Fast- and slower-growing broilers respond similarly to a reduction in stocking density with regard to gait, hock burn, skin lesions, cleanliness, and performance

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ABSTRACT There is an increasing trend toward broiler production systems with higher welfare requirements. Breed and stocking density are considered key factors for broiler welfare that are often specified as criteria for such higher welfare systems. However, it remains unknown how slower-growing broilers respond to a reduction in stocking density with regard to their welfare and performance, and whether this response differs from fast-growing broilers. Therefore, we compared fast- (\mathbf{F}) and slower-growing broilers (\mathbf{S}) housed at 4 different stocking densities (24, 30, 36, and 42 kg/m², based on slaughter weight) and measured their welfare scores (i.e., gait, footpad dermatitis, hock burn, skin lesions and cleanliness), litter quality and performance. The experiment had a 2×4 factorial design with 4 replicates (pens) per treatment (32 pens in total). Thinning (15%)was done in a 50/50 male/female ratio at 38 (F) and 44 (S) d of age (estimated body weight of 2.2 kg). We hypothesized that breeds would respond differently to a reduction in stocking density. Contrary to our hypothesis, only one interaction between breed and stocking density was found on footpad dermatitis, indicating that fast- and slower-growing broilers generally showed similar responses to a reduction in stocking density. F broilers showed a steeper decline in the prevalence of footpad dermatitis with reducing stocking density compared to S broilers. Broilers housed at lower stocking densities $(24 \text{ and/or } 30 \text{ kg/m}^2)$ showed improved welfare measures, litter quality and performance compared to those housed at higher stocking densities (36 and/or 42 kg/m²). S broilers had better welfare scores (gait, footpad dermatitis and skin lesions), litter quality and lower performance compared to F broilers. In conclusion, reducing stocking density improved welfare of both F and S broilers, but more for F broilers in case of footpad dermatitis, and using S broilers improved welfare compared to F broilers. Reducing stocking density and using slower-growing broilers benefits broiler welfare, where combining both would further improve broiler welfare.

Key words: broiler, performance, welfare, breed, stocking density

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INTRODUCTION

There is an increasing trend to implement broiler production systems with higher welfare requirements in several countries, mainly in North-West Europe (e.g., the Netherlands, Denmark, and Germany), caused by increased pressure of NGO's (Vissers et al., 2019; de Jong et al., 2022). This trend is expected to continue as a result of the European Chicken Commitment ("European Chicken Commitment. Accessed November 2022. https://welfarecommitments.com/europeletter/.") or

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Better Chicken Commitment (US) ("Better Chicken Commitment. Accessed November 2022. https://betterchickencommitment.com/en/."). These "higher-welfare" systems generally apply a reduced stocking density as compared to conventional broiler production, and also include a slow(er)-growing breed. Both are considered as key factors to improve broiler welfare (de Jong et al., 2012; Dixon, 2020; Rayner et al., 2020).

Reducing stocking density in general improves litter quality and welfare measures, such as gait score, hock burn and footpad dermatitis (Hall, 2001; Thomas et al., 2004; Buijs et al., 2009), although some studies report no effect on gait score (McLean et al., 2002; Hongchao et al., 2014; Bailie et al., 2018) or litter quality (Mocz et al., 2022). Furthermore, most studies indicate that reducing stocking density improves performance, such as body weight, body weight gain, feed intake, although

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feed conversion ratio (FCR) and mortality are generally not affected (McLean et al., 2002; Thomas et al., 2004; Dozier et al., 2005; Villagrá et al., 2009; Abudabos et al., 2013; Nasr et al., 2021). Yet, some studies report lower FCR (Guardia et al., 2011; Cengiz et al., 2015) or no effect on body weight (Buijs et al., 2009; Bailie et al., 2018) with reducing stocking density. For carcass yields, reducing stocking densities resulted in higher breast yield and lower thigh yields (Cengiz et al., 2015; Costa et al., 2021; Nasr et al., 2021), although some studies reported no effect on carcass yields (Thomas et al., 2004; Dozier et al., 2005). Thus, reducing stocking density seems to improve welfare, litter quality and performance of broilers. However, most studies to date have focused on identifying effects of stocking density on welfare and performance of fast-growing broilers, while the effect of stocking density on slower-growing broilers remains relatively unknown.

It is important to study the effects of stocking density on slower-growing broilers, as fast- and slower-growing broilers differ with regard to their behavior, welfare and performance. Here, we define slower-growing broilers as growing ≤ 50 g/d, while fast-growing broilers grow \geq 60 g/d (de Jong et al., 2022). In general, slower-growing broilers are more active and have improved welfare measures, such as gait score, footpad dermatitis, hock burn, cleanliness (Kjaer et al., 2006; Wilhelmsson et al., 2019; Dixon, 2020; Rayner et al., 2020; Güz et al., 2021; Santos et al., 2022; van der Eijk et al., 2022a), although differences are not always found (de Jong et al., 2021). Similarly, litter quality is often better when housing slower- compared to fast-growing broilers under similar conditions (Rayner et al., 2020; Santos et al., 2022; van der Eijk et al., 2022a), although here too differences are not always found (Wilhelmsson et al., 2019). With regard to performance, slower-growing broilers generally have a lower daily body weight gain, daily feed intake and mortality, and higher FCR compared to fast-growing broilers (Dixon, 2020; Rayner et al., 2020; de Jong et al., 2021; Güz et al., 2021; Torrey et al., 2021; van der Eijk et al., 2022a). Furthermore, slower-growing broilers had lower carcass yield, breast yield, and higher thigh, drumstick and wing yield relative to carcass weight (Santos et al., 2021). Thus, slower-growing broilers differ from fast-growing broilers with regard to behavior, welfare measures and performance. These differences may cause slower-growing breeds to respond differently to a reduction in stocking density. Therefore, it is important to know how stocking density affects welfare and performance of slower-growing broilers and to determine whether fast- and slower-growing broilers respond differently to a reduction in stocking density.

