



## OPEN Performance, meat quality and blood parameters in four strains of organic broilers differ according to range use

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Chicken meat production in organic systems involves free-range access where animals can express foraging and locomotor behaviours. These behaviours may promote outdoor feed intake, but at the same time energy expenditure when exploring the outdoor area. More generally, the relationship of range use with metabolism, welfare including health, growth performance and meat quality needs to be better understood. We studied four strains of intermediate (JA757) to slow-growing (S757N, White Bresse and a dual-purpose strain) meat-type chickens with outdoor access. We selected 25 males high- (HR) and low-rangers (LR) per strain. Only in JA757, HR exhibited lower body weight before range access, which may have predisposed them to use the range more. Carcass weight and/or carcass yield were significantly lower in HR compared to LR, showing a negative trade-off between range use and growth performance in all strains. Breast meat yellowness was higher in HR compared to LR in JA757 and the dual-purpose strain, probably due to carotenoids intake from the grass. No relationship between range use and welfare indicators at slaughter was reported whatever the strain. Chicken metabolism differed by range use as HR and LR diverged for blood biomarkers of oxidative and metabolic status, immune and inflammatory system response.

**Keywords** Poultry, Free-range, Range use behaviour, Growth performance, Biomarkers

Consumption per capita of meat issued from free-ranging livestock has increased by 250% between 2009 and 2019 in Europe<sup>1</sup>, driven by the European consumer's perception that free range and animal welfare are related<sup>2</sup>. By providing an outdoor range, broilers have the possibility to more frequently express natural behaviours such as locomotor behaviour, expressed 91 times more in a free-range system than in a conventional system<sup>3</sup>. Nevertheless, outdoor range use is reported to vary from 4% to more than 50% of the chickens accessing the range daily<sup>4–6</sup>. While climatic conditions and outdoor range design influences range use<sup>6,7</sup>, several studies also showed that range use differs between chickens and is consistent within an individual over time and across contexts or situations<sup>8,9</sup>, therefore qualifying ranging behaviour as a potential personality trait<sup>10</sup>.

Free-range access can influence chicken meat quality and composition but also blood physiological parameters due to animals' foraging and locomotor activities<sup>11,12</sup>, but results are sometimes inconsistent. In fast-growing strains, ranging birds significantly gained less weight than that of non-ranging birds<sup>13,14</sup>. Accordingly, the slaughter weight of slow-growing indoor birds was significantly higher (4% to 5%) compared to slow-growing birds with outdoor access<sup>12</sup>. However, higher body weight (BW) was related to more time spent outdoors in another slow-growing strain studied by Marchewka et al<sup>15</sup>. Several studies also showed an effect of

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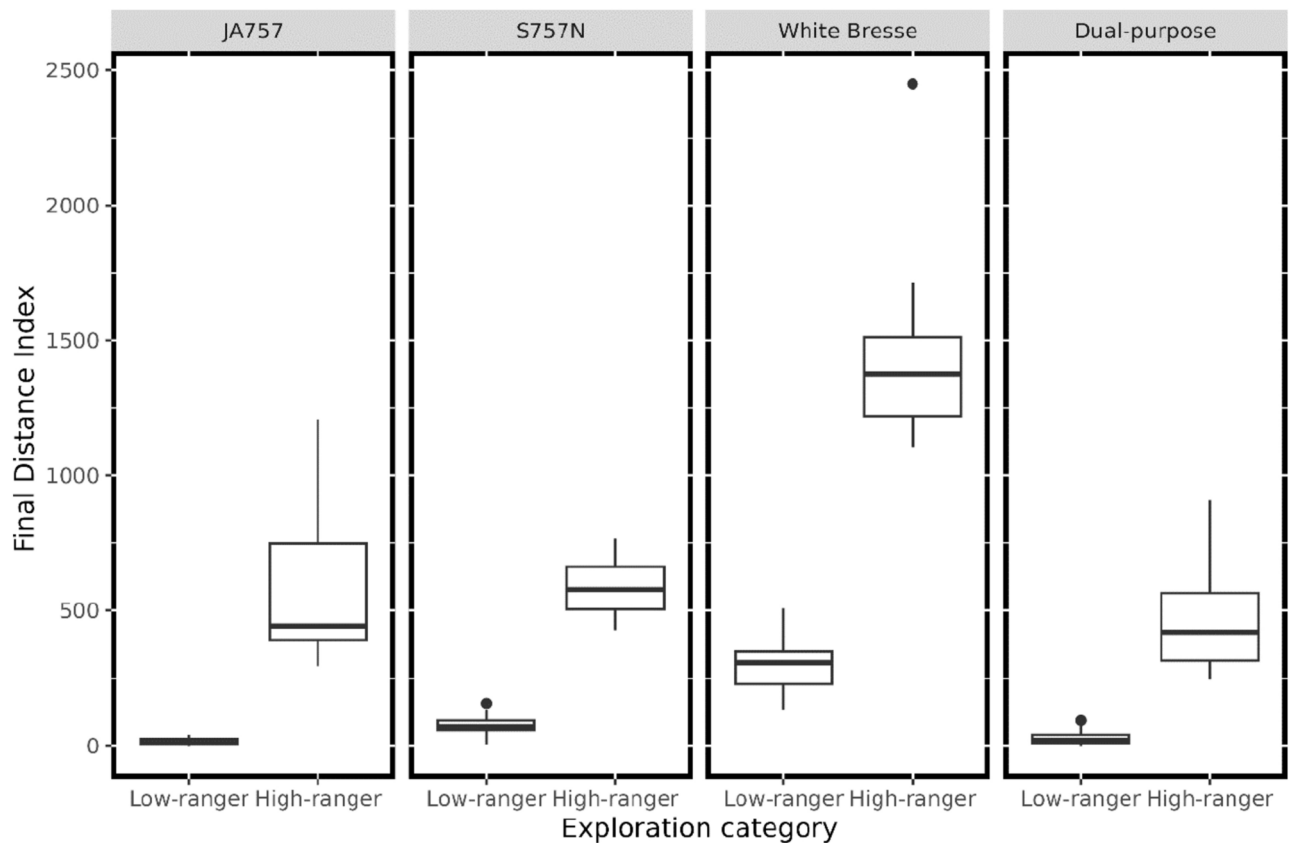
range use on slow-growing breast meat colour with darker, redder and yellower breast meat<sup>12,16</sup> while no effect was reported in other studies<sup>17,18</sup>. The inconsistency of these findings on the variability of growth rate and meat quality in relation to the range use might be due to the diversity of slow-growing chicken genotypes studied and of the environmental conditions, the varying level of free-range use and the lack of individual assessment of chickens' ranging behaviours. It is thus needed to provide a detailed characterization of broiler strains, not only on performance indicators, but also on other health and metabolic criteria considering the great diversity of genetic strains used in Europe outdoor systems.

To improve the sustainability of free-range systems considering economic, welfare and product quality impacts, a better understanding of the consequences and mechanisms activated by range use is needed. The objective of the current study was to compare indicators of performance, including meat quality, as well as animal welfare including health and metabolism between high-rangers and low-rangers from four strains of broilers. We chose four strains of various growing rate to reflect a wide range of the chicken genotypes used in free-range systems in Europe. The analysis of the four strains from very low to medium growth rate, and from local to widely used strain, and especially of broilers from these genotypes selected on their range use phenotype, will provide information to practitioners and citizens in Europe on the suitable genotypes adapted to the range use and their current internal variability. To overcome the limitations of group range use assessment, we have previously carried out an individual visual observation (scan sampling<sup>9</sup>) and high-throughput phenotyping (Radio Frequency Identification<sup>19</sup>) of range use in 100 male chickens per assessed strain. These first studies allowed us to identify high and low ranging chickens within each strain, and to show that range use is consistent with time at the individual level. In the present study, we compare performance and meat quality traits, indicators of leg health and blood parameters at slaughter of high-ranger (HR) and low-ranger (LR) birds in different strains to address the important issue of the trade-off between range use propensity and economic impact, but also physiology, health and welfare of the birds (Fig. 1).

