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Reducing stocking density benefits behaviour of fast- and slower-growing broilers

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A B S T R A C T

The broiler industry has come under sustained pressure from welfare organizations to improve broiler welfare. Breed and stocking density are important factors for broiler welfare and are often specified as criteria for higher welfare systems. However, it remains unknown how slower-growing broilers respond to a reduction in stocking density with regard to their behaviour, and whether this response differs from fast-growing broilers. Therefore, we compared fast- (Ross 308) and slower-growing broilers (Ranger Classic) housed at 4 different stocking densities (24, 30, 36 and 42 kg/m^2, based on slaughter weight) with regard to their behaviour, responses to behavioural tests (i.e., novel object (NO), human approach (HA) and free-space (FS) test) and enrichment use at 4 target body weights (TBW's, 0.4, 1.1, 1.7 and 2.1 kg). The experiment had a 2 × 4 factorial design with 4 replicates (pens) per treatment (total of 32 pens). Thinning (15%) was done in a 50/50 male/female ratio at 38 (Ross 308) and 44 (Ranger Classic) days of age (estimated body weight of 2.2 kg). We hypothesized that slower-growing broilers would respond more strongly to a reduction in stocking density with regard to their behaviour. As slower-growing broilers are more active and show more comfort and foraging behaviours, it is expected that with increasing space they will respond with a larger increase in these types of behaviours compared to fast-growing broilers. Contrary to our hypothesis, hardly any interactions between breed and stocking density were found, indicating that fast- and slower-growing broilers showed similar responses to a reduction in stocking density. Broilers housed at lower stocking densities (24 and/or 30 kg/m^2) showed more comfort and foraging behaviour, and showed more frolicking, running and sparring in the FS test compared to those housed at higher stocking densities (36 and/or 42 kg/m^2). Slower-growing broilers showed less ingestion, more locomotion, standing, comfort and foraging behaviour, made better use of the enrichment by sitting on the bale, and more slower-growing broilers approached a human and NO compared to fast-growing broilers. In conclusion, reducing stocking density positively affected performance of comfort, foraging and play behaviours, indicating improved welfare. Slower-growing broilers showed more locomotive, comfort and foraging behaviours, less fear and a better use of enrichments, suggesting improved welfare compared to fast-growing broilers. Thus, reducing stocking density and using slower-growing broilers would benefit broiler welfare, where combining both would further improve broiler welfare with regard to their behaviour.

1. Introduction

The broiler industry has come under sustained pressure from welfare organizations to improve broiler welfare, causing a trend towards broiler production systems with higher welfare requirements ("Better Chicken Commitment," n.d.; Vissers et al., 2019). To improve broiler welfare several factors can be adapted, such as breed, stocking density, light schedule or environmental enrichment (Bracke et al., 2019). Breed (slower-growing breeds) and (reducing) stocking density are considered important factors for welfare (de Jong et al., 2012; Dixon, 2020; Rayner et al., 2020) and are often included in criteria for higher welfare systems. For example, the European Chicken Commitment (ECC) standard includes, among others, implementing a maximum stocking density of 30 kg/m^2 and adopting breeds that demonstrate higher welfare outcomes compared to fast-growing breeds, i.e. slower-growing breeds by 2026 ("European Chicken Commitment," n.d.).

With regard to stocking density, previous studies have reported that stocking density affects broiler behaviour, but findings are not always

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consistent. Locomotion was found to decrease with increasing density (Hall, 2001; Hongchao et al., 2014; Leone and Estevez, 2008; Ventura et al., 2012), or was not affected by stocking density (Collins, 2008; Cornetto and Estevez, 2001; McLean et al., 2002). Litter directed behaviour was also found to decrease with increasing density (Buijs et al., 2011a; Hall, 2001; Ventura et al., 2012), or was not affected by stocking density (Cornetto and Estevez, 2001; Febrer et al., 2006; McLean et al., 2002). More consistent results were found regarding broiler disturbance. Increasing density leads to broilers being increasingly disturbed during resting and preening bouts (Buijs et al., 2010; Cornetto et al., 2002; Dawkins et al., 2004; Febrer et al., 2006; Hall, 2001; Ventura et al., 2012) with one recent exception (Bailie et al., 2018). Also fear levels can be elevated by increasing stocking density. The duration of tonic immobility (TI) was found to increase from a stocking density of 18 or 22 birds/m^2 onwards (Buijs et al., 2009; Onbasilar et al., 2008), while no relationship was found between TI and densities from 8 to 18 birds/m^2 (Ventura et al., 2010) or from 8 to 30 birds/m^2 (Villagrá et al., 2009). Furthermore, stocking density did not affect responses to a novel object (NO) test at densities from 30 to 36 kg/m^2 (Bailie et al., 2018). Thus, reducing stocking densities could improve broiler welfare, by increasing locomotion and litter directed behaviour, and reducing fear levels. Yet, studies to date have focused on identifying effects of stocking density on behaviour of fast-growing broilers, while the effect of stocking density on the welfare of slower-growing broilers remains unknown.