A recent study compared fast- and slower-growing broilers housed at 29 and 37 kg/m² stocking density (Weimer et al., 2020). Reducing stocking density did not affect footpad dermatitis, probably because of low prevalence, and increased performance, such as body weight, FCR and mortality, of fast- and slower-growing broilers. Furthermore, reducing stocking density improved the prevalence of hock burn in slower-growing broilers but

not in fast-growing broilers. These findings indicate that breeds may differ in their response to a reduction in stocking density, but more research is needed. Therefore, the aim of this study was to determine how slower-growing broilers respond to a reduction in stocking density, and whether this response differs from fast-growing broilers, with regard to welfare, litter quality and performance. We hypothesized that fast-growing broilers would benefit more from a reduction in stocking density, as the extra space could improve their activity, welfare and litter quality, while slower-growing broilers already are more active and show better welfare compared to fast-growing broilers. However, an alternative hypothesis would be that slower-growing broilers would benefit more from a reduction in stocking density, as they would be more able to "use" the extra space because they are more active and have a better walking ability compared to fast-growing broilers.

MATERIALS AND METHODS

Experimental Design and Ethical Approval

The experiment had a 2×4 factorial design with 2 broiler breeds, fast-growing $(\mathbf{F}, \text{Ross } 308)$ and slowergrowing broilers (S, Ranger Classic), that were housed at 4 stocking densities (24, 30, 36, and 42 kg/m²). We included 42 kg/m² as maximum stocking density (EU regulations, see 2007/43/EC, article 3 (2-5)) and 24 kg/m^2 as minimum stocking density (close to stocking) density of the Better Life one star production system in the Netherlands (Vissers et al., 2019)). Furthermore, 30 kg/m^2 (maximum according to ECC for higher welfare systems ("European Chicken Commitment")) and 36 kg/m^2 were chosen as steps between this maximum and minimum. In order to identify responses for both breeds these stocking densities were the same for both breeds. The experiment was conducted in a semicommercial setting in the experimental facility of Schothorst Feed Research (Lelystad, the Netherlands). The housing and management and the experimental procedures were approved by the institutional Animal Welfare Body. Because the procedures were noninvasive, this study was not considered to be an animal experiment under the Law on Animal Experiments, as confirmed by the institutional Animal Welfare Body (9th of March, 2021, Lelystad, The Netherlands).

Animals, Housing, and Diets

Day-old broiler chicks, originating from a parent stock of 44 wk of age (for both F and S broilers), were obtained from a commercial hatchery (Probroed & Sloot, Meppel, the Netherlands). A total of 11,360 F and 11,360 S broilers were randomly allocated to the 4 stocking densities, resulting in 8 experimental groups (F24, F30, F36, F42, S24, S30, S36, and S42). See Table 1 for an overview of the treatments. A split-plot design was used with blocks of 4 pens (of 47.5 m² each) next to each other per breed, and densities being randomly distributed

Group	Breed	Density (kg/m^2)	${\it Males/pen}$	${\rm Females}/{\rm pen}$	$\mathrm{Total}/\mathrm{pen}$	${\rm Feeders}/{\rm pen}$	Drinkers /pen	Bales /pen
F24	Ross 308	24	258	259	517	6	41	1
F30	Ross 308	30	322	323	645	8	52	1
F36	Ross 308	36	387	388	775	9	62	2
F42	Ross 308	42	451	452	903	11	72	2
S24	Ranger Classic	24	258	259	517	6	41	1
S30	Ranger Classic	30	322	323	645	8	52	1
S36	Ranger Classic	36	387	388	775	9	62	2
S42	Ranger Classic	42	451	452	903	11	72	2

Table 1. Overview of different treatments.

F = fast-growing broilers; S = slower-growing broilers; 24, 30, 36, and 42 correspond to stocking density in kg/m².

within a block. At the start of the experiment broilers were housed in groups of 517, 645, 775, and 903, for 24, 30, 36, and 42 kg/m², respectively. Pens had an exact 50/50 male/female distribution (i.e., straight run) and chicks were sexed at the commercial hatchery. Each experimental group was replicated 4 times, with a total of 32 experimental pens divided over 2 climate controlled rooms (16 pens per room). Each experimental group was equally assigned to the 2 rooms with 2 replicates per room. Thinning was done by taking out 15% of the broilers from each pen in a 50/50 male/female ratio at 38 d of age for F and 44 d of age for S broilers (estimated body weight 2.2 kg). Thinning was performed as this is standard practice in the Dutch broiler sector, making our results better transferable to commercial practice. Depopulation occurred at 41 d of age for F and 50 d of age for S broilers (estimated body weight 2.6 kg).

Ambient temperature was gradually decreased from 34.5°C at arrival to a constant temperature of 20°C from 40 d of age onward. The lighting program used was 24L:0D at arrival, 20L:4D from d 1 to 6 and 18L:6D from d 7 onward. Light intensity at chick height (\pm 25 cm) was 40 lux between d 0 to 6 and 20 lux from d 7 onward. Floor pens (47.5 m^2 , length 9.5, width 5, and height 0.75 m) had fresh wood shavings as litter. Number of pan feeders and nipple drinkers in each pen was adjusted depending on stocking density (number of feeders: 6, 8, 9, 11; number of drinkers: 41, 52, 62, 72, respectively). Furthermore, firmly pressed straw bales (length 50, width 30, and height 40 cm) were provided as enrichment with 1 bale per pen for 24 and 30 kg/m² and 2 bales per pen for 36 and 42 kg/m². For S broilers, pens included a net up to 1.6m high to avoid them from escaping to other pens and at 14 d of age a 0.3 m high solid barrier was placed in between the blocks to avoid S broilers being disturbed by the depopulation of F broilers. Broilers had ad libitum access to feed and water. A 4-phase feeding schedule was applied with similar intermediate diets for both breeds: starter diet (d 0-10), grower diet 1 (d 10-20), grower diet 2 (d 20-30), and finisher diet (d 30 onward). The starter diet was crumbled, while the other diets were pelleted (3 mm diameter). All diets were produced and pelleted by ABZ Diervoeding (Nijkerk, the Netherlands) and analyzed for moisture, ash, dry matter, crude fiber, crude fat, crude protein using the Weende (proximate) analysis by Schothorst Feed Research (Lelystad, the Netherlands). Diet compositions, calculated and analyzed nutrient values are shown in Table 2. Broiler chicks were

vaccinated against Infectious Bronchitis at the hatchery, against Newcastle Disease at 7 d of age via spray and against Gumboro at 21 d of age via the drinking water. A positive *Salmonella* sample was taken prior to slaughter, but this was identified as *Salmonella Infantis* C1 group which is considered noninvasive (Drauch et al., 2021).