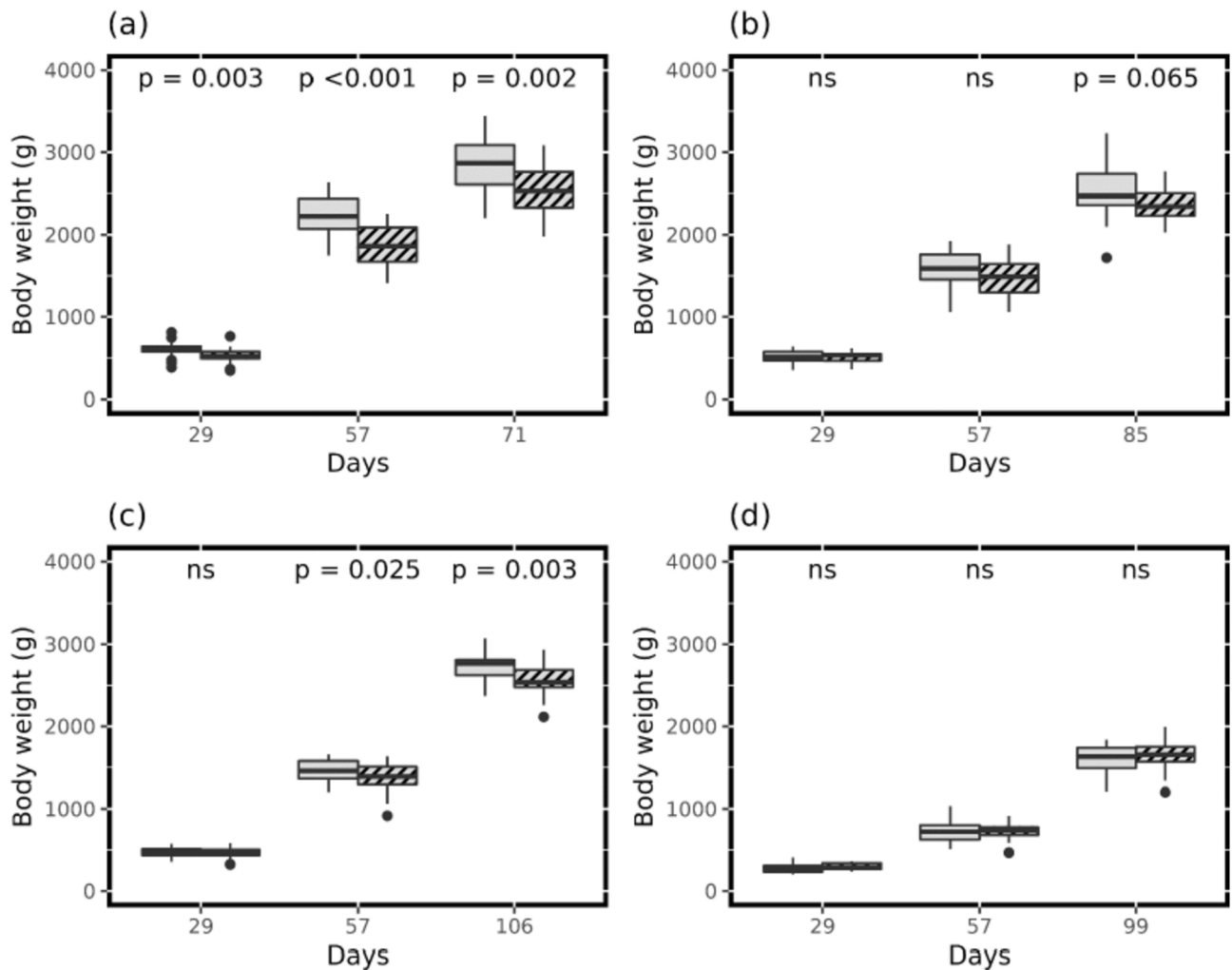
## Results

### Variation of BW according to range use

In the JA757 strain, HR exhibited a 14% lower live body weight than LR at 29 days of age, before range access (Fig. 2). After 21 days of range access, the body weight of HR was about 16% and 7% lower compared to LR in JA757 and White Bresse strains, respectively. The differences were broadly maintained at slaughter, with BW



**Fig. 1.** Boxplots showing the FDI of JA757 strain (n = 25 per range use group), S757N strain (n = 25 per range use group), White Bresse strain (n = 25 per range use group) and dual-purpose strain (n = 25 per range use group) indicating minimum, 25th percentile values of FDI, median, 75th percentile values of FDI, maximum of FDI of broilers.



**Fig. 2.** Boxplots showing the BW of (a) JA757 strain (n = 25 per range use group), (b) S757N strain (n = 25 per range use group), (c) White Bresse strain (n = 25 per range use group) and (d) dual-purpose strain (n = 25 per range use group) at three weighing times (29 days old, 57 days old and slaughter age), indicating minimum, 25th percentile values of BW, median, 75% percentile values of BW, maximum of BW of broilers depending on their range use indicated by no pattern for low-rangers and patterns with stripes for high-rangers and the p-value after t-test to compare range use categories by age for each strain.

around 11% and 7% lower in HR compared to LR in JA757 and White Bresse strains, respectively. Live body weight was not significantly influenced by range use in the dual-purpose strain, and only a tendency ( $p = 0.065$ ) was observed in the S757N strain with a reduction of about 6% in the HR compared to the LR group at 85 days old.

### Range use relationship with carcass, tibial and meat quality traits

#### Carcass traits

Range use was negatively related to the carcass traits in all the four studied strains. A reduction of 12%, 8% and 7% of carcass weight was observed in the HR compared to the LR in JA757, S757N and the White Bresse, respectively (Table 1). HR carcass yield was 2% and 1% lower than in LR in S757N and the dual-purpose strains, respectively (Table 1). Similarly, HR thigh and breast weights were lower than in LR in JA757 and S757N chickens and LR thigh weight in White Bresse chickens. Moreover, a reduction of 3% and 2% of thigh yield was associated to greater range use in S757N and the dual-purpose strain, respectively.

#### Leg health and behaviour at slaughter

None of the welfare indicators measured (hock burns and pododermatitis) nor the behavioural traits measured at slaughter (struggling activity on the shackle line and total duration of wing flapping) were significantly different depending on range use whatever the strain (Table 2). Tibial bone was 4% shorter, 5% thinner ( $p$ -value = 0.053) and 2% stronger in HR than in LR in JA757 chickens. It was also 7% thinner in HR than LR in dual-purpose chickens (Table 3). declarations

