$). In each method, no difference was detected between lines so a unique relationship could be used.

Based on CT volumes, we observed, as expected, that scaling relationships with BW were hypometric for muscle ($b = 0.77 \pm 0.11$) whereas they were hypermetric for fat ($b = 1.88 \pm 0.35$) (Fig. 6B). Scaling exponents and coefficients were not statistically different between lines although the nine R sheep tended to show a lower Vfat (Line_R – Line_S = -0.070 dm^3 , $t_{(12)} = -1.61$, $P = 0.133$) and a slightly higher Vmuscle (Line_R – Line_S = $+0.029 \text{ dm}^3$; $t_{(12)} = 2.123$, $P = 0.055$) than the nine S sheep. Put differently, the ratio Vfat:Vmuscle increased linearly with BW (Fig. 6C) and was significantly lower in the R line compared with the S line (Line_R – Line_S = -0.096 , $t_{(12)} = -2.432$, $P = 0.031$), in accordance with the BFT differences measured on farm (Fig. 4B).

4. Discussion

This study makes a new contribution to long-standing debate about the costs of breeding for host resistance on production traits (Bishop and Stear, 2003; Colditz, 2007; Greer, 2008; Williams, 2011; Doeschl-Wilson and Kyriazakis, 2012; Douhard et al., 2022; Hayward, 2022; Mucha et al., 2022). While most empirical studies in meat sheep have discussed those costs based on genetic correlations between FEC and BW or BW gains, the lack of a clear general pattern calls for more comprehensive assessments (Doeschl-Wilson et al., 2008; Hayward, 2022). Similar to other experimental designs (e.g. Greer et al., 2018), the use of selected lines experimentally infected or not under controlled conditions allowed us to disentangle genetic effects from environmental effects on infection responses and body growth. Specifically, over

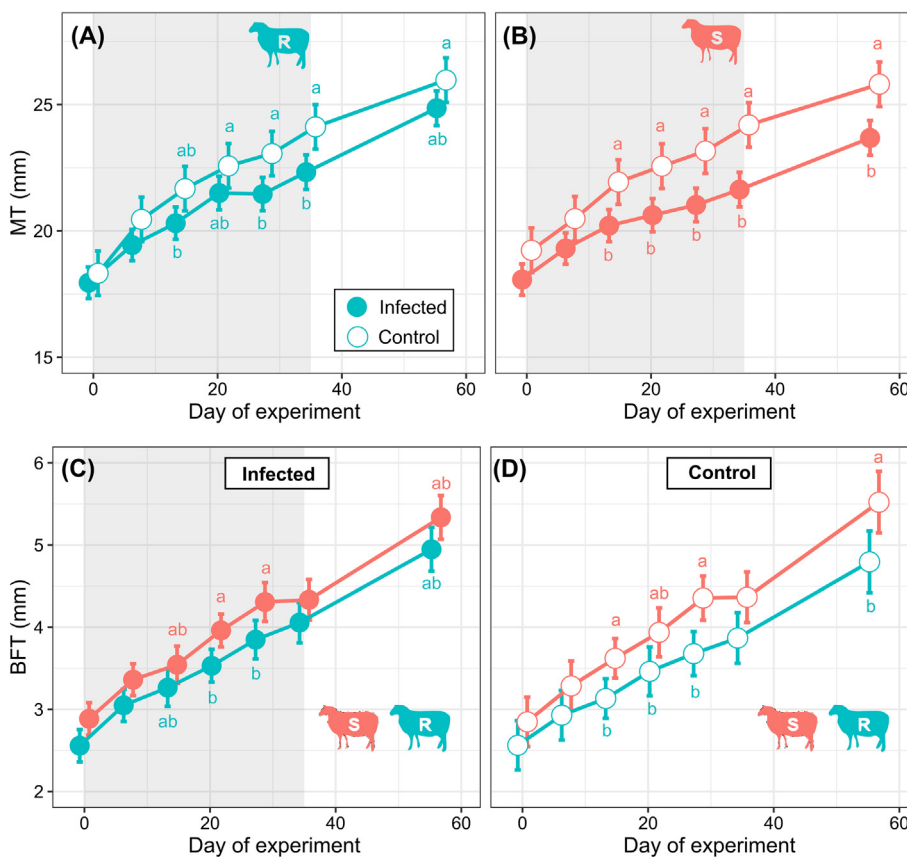


Fig. 4. Changes in muscle thickness (MT) in back fat thickness (BFT) in lambs from lines selected for parasite resistance (R) or susceptibility (S) and infected or not with *Haemonchus contortus*. Effect of infection on MT (A) in R lambs and (B) in S lambs. Effect of selection line on BFT (C) in infected lambs and (D) in uninfected lambs. Grey areas indicate the time period of secondary infection (10,000 L3s of *H. contortus* per lamb at Day 0; drenching at Day 35). Circles are adjusted means with error bars representing 95% confidence intervals. At each day where at least a difference exists among the four groups means, compact letter display is reported (means followed by different letters differ at $P < 0.05$ in A and B, and in C and D).