It is important to study stocking density effects on slower-growing broilers, as fast- and slower-growing broilers differ with regard to their behaviour. Here, we define slower-growing broilers as growing $\leq$ 50 g/day, while fast-growing broilers grow $\geq$ 60 g/day (de Jong et al., 2022). Slower-growing broilers show more walking, standing, foraging and aggressive behaviour, and less eating and sitting compared to fast-growing broilers (Dawson et al., 2021; de Jong et al., 2021; Dixon, 2020; Güz et al., 2021; Rayner et al., 2020; van der Eijk et al., 2022; Wallenbeck et al., 2016). With regard to fearfulness, results are less consistent. One study reported reduced fear levels in slower- compared to fast-growing broilers, as indicated by more broilers approaching a NO (Lindholm et al., 2016). However, others reported opposite effects, e.g. reduced ability of an observer to touch slower-growing broilers (Wilhelmsson et al., 2019), longer TI duration (Lindholm et al., 2016) and being more reactive to an observer (Baxter et al., 2021). Several studies found no differences between slower- and fast-growing broilers, e.g. in response to social isolation (Lindholm et al., 2016) or to a NO test (Baxter et al., 2021). One recent study reported that slower-growing broilers showed more play behaviour (frolicking and sparring) (Baxter et al., 2021). Thus, slower-growing broilers differ from fast-growing broilers with regard to their behaviour, including fear and play behaviour, although results are not always consistent, which might depend on different strains of slower-growing breeds or pen size used. These differences might cause slower-growing breeds to respond differently to a reduction in stocking density. As reducing stocking density is an important factor in higher welfare systems, it is important to know how it affects behaviour of slower-growing broilers and to determine whether they respond differently to a reduction in stocking density as compared to fast-growing broilers.

A recent study compared one fast-growing breed housed at 34 kg/m^2 to one slower-growing breed housed at 30 and 34 kg/m^2 and to another slower-growing breed housed at 30 kg/m^2. They reported that fast-growing broilers housed at 34 kg/m^2 showed lower levels of behaviours indicative of positive welfare (bale occupation, qualitative happy/active scores, play, ground scratching) than slower-growing broilers housed at 30 or 34 kg/m^2 (Rayner et al., 2020). Interestingly, slower-growing broilers housed at 30 kg/m^2 approached an observer more compared to the slower- and fast-growing broilers housed at 34 kg/m^2 (Rayner et al., 2020). These findings suggest that broiler breeds may differ in their behavioural response to reducing stocking density. Therefore, the aim of this study was to identify how slower-growing broilers respond to a reduction in stocking density with regard to their behaviour, and whether this response differs from fast-growing broilers. We hypothesized that slower-growing broilers respond more strongly to a reduction in stocking density with regard to their behaviour compared to fast-growing broilers. As we know that slower-growing broilers are more active and show more comfort and foraging behaviours (Dawson et al., 2021; de Jong et al., 2021; Dixon, 2020; Güz et al., 2021; Rayner et al., 2020; van der Eijk et al., 2022; Wallenbeck et al., 2016), it is expected that with increasing space they will respond with a larger increase in these types of behaviours as compared to fast-growing broilers, which are relatively inactive, especially at older ages.

2. Material and Methods

2.1. Experimental design

The experiment had a 2 × 4 factorial design with two broiler breeds, fast- (Ross 308, F) and slower-growing broilers (Ranger Classic, S) that were housed at 4 stocking densities (24, 30, 36 and 42 kg/m^2). We included 42 kg/m^2 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 quality label Better Life one star (BLS) in the Netherlands), 30 kg/m^2 (maximum according to ECC for higher welfare systems) and 36 kg/m^2 were chosen as steps between this maximum and minimum. The experiment was carried out in a semi-commercial environment in the experimental facility of Schothorst Feed Research (Lelystad, the Netherlands) to make it comparable to commercial practice.

2.2. Ethical statement

The housing and management and the experimental procedures were conducted in accordance with the national legislation on animal welfare and animal experiments, and approved by the institutional Animal Welfare Body. Because the procedures were non-invasive, 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).

2.3. Animals and housing

Day-old broiler chicks, originating from a parent stock of 44 weeks of age (for both F and S), 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 next to each other per breed, and densities being randomly distributed 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/m^2 respectively. We assumed that within these ranges (517−903) group size would not affect behaviour since hierarchy establishment decreases with increasing group size (Estevez et al., 2003) and group dynamics would therefore be similar between the stocking densities. 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 two houses (16 pens per house) and each experimental group having 2 replicates per house. Thinning was done by taking out 15% of the broilers from each pen in a 50/50 male/female ratio at 38 days of age for F and 44 days of age for S (at an estimated body weight of 2.2 kg for both breeds). Depopulation was at 41 days of age for F and 50 days of age for S (estimated body weight of 2.6 kg for both breeds). Actual slaughter weight was on average 2.68 and 2.55 kg, and average body weight gain per day was 64 and 50 g/day for F and S, respectively. Performance and welfare data are reported elsewhere (van

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/m^2.

Group	Breed	Density (kg/m^2)	Males /pen	Females /pen	Total /pen	Feeders /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

der Eijk et al., in prep).

Both houses were identical and climate controlled with 34.5 °C at arrival which gradually decreased to a constant temperature of 20 °C at 40 days of age. The lighting program used was 24L:0D at arrival, 20L:4D from day 1–6 and 18L:6D from day 7 onwards. Light intensity at chicken height (± 25 cm) was 40 lux between day 0–6 and 20 lux from day 7 onwards. Floor pens (47.5 m^2, length 9.5, width 5 and height 0.75 m) had wood shavings as litter and further included pan feeders and nipple drinkers with cups. Number of feeders and drinkers per pen were adjusted to account for stocking density (number of feeders: 6, 8, 9, 11; number of drinkers: 41, 52, 62, 72, respectively). Furthermore, firmly pressed straw bales were provided (length 50, width 30 and height 40 cm) with 1 bale per pen for 24 and 30 kg/m^2 and 2 bales per pen for 36 and 42 kg/m^2. For S broilers, pens included a net up to 1.6 m high to avoid them from escaping to other pens as they are more active than F broilers. At 14 days of age a 0.3 m high 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. An intermediate diet for both breeds was provided with a four-phase feeding schedule: starter diet from day 0–10 (3000 kcal/kg), grower diet 1 from day 10–20 (3100 kcal/kg), grower diet 2 from day 20–30 (3150 kcal/kg), and finisher diet from day 30 onwards (3200 kcal/kg). The starter diet was crumbled, while the other diets were pelleted. Coccidiostats were added to the diet (Maxiban® for starter and grower 1, Sacox® for grower 2 and finisher).