Item	JA757			S757N			White Bresse			Dual-purpose		
	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value
Carcass weight (g)	1973 ± 268	1748 ± 227	<b>0.006</b>	1740 ± 243	1606 ± 150	<b>0.047</b>	1802 ± 131	1672 ± 145	<b>0.006</b>	997 ± 120	1026 ± 125	0.605
Carcass yield (% of BW)	69.4 ± 1.3	68.6 ± 1.5	0.072	69.0 ± 1.6	67.6 ± 1.3	<b>0.006</b>	65.7 ± 1.4	65.1 ± 1.4	0.176	63.2 ± 1.3	62.3 ± 1.1	<b>0.009</b>
Breast weight (g)	233 ± 37	201 ± 31	<b>0.006</b>	183 ± 30	168 ± 18	<b>0.047</b>	176 ± 15	165 ± 19	0.068	83 ± 13	84 ± 12	0.702
Breast yield (% of BW)	16.4 ± 1.1	15.8 ± 1.0	0.072	14.5 ± 1.1	14.1 ± 1.0	0.236	12.8 ± 0.7	12.8 ± 0.8	0.994	10.5 ± 0.9	10.3 ± 0.6	0.605
Thigh weight (g)	351 ± 48	315 ± 37	<b>0.012</b>	322 ± 39	300 ± 33	<b>0.047</b>	358 ± 27	332 ± 27	<b>0.006</b>	195 ± 23	199 ± 24	0.653
Thigh yield (% of BW)	24.7 ± 0.9	24.8 ± 0.5	0.518	26.0 ± 1.3	25.2 ± 1.0	<b>0.047</b>	26.2 ± 0.9	25.9 ± 0.8	0.316	24.8 ± 0.5	24.2 ± 0.6	<b>0.018</b>
	LR (n = 11)	HR (n = 12)	P-value	LR (n = 10)	HR (n = 12)	P-value	LR (n = 11)	HR (n = 12)	P-value	LR (n = 13)	HR (n = 12)	P-value
Tibia length (mm)	134.0 ± 5.6	128.2 ± 4.5	<b>0.006</b>	137.7 ± 5.3	137.0 ± 6.5	0.765	153.0 ± 2.3	150.5 ± 4.3	0.288	126.8 ± 5.8	128.1 ± 5.9	0.591
Tibia diameter (mm)	8.2 ± 1.1	7.8 ± 0.6	0.053	8.1 ± 0.8	7.8 ± 0.9	0.758	8.9 ± 0.8	8.8 ± 0.7	0.786	7.6 ± 0.6	7.1 ± 0.4	<b>0.042</b>
Tibia bone-breaking strength (N)	238.4 ± 54.7	242.6 ± 29.8	<b>0.021</b>	248.2 ± 33.6	230.6 ± 50	0.758	311.6 ± 61.5	296.8 ± 46.5	0.786	189.3 ± 34.5	173.2 ± 26.2	0.302

**Table 1.** Effects of range use on growth and tibial quality traits (mean ± SD) in four different strains of organic broilers. Significant values are in bold.

	JA757			S757N			White Bresse			Dual-purpose		
	LR (n = 25)	HR (n = 25)	P value	LR (n = 25)	HR (n = 25)	P value	LR (n = 25)	HR (n = 25)	P value	LR (n = 25)	HR (n = 25)	P value
Total duration of wing flapping (mean ± SD)	4.11 ± 4.82			3.65 ± 4.85			1.64 ± 4.43			3.79 ± 4.97		
Scores of leg damage	0	1	2	0	1	2	0	1	2	0	1	2
Hock burn (% of flock)	60	32	8	92	8	0	52	44	4	100	0	0
Pododermatitis (% of flock)	4	48	48	28	56	16	16	72	12	25	75	0
Activity on the slaughter line (Yes)	28			28			12			29		
Chickens (%)	36			0.880			1.000			1.000		

**Table 2.** Effects of range use on welfare indicators in four different strains of organic broilers.

Item	JA757			S757N			White Bresse			Dual-Purpose		
	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value
<b>Muscle characteristics</b>												
Pectoralis major muscle weight (g)	181 ± 29	156 ± 25	<b>0.006</b>	139 ± 24	126 ± 14	0.225	128 ± 12	120 ± 14	0.243	62 ± 9	63 ± 9	0.802
pH 15 min post-mortem (pH15)	6.56 ± 0.13	6.49 ± 0.12	0.053	6.40 ± 0.14	6.38 ± 0.15	0.837	6.46 ± 0.13	6.48 ± 0.13	0.903	6.15 ± 0.15	6.17 ± 0.14	0.776
Ultimate pH (pHu)	5.67 ± 0.07	5.78 ± 0.12	<b>0.004</b>	5.77 ± 0.10	5.76 ± 0.11	0.837	5.49 ± 0.11	5.46 ± 0.10	0.903	5.65 ± 0.08	5.61 ± 0.11	0.495
<b>Meat quality traits</b>												
Lightness (L*)	48.1 ± 1.6	46.5 ± 2.1	<b>0.007</b>	45.1 ± 2.0	46.0 ± 1.8	0.504	47.7 ± 3.1	48.5 ± 3.5	0.903	44.9 ± 2.5	45.4 ± 2.8	0.776
Redness (a*)	-0.4 ± 0.5	0.3 ± 0.5	< <b>0.001</b>	0.7 ± 1.0	0.6 ± 0.9	0.921	0.7 ± 0.8	0.7 ± 1.1	0.947	1.8 ± 1.5	2.1 ± 1.2	0.776
Yellowness (b*)	10.2 ± 1.2	11.3 ± 1.4	<b>0.009</b>	11.1 ± 1.4	11.1 ± 1.7	0.973	11.9 ± 1.3	11.8 ± 1.0	0.903	10.2 ± 1.4	12.0 ± 1.7	<b>0.002</b>
Drip loss (DL, %)	1.1 ± 0.4	1.1 ± 0.3	0.487	0.9 ± 0.3	0.9 ± 0.3	0.837	1.1 ± 0.3	1.2 ± 0.3	0.903	1.5 ± 0.5	1.5 ± 0.7	0.776
Thawing-cooking loss (TCL, %)	14.3 ± 2.6	15.6 ± 2.8	0.121	13.5 ± 4.7	14.7 ± 7.4	0.837	11.0 ± 1.7	11.1 ± 1.8	0.903	17.0 ± 3.4	16.4 ± 3.3	0.776
Warner Bratzler shear force (WB, N/cm <sup>2</sup> )	17.0 ± 2.5	16.3 ± 2.1	0.323	16.2 ± 2.9	16.7 ± 2.2	0.837	16.3 ± 1.7	16.2 ± 2.1	0.903	22.4 ± 5.5	20.6 ± 7.6	0.776

**Table 3.** Effects of range use on *Pectoralis major* weight, pH and quality measurements (mean ± SD) in four different strains of organic broilers. Significant values are in bold.

### Breast and thigh meat quality and composition

In the JA757 strain, HR chickens had a higher pHu (+0.11 pH units) associated with darker, redder and more yellow meat than LR chickens. In the dual-purpose strain, breast meat was also yellower in HR compared to LR chickens (Table 3).

Regarding thigh meat, whatever the strain and the level of range use, the main fatty acids were palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2) acids. Range use only increased  $\alpha$ -linolenic acid (C18:3 n-3) content in the dual-purpose strain, with a 22% higher percentage observed in HR compared to LR chickens (Table 4).

### Range use and blood physiological parameters at slaughter

As observed in thigh meat, the main fatty acids of plasma in the four studied strains were palmitic (C16:0), stearic (C18:0), oleic (C18:1) and linoleic (C18:2). The n-6/n-3 ratio was reduced by 26% with greater range use in the S757N strain, while an increase by 62% of this ratio with greater range use was observed in the White Bresse strain (Table 5). In the White Bresse strain, HR chickens also had a 24% higher triglyceride concentration than LR chickens. Additionally, their Total Antioxidant Status was increased by 10%. In the S757N strain, HR chickens had lower blood concentration or activity for uric acid (−19%), creatine kinase (−17%), haptoglobin-like (−30%) and lysozyme (−25%) than LR chickens. Similar effects were observed in the JA757 strain for creatine kinase (−31%) and lysozyme (−29%). In this genetic strain, the HR birds were also characterized by lower Total Antioxidant Status (−8%) and tocopherols concentration (−35%), but a higher value of hydrogen peroxide (+22%). Regarding the dual-purpose strain, the only significant difference was in creatine kinase activity, decreased by 22% with range use.

### Discussion

Given the high societal expectations for animal welfare and the development of production systems offering animals access to the outdoors, it is important to acquire knowledge about their range use capabilities and the consequences on physiological and productive functions. Because outdoor access can relate to different types of production, our study considered 4 strains of chickens with different growth rates (from 16 g/d to 36 g/d) and different rearing durations (from 71 to 106 days).