Performance

Body weight (**BW**) at pen level was measured using the container weights at final depletion. Feed intake (**FI**) and FCR were determined for the total rearing period and corrected for the weight at mortality (**MRT**) as previously proposed by Dersjant-Li et al. (2013) with slight modifications. FCR was calculated using the following formula:

 $\frac{\text{total feed intake in period } x - y}{(\text{total live weight} + \text{weight dead birds})y - (\text{total live weight})x}$

Weight of dead birds was calculated by taking the number of dead birds on d x * 0.8 * average weight on d x based on the weighing plateaus. The factor 0.8 was used to account for the generally lower body weight of weak (er) birds that have a higher likelihood to die. MRT was noted daily at pen level.

Slaughter Yield

Slaughter yield measurements were done of 15 males and 15 females, randomly selected per pen at 41 d of age for F and 50 d of age for S. Broilers were individually tagged, weighed and transported to a commercial slaughter plant. Broilers were slaughtered and eviscerated by hand by trained slaughter-plant personnel. Carcass weight and processing yields (expressed as a percentage of carcass weight) of the different commercial parts (wings, legs, breast filet) were determined.

Litter Quality

Litter quality was assessed in each pen at 4 ages (d 14, 24, 31, and 35 for F and d 16, 27, 36, and 42 for S) by a panel of 2 trained assessors. These ages were chosen based on similar target body weights (**TBWs**) of F and

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Table 2. (A) Ingredients (%), (B) calculated and analyzed nutrients of the experimental diet (g/kg, as-fed basis).

A Ingredients (%)	Starter $(0-10 \text{ ds})$	Grower 1 $(10-20 \text{ d})$	Grower 2 (20 -30 d)	Finisher (30 d to end)
Corn	40.000	40.000	40.000	35.000
Wheat	20.716	23.844	23.572	31.761
Soybean meal $(>48\% \text{ CP})$	27.106	22.571	21.922	17.608
Rape seed meal	1.000	2.000	3.000	4.000
Oat hulls	2.000	2.000	2.000	2.000
Potato protein – Protamyl	1.000	1.000	—	—
Soya oil	3.412	4.469	5.537	6.011
Limestone	1.290	1.109	1.081	0.961
Monocalcium phosphate	1.028	0.743	0.587	0.392
Sodium bicarbonate	0.382	0.344	0.330	0.335
Salt	0.121	0.097	0.107	0.103
Lysine HCL (79%)	0.258	0.226	0.211	0.218
Methionine L/DL (99%)	0.289	0.247	0.228	0.194
Threenine L (98%)	0.127	0.097	0.090	0.081
Valine L (99%)	0.017	_	_	—
Premix Sacox	—	_	0.583	0.583
Premix Maxiban	0.500	0.500	_	—
Glucanase/xylanase premix	0.250	0.250	0.250	0.250
Phytase	0.003	0.003	0.003	0.003
Vitamin & mineral premix	0.500	0.500	0.500	0.500
Total	100.000	100.000	100.000	100.000
B Calculated nutrients and (analyzed nutrients) (g/kg)	Starter $(0-10 \text{ d})$	Grower 1 (10-20 d)	Grower 2 $(20-30 \text{ d})$	Finisher (30 d to end)
Moisture	118 (121)	118 (129)	117 (123)	117 (121)
Crude ash	55(54)	48(45)	45(45)	41(41)
Crude protein	203(198)	187(181)	179(175)	167(164)
Crude fat (AH)	62(61)	72(73)	83 (94)	87 (93)
Crude fiber	28(34)	28(29)	29(28)	29(34)
Starch (AM)	377	395	396	412
Sugar	29	28	29	28
Ca	7.96	6.76	6.16	5.36
P	5.72	4.99	4.66	4.16
Mg	1.50	4.55	1.44	1.40
K	8.44	7.70	7.63	6.96
Na	1.60	1.40	1.40	1.40
Cl	1.80	1.40	1.40	1.60
Av Ca	9.60	8.40	7.80	7.00
Retainable P	4.60	4.00	3.70	3.30
IP	0.32	0.26	0.29	0.26
C18:2	29.43	34.73	40.11	42.04
ME-WPSA (kcal/kg)	3,000	3,100	3,150	3,200
SID lysine	11.30	10.10	9.40	8.60
SID methionine	5.70	5.12	4.74	4.27
SID cysteine	2.62	2.51	2.46	2.40
SID met + cys	8.36	7.68	7.24	6.71
SID threonine	7.57	6.77	6.30	5.76
SID tryptophane	2.05	1.87	1.77	1.65
SID valine	8.48	7.68	7.24	6.71
SID isoleucine	7.57	6.92	6.53	5.98
SID leucine	14.72	13.66	12.95	11.88
SID arginine	11.67	10.54	10.18	9.20
SID histidine	4.75	4.39	4.23	3.91
SID tyrosine	6.16	5.62	5.22	4.73
SID phenylalanine	8.91	8.18	7.73	7.11
SID proline	11.15	10.66	10.34	10.13
SID glutamic acid	34.36	32.28	31.59	30.55
SID aspartic acid	16.73	14.96	14.07	12.42
SID alanine	8.01	7.45	7.14	6.52
SID glycine	7.09	6.57	6.27	5.86
SID serine	8.69	7.97	7.60	7.00
NTE NUTITIV	0.00	1.01	1.00	1.00

Abbreviation: SID, standardized ileal digestible.

S broilers (e.g., 0.4, 1.1, 1.7, and 2.1 kg, respectively). Litter was scored on a scale of 1 to 10 for friability and wetness according to van Harn et al. (2009), where litter score 1 corresponded with low litter quality (very wet, completely caked) and score 10 corresponded with high litter quality (dry, completely friable).

Welfare

Welfare measurements were recorded at 2 ages (d 34 and 38 for F, and d 41 and 45 for S). These ages were chosen based on similar TBW's of F and S broilers (2.0 and 2.3 kg, respectively). Welfare measurements included lameness,

footpad dermatitis, hock burn, cleanliness and injuries of 15 males and 15 females per pen (n = 30 per pen) that were assessed according to Welfare Quality protocol (Welfare Quality[®], 2009) by one trained observer. Lameness was recorded using a gait score between 0 (perfect) and 5 (unable to walk). Footpads and hocks were assigned a score between 0 (no lesions) and 4 (severe lesions). Cleanliness was scored by inspection of the breast area and assigned a score between 0 (completely clean) and 3 (very dirty). Skin lesions were assigned a score 0 (no scratches or wounds), 1 (single scratch or small wound $\leq 0.5 \text{ cm}^2$), or 2 (multiple scratches and/or large wounds > 0.5 cm^2).