Chickens were spray vaccinated against Infectious Bronchitis at the hatchery, against Newcastle Disease at 7 days of age via spray and against Gumboro at 21 days of age via the drinking water. A positive *Salmonella* sample was taken prior to slaughter, but this was identified as *Salmonelle Infantis* C1 group which is considered non-invasive (Drauch et al., 2021). Mortality was on average 1.79%, where F had a mortality of 2.23% (1.15–3.30%) and S of 1.34% (0.78–2.26%).

2.4. Behaviour observations and enrichment use

Behaviour was observed at pen level using instantaneous scan sampling at four ages (day 14, 24, 31 and 35 for F and day 16, 27, 36 and 42 for S). These ages were chosen based on similar target body weights (TBW's) of F and S broilers (0.4, 1.1, 1.7 and 2.1 kg, respectively). Each pen was observed once in the morning (08:30 – 13:00) and once in the afternoon (13:00 – 17:30) on each observation day. Each observation consisted of scoring two fixed areas (of ± 3 m^2 each) that ranged from the centre to the wall/net, and included feeders and drinkers, but no bale. Each area was scanned 5 times (approximately 1 min per scan) after a 5 min habituation period. Per scan, the behaviour of all broilers in the area was scored according to the ethogram in Table 2. Behavioural observations were performed by two observers. Reliability between the two observers (inter-observer agreement) was high (index of concordance: 0.74), which was assessed via an inter-observer reliability test using a set of videos prior to behavioural observations. After behavioural observations, the use of the enrichments was scored by counting the number of chickens on and 0.5 m around one bale and the number of birds pecking at the bale (positioned closest to the front).

Table 2
Ethogram used during behavioural observations.

Behaviour	Description
Eating	Having the head above or in the feeder or pecking at feed in the feeder
Drinking	Pecking at the drinking nipples or cup beneath the drinking nipple
Locomotion	Walking, running, jumping or hopping without performing any other type of behaviour
Inactive	Sitting or lying while not engaged in any other activities
Standing	Standing without performing any other type of behaviour
Ground pecking	Pecking at the ground or litter while sitting or lying
Ground scratching	Pecking and/or scratching at the ground or litter while standing
Preening	Preening (manipulating own feathers with the beak or paws), stretching, wing flaps, feather ruffles, shakes (outside context of dust bathing)
Dustbathing	Rubs head and body against the ground, pecks and scratches while lying on the side, distributes substrate over body or shakes off substrates from feathers
Aggressive behaviour	All elements of aggressive behaviour, such as hopping oriented towards another chicken, threatening (both upright position), leaping, kicking, wing flapping or aggressive pecking (pecking directed to the head)
Other	All other behaviours not described above

2.5. Behaviour tests

2.5.1. Human approach test

This test was performed during the morning sessions after performing the behavioural observations. The observer walked to a fixed location in a normal pace, turned around and directly started the observations. The number of chickens within 0.5 m in front of the observer was recorded every 30 s during 3 min. In addition, the latency of the first chicken to enter the circle of 0.5 m around the observer was recorded.

2.5.2. Novel object test

This test was performed directly after the human approach test. The observer presented a novel object to the chickens by placing it in the litter, rising slowly and walking backwards for appr. 2 m and directly started observations. The number of chickens within 0.3 m of the object was recorded every 30 s during 3 min. In addition, the latency of the first chicken to enter the circle of 0.3 m around the object was recorded. The novel object differed for each age with the following order: a golf ball wrapped in aluminium foil (diameter 4.5 cm), a rubber duck (length 6.5, width 5, height 6 cm), a PVC block wrapped in coloured tape (length 5, width 2 and height 10 cm) and round box wrapped in orange tape (height 7, diameter 9.5–11.5 cm), respectively.

2.5.3. Free-space test

The free-space test to identify play behaviour was adapted from Liu et al. (2020). This test was performed directly after the novel object test. The observer walks towards the back of the pen, turns around and walks towards the front of the pen and tries to drive as much chickens away from the area between the feeder line and wall by making sounds and spreading their arms. The area was video recorded for 5 min using a camcorder on a tripod. From the recorded videos, the observer used continuous all-occurrences sampling of specific behaviours (Table 3) over the whole 5 min observation period.

2.6. Statistical analysis

SAS Software version 9.4 was used for statistical analysis (SAS Institute Inc., Cary, USA) and data was analysed at pen level on logscale (except for inactive behaviour). Normality of the data was assessed based on model residuals. Behavioural data was aggregated and expressed as percentage of broilers performing a certain behavioural category compared to total number of birds observed: ingestion (eating and drinking), locomotion, inactive, standing, comfort (preening and dust bathing), foraging (ground pecking and scratching). Behavioural data were analysed using linear mixed models consisting of fixed effects of breed, density, TBW and the interactions between breed*density*TBW, breed*TBW, density*TBW and breed*density. A backward regression procedure was used when fixed effects (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. Pen (1−32) within breed and density, and block (1−8) were included as separate random effects. Occurrences of aggressive and other behaviour were very low, therefore these behaviours were excluded from statistical analysis. Post hoc pairwise comparisons were corrected by Tukey-Kramer adjustment.