It should be noted that the intra-population variability in range use (as assessed by the FDI calculation) differed between the strains studied. All birds of one genetic strain were kept in one barn and one outdoor range; while the design of all the barns was similar, the ranges differed, particularly in the location of trees (Supplementary Figure S1). Not only genetics but also these confounding environmental factors may have

Item	JA757			S757N			White Bresse			Dual-Purpose		
	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value
<b>Muscle characteristics</b>												
pHu in the thigh muscle	6.02 ± 0.10	6.02 ± 0.09	0.808	5.87 ± 0.12	5.86 ± 0.07	0.977	5.75 ± 0.11	5.73 ± 0.09	0.562	5.90 ± 0.09	5.90 ± 0.09	0.922
<b>Chemical (%) and fatty acids composition (% of total fatty acids)</b>												
	LR (n = 11)	HR (n = 12)	P-value	LR (n = 10)	HR (n = 12)	P-value	LR (n = 11)	HR (n = 12)	P-value	LR (n = 13)	HR (n = 12)	P-value
Lipids	6.8 ± 1.1	6.2 ± 1.0	0.399	5.5 ± 2.3	5.5 ± 2.0	0.999	4.5 ± 0.6	4.1 ± 0.8	0.405	4.2 ± 0.9	4.3 ± 0.7	0.859
Myristic (C14:0)	0.5 ± 0.0	0.4 ± 0.1	0.223	0.4 ± 0.0	0.4 ± 0.0	0.999	0.5 ± 0.1	0.4 ± 0.1	0.662	0.5 ± 0.0	0.5 ± 0.1	0.393
Palmitic (C16:0)	20.7 ± 1.2	19.5 ± 0.8	0.095	19.6 ± 0.8	19.8 ± 0.9	0.999	19.7 ± 1.0	19.3 ± 1.6	0.664	19.6 ± 1.2	18.6 ± 1.3	0.300
Stearic (C18:0)	6.0 ± 1.0	6.9 ± 1.3	0.223	8.1 ± 1.6	8.2 ± 1.0	0.999	8.4 ± 0.8	9.1 ± 1.2	0.348	8.5 ± 0.8	9.0 ± 0.6	0.393
Palmitoleic (C16:1)	3.5 ± 0.8	2.6 ± 0.6	0.095	1.9 ± 0.3	1.7 ± 0.3	0.525	2.0 ± 0.3	1.6 ± 0.3	0.150	1.4 ± 0.3	1.3 ± 0.3	0.393
Oleic (C18:1)	37.3 ± 1.4	37.1 ± 1.6	0.828	36.5 ± 2.3	36.7 ± 1.6	0.999	36.3 ± 1.4	35.4 ± 1.1	0.348	35.4 ± 1.5	34.9 ± 1.5	0.526
Paullinic (C20:1)	0.34 ± 0.02	0.35 ± 0.04	0.593	0.37 ± 0.06	0.39 ± 0.04	0.525	0.35 ± 0.06	0.38 ± 0.08	0.365	0.36 ± 0.02	0.37 ± 0.02	0.300
Linoleic (C18:2n-6)	27.3 ± 1.9	28.6 ± 1.3	0.223	27.8 ± 0.9	28.1 ± 1.3	0.999	28.1 ± 1.6	28.1 ± 3.1	0.948	29 ± 1.9	29.9 ± 1.6	0.393
Alpha-linolenic (C18:3n-3)	2.1 ± 0.2	2.3 ± 0.1	0.095	1.9 ± 0.1	2.0 ± 0.2	0.904	1.7 ± 0.2	1.7 ± 0.3	0.674	1.8 ± 0.2	2.2 ± 0.2	<b>0.007</b>
Saturated fatty acids	28.2 ± 2.1	28.5 ± 2.5	0.828	27.7 ± 0.9	29.5 ± 1.7	0.270	28.7 ± 1.4	28.6 ± 1.8	0.948	28.8 ± 1.3	28.2 ± 1.7	0.526
Monounsaturated fatty acids	40.3 ± 2.6	39.8 ± 2.8	0.828	39.8 ± 2.2	38.5 ± 2.7	0.999	38.9 ± 1.6	37.4 ± 1.3	0.170	36.9 ± 1.5	36.7 ± 1.7	0.827
Polyunsaturated fatty acids	31.8 ± 2.9	33.1 ± 3.2	0.728	33.2 ± 2.1	33 ± 2.8	0.999	32.1 ± 2.0	32.9 ± 3.4	0.638	34.4 ± 1.1	35.3 ± 2	0.393
n-6/ n-3	6.8 ± 1.1	7.0 ± 1.3	0.828	6.8 ± 1.1	6.6 ± 1.4	0.999	7.2 ± 1.7	6.7 ± 1.1	0.663	6.7 ± 0.7	6.3 ± 0.5	0.393

**Table 4.** Effects of range use on thigh muscle pH and meat quality measurements (mean ± SD) in four strains of organic broilers. Significant values are in bold.