Statistical Analysis

SAS Software version 9.4 was used for statistical analysis of performance and slaughter yield measurements (SAS Institute Inc., Cary, NC). Data were analyzed at pen level. Normality of the data was assessed based on model residuals. We used linear mixed models consisting of fixed effects of breed, density and the interaction between breed*density. For performance, block (1-8) was included as random effect. For slaughter yield, pen (1-32) within breed and density, and block (1-8) were included as random effects. Post hoc pairwise comparisons were corrected by Tukey-Kramer adjustment. Performance and slaughter yield data are presented as least square means \pm pooled standard error of the mean (SEM).

GenStat version 19.1 (VSN International, Hemel Hempstead, UK) was used for the analysis of welfare and litter scores at pen level. We used generalized linear mixed models with a multinomial distribution (except for hock burn which was binomial) consisting of fixed effects of breed, density, TBW and the interactions between breed*density, breed*TBW, density*TBW and breed*density*TBW. A backward regression procedure was used when fixed interactions (i.e., breed*density*TBW, breed*TBW, density*TBW) had P > 0.1. The interaction between breed and density was always included, as this was the primary aim of the study. Block and pen within block were included as separate random effects. All data are presented as means, unless otherwise specified. For gait score, 0 to 1 and 3 to 5 scores were grouped because of low prevalence of scores 0 (n = 1), 4 (n = 1), and 5 (n = 2). For footpad dermatitis, 3 and 4 scores were grouped because of low prevalence of score 4 (n = 1). For hock burn, 1 to 4 scores were grouped because of low prevalence of scores 2 (n = 3), 3 (n = 6), and 4 (n = 0). For cleanliness, 2 and 3 scores were grouped because of low prevalence of score 3 (n = 1).

To determine the response of welfare and litter scores to stocking density, these were further analyzed at pen level on logscale, using a generalized linear mixed model with a multinomial distribution consisting of fixed effects of breed, linear density, quadratic density, breed*linear density and breed*quadratic density. Block was included as random effect. For model simplification. nonsignificant (P > 0.05) terms were removed.

RESULTS

Performance

Performance results are summarized in Table 3. No interactions between breed and stocking density were found for performance. Breed effects were found for BW, average daily body weight gain (ADBWG), average daily feed intake (**ADFI**), FCR, and MRT. S broilers had lower BW ($\Delta = -133$ g, P < 0.001), ADBWG

Table 3. Effects of breed, stocking density and their interaction on performance.

Performance		BW^1	$ADBWG^2$	$ADFI^3$	FCR^4	MRT^5
Breed	F	2682 ^a	64.4^{a}	$107.1^{\rm a}$	1.66^{b}	2.2^{a}
	S	2549^{b}	50.2^{b}	87.8^{b}	1.75^{a}	1.3^{b}
	SEM	18	0.4	0.4	0.01	0.1
Density	24	2654^{a}	58.1^{a}	98.7^{a}	$1.70^{\rm ab}$	1.6
·	30	2679^{a}	58.7^{a}	98.9^{a}	1.69^{b}	2.1
	36	2588^{b}	56.7^{b}	96.9^{ab}	1.72^{a}	1.8
	42	2541^{b}	55.6^{b}	95.3^{b}	1.72^{a}	1.6
	SEM	19	0.4	0.6	0.01	0.2
Breed * Density	F24	2719	65.3	108.6	1.66	1.8
v	F30	2731	65.6	108.1	1.65	2.8
	F36	2681	64.4	107.2	1.67	2.3
	F42	2598	62.3	104.4	1.67	2.1
	S24	2589	51	88.7	1.74	1.5
	S30	2628	51.7	89.7	1.73	1.4
	S36	2494	49.1	86.6	1.77	1.3
	S42	2485	48.9	86.2	1.76	1.2
	SEM	27	0.6	0.8	0.01	0.2
P values	Breed	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
	Density	< 0.001	< 0.001	< 0.001	0.02	0.21
	Breed * Density	0.29	0.28	0.37	0.59	0.21

F = fast-growing broilers (41 d of age); S = slower-growing broilers (50 d of age); 24, 30, 36 and 42 correspond to stocking density in kg/m² (n = 4pens). ^{a–b}Per factor, values in a column lacking a common superscript differ significantly (P < 0.05).

 $^{1}\mathrm{BW} = \mathrm{body} \mathrm{ weight} \mathrm{ at} \mathrm{ final depletion (g)}.$

²ADBWG = average daily body weight gain (g/d) based on final depletion weight.

 ${}^{3}ADFI = average daily feed intake (g/d).$

 ${}^{4}\text{FCR} = \text{feed conversion ratio.}$

 ${}^{5}MRT = mortality in \%$.

 Table 4. Effects of breed, stocking density, and their interaction on slaughter yield.

Slaughter yield		Carcass weight (g)	Wing $\%$	Leg %	Filet $\%$
Breed	F 1795		$10.7^{\rm b}$	33.8	32.3 ^a
	S	1797	11.4^{a}	33.7	$30.4^{\rm b}$
	SEM	12	0.02	0.07	0.1
Density	24	1865^{a}	10.9^{c}	33.6	31.6
·	30	1789^{b}	11.0^{bc}	33.7	31.4
	36	1781^{b}	11.1^{ab}	33.8	31.3
	42	1749^{b}	11.1^{a}	33.9	31.1
	SEM	18	0.03	0.09	0.15
Breed * Density	F24	1874	10.6	33.5	32.8
	F30	1793	10.6	33.7	32.3
	F36	1773	10.7	33.8	32.2
	F42	1741	10.8	34.1	31.8
	S24	1856	11.3	33.7	30.4
	S30	1786	11.4	33.6	30.5
	S36	1789	11.4	33.8	30.4
	S42	1757	11.5	33.6	30.4
	SEM	25	0.05	0.13	0.21
P values	Breed	0.93	< 0.001	0.53	< 0.001
	Density	< 0.001	< 0.001	0.17	0.13
	Breed * Density	0.88	0.62	0.09	0.2

F = fast-growing broilers (41 d of age); S = slower-growing broilers (50 d of age); 24, 30, 36 and 42 correspond to stocking density in kg/m² (n = 4 pens). For wing, leg and breast filet, values are expressed as a percentage of carcass weight.