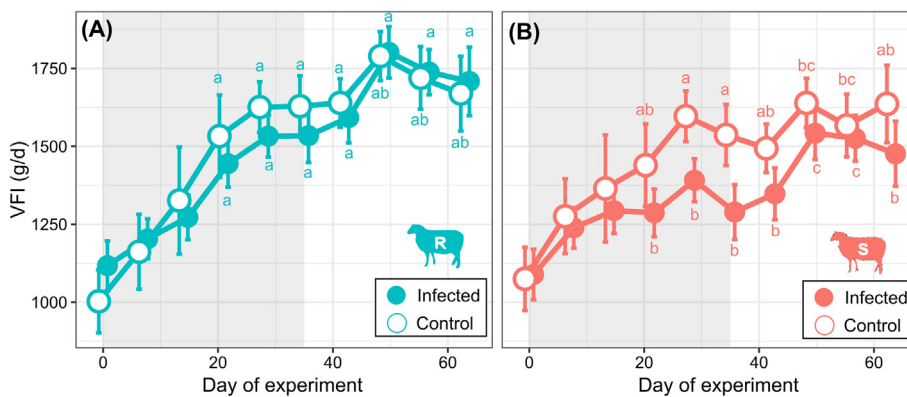


Fig. 5. Changes in voluntary feed intake (VFI) of concentrates in lambs from lines selected for parasite resistance (R) or susceptibility (S) and infected or not with *Haemonchus contortus*. Effect of infection on VFI (A) in R lambs and (B) in S lambs. Grey areas indicate the time period of secondary infection (10,000 L3s of *H. contortus* per lamb at Day 0; drenching at Day 35). Circles are adjusted means with error bars representing 95% confidence intervals. At each day where at least a difference exists among the four groups means, compact letter display is reported (means followed by different letters differ at $P < 0.05$).