	JA757			S757N			White Bresse			Dual-Purpose		
	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value	LR (n = 25)	HR (n = 25)	P-value
Uric acid <sup>b</sup> (mg/L)	41 ± 15	33 ± 12	0.108	43 ± 11	35 ± 7	<b>0.020</b>	28 ± 9	32 ± 8	0.269	28 ± 6	32 ± 10	0.586
Glucose <sup>b</sup> (mg/L)	2282 ± 110	2296 ± 115	0.747	2454 ± 218	2384 ± 173	0.325	2514 ± 193	2584 ± 124	0.274	2433 ± 138	2392 ± 107	0.516
Carbonyl <sup>a</sup> (nmol /mg of protein)	0.9 ± 0.4	0.9 ± 0.5	0.765	0.8 ± 0.2	0.7 ± 0.2	0.325	0.7 ± 0.2	0.8 ± 0.2	0.269	1.2 ± 0.5	1.0 ± 0.5	0.468
Triglycerides <sup>b</sup> (mg/L)	231 ± 51	199 ± 54	0.068	183 ± 49	165 ± 38	0.285	209 ± 45	259 ± 64	<b>0.024</b>	163 ± 36	187 ± 29	0.060
Creatine Kinase activity <sup>a</sup> (U/L)	5801 ± 1984	4013 ± 1045	<b>0.003</b>	3871 ± 951	3228 ± 912	0.054	3195 ± 839	3765 ± 922	0.120	4848 ± 1621	3775 ± 1274	<b>0.007</b>
Infectious bronchitis antibody titres <sup>b</sup>	6363 ± 1580	5988 ± 1699	0.679	3668 ± 1978	3232 ± 1870	0.482	5174 ± 2026	5769 ± 1660	0.393	4101 ± 2450	3410 ± 2671	0.516
Haptoglobin-like activity <sup>b</sup> (mg/ml)	2.2 ± 0.4	2.0 ± 0.6	0.549	2.3 ± 0.6	1.6 ± 0.6	<b>0.003</b>	2.3 ± 0.7	2.2 ± 0.5	0.628	1.4 ± 0.3	1.3 ± 0.3	0.707
Lysozyme activity <sup>a</sup> (mg/L)	5.5 ± 1.9	3.9 ± 1.2	<b>0.002</b>	4.0 ± 1.1	3.0 ± 0.7	<b>0.003</b>	4.0 ± 1.3	3.9 ± 1.4	0.690	2.8 ± 1	2.9 ± 0.9	0.707
H <sub>2</sub> O <sub>2</sub> <sup>a</sup> (µM)	10.2 ± 2.8	12.4 ± 3.7	<b>0.046</b>	9.2 ± 4	11.4 ± 4.5	0.144	11.0 ± 2.4	10.5 ± 2	0.626	18.5 ± 2.6	18.4 ± 3.3	0.946
Total Antioxidant Status <sup>b</sup> (mmol/L)	1.2 ± 0.2	1.1 ± 0.1	<b>0.003</b>	1.1 ± 0.2	1.1 ± 0.1	0.373	1.0 ± 0.1	1.1 ± 0.1	<b>0.024</b>	1.0 ± 0.1	1.0 ± 0.1	0.801
Σ Tocols <sup>b</sup> (µmol/L)	2.6 ± 0.8	1.7 ± 0.7	<b>&lt;0.001</b>	2.8 ± 1.4	2.3 ± 1.4	0.146	2.2 ± 1.5	2.3 ± 1	0.404	4.9 ± 4.0	3.7 ± 2.4	0.516
Glutathione Peroxidase activity <sup>b</sup> (U/L)	15.433 ± 2258	15.205 ± 1640	0.747	17,206 ± 2747	16,716 ± 3050	0.561	18,393 ± 2417	17,577 ± 2020	0.346	17,135 ± 2053	16,666 ± 1979	0.754
Fatty acids <sup>a</sup> (% of total fatty acids)												
Myristic (C14:0)	0.15 ± 0.11	0.20 ± 0.11	0.204	0.12 ± 0.06	0.14 ± 0.04	0.369	0.17 ± 0.11	0.17 ± 0.10	1	0.14 ± 0.05	0.12 ± 0.05	0.468
Palmitic (C16:0)	16.8 ± 1.8	16.4 ± 1.5	0.677	16.8 ± 2.1	17.3 ± 1.4	0.476	15.3 ± 2.2	15.5 ± 1.7	1	15.6 ± 1.4	15.5 ± 1.3	0.861
Stearic (C18:0)	18.9 ± 3.3	19.2 ± 3.1	0.854	18.9 ± 2.9	20.2 ± 2.6	0.221	12.8 ± 1.9	12.9 ± 2.1	1	19.2 ± 3.4	19.3 ± 3.9	0.861
Palmitoleic (C16:1)	0.54 ± 0.24	0.48 ± 0.25	0.654	0.34 ± 0.13	0.26 ± 0.09	0.138	0.36 ± 0.36	0.27 ± 0.21	0.810	0.19 ± 0.08	0.21 ± 0.08	0.512
Oleic (C18:1)	12.2 ± 3.7	12.7 ± 3.6	0.960	11.3 ± 1.8	10.5 ± 1.2	0.221	9.9 ± 3.1	10.5 ± 2	0.891	9.4 ± 1.6	9.7 ± 1.1	0.468
Paullinic (C20:1)	0.13 ± 0.16	0.16 ± 0.19	0.960	0.12 ± 0.15	0.09 ± 0.13	0.554	-	-	NA	0.10 ± 0.10	0.11 ± 0.14	0.468
Linoleic (C18:2n-6)	16.4 ± 2.9	17.6 ± 4	0.654	18.2 ± 5.1	15.4 ± 3.4	0.221	21.6 ± 5.4	23.5 ± 3.3	0.891	18.5 ± 4.6	19.1 ± 4.2	0.790
Alpha-linolenic (C18:3n-3)	0.57 ± 0.33	0.64 ± 0.3	0.654	0.39 ± 0.16	0.37 ± 0.17	0.320	0.51 ± 0.13	0.53 ± 0.10	0.891	0.57 ± 0.31	0.60 ± 0.35	0.861
Saturated fatty acids	36 ± 4	36 ± 4	0.960	36 ± 5	38 ± 3	0.221	28 ± 3	28 ± 4	0.891	35 ± 4	35 ± 5	0.861
Monounsaturated fatty acids	13 ± 4	13 ± 4	0.960	12 ± 2	11 ± 1	0.221	10 ± 3	11 ± 2	0.891	10 ± 2	10 ± 1	0.468
Polyunsaturated fatty acids	36 ± 4	40 ± 8	0.204	36 ± 5	35 ± 3	0.554	40 ± 7	39 ± 5	0.891	40 ± 3	41 ± 4	0.861
n-6/ n-3	4.3 ± 2.5	3.6 ± 2.2	0.654	7.5 ± 1.7	5.6 ± 2.1	<b>0.002</b>	4.4 ± 2.1	7.1 ± 1.2	<b>&lt;0.001</b>	4.3 ± 1.3	4.0 ± 1.7	0.468

**Table 5.** Effects of range use on broiler's blood physiological parameters (mean ± SD) at slaughter time in four different strains of organic broilers. <sup>a</sup>Blood analysis on serum samples. <sup>b</sup>Blood analysis on plasma samples. Significant values are in bold.

contributed to the gap between high and low rangers among the four flocks we studied. This is a limitation of the current study, which should be repeated in different situations, such as seasons and free range or barn design, to gain knowledge on how each strain uses the outdoor range. Nevertheless, we observed evidence of trade-offs between range use, performance, health and welfare, which will be discussed in the light of previous research on the impact of outdoor access and knowledge of bird's physiology.

### Relationship between range use and growth performance

High-rangers of the JA757 strain had lower BW after range access but also before range access than low-rangers. These results are in line with previous studies on fast-growing strains that already reported weight difference prior to range access<sup>14</sup>. When studying behavioural budget before range access, we reported a positive correlation between active behaviours (locomotion and foraging) and the later range use in the JA757 strain<sup>9</sup>. This suggests that, in fast- or intermediate-growing strains, higher activity levels of the birds may be a reason for their lower weight gain, but also that lower weights may be less of a hindrance to mobility<sup>14</sup>. This does not seem to be the case in the studied slower-growing strains for which we did not report any difference in BW before range access between high and low rangers, nor any correlation between active behaviours indoors before range access and later range use<sup>9</sup>. When considering the final body weight or the carcass weight at slaughter, a negative trade-off with range use was obvious for all the genotypes except the slowest-growing dual-purpose strain. Considering that locomotor activity on the range and range use are positively correlated<sup>20</sup>, our results seem consistent but highlight the economic challenge of allowing free ranging to express natural behaviour.

### Range use, welfare and behavioural indicators at slaughter

We scored hock burns and pododermatitis as welfare indicators, considering them as potential markers of pain and injury<sup>21</sup>. We observed few differences for leg health between HR and LR in the four studied strains. This is consistent with previous studies showing that outdoor range use did not impact foot pad dermatitis and/or hock burns scores in fast-growing strains<sup>14</sup> or in slow-growing strains<sup>12,15</sup>. Although our results showed that the birds studied were more affected than in the study by Marchewka et al<sup>15</sup>, it is consistent with the results of Stadig et al<sup>12</sup>, who found that most chickens were not affected by hock burns but had a mild degree of pododermatitis. The pododermatitis incidence of our study may be related to the presence of mud in front of the poultry house due to a rainy spring. We observed no difference on tibia bone parameters by range use groups in S757N and White Bresse strains which is in accordance with a previous study comparing slow-growing strains indoor and outdoor<sup>22</sup>. However, one study observed shorter tibia bones in a slow-growing strain reared outdoors compared to indoors<sup>23</sup> and studying females of fast-growing strains, decreased tibial length and midpoint cross-sectional area was associated with forced exercise<sup>24</sup>. Shorter, thinner and stronger tibia bone in JA757 and thinner tibia bone in dual-purpose crossbreed in HR compared to the LR may be due to their higher locomotor activity on the range. Therefore, our results indicate that higher range use interlinked with a supposedly higher locomotor activity may positively affect bone health in the intermediate-growing line that was slaughtered at the younger age (around ten weeks) compared to other strains. Finally, it has been previously shown that struggling on the shackle line was more intense in slow-growing than in fast-growing lines<sup>25</sup>. The duration of wing flapping was even shown to be heritable in a Label Rouge type line<sup>26</sup> whose growing rate corresponded to the strain S757N studied here. As the present study did not find any relationship between range use and struggling behaviour at slaughter, the two traits appear to have different determinants.