^{a-c}Per factor, values in a column lacking a common superscript differ significantly (P < 0.05).

 $(\Delta = -14.2 \text{ g/d}, P < 0.001)$, ADFI ($\Delta = -19.3 \text{ g/d}, P < 0.001$) and MRT ($\Delta = -0.9\%$, P < 0.001), and higher FCR ($\Delta = +0.09, P < 0.001$) compared to F broilers.

Density effects were found for BW, ADBWG, ADFI and FCR. BW and ADBWG were higher for 24 kg/m² and 30 kg/m² broilers compared to 36 kg/m² and 42 kg/m² broilers (P < 0.001). ADFI was higher for 24 kg/m² and 30 kg/m² broilers compared to 42 kg/m² broilers, with 36 kg/m² broilers not differing from other stocking densities (P < 0.001). FCR was lower for 30 kg/m² broilers compared to 36 kg/m² broilers (P < 0.05), with 24 kg/m² broilers not differing from other stocking densities.

Slaughter Yield

Slaughter yield results are summarized in Table 4. No interactions between breed and stocking density were found for slaughter yield. Breed effects were found for wing and filet yield. S broilers had higher wing % ($\Delta = +0.7\%$, P < 0.001) and lower filet % ($\Delta = -1.9\%$, P < 0.001) compared to F broilers.

Stocking density effects were found for carcass weight and wing yield. Carcass weight was higher for 24 kg/m² broilers compared to 30 kg/m², 36 kg/m² and 42 kg/m² broilers (P < 0.001). Wing % was lower for 24 kg/m² broilers compared to 36 kg/m² and 42 kg/m² broilers, and was also lower for 30 kg/m² broilers compared to 42 kg/m² broilers (P < 0.001).

Litter Quality

Actual weights during litter quality observations slightly differed from TBWs (Table 5). There were no interactions between density, breed and TBW, between breed and TBW, nor between breed and density for

Table 5. Target body weight (TBW), actual BW (in kg) for both fast- (F) and slower-growing broilers (S), and BW difference (in kg and % of actual BW) of both breeds.

TBW (kg)	0.4	1.1	1.7	2.1
F	0.43	1.18	1.75	2.13
S	0.43	1.00	1.55	1.98
Difference in kg $(F - S)$	0.00	0.18	0.20	0.15
Difference in % vs. F weight	0.0%	15.3%	11.4%	7.0%
Difference in % vs. S weight	0.0%	18.0%	12.9%	7.6%

both friability and wetness. For wetness an interaction between stocking density and TBW was found (P < 0.001). A higher score for friability and wetness means more friable and dryer (i.e., better) bedding material. At 1.1kg, 24 kg/m² pens had higher wetness scores compared to 30, 36 and 42 kg/m² pens, and 30 kg/m² pens also had higher scores compared to 42 kg/m² pens (P < 0.05). At 1.7kg, 24 and 30 kg/m² pens had higher scores compared to 42 kg/m² pens (P < 0.05), with 36 kg/m² pens not differing from other stocking densities. At 2.1kg, 24 and 30 kg/m² pens had higher scores compared to 36 and 42 kg/m² pens (P < 0.05). For the remainder of this section we focus on describing main effects of breed and stocking density.

Breed had an effect on friability (P < 0.05) and wetness (P < 0.01). For pens with S broilers litter scores for friability and wetness were significantly higher compared to pens with F broilers. Predicted means per breed (on logit scale) were as follows: for friability, S = 2.14 and F = 1.31, SE = 0.32, and for wetness, S = 2.58 and F = 1.42, SE = 0.33.

Stocking density influenced friability and wetness (both P < 0.001). Scores significantly decreased and thus became worse with increasing stocking density (Figure 1). Predicted means per density from 24 to 42 kg/m² (on logit scale) were as follows: friability, 2.85,

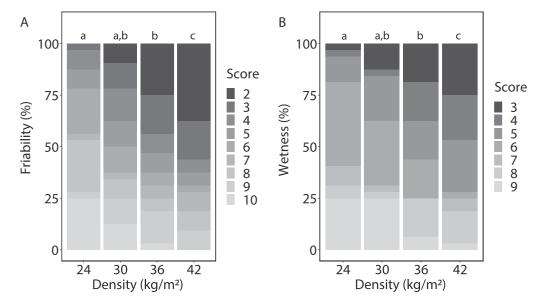


Figure 1. Mean percentage of pens with a specific litter score for A) friability and B) wetness at different stocking densities (kg/m^2) and averaged over target body weights. Scores range from very bad (1) to very good (10). Scores that are not included in the legend were not observed. ^{a-c} values lacking a common superscript differ significantly (P < 0.05).

2.00, 1.29 and 0.75, SE = 0.44 to 0.47 and for wetness, 2.94, 2.39, 1.59, and 1.07, SE = 0.45 to 0.47. Where 24 kg/m² pens had higher scores for friability and wetness compared to 36 and 42 kg/m² pens (P < 0.05), and 30 kg/m² pens had higher scores for friability and wetness compared to 42 kg/m² pens (P < 0.05).

There was no quadratic relation between friability or wetness and density, nor an interaction effect between breed and quadratic density on friability or wetness. We did find clear linear relationships between friability or wetness and density (both P < 0.001, see Supplementary Figure 1), where breeds did not differ, that is, for both breeds friability and wetness scores decreased with increasing density, indicating a reduced litter quality with increasing density.

Welfare

Actual weights during welfare measures slightly differed from TBW's. For TBW 2.0kg, F broilers had an average weight of 2.0 kg and S broilers of 1.9 kg. For TBW 2.3 kg, F had an average weight of 2.4 kg and S of 2.2 kg. There were no interactions between stocking density, breed and TBW, nor between stocking density and TBW for any of the welfare scores. For hock burn, skin lesions and cleanliness, interactions between breed and TBW were found (P < 0.05, P < 0.001, and P < 0.01, respectively). At 2.0 kg, S broilers had lower skin lesions scores compared to F broilers (P < 0.05). At 2.3 kg, S broilers had lower hock burn, skin lesions and cleanliness scores compared to F broilers (P < 0.05). No interactions between breed and stocking density were found on welfare measurements, except for footpad dermatitis (P <0.001) (Figure 2). Where S broilers housed at 24 and 30 kg/m^2 had lower footpad dermatitis scores compared to those housed at 42 kg/m², and compared to F broilers housed at 24, 30, 36, and 42 kg/m² (P < 0.05). Further,

S broilers housed at 36 and 42 kg/m², and F broilers housed at 24 kg/m² had lower footpad dermatitis scores compared to F broilers housed at 36 and 42 kg/m² (P < 0.05). Lower footpad dermatitis scores mean less severe footpad lesions and a better footpad health. For the remainder of this section we focus on describing main effects of breed and stocking density.