GenStat version 19.1 (VSN International, Hemel Hempstead, UK) was used for the analysis of enrichment use and behavioural test data at pen level. For enrichment use, calculations were made to obtain percentages of birds using the enrichment compared to total number of birds in the pen (on, 0.5 m around or pecking at the bales). Behavioural test data was expressed as percentage of birds performing a certain behaviour compared to total number of birds in the pen, percentage of time for latencies compared to duration of tests (3 min) and average percentage of birds approaching the human or novel object compared to the total number of birds in the pen. Enrichment use and behavioural test data were analysed using generalized linear mixed models with a binomial distribution consisting of fixed effects of breed, density, TBW and the interactions between breed*density*TBW, breed*TBW, density*TBW and breed*density. A backward regression procedure was used when fixed effects (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, pen within block and TBW within pen was added as random effect for enrichment use data. Post hoc pairwise comparisons were corrected by Bonferroni adjustment. All data is presented as means ± standard error (SE), unless otherwise mentioned.

To determine the response of foraging and comfort behaviour to stocking density, these behaviours were further analysed at pen level on logscale, using a mixed model with a 2nd polynomial model [1]:

Table 3
Ethogram used in the free-space test as adapted from Liu et al. (2020).

Behaviour	Description
Running	Forward movement, often including rapid direction change, at least 2–3 times normal walking speed. No wing flapping involved.
Frolicking	Forward movement, at least 2–3 times normal walking speed, with wings extended to each side or flapping, often includes sudden direction change
Wing flapping	Rapid bilateral up and down movements of wings while standing still or normal walking speed. Excludes wing flaps performed by a bird to balance itself on the drinker or feeder line/weighing scale.
Sparring	Two birds interact face to face as in fighting. May include hopping or chest bumping but no physical contact necessary. Brief, with no aggressive pecking. Each interaction between two birds was counted once.

$$Y_{ijkl} = \beta 0_{ik} + \beta 1_{ik} * X + \beta 2_{ik} * X^2 + \alpha_{ijk} + \mathcal{E}_l + \mathcal{E}_{lm} + \mathcal{E}_{klm} + \mathcal{E}_{klmo} \quad (1)$$

with Y = dependent variable, $\beta 0$ = intercept, $\beta 1$ = linear, $\beta 2$ = quadratic, X = (stocking density -/- 24), and α_{ijk} = lack of fit of polynomial function, $_i$ = breed, $_j$ = stocking density, $_k$ = TBW measurenumber, $_l$ = block, $_m$ = pen, and $_o$= observer (within TBW measurenumber), $\mathcal{E}_l$,$\mathcal{E}_{lm}$,$\mathcal{E}_{klm}$ =random effects for block, pen and TBW number within pen,$\mathcal{E}_{klmo}$ = residual error term. For model simplification, non-significant ($P > 0.1$) terms from model [1] were removed.

3. Results

3.1. Behaviour observations and enrichment use

Actual weights during observations slightly differed from TBW's (see Table 4) and there was a difference between breeds in actual weight, especially at TBW's 1.1 and 1.7 kg. Behaviour results are summarized in supplementary Table 1. No interactions between breed, density and TBW, density and TBW or density and breed were found. Interactions between breed and TBW were found for ingestion ($P < 0.05$) and inactive behaviour ($P < 0.01$), with slower-growing broilers (S) showing less ingestion compared to fast-growing broilers (F) at 1.7 and 2.1 kg ($P < 0.01$). For inactive behaviour no significant differences were found between breeds at the same TBW. Breed effects were found for ingestion, locomotion, standing, comfort and foraging. S broilers showed less ingestion ($\Delta = -5.6\%$, $P < 0.001$), and more locomotion ($\Delta = +1.0\%$, $P < 0.01$), standing ($\Delta = +1.6\%$, $P < 0.001$), comfort ($\Delta = +1.2\%$, $P < 0.05$) and foraging ($\Delta = +1.8\%$, $P < 0.001$) compared to F broilers.

Density effects were found for ingestion ($P < 0.01$), locomotion ($P < 0.001$), standing ($P < 0.05$), comfort ($P < 0.001$) and foraging behaviour ($P < 0.001$) (Fig. 1). 30 kg/m^2-broilers showed less ingestion compared to 36 and 42 kg/m^2-broilers ($P < 0.01$), with 24 kg/m^2-broilers not differing from other densities. 24 and 36 kg/m^2-broilers showed more locomotion compared to 42 kg/m^2-broilers ($P < 0.05$), with 30 kg/m^2-broilers not differing from other densities. 24 kg/m^2-broilers showed less standing compared to 30 kg/m^2-broilers ($P < 0.05$), with 36 and 42 kg/m^2-broilers not differing from other densities. 24 kg/m^2-broilers showed more comfort behaviour compared to 36 and 42 kg/m^2-broilers ($P \leq 0.01$), and 30 kg/m^2-broilers showed more comfort compared to 42 kg/m^2-broilers ($P < 0.01$). 24, 30 and 36 kg/m^2-broilers showed more foraging compared to 42 kg/m^2-broilers ($P < 0.05$).

For foraging and comfort behaviour, we further investigated whether there was a linear or quadratic relationship with density, and whether this relationship depended on breed and TBW. There was no quadratic relation between foraging or comfort behaviour and density. We did find a clear linear relationship for both foraging and comfort behaviour with density (both $P < 0.001$, Fig. 2). Breeds did not differ in their response, i.e., for both breeds foraging and comfort behaviour decreased with increasing density, and the relationship (slope) did not differ for the different TBW's.

Enrichment use is summarized in supplementary Table 2. An interaction between breed, density and TBW was found for percentage of broilers on the bale ($P < 0.05$). At 0.4 kg, S broilers housed at 30 kg/m^2 were sitting more on the bale compared to those at 24 and 36 kg/m^2, and compared to F broilers at 30 kg/m^2. Furthermore, more S broilers

Table 4
Target weight, actual weights in kg for both breeds, and difference in kg and % of actual weights of both breeds.