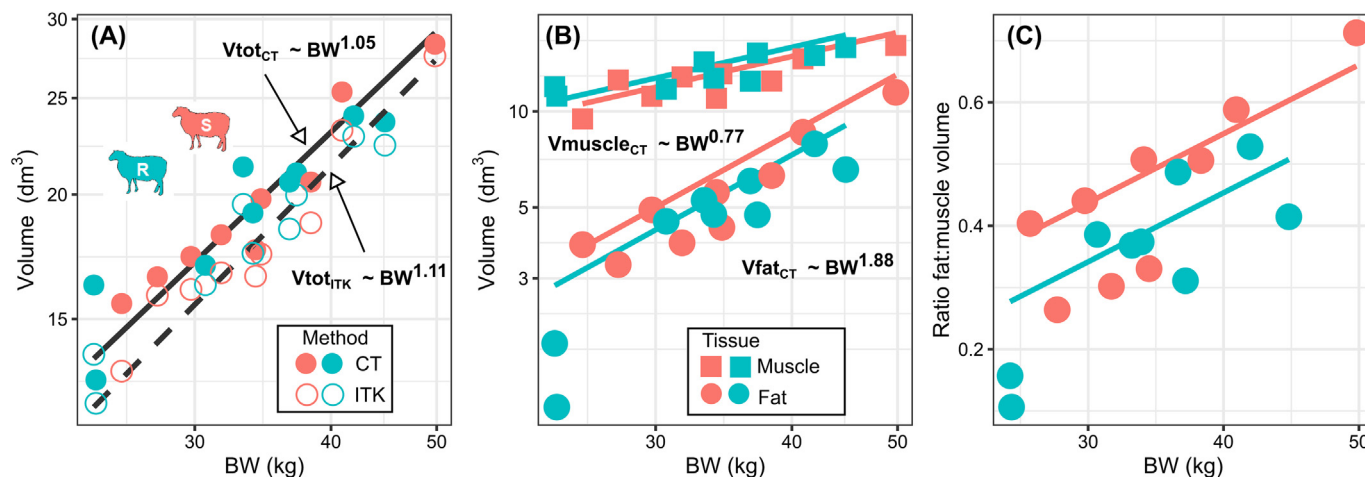


Fig. 6. Relationships between volumetric traits based on image analysis and body weight (BW) in the 18 infected lambs from lines selected for parasite resistance (R) or susceptibility (S) and slaughtered at Day 8, 15, and 35 of infection with *Haemonchus contortus*. Relationships between BW and (A) total volume (Vtot; that is the sum of fat volume (Vfat) and muscle volume (Vmuscle) according to the two methods of calculation (Vtot_{CT} = using the volume tool of the computed tomography (CT) console versus Vtot_{ITK} = using the ITK-SNAP free software dedicated to image processing (Yushkevich et al., 2006)), and based on the CT method: (B) Vfat and Vmuscle, and (C) Ratio Vfat:Vmuscle. Note that (A) and (B) describe scaling relationships with a log-scale in the two axes whereas (C) is in linear scale.

the relatively short period of a single-dose infection, we showed a clear distinction between the effects of selection line impacting fat deposition (BFT) and the effects of infection impacting muscle growth (MT).

The effects of infection are consistent with the well-known pathophysiological consequences of *H. contortus* and its blood-feeding activity (Dargie and Allonby, 1975; Parkins and Holmes, 1989). In particular, the strong HcT responses to adult worm burden (i.e., large drop after the prepatent period and quick recovery after treatment) is consistent with the massive expected loss of endogenous protein in the gastro-intestinal tract. This loss of protein likely limits muscle growth as suggested by the reduction in MT gain in infected lambs compared with their non-infected counterparts. Hence, lambs could not fully compensate for the protein deficit caused by infection despite the protein-rich diet provided ad libitum even though they demonstrated some degree of resilience in HcT during the last 2 weeks of infection. Simultaneous with the reduction in MT gain, the VFIs of infected animals tended to slow down too. In our study, this infection-induced anorexia seems then more likely to first explain muscle growth reduction compared with the hypothesis of a reallocation of proteins away from body growth and towards the gastro-intestinal tract (Coop and

Kyriazakis, 1999). Negative impacts of infection on BW appeared relatively slowly and were consistent with changes observed over several weeks of infection in other systems (Greer et al., 2018).

A more intriguing outcome of this study that has, to our knowledge, not been reported so far, is that selection for host resistance can affect body fat deposition in growing lambs. This strengthens a line of evidence for a genetic cost of host resistance on fat deposition in our selection experiment. Previously, differences in body fat between lines have been indicated by dorsal ultrasound scan measurements in infected female lambs of the second generation (Douhard et al., 2022). Here, BFT differences between lines were confirmed in females of the fourth generation, regardless of their infection status. In addition, our analysis of volumetric traits does not indicate that those genetic differences are due to a different distribution of adipose tissues in the body (e.g. subcutaneous fat on the back versus abdominal fat). Instead, it seems that the ratio body fat:muscle is lower in R lambs than in S lambs despite their similar BW. Interestingly, Greer et al. (2018) found that a Romney line selected for resistance to GIN had a lower growth potential in BW compared with a line selected on resilience. Although this last study did not provide information about body composition, an earlier physical maturity promoting an earlier acquisition of immune-

competence may be consistent with differences in body fat (Greer and Hamie, 2016).

Contrary to our expectation, our results indicate that the line effect on body fat does not depend on the infection status. In the second generation (G2), R female lambs had a slower growth in BFT than those from the S line during secondary infection, but their initial level was equal and BFT became equal again during subsequent infections during pregnancy and lactation (cf. in Douhard et al. (2022) Fig. 5 and Supplementary Fig. S3). Based on those results, we could reasonably expect an absence of constitutive difference in BFT between lines – although no BFT measurements were simultaneously performed in uninfected G2 lambs. However, in the present study as in Greer et al. (2018), the potential costs of host resistance seem to reflect inherent genetic differences between selected lines rather than different costs of infection. The experimental conditions of our earlier experiment (i.e. age of animals, rearing conditions, feed, doses of L3s) were nearly identical to those of this study, except the isolates of *H. contortus*. However we cannot exclude that those two susceptible isolates have different pathogenicity and fecundity. Moreover the current experiment does not allow us to distinguish the effects of infection from those related to immune priming. Further research would be needed to more precisely investigate if the infection status may affect the expression and the costs of host resistance in our selected lines, for instance through the simultaneous infection of naïve and primed lambs (Lacroux et al., 2006).