Breed effects were found for gait (P < 0.001), footpad dermatitis (P < 0.001), and skin lesions (P < 0.001). Predicted means per breed (on logit scale) were as follows:

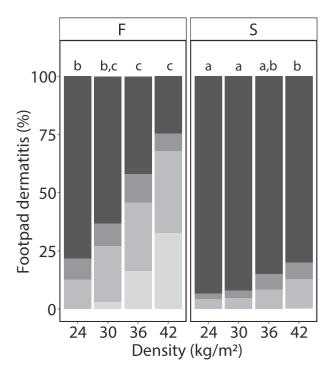


Figure 2. Mean percentage of birds with a specific score for footpad dermatitis (black = 0, gray = 1, middle gray = 2, light gray = 3-4) for different stocking densities (kg/m²) and for fast- (F) and slower-growing broilers (S) averaged over target body weights. ^{a-c} values lacking a common superscript differ significantly (P < 0.05).

gait, S = 2.45 and F = 5.09, SE = 0.18, footpad dermatitis S = -2.15 and F = -0.05, SE = 0.44, and for skin lesions, S = -3.20 and F = -0.43, SE = 0.27. S broilers had significantly lower scores for gait, footpad dermatitis, and skin lesions, indicating better welfare, compared to F broilers. Breed had no effect on hock burn and cleanliness.

Density effects were found for gait (P < 0.05), footpad dermatitis (P < 0.001), hock burn (P = 0.003), skin lesions (P < 0.001), and cleanliness (P < 0.001). Predicted means per density from 24 to 42 kg/m² (on logit scale) were as follows: gait, 3.59, 3.69, 3.75, and 4.05, SE = 0.16, footpad dermatitis, -2.13, -1.59, -0.71, and 0.02, SE = 0.27 to 0.31, hock burn, -4.06, -4.80, -3.33, and -3.29, SE = 0.42 to 0.71, skin lesions, -3.05, -1.93, -1.92, and -0.35, SE = 0.24 to 0.39, and for cleanliness, 1.46, 2.08, 2.41, and 2.94, SE = 0.19 to 0.20. Where, 24, 30, and 36 kg/m² broilers had lower scores for footpad dermatitis, skin lesions and cleanliness compared to 42 kg/m² broilers (P < 0.05), 24 and 30 kg/m² broilers further had lower scores for gait compared to 42 kg/m² broilers and for footpad dermatitis compared to 36 kg/m² broilers (P < 0.05). In addition, 24 kg/m² broilers had lower scores for skin lesions and cleanliness compared to 30 and 36 kg/m² broilers (P < 0.05). For hock burn, 30 kg/m² broilers had lower scores compared to 36 and 42 kg/m² broilers (P < 0.05) (Figure 3). Thus, with reducing stocking density improved welfare measures were found.

There was no quadratic relation between any of the welfare measures and density. Nor an interaction between breed and quadratic density on any of the welfare measures. We did find a significant interaction between breed and linear density on footpad dermatitis (P < 0.001) (see Supplementary Figure 2), but not for the other welfare measures. Significant linear relationships were found between gait (P < 0.01), footpad dermatitis (P < 0.001), hock burn (P < 0.01), skin lesions (P < 0.001), cleanliness (P < 0.001), and density (see Supplementary Figure 3).

DISCUSSION

The aim of this study was to determine whether fastand slower-growing broilers respond differently to a

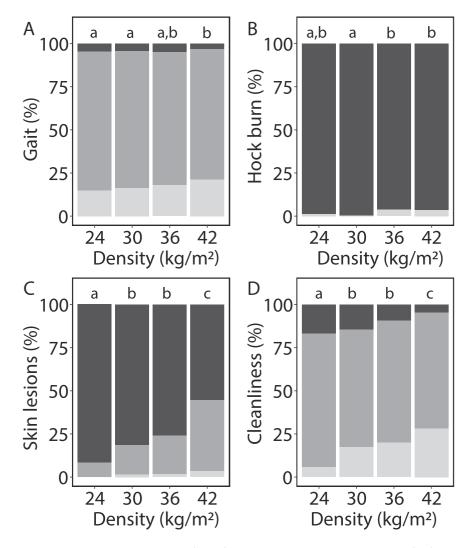


Figure 3. Mean percentage of birds with a specific score for A) gait (black = 0-1, gray = 2, light gray = 3-5), B) hock burn (black = 0, gray = 1-4), C) skin lesions (black = 0, gray = 1, light gray = 2), D) cleanliness (black = 0, gray = 1, light gray = 2-3) for different stocking densities (kg/m²) and averaged over target body weights. ^{a-c} values lacking a common superscript differ significantly (P < 0.05).

reduction in stocking density with regard to their welfare and performance. We hypothesized that fast-growing broilers would benefit more from a reduction in stocking density, as the extra space could improve their activity, welfare and litter quality. Alternatively, it was possible that slower-growing broilers would benefit more from a reduction in stocking density, as they would be more able to "use" the extra space provided. In contrast to both hypotheses, we found only one interaction between breed and stocking density, indicating that fast- and slower-growing broilers mostly showed similar performance, litter quality and welfare responses to reducing stocking density. The only exception was footpad dermatitis. Fast-growing broilers showed a steeper decline in the prevalence of footpad dermatitis with a reduced stocking density compared to slower-growing broilers. This suggests that reducing stocking density led to a greater relative improvement in welfare of fastgrowing broilers compared to slower-growing broilers, which supports our first hypothesis. In contrast to our finding, reducing stocking density did not affect footpad dermatitis and improved the prevalence of hock burn in slower-growing broilers but not in fast-growing broilers (Weimer et al., 2020), which supports our second hypothesis. This discrepancy might be explained by different types of slower-growing breeds being used. Differences between fast- and slower-growing breeds in the current study may result from slower-growing broilers being less sensitive to develop footpad dermatitis compared to fast-growing broilers (Kjaer et al., 2006; Ask, 2010; Shepherd and Fairchild, 2010). It could further be related to the better litter quality seen in slower- compared to fast-growing pens, as previously it was suggested that effects of stocking density on broiler welfare, especially on contact dermatitis, are a response to factors related to stocking density, such as litter quality, temperature and humidity (Dawkins et al., 2004). This may further be related to slower-growing broilers showing more locomotion and foraging behavior compared to fast-growing broilers (Dixon, 2020; Rayner et al., 2020; de Jong et al., 2021; Güz et al., 2021; van der Eijk et al., 2022a), thereby improving litter quality (de Jong et al., 2014). In addition, for fast-growing broilers feed intake per day was higher, resulting in a larger amount of excreta deposited over a shorter period of time compared to slower-growing broilers. This likely accelerated the decrease in litter quality of fast-growing broiler pens. It should be noted that we did not adapt the ventilation rate to the different stocking densities as treatments (breed \times stocking density) were distributed equally within rooms. This may have caused a too high ventilation rate for low stocking density pens and a too low ventilation rate for high stocking density pens. Still, although fast-growing broilers showed a greater improvement in footpad dermatitis than slower-growing broilers with reducing stocking density, slower-growing broilers had a lower prevalence of footpad dermatitis compared to fast-growing broilers at 36 and 42 kg/m² and when comparing breeds at similar densities.