### Range use relationship with meat quality

Regarding breast meat quality, we observed a higher pHu and a darker meat in HR than in LR in the JA757 strain only. Darker meat has already been reported in slow-growing birds with outdoor access compared to those reared indoors<sup>12</sup>. Previous studies reported that in chickens glycolytic potential negatively correlated with ultimate pH<sup>27</sup> and positively with pH<sub>15</sub> while pH<sub>15</sub> is negatively correlated to struggling activity on the shackle line<sup>28,29</sup>. Therefore, even though we did not observe significant differences in struggling activity on the shackle line, decreased pH<sub>15</sub> reported in high-ranger chickens may suggest higher muscle glycolytic activity before slaughter, which may have contributed to the observed higher pHu in HR than in LR in JA757 chickens. The differences in pHu and pH<sub>15</sub> observed between HR and LR in the strain JA757 were neither related to differences in drip and cooking loss, nor in tenderness. No effect of range use on these traits was observed in the other three strains, which is consistent with previous results<sup>16</sup>. Additionally, breast meat of HR was yellower than that of LR in both JA757 and dual-purpose strains. Increased yellowness has been reported in several other studies<sup>12,30,31</sup> and could be caused by the intake of fresh plants (e.g., grass or clover) containing carotenoids<sup>11,32</sup>. We can hypothesize that JA757 and dual-purpose high-rangers dedicated a higher percentage of their time to foraging activities, and/or their metabolism is able to assimilate more pigments than other strains from the ingested plants, leading to a higher enrichment of their muscles in carotenoids. Overall, range use and breast meat quality of slow-growing strains were barely associated while, even though non-detrimental, more pronounced colour differences were observed in strain JA757, which may be related to differences in pigment content or energy metabolism in the muscle.

Whatever the strain, no difference in thigh ultimate pH (proxy of glycogen reserve) and proximal composition between high and low-ranger were reported, which is in line with previous results<sup>12,18</sup>. However, when looking at the fatty acid composition, higher percentage of  $\alpha$ -linolenic acid (C18:3 n-3) in the thigh muscle of HR compared to LR of the dual-purpose strain were observed. As  $\alpha$ -linolenic acid is an omega 3 precursor, there could be a nutritional interest to its increase. Although the pasture fatty acids profile was not investigated,  $\alpha$ -linolenic acid increase could be due to higher grass ingestion as observed in previous studies<sup>11</sup>.

### Broilers' blood parameters divergence with range use

Several biomarkers of the metabolism, inflammation, immunity and redox status were measured to assess the relationship between range use and chicken's physiological and health status at slaughter age. Most of the differences between HR and LR were observed in the most productive strains of this study, JA757 and S757N. Indeed, we reported a reduced lysozyme activity in the two strains, coupled with a reduced haptoglobin-like activity in the S757N strain. Lysozyme is an innate immunity protein indicative of acute or chronic inflammation<sup>33</sup> and the haptoglobin-like activity is indicative of an inflammatory status<sup>34</sup>. Our results are in accordance with previous results showing lower levels of lysozyme activity in strains spending more time outdoors<sup>35</sup> and in rearing systems with outdoor access compared to indoor rearing<sup>36</sup>. Whether range use has an immunosuppressing effect or a protective effect on anti-inflammatory status depends on the level of expression and therefore concentration in the serum or plasma but also on the level of expression before access. Additional data showed similar activity of haptoglobin-like before range access and a reduced increase of haptoglobin-like in the HR compared to the LR (Supplementary Figure S2). Therefore, we suggest a possible positive impact of range use with lower acute and chronic inflammation in the S757N strains but we cannot draw a clear conclusion regarding the lysozyme activity as we do not have measurements before range access.

Creatine kinase is a muscle enzyme that plays a role in the production of energy and is closely associated with muscle growth rate and activity<sup>37</sup>. We observed a reduction in creatine kinase activity with range use in all the strains except the White Bresse. This result seems consistent with the reduction in breast muscle mass observed in high rangers from the JA757 and S757N strains but suggests another physiological determinant for the dual-purpose strain for which no difference on breast muscle mass or yield with range use was found.

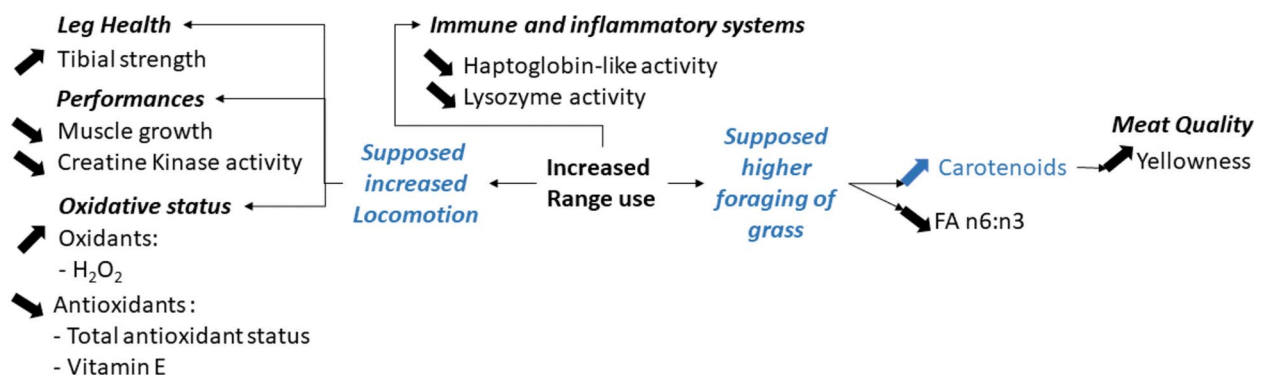
Additionally, high range use also reduced blood concentrations of antioxidants. In the strain JA757, total antioxidant status (TAS) and the sum of tocopherols (including tocopherols) decreased with range use, while pro-oxidative substances ( $H_2O_2$ ) increased. Following similar trends, uric acid concentration, which acts as an antioxidant<sup>38,39</sup>, also decreased with range use in the strain S757N. Our observations are quite consistent with previous results that showed that locomotor activity (which is likely higher in HR chickens than in LR chickens) of Ross 308 broilers and other slow-growing strains are correlated to higher oxidative indicators and lower antioxidant concentration in the blood<sup>11,20,40</sup>. Therefore, range use seems to induce an imbalanced redox status in the JA757 strain, characterized by the highest growth rate among the studied strains.

Regarding blood lipids, it is worth noting that although no changes in FA concentration and profile was observed between high- and low-rangers, n-6/n-3 ratio decreased with greater range use in S757N. It has been shown that pasture composition contains lower n-6/n-3 ratio and higher tocopherol concentration than diets classically provided to chickens<sup>11,41</sup>, which can explain changes in n-6/n-3 observed in the blood of S757N. In the White Bresse strain, an increase in total antioxidant status, triglyceride and n-6/n-3 ratio was observed in high-ranger chickens, which is difficult to explain. Because additional data reported no difference in triglycerides concentration between HR and LR of the White Bresse strain earlier during rearing (64 days of age) (Supplementary Figure S3), it is difficult to conclude if lipid changes observed at slaughter in this strain are related to differences in range use or as a response to fasting or other stressful conditions that may affect bird metabolism before slaughter.

To summarize our results, we reported in Fig. 3 some of the main relationships evidenced between range use and indicators including performance, health, welfare and meat quality traits.

### Conclusions

In our experimental conditions of spring rearing on free ranges, we showed that increased range use was generally associated with reduced growth performance, except for the dual-purpose strain. We did not observe any effect on the various welfare indicators studied, except for an improvement in leg health with increased range use in the fastest growing strain. The latter also showed the most variability between high and low rangers for parameters related to redox, inflammatory and immune status and meat quality. This study provides a multifactorial assessment of the potential effects of range use which can be used to guide the development of



**Fig. 3.** Graphical conclusion summarizing the potential relationships that were outlined in our study in the JA757, S757N and the dual-purpose strains. In black are the observed results in our study and in blue are the suppositions.

breeding practices, such as enriching the range with plants with antioxidant to counteract the adverse effects of increased range use on redox balance status. Further research is also needed to characterise the effects of seasons and variation with geographical regions, which could have a more or less pronounced impact on range use and bird physiology depending on genetics.