Target body weight	0.4	1.1	1.7	2.1
Ross 308 (F)	0.43	1.18	1.75	2.13
Ranger Classic (S)	0.43	1.00	1.55	1.98
Difference in kg	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%

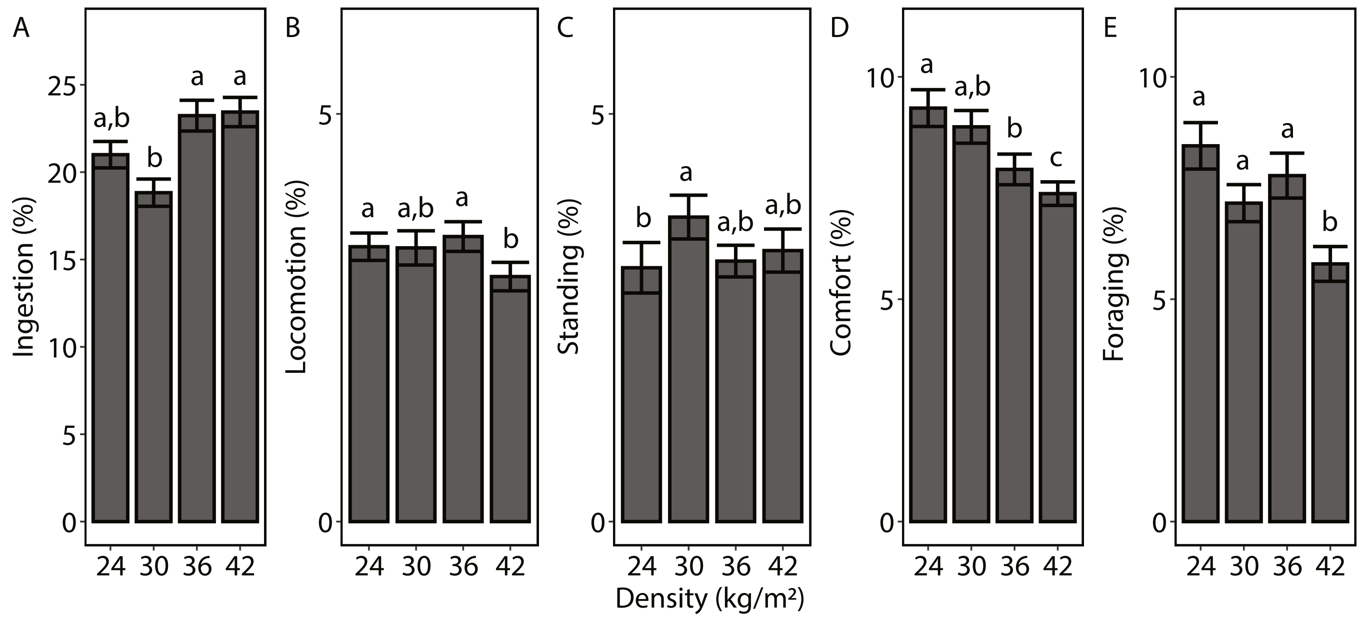

Fig. 1. Mean percentage of birds showing A) ingestion, B) locomotion, C) standing, D) comfort or E) foraging behaviour (± SE) for different stocking densities (kg/m^2). [a-c] values lacking a common superscript differ significantly (P < 0.05).

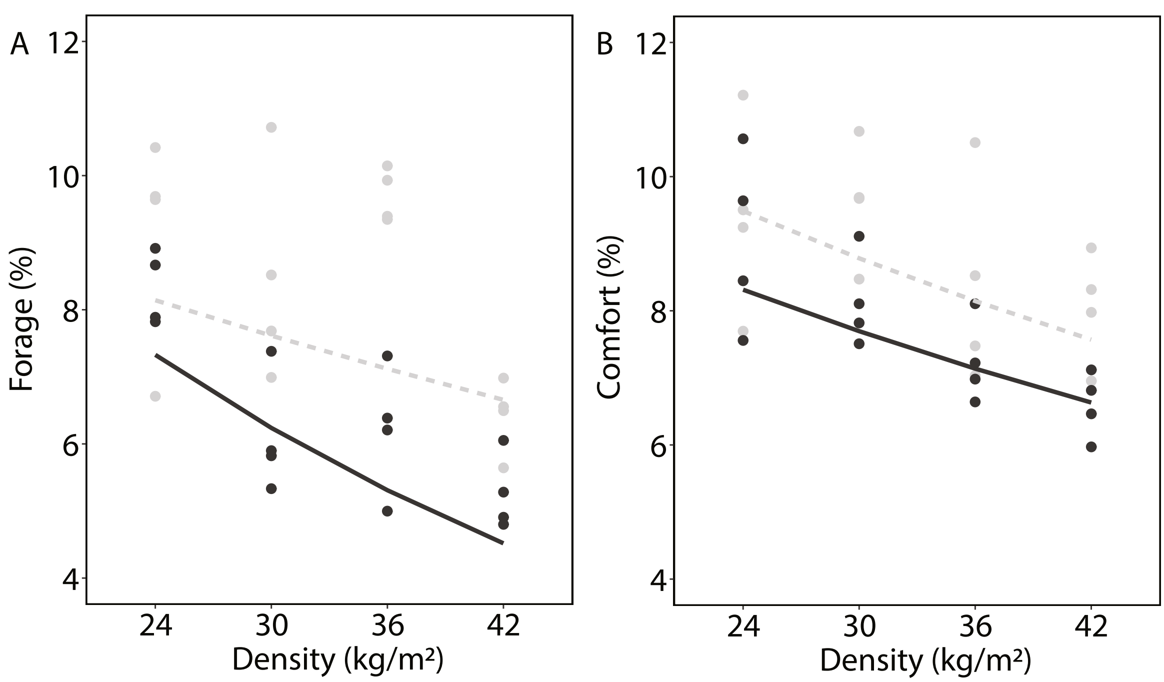

Fig. 2. Percentage of birds showing A) foraging or B) comfort behaviour. Slower-growing broilers: light grey dots and line, fast-growing broilers: black dots and line. Dots represent average percentage per pen per target stocking density, and lines represent linear model predictions (for both foraging and comfort, P < 0.001).