Stocking Density

Broilers housed at lower stocking densities (24 and 30 kg/m^2) had higher final depletion body weights, daily body weight gain, daily feed intake and carcass weight, and a lower FCR and wing yield compared to broilers housed at higher stocking densities (36 and 42 kg/m²). There was no effect of stocking density on mortality nor on leg or filet yield. Our findings are supported by previous studies showing that reducing stocking density increases body weight, body weight gain and feed intake, although FCR, mortality and carcass yields are usually not affected by stocking density (Sorensen et al., 2000; McLean et al., 2002; Thomas et al., 2004; Dozier et al., 2005; Villagrá et al., 2009; Abudabos et al., 2013). Similar to our finding. Guardia et al. (2011) and Cengiz et al. (2015) also found lower FCR with reducing stocking density. We adjusted the number of feeders and drinkers for each stocking density, to make sure that performance results could not be related to more space at the feeders or drinkers. However, broilers at lower stocking densities of course had more space to move around freely, and hence to obtain access to feeders and drinkers, which may have caused differences in performance. Performance may further be influenced by birds at lower stocking densities having better walking ability, as was assessed via gait score (Kestin et al., 1992), which is further supported by broilers housed at lower stocking densities (24 and 36 vs. 42 kg/m^2) showing more locomotion behavior in the current study (van der Eijk et al., 2022b). Another explanation may be that broilers at lower stocking densities were less likely to be disturbed during feeding compared to those at higher stocking densities, as was found previously for resting behavior (Hall, 2001; Febrer et al., 2006). Interestingly, we did find that broilers housed at lower stocking densities (30 vs. 36 and 42 kg/m^2) actually showed less ingestion behavior. Birds at lower stocking densities may have shown shorter but more frequent feeding bouts compared to broilers at higher stocking densities, as birds with gait score 3 (obvious abnormality, affects ability to move) reduced the amount of visits to the feeder but increased feeding duration compared to birds with gait score 0 (normal, dexterous, and agile) (Weeks et al., 2000). Yet, total time spent feeding and feeding bout length did not differ with stocking density (Buijs et al., 2010). It has also been suggested that the reduced performance at higher stocking densities is most likely caused by problems of dissipating metabolic heat (i.e., heat stress) (Bessei, 2006). This is supported by McLean et al. (2002) who showed that broilers showed more deep panting at high compared to low stocking densities. Thus, the requirement for food energy may decline as a result of reduced ability to dissipate heat at higher densities causing reduced performance. Reduced performance could further be related to poor litter quality in high stocking density pens, as wet litter is an ideal environment for microbial activity and ammonia production both of which are known to reduce performance (Thomas et al., 2004; Jones et al., 2005; de Jong et al., 2014). Interestingly, we found lower wing yields at lower stocking densities and no effect on breast or leg yields, while previous studies found effects of stocking density only on breast and thigh yield (Cengiz et al., 2015; Costa et al., 2021; Nasr et al., 2021) or no effect on carcass yields (Thomas et al., 2004; Dozier et al., 2005). It is unclear why we found lower wing yield with reducing stocking densities.

Reducing stocking density improved litter quality (friability and wetness) and welfare measures (gait, footpad dermatitis, hock burn, skin lesions, and cleanliness), and linear relations with stocking density were also found for litter quality and welfare measures. Our findings are supported by previous studies showing that reducing stocking density in general improves litter quality and welfare measures, such as gait, footpad dermatitis, hock burn, skin lesions and cleanliness (Frankenhuis et al., 1991; Sorensen et al., 2000; Hall, 2001; Dozier et al., 2005; Allain et al., 2009; Buijs et al., 2009; Villagrá et al., 2009; Guardia et al., 2011).

Litter quality is influenced most by outdoor climate (temperature and relative humidity), humidity in the room, ventilation rate, air speed over the litter, and quality of the excreta. Reducing stocking density of course results in less broilers per m^2 and consequently in more open space and less excreta per m². Although feed intake per bird was higher at low stocking densities, the overall feed intake was lower for low stocking density pens, likely resulting in less excreta being deposited and better litter quality. Indeed, reducing stocking densities resulted in lower litter pH, moisture (Petek et al., 2014) and temperature (Reiter and Bessei, 2000), which might reduce microbial activity and ammonia levels (Bessei, 2006). All these factors are thought to contribute to contact dermatitis (Bradshaw et al., 2002). Furthermore, broilers housed at low stocking densities showed more foraging and comfort behavior (van der Eijk et al., 2022b), which could improve litter quality (De Jong et al., 2013) and as mentioned earlier ventilation was likely too high for low stocking density pens and too low for high stocking density pens.