## Materials and methods

### Animals, housing and experimental design

The four slow to medium growing breeds studied were Hubbard JA757 (used for French certified chicken production), naked-neck Hubbard S757N (used for Label Rouge production), White Bresse (a local breed used for the French Bresse production) and an experimental dual-purpose crossbreed (developed from a crossing between two laying strains). Their respective growth rates (GR) in this experiment were 36, 26, 23 and 16 g/d and rearing duration was 71, 85, 106 and 99 days, respectively. Two batches of one-day-old chicks (male/female ratio of 1), issued from three different hatcheries (Hubbard chicks were from the same hatchery), were received 15 days apart. They were all vaccinated against coccidiosis, Marek's and Gumboro diseases and infectious bronchitis. Each strain was reared in one identical barn with straw litter in spring 2021. All chickens were identified by a wing tag at 7 days old but only 100 males per strain were randomly selected and equipped with a plastic poncho around the neck with a unique acronym to easily follow animals during scan samplings. Chickens housing, lighting programme and identification is fully detailed in Bonnefous et al.<sup>9</sup>. We chose to study males due to a potential favourable effect of this sex for range use, as male chickens have been reported to have higher number of exits than females<sup>42,43</sup>. Range access was provided to all chickens from 36 days of age. Ranges measured 2500 m<sup>2</sup> (50 m × 50 m) and were planted with grass and unevenly distributed trees (density of 60 to 76 trees per hectare). We provided water and 100% organic feed (including 3 diets based on soya meal, maize, wheat and sunflower meal with in addition barley, alfalfa and triticale from day 29 to day 57 or with faba beans and triticale in addition from day 57 to the end of the rearing period) and characterized by 15.8 MJ/kg and 20.8% Crude Protein (CP) from day 1 to day 28, 15.6 MJ/kg and 18.7% CP from day 29 to day 57, and 15.3 MJ/kg and 17.4% CP from day 57 to the end of the fattening period) ad libitum. All strains had access to the same feed formulated according to the S757N strain nutritional needs, which is commonly used in organic rearing in France.

### Measurements

The definition of the measures according to the ontologies is available in Supplementary Table S1, except for the range use indicator. All chickens fasted since 5 am, were captured and loaded in a small truck at 8 am in the morning of the slaughter. After a two and a half hour driving, the chickens were slaughtered at 1 pm.

### Individual range use

Individual range use was assessed according to the method validated by Ferreira et al.<sup>42</sup> and fully described in Bonnefous et al.<sup>9</sup>. Briefly, we located chickens on the range by visual observation and reported the distance between the broiler and the poultry house to calculate a distance index per day of observation. We calculated the final distance index (FDI) of each individual by adding the distance index from the first to the last day of scan sampling observation. Based on the FDI of each of the 100 individuals studied per strain, we ranked them and divided them into two extreme groups of 25 birds per strain. We named the group with the highest FDI 'high-ranger' (HR) and the group with the lowest FDI 'low-ranger' (LR) (Fig. 1). The average difference between the two groups was equivalent to 1.6–1.9 standard deviation of the trait depending on the strain.

### Live body weight

Chicken live body weights were measured before range access at 29 days of life, after range access at 57 days of life and at slaughter at 71, 85, 106 and 99 days for the JA757, S757N, White Bresse and dual-purpose breeds, respectively, following a fasting period of 8 h.

### Struggling activity, foot pad dermatitis and hock burn evaluation on the slaughter line

Following the measurement of live body weight, birds were hung on the slaughter line and electrically stunned in a water bath before bleeding. Struggling activity between hanging and stunning was evaluated by two measurements: straightening up (SU) of the body (head over the legs) observed by one person as a binary score equal to 0 when the bird stayed immobile and 1 otherwise, and the total duration of wing flapping (TDWF) also observed by one person<sup>29</sup>. After stunning, bleeding and scalding, foot pad dermatitis and hock burns were evaluated by visual inspection with a 3-point system<sup>44</sup>. Briefly, one observer for both foot pad dermatitis and hock burns scaled as 0 for no lesion present, 1 for a mild lesion (lesion ≤ 7.5 mm) and 2 for severe lesion (lesion > 7.5 mm).

### Carcass traits

Carcass weight was measured 1-day post-slaughter and carcass yield was calculated in relation to live body weight measured at the slaughterhouse before stunning. Left drumsticks of 23, 22, 23 and 25 birds of the JA757, S757N, White Bresse and dual-purpose strain respectively, were deboned. Tibia diameter and length were measured with an e-calliper and bone-breaking strength of the tibia was determined with an Instron Universal Testing Instrument (model 5543, INSTRON, Norwood, USA)<sup>45</sup>.

### Weight and quality indicators of breast meat

At 15 min post-mortem, the pH (pH15) was measured in the left *Pectoralis (P.) major* muscle as described in Berri et al.<sup>37</sup>. Two grams of fresh muscle were immediately homogenized in 18 ml of 5 mM iodoacetate/ 15 M KCl solution. The pH of the homogenate was measured with a portable pH meter (Model ph330i, WTW GmbH,

Vienna, Austria) equipped with a combined glass electrode. After carcass dissection 1-day post-slaughter, the right breast muscle (*P. major* and *minor* muscles) weight was measured. The corresponding total breast yield was calculated in relation to live body weight measured at the slaughterhouse before stunning. At 24 h post-mortem, the pH (pHu) was measured by direct insertion of the electrode in the *P. major* muscle (Model ph330i, WTW GmbH, Vienna, Austria). Colour was measured at 24 h post-mortem on the upper ventral side of this muscle by using a spectrophotometer (CM 700 D, Konica Minolta Inc, Tokyo, Japan), applying the CIELAB trichromatic system as lightness ( $L^*$ ), redness ( $a^*$ ) and yellowness ( $b^*$ ) values. Drip loss (DL) of the same *P. major* muscle between 24 and 72 h post-mortem was measured as described in Le Bihan-Duval et al.<sup>46</sup>. After weighing, whole muscle was placed in a plastic bag, hung from a hook, and stored at 2 °C for 48 h. After hanging, the muscle was wiped with absorbent paper and weighed again. The difference in weight corresponded to the DL and was expressed as the percentage of the initial muscle weight. After DL measurement, *P. major* was vacuum-packed and stored at -20 °C until further analyses. Following Le Bihan-Duval et al.<sup>27</sup>, breast muscle was thawed overnight at 4 °C, cooked in a water-bath at 85 °C for 7 to 10 min depending on the muscle weight to obtain an internal endpoint temperature of 72 °C, and cooled in crushed ice for 10 min. The thawing-cooking loss was expressed as a percentage of the fresh muscle weight. The toughness of cooked meat was evaluated by the Warner Bratzler (WB) shear test using an Instron Universal Testing Instrument (model 5543, INSTRON, Norwood, USA).

### Weight and quality indicators of thigh meat

Thigh muscle weight was measured after carcass dissection, 1-day post-slaughter. Corresponding ratios were calculated in relation to live body weight measured at the slaughterhouse before stunning. At 24 h post-mortem, the pH (pHu) was measured by direct insertion of the electrode in the muscle. Proximate and fatty acids composition of a mix of all thigh muscles were calculated from chemical measurements on 23, 22, 23 and 25 birds of the JA757, S757N, White Bresse and dual-purpose strains, respectively. Dry matter, protein and lipid contents were analysed as follows: oven method<sup>47</sup> for moisture and minerals, Kjeldahl copper catalyst method<sup>47</sup> for protein, and chloroform/ methanol procedure of Folch et al.<sup>48</sup> for total lipid content. Fatty acid composition was determined after transmethylation of lipids<sup>49</sup> by gas chromatography (Perkin Elmer Autosystem, St Quentin en Yvelines, France). Injector and detector (FID) temperatures were 250 °C, and the carrier gas was nitrogen with a head column pressure of 16.5 psi using a capillary column (25 m × 0.22 mm, BPX, SGE, Villeneuve St Georges, France). Finally, methyl esters were identified and quantified by comparison with standards (Sigma, St Quentin Fallavier, France).