housed at 42 kg/m^2 were sitting on the bale compared to F broilers at 42 kg/m^2. At 1.1 kg, less F broilers housed at 36 kg/m^2 were sitting on the bale compared to those at 24 and 30 kg/m^2, and compared to S broilers at 36 kg/m^2. In addition, less F broilers at 24 kg/m^2 were sitting on the bale compared to S broilers at 24 kg/m^2. At 1.7 and 2.1 kg, more S broilers at 24, 36 and 42 kg/m^2 were sitting on the bale compared to F broilers housed at the same density. However, differences were not significant after correction for multiple comparisons. No interactions between breed and density were found on enrichment use. A breed effect was found for the percentage of broilers on the bale (P < 0.001), with a higher percentage of S broilers being on the bale ($\Delta = +0.37\%$) compared to F broilers.

An interaction between density and TBW was found for the percentage of broilers around the bale (P < 0.05), with more 30 kg/m^2-broilers sitting around the bale compared to 42 kg/m^2-broilers at 0.4 kg, 36 kg/m^2-broilers compared to 30 and 42 kg/m^2-broilers at 1.1 kg, and 24 kg/m^2-broilers compared to 42 kg/m^2-broilers at 1.7 kg. However, differences were not significant after correction for multiple comparisons. A density effect was found for the percentage of broilers on the bale (P < 0.05), with more broilers sitting on the bale at 24 kg/m^2 compared to 36 kg/m^2. However, differences were not significant after correction for multiple comparisons.

3.2. Behaviour tests

Responses to behaviour tests are shown in supplementary Table 3. No interactions between breed, density and TBW or density and breed were found for responses to the human approach (HA), novel object (NO) or free-space (FS) tests. Interactions between breed and TBW were found for latency to HA ($P < 0.01$), percentage of birds approaching a human ($P < 0.001$) and NO ($P < 0.01$). Breeds did not differ significantly from each other at the same TBW for latency to HA. More S broilers approached a human compared to F broilers at 0.4 and 1.7 kg ($P < 0.05$), and the same was found for broilers approaching a NO at 1.7 kg ($P < 0.05$). Breed effects were found for percentage of birds approaching a human ($P < 0.001$) and NO ($P < 0.01$), where S broilers approached the human and NO with higher percentages ($\Delta = +0.17\%$, $\Delta = +0.17\%$, $\Delta = +0.60\%$ and $\Delta = +0.31\%$) compared to F broilers. For the FS test, interactions between breed and TBW were found for sparring ($P < 0.05$) and wing flapping ($P < 0.01$), but breeds did not differ significantly from each other at the same TBW. Breed did not affect responses to the FS test.

Interactions between density and TBW were found for latency to approach the NO ($P < 0.05$) and running during the FS test ($P < 0.05$). At 0.4 kg, 24 kg/m^2-broilers approached the NO sooner compared to 36 kg/m^2-broilers, and at 1.1 kg 30 kg/m^2-broilers approached it sooner compared to 42 kg/m^2-broilers. 42 kg/m^2-broilers showed less running compared to 24 and 30 kg/m^2-broilers at 0.4 kg, compared to 24, 30 and 36 kg/m^2-broilers at 1.1 kg, and compared to 24 and 30 kg/m^2-broilers at 1.7 kg. At 2.1 kg, 24 kg/m^2-broilers showed more running compared to 30, 36 and 42 kg/m^2-broilers. However, differences were not significant after correction for multiple comparisons. Density effects were found on latency to approach the NO ($P < 0.05$), but densities did not differ significantly from each other. Density effects were also found on the percentage of birds showing frolicking ($P < 0.01$), running ($P < 0.05$), sparring ($P < 0.01$) and total play ($P < 0.001$) during the FS test (Fig. 3). Where 24 and 30 kg/m^2-broilers showed more frolicking and running compared to 42 kg/m^2-broilers, and 24 kg/m^2-broilers also showed more frolicking and running compared to 36 kg/m^2-broilers. For sparring, 24 kg/m^2-broilers showed more sparring compared to 36 and 42 kg/m^2-broilers, with 30 kg/m^2-broilers not differing from other densities. For total play, 24 and 30 kg/m^2-broilers showed more play compared to 36 and 42 kg/m^2-broilers.

4. Discussion

The aim of this study was to identify how slower-growing broilers respond to a reduction in stocking density with regard to their behaviour, and whether this response differs from fast-growing broilers. We hypothesized that slower-growing broilers would respond more strongly to a reduction in stocking density because of their higher activity level compared to fast-growing broilers. In contrast to our hypothesis, we found limited interactions between breed and stocking density, indicating that fast- and slower-growing broilers showed similar behavioural responses to reducing stocking density. For foraging and comfort behaviour a linear relation with stocking density was found, with more foraging and comfort behaviour when reducing stocking density. Slower-growing broilers are consistently showing more of these behaviours compared to fast-growing broilers, which might be related to their better locomotion, walking ability and litter quality as discussed below.