Effects of stocking density on broiler welfare, especially on contact dermatitis, are suggested to be a response to factors related to stocking density, such as litter quality (Dawkins et al., 2004). This suggests that the lower prevalence of contact dermatitis is related to the better litter quality as a result of reducing stocking density. Better litter quality likely also results in improved cleanliness (Saraiva et al., 2016; Louton et al., 2018) and broilers housed at low stocking densities also showed more comfort behavior (van der Eijk et al., 2022b), which could be a result of the better litter quality (dryer and more friable litter), ideal for showing comfort behavior, and also in itself can improve cleanliness. Contact dermatitis and walking ability interact with each other, where improved walking ability might result in less contact with the litter when sitting or lying resulting in a reduced risk of contact dermatitis (Bessei, 2006). In turn, contact dermatitis often causes poorer walking ability (Bradshaw et al., 2002). Broilers at low stocking

densities of course had more space to move and be active and this is supported by our finding that broilers at low stocking densities showed more locomotion and foraging behavior (van der Eijk et al., 2022b), which could contribute to improved walking ability. It is interesting to note that poor walking ability is usually related to a higher body weight in broilers. In our study, however, reducing stocking density resulted in increased performance (i.e., body weight) and improved walking ability. With regard to skin lesions, at low stocking densities there are less birds and more space to move around, which likely results in less overcrowding and thereby less chance of receiving scratches from conspecifics (Dozier et al., 2005; Allain et al., 2009).

Overall, reducing stocking density increased performance and improved litter quality and welfare measures, indicating improved broiler welfare at low stocking densities. Performance showed highest increase by a reduction to 24 and/or 30 kg/m² in comparison to 42 kg/m². It should be determined what the economic revenue is of reducing stocking density, whether the improvements in relation to welfare and performance outweigh the costs of reducing stocking density. Litter quality was most positively affected by a reduction to 24 kg/m² in comparison to 36 and 42 kg/m². Welfare measures were most positively affected by a reduction to 24 and/or 30 kg/m^2 in comparison to $42 kg/m^2$. Although we used a semicommercial setting, pens were still relatively small as compared to commercial houses and effects might have been larger in a commercial setting as relatively more space is created because of broilers clustering together when resting. Further research should identify whether reducing stocking densities even more, considerably improves broiler welfare and when a threshold is reached at which no further beneficial effect on welfare is observed.

Breed

Slower-growing broilers had lower daily body weight gain, daily feed intake, mortality and filet yield, and a higher FCR and wing yield. Previous studies support our findings where slower-growing broilers had lower performance, such as daily body weight gain, daily feed intake, mortality and higher FCR, compared to fastgrowing broilers (Dixon, 2020; Rayner et al., 2020; de Jong et al., 2021; Güz et al., 2021; Torrey et al., 2020; de Jong et al., 2021; Güz et al., 2021; Torrey et al., 2021; van der Eijk et al., 2022a). With regard to carcass characteristics, slower-growing broilers had lower carcass yield, breast yield, higher leg (thigh and drumstick) and wing yield based on carcass weight compared to fastgrowing broilers (Santos et al., 2021). These performance and carcass yield findings are most likely related to genetic selection.

Slower-growing broilers had better litter quality (friability and wetness) and welfare measures (gait, footpad dermatitis, and skin lesions) compared to fast-growing broilers. This is supported by previous studies showing that using slower-growing broilers results in better litter quality and welfare measures, such as gait, footpad dermatitis and hock burn (Kjaer et al., 2006; Dixon, 2020; Rayner et al., 2020; Güz et al., 2021; Santos et al., 2022; van der Eijk et al., 2022a), although differences between breeds are not always observed (Wilhelmsson et al., 2019; de Jong et al., 2021).

Similarly as for reducing stocking density, better litter quality in slower-growing broiler pens is probably related to their lower feed intake and amount of excreta. Furthermore, we created 30 cm high walls between the pens to prevent visual contact of fast- to slower-growing broiler pens. This might have hindered air circulation at block level. This may have affected fast-growing broilers to a greater extent than slower-growing broilers due to their fast growth and greater amount of excreta produced at an earlier age. We tried to limit this effect as much as possible by placing these walls after 14 d of age. In addition, as mentioned earlier ventilation was not adapted per treatment, therefore it might have been too high for slower-growing broilers and too low for fastgrowing broilers.

The lower prevalence of footpad dermatitis and skin lesions in slower-growing broilers is likely caused by better litter quality (Bessei, 2006; Saraiva et al., 2016; Louton et al., 2018), their skin integrity (Kjaer et al., 2006), more locomotor activity and use of enrichments (van der Eijk et al., 2022b), reducing contact with the litter (Bessei, 2006). The better walking ability of slower-growing broilers might also be related to more locomotor activity (Kestin et al., 1992; Reiter and Bessei, 2009) and lower prevalence of contact dermatitis (Bradshaw et al., 2002; de Jong et al., 2014). Interestingly, it is often suggested that higher activity is related to more skin scratches or lesions, as broilers are more likely to climb on conspecifics and inflict scratches (Louton et al., 2019). Furthermore, slower-growing broilers had more skin scratches compared to fast-growing broilers (de Jong et al., 2022), although this was confounded with low vs. high stocking density. However, we found the opposite with slowergrowing broilers showing more locomotor activity and less skin lesions compared to fast-growing broilers (van der Eijk et al., 2022b). As welfare indicators were scored at similar TBW, differences in welfare measures were more likely related to genetic background or ontogeny (i.e., age) than to body weight. However, it should be noted that breeds differed in actual body weights for TBW 2.0 and 2.3 kg with fast- being heavier than slower-growing broilers and variation between breeds being higher for 2.3 than 2.0 kg. We cannot exclude that differences in body weights might have affected our results.

Overall, slower-growing broilers showed lower performance, better litter quality and welfare measures compared to fast-growing broilers, indicating improved welfare. Differences between both breeds are likely caused by their genetic background or ontogeny, and might further be related to differences in performance of locomotor, comfort and foraging behaviors. However, it should be noted that there is variation in specific growth-rate of slower-growing breeds in addition to a different genetic background (Dawson et al., 2021). Improved broiler welfare may therefore be related to a breed's specific growth rate, next to breed-specific behavior, that is, genetic differences, which merits further study.

CONCLUSIONS

In conclusion, fast- and slower-growing broilers responded similarly to reducing stocking density, except for footpad dermatitis, where fast-growing broilers showed a steeper decline in the prevalence of footpad dermatitis with reducing stocking density compared to slower-growing broilers. Litter quality and welfare measures were positively affected by a reduction in stocking density, indicating that reducing stocking density improved welfare of both fast- and slower-growing broilers. Although performance (daily body weight gain, FCR, and filet yield) was negatively affected, litter quality and welfare measures (gait, footpad dermatitis, and skin lesions) were positively affected by using slowergrowing broilers. Thus, reducing stocking density and using slower-growing broilers benefits broiler welfare, where combining both would further improve broiler welfare.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the present study.

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j. psj.2023.102603.

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