### Serum and plasma analyses

We measured blood parameters to gain knowledge on the physiology and health of chickens of HR and LR groups. First, because high-rangers are more prone to be exposed to pathogens outdoors than low-rangers, we measured blood parameters related to the immune and inflammatory system responses. Second, range use by chickens may also be linked to their activity<sup>19</sup>. This may impact on how they spend and store energy as reflected by their lipid, creatine kinase and glucose blood parameters measurements and on their redox status indicators. Third, high-rangers may ingest more grass than low-rangers<sup>50</sup>, which may impact chicken blood metabolic indicators.

We collected blood just after stunning from the carotid bloodletting, half in a heparinized tube and the other half in a dry tube. Blood samples were then centrifuged, after one hour in ice or at room temperature respectively for plasma and serum, then kept at -80 °C until further analysis. Details on coefficient of variation intra and inter-assay are present in Supplementary Table S2.

Commercial kits (Thermo Fisher Diagnostics SAS, France) were used to determine plasma glucose (mg/L), uric acid (mg/L) and triglyceride (mg/L) concentrations. Total plasma antioxidant activity was determined through total antioxidant status (TAS) measurement (mmol/L) (Randox Laboratories, UK). The activities of serum creatine kinase (CK) and plasma glutathione peroxidase (GPx) were measured with commercial kits (Biolabo, Malzy, France and Randox Laboratories, London, United Kingdom respectively). The plasma haptoglobin-like activity (mg/mL), that increases in response to acute infection or inflammation<sup>51</sup>, was measured using a commercial kit (Tridelta Development Limited, Maynooth, Ireland). Protocols listed above were used in accordance with the supplier instructions and adapted to the Thermo Scientific Arena 20XT photometric analyzer (Thermo Fisher Scientific, Courtaboeuf, France). The commercial kit (OxiSelect™, Cell Biolabs, San Diego, USA) was used to quantify serum hydrogen peroxide (μM).

The commercial ID Screen® IBV Indirect kit (IDVET, Grabels, France) was used to qualify the vaccine response to infectious bronchitis by ELISA in plasma. The concentration of antibodies specifically targeting infectious bronchitis antigen was evaluated spectrophotometrically at 450 nm and thus calculated and expressed in antibody titres in order to assess the immune system activity.

The detection of protein carbonyl groups in serum was made following the method of Dalle-Donne et al., (2003), using 2,4-dinitrofenilhidrazina (DNPH) as reactive. Briefly, 500 μL of serum was diluted to 1:40 with phosphate-buffered saline (PBS) before two centrifugations at 1,317 g for 5 min. The solution was extracted after each centrifugation. One mL of trichloroacetic acid (TCA) was added to the extracted solution before centrifugation at 1,317 g for 3 min. The pellets from trichloroacetic acid (TCA) extracts were mixed with 1 mL of 10 mM DNPH in 2 M hydrochloric acid (HCl). Samples were incubated for 1 h at room temperature and then centrifuged at 1,317 g for 5 min. To remove unreacted DNPH, supernatants were discarded and the pellets were washed 3 times with 1 mL of ethanol – ethylacetate (1:1, v/v). The pellets were then dissolved in 1.5 mL of 6 M guanidine-HCl and centrifuged as above to pellet insoluble particles. The carbonyl content of the resulting supernatants was evaluated spectrophotometrically at 366 nm using a molar extinction coefficient of 22,000 1/M\*cm. Protein concentrations were measured using the Bradford method with Coomassie Brilliant Blue

G-250<sup>53</sup>, with bovine serum albumin as the standard. Finally, carbonyl proteins values were expressed as nmol of carbonyl/ mg of protein in the guanidine chloride solution.

The tocopherols (i.e.,  $\alpha$ -tocopherol and its isoforms  $\gamma$  and  $\delta$ , and  $\alpha$  and  $\gamma$ -tocotrienol) levels were measured in plasma according to Schüep and Rettenmaier<sup>54</sup>. Briefly, 0.5 mL of plasma was mixed with 0.5 mL of ethanol solution, 1 mL of saline solution and 4 mL of hexane / ethanol solution of 0.06% butylated hydroxytoluene (BHT). The mixture was sonicated in a sonication bath at intensity 30 for 10 min. Following centrifugation, 3.5 mL of the supernatant was transferred into a glass tube, dried under nitrogen flow, and resuspended in 200 mL of acetonitrile. The solution was reextracted twice and 50  $\mu$ L of final acetonitrile solution was then injected into the High-Performance Liquid Chromatography with Fluorescence Detection (HPLC-FD) (pump model Perkin Elmer series 200, equipped with an autosampler system, model AS 950–10, Jasco, Tokyo, Japan) on a Sinerhy Hydro-RP column (4 mm, 4.6  $\times$  100 mm; Phenomenex, Bologna, Italy). The flow rate was 2 mL/min. All tocopherols and tocotrienols were identified using a Fluorescence Detector (model Jasco, FP-1525—excitation and emission wavelengths of 295 and 328 nm, respectively) and quantified using external calibration curves prepared with increasing amounts of pure standard solutions (Sigma-Aldrich, Bornem, Belgium) in ethanol. The tocopherols were expressed as  $\mu$ mol/L and their sum was used for statistical analysis.

The lipid fraction for fatty acid evaluation was extracted from serum following the method reported by Folch et al.<sup>48</sup>. To obtain the fatty acid methyl esters, the lipid extract was dried with a rotavapor and 300  $\mu$ L of n-hexane was added. Finally, the transesterification procedure was performed with 150  $\mu$ L of 2 M potassium hydroxide (KOH) methanol solution at 60 °C for 10 min, following the method reported by Glass and Christopherson<sup>55</sup>. For fatty acid quantity calculation, heneicosanoic acid was used as the internal standard (C21:0, Sigma-Aldrich analytical standard). To calculate the sum of the total saturated (SFA), total monounsaturated (MUFA), and total polyunsaturated (PUFA) acids from the n-3 and n-6 series, we used the average amount of each fatty acid. We then calculated the n-6/n-3 fatty acid ratio. Fatty acids results were expressed as % of total fatty acids.

Lysozymes in serum were evaluated following the method of Osserman and Lawlor<sup>56</sup>. Briefly, 40  $\mu$ L of serum were distributed in a well within a petri dish containing a micro-organism sensitive to lysozymes' lytic activity (*Micrococcus lysodeikticus*). After an incubation of 18 h at 37 °C, the diameter of the ring was measured with an e-calliper. The activity of lysozymes in the serum was calculated and expressed in  $\mu$ g/ml (or mg/L), using a petri dish with known activity of lysozymes as reference.

### Statistical analysis

In order to evaluate the relationship between range use groups and body performances, meat quality or blood quantitative parameters within each line, we compared the means of the two groups using Student's test or Wilcoxon–Mann–Whitney's test depending on the normality that were evaluated by Shapiro's test. For qualitative parameters, we used a Fisher exact test. We applied a false discovery rate correction to our p-values per category of parameters as presented in the result tables. Significance was held when  $P < 0.05$  and only significant differences have been discussed. We carried out our analyses using R version 4.1.2.

### Data availability

The data were deposited in an official repository [entrepot.recherche.data.gouv.fr/dataverse/inrae](https://entrepot.recherche.data.gouv.fr/dataverse/inrae). The data-sets generated and analysed during the current study are available on this website: <https://entrepot.recherche.data.gouv.fr/dataverse/inrae>. The data-sets digital object identifier is <https://doi.org/10.57745/JS5HLR>.

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