4.1. Stocking density

Regardless of breed, broilers housed at lower stocking densities (24 and/or 30 kg/m^2) showed less ingestion and more locomotion, comfort and foraging behaviours compared to broilers housed at higher stocking densities (36 and/or 42 kg/m^2). Although it should be noted that locomotion and foraging was also shown more by broilers housed at 36 kg/m^2 compared to 42 kg/m^2. Furthermore, broilers housed at 24 kg/m^2 showed less standing compared to 30 kg/m^2. Contrary to our findings, previous studies found no effects of density on eating or drinking behaviour (Buijs et al., 2011b, 2010; Febrer et al., 2006; Hall, 2001; Ventura et al., 2012) and on locomotion, although others have found increased locomotion with reducing density (Hall, 2001; Hongchao et al., 2014; Leone and Estevez, 2008; Ventura et al., 2012) like in our study. In support of our findings, litter-directed behaviour was shown to increase with reducing density in several studies (Buijs et al., 2011a; Hall, 2001; Ventura et al., 2012), but not all (Cornetto and Estevez, 2001; Febrer et al., 2006; McLean et al., 2002). Similarly, increasing density seems to hamper preening (Buijs et al., 2010; Febrer et al., 2006; Hall, 2001), which is in accordance with our findings of reduced comfort behaviour at higher densities. Discrepancies between our and previous studies might be related to genetic selection, as most studies have been conducted 10 or even 20 years ago. Furthermore, we included both fast- and slower-growing broilers, while previous studies only identified

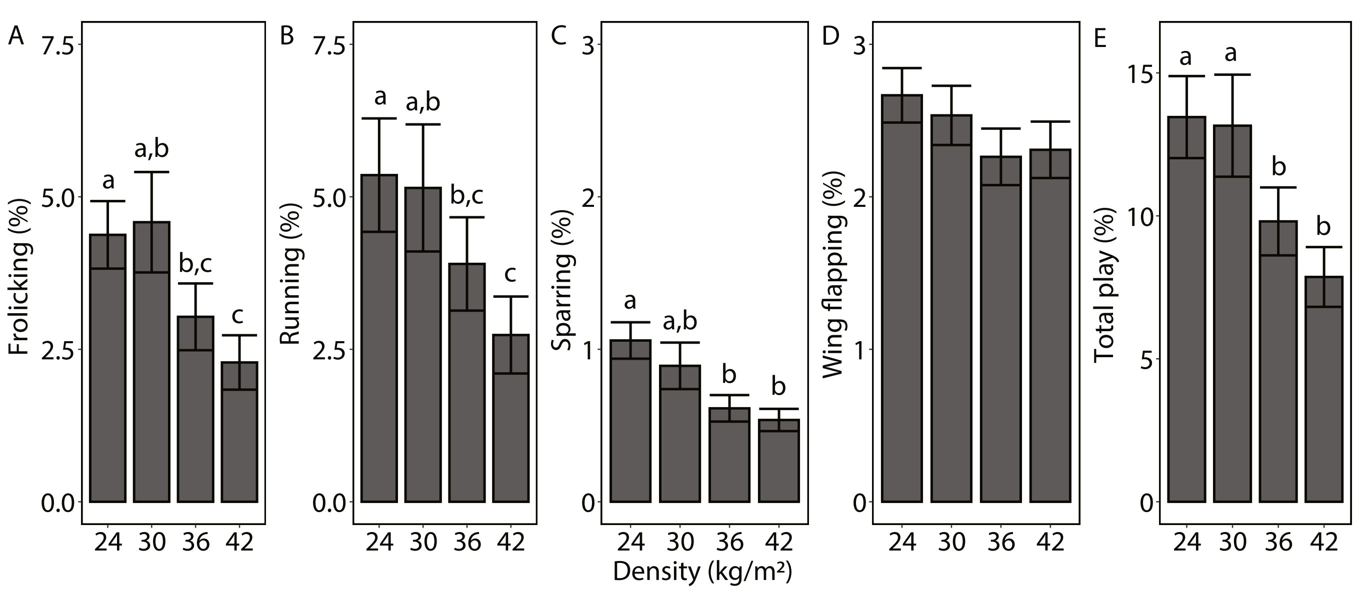

Fig. 3. Mean percentage of birds showing A) frolicking, B) running, C) sparring, D) wing flapping or E) total play (± SE) during the free-space test for different stocking densities (kg/m^2). [a-c] values lacking a common superscript differ significantly ($P < 0.05$).

effects on fast-growing broilers. In addition, most studies used relatively small pens (group sizes 8–208 birds per pen) (Buijs et al., 2011a, 2011b, 2010; Cornetto and Estevez, 2001; Hongchao et al., 2014; Leone and Estevez, 2008; McLean et al., 2002; Ventura et al., 2012), while we used a semi-commercial setting (group size 517–903 birds), which should make results more comparable to commercial practice, like studies performed in commercial farm houses (Collins, 2008; Febrer et al., 2006; Hall, 2001). Related to this, in larger pens there is relatively more free space available as broilers tend to cluster when resting. Moreover, studies compared different stocking densities, ranging between 6 and 56 kg/m^2, with most comparing 3 or 4 densities ranging between 20 and 48 kg/m^2.

Broilers housed at low stocking densities showed more locomotion, comfort and foraging behaviour. This may result from more free space and/or a better litter quality and/or walking ability that is often related to lower stocking densities (Bessei, 2006; van der Eijk et al., in prep.). Comfort and foraging behaviour are pleasurable and promote biological functioning (Bracke and Hopster, 2006; Lawrence et al., 2019), and are further categorised as behaviours performed after fulfilment of basic needs and when birds are free from suffering (Duncan and Mench, 1993). This could be indicative of a more positive emotional state experienced by broilers housed at low stocking densities. This is also supported by our finding that broilers housed at lower stocking densities showed more play behaviour during the free-space test. Interestingly, a recent study found that broilers from high density pens tended to approach all cues faster in a judgment bias test compared to broilers from low density pens. This could be explained, however, by an increased food motivation because of resource competition in high-density pens, in which case it would not necessarily indicate a more positive affective state (Anderson et al., 2021).