With increasing selection pressure on host defense (e.g. due to high parasites exposure), evolutionary theory predicts that inducible immune responses (only activated upon infection) should evolve towards constitutive responses (activated even in the absence of infection) (Boots and Best, 2018). Support for the theory has been recently presented in a well-known host-parasite system in *Drosophila* using gene expression profiles (Leitão et al., 2020; Zhou et al., 2024). However, we are not aware of similar studies in a vertebrate host. Although our results suggest that a similar phenomenon may occur in sheep, its investigation might be particularly challenging. In particular, a main roadblock would be knowledge of the precise immune mechanisms involved in sheep resistance to *H. contortus*. In our specific host-parasite system, a previous study has clearly shown a Th2-oriented immune response to *H. contortus* (Lacroux et al., 2006), however no precise effector mechanisms or quantitative trait loci (QTL) with major effects have been identified (Sallé et al., 2021, 2012). From the limited number of worm counts obtained in this study, it was not clear that the selected mechanisms of host resistance target a particular stage of parasitic phase such as L3 establishment in the abomasum.

Before discussing a potential genetic association between BFT and host resistance, one must keep in mind that alternative causes of the BFT divergence may exist. In particular, founder effects or random genetic drift can occur during selection experiments (Garland, 2003). This risk was relatively small here as animals from the initial population were chosen from different families. To fully discard this effect we would need to replicate our experimental lines using the same protocol. However this is hardly feasible in terms of costs and logistics, as for the large majority of selection experiments in large animals.

A link between adipose tissue homeostasis and type 2 immunity against parasitic helminths may exist in vertebrates (Wu et al., 2011; Kabat et al., 2022). However, the possibility that such a link could mediate a cost of host resistance has not been investigated to our knowledge. In sheep, body fat seldom has been included in estimations of genetic correlations with host resistance (contrary to BW) but the rare available estimates actually suggest an opposite trend compared with our findings, i.e. a positive association (Bishop et al., 2004; Brown and Fogarty, 2017). A favourable correlation is congruent with other findings at the phenotypic level

showing a higher resistance in ewes with high fat reserves compared with others around lambing (Macarthur et al., 2013). However, in all those different cases, levels of body fat were considerably lower than in our case (BFT approximately 2–2.5 mm versus 3.5–4 mm in this study). Here, feeding and housing conditions were similar to those used for the evaluation of unrestricted post-weaning growth in the breeding scheme (Tortereau et al., 2020) so female lambs in this experiment quickly grew and gained fat, even when they were heavily infected. Further research would then be needed to confirm that S sheep are also more susceptible to excess fat accumulation than R sheep. In line with this hypothesis, it has been shown that a mouse strain resistant to obesity expressed a stronger Th2 immune response and a better resistance to *Heligmosomoides polygyrus* compared with a strain susceptible to obesity (Wong et al., 2007).

Finally, this study has shown that selection for host resistance to a highly pathogenic helminth can entail a cost on body fat growth, even in the absence of infection. Those findings advance our current understanding of potential trade-offs between host resistance and production traits by highlighting a genetically fixed constitutive aspect of host defense on body composition. Addressing the physiological mechanisms underlying this phenotype will be needed to confirm its link with host resistance. Thereby this research may ultimately contribute to a better understanding of how different parasitic pressures may favor genetic changes in host metabolism. From a breeding perspective, a lower predisposition to accumulate fat under intensive farming conditions is usually not disadvantageous but potential adverse consequences in under-feeding situations should be addressed given the critical role of body reserves on sheep robustness, especially in parasitized environments. With an increasing variability in pasture productivity due to climate change, this will be critical to confirm that breeding for host resistance based on artificial infection indoors is an effective strategy to support sustainable parasite control (Aguerre et al., 2018).

CRedit authorship contribution statement

Frédéric Douhard: Writing – review & editing, Resources, Investigation, Formal analysis, Conceptualization, Methodology, Software, Validation, Data curation, Visualization, Supervision, Project Administration, Funding acquisition. **Xavier Matthey:** Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. **Didier Marcon:** Writing – review & editing, Resources, Investigation. **Camille Coffre-Thomain:** Writing – review & editing, Resources, Investigation. **Lucie Estivalet:** Writing – review & editing, Resources, Investigation. **Delphine Serreau:** Writing – review & editing, Resources, Investigation. **Fabrice Guégnard:** Writing – review & editing, Resources, Methodology, Investigation. **Guillaume Sallé:** Writing – review & editing, Methodology, Investigation. **Papa Moussa Drame:** Writing – review & editing, Software, Investigation, Formal analysis. **Frédéric Elleboudt:** Writing – review & editing, Resources, Investigation, Formal analysis. **François Lecompte:** Writing – review & editing, Resources, Investigation, Formal analysis. **Hans Adriaensen:** Writing – review & editing, Supervision, Software, Resources, Methodology, Investigation, Formal analysis.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.ijpara.2024.09.004>.

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