Density did not affect responses to the human approach or novel object tests, indicating no differences in fear level. Previously, reducing stocking density resulted in reduced fear levels (Buijs et al., 2009; Onbasilar et al., 2008). Yet, similar to our findings, other studies report no relationship between stocking density and fearfulness (Bailie et al., 2018; Ventura et al., 2010; Villagrá et al., 2009). Thus, results regarding effects of stocking density on fear levels are inconsistent, which might further be related to other factors influencing birds' responses, such as exploration (Forkman et al., 2007). As they are confronted with a novel object, the situation creates an approach-avoidance conflict as it stimulates a type of exploration that may be essential to survive (e.g., foraging) as well as the avoidance of a potentially threatening situation (Hughes, 1997). With regard to play behaviour, results are consistent as broilers housed at low stocking densities showed more total play behaviour during the free-space test compared to broilers housed at high stocking densities, specifically frolicking, running and sparring. It is interesting to note that even though broilers at low stocking densities had more space, they also showed more play behaviour compared to broilers housed at high stocking densities when space was created during the free-space test. This may indicate that broilers housed at high stocking densities did not show a greater rebound of play behaviour than broilers housed at low stocking densities when free space was provided temporarily. Previously, stocking density did not affect play behaviour, although slower-growing broilers housed at 30 or 34 kg/m^2 showed more play behaviour compared to fast-growing broilers housed at 34 kg/m^2 (Rayner et al., 2020). This was potentially related to poor walking ability, contact dermatitis and poor litter quality of fast-growing broilers (Rayner et al., 2020). Similarly, in our study, broilers housed at low stocking densities had better walking ability (as measured via the gait score), lower prevalence of contact dermatitis and better litter quality (van der Eijk et al., in prep.), this may result in them being better able to show play behaviour.

Stocking density did not affect enrichment use. Although we did see that broilers housed at low stocking densities made better use of enrichments, by sitting on and around the straw bale compared to those housed at high stocking densities. In support, slower-growing broilers housed at 30 kg/m^2 occupied bales more compared to those housed at 34 kg/m^2 (Rayner et al., 2020). Better use of enrichments at low stocking densities might be explained by less blockade or being limited by other broilers surrounding the bale. Better use of enrichments, especially sitting on bales, may also be related to better walking ability at low stocking densities (van der Eijk et al., in prep.).

Overall, reducing stocking density increased performance of locomotion, comfort, foraging and play behaviours, which could be indicative of a more positive emotional state and thus improved broiler welfare at low stocking densities. Performance of such behaviours was most positively affected by a reduction to 24 kg/m^2 in comparison to 42 kg/m^2. However, it should be noted that although statistically significant, differences over all TBW's were quite small, although differences at specific TBW's were larger. At low stocking densities, an increase of 0.5–0.6% was found for locomotion behaviour, of 1.5 – 2.8% for comfort and foraging behaviour, and of 3.4–5.6% for play behaviour, compared to high stocking densities. These relatively small differences might be explained by broilers being inactive for the largest part of the day, even slower-growing broilers (i.e. we found no difference in inactivity between breeds). Thus, creating more space to move around might not be as beneficial because broilers are mostly inactive. Still, reducing stocking density did increase performance of locomotion, comfort, foraging and play behaviour, indicating broiler's at low stocking densities experienced a more positive emotional state. Further research should identify whether reducing stocking densities even more, considerably improves broiler welfare with regard to behaviour. In addition, differences might become larger in a commercial setting as relatively more space is created because of broilers clustering together when resting. Although we used a semi-commercial setting, pens were still relatively small as compared to commercial houses and effects might have been larger in a commercial setting.

4.2. Breed

Slower-growing broilers showed more locomotion, standing, comfort and foraging, and less ingestion compared to fast-growing broilers. These findings are supported by previous studies showing more walking, standing, foraging, aggressive behaviour, and less eating and sitting in slower-growing broilers (Dawson et al., 2021; de Jong et al., 2021; Dixon, 2020; Güz et al., 2021; Rayner et al., 2020; van der Eijk et al., 2022; Wallenbeck et al., 2016). Differences between slower- and fast-growing broilers may be related to differences in growth rate and/or body weight. In the current study we compared breeds at similar TBW's, like was done by de Jong et al. (2021), Güz et al. (2021) and van der Eijk et al. (2022). This means that differences in behaviour are more likely related to genetic background or ontogeny (development, i.e. age) than body weight. However, it should be noted that breeds differed in actual body weights for TBW 1.1, 1.7 and 2.1 kg with fast- being heavier than slower-growing broilers and variation in body weight being highest for 1.1 kg and lowest for 2.1 kg. We cannot exclude that differences in body weights might have affected our results. Slower-growing broilers showed more locomotion and foraging, which might be related to them having better leg health, as indicated by better walking ability and lower prevalence of contact dermatitis compared to fast-growing broilers at similar body weights (Dixon, 2020; Güz et al., 2021; Rayner et al., 2020; van der Eijk et al., 2022). This was also found in the current study (van der Eijk et al., in prep.), although differences between breeds are not always found (de Jong et al., 2021). Slower-growing broilers also showed more comfort and foraging behaviours, which may indicate a higher intrinsic motivation to perform such behaviours or a more positive emotional state experienced by slower-growing broilers, as these types of behaviours are performed after fulfilment of basic needs and when birds are free from suffering (Duncan and Mench, 1993). However, especially for foraging and dustbathing as part of comfort behaviour, it could also be related to the better litter quality and leg health, which are often seen in slower- compared to fast-growing broilers

(Rayner et al., 2020; van der Eijk et al., 2022; Wilhelmsson et al., 2019; van der Eijk et al., in prep.).

More slower-growing broilers approached a human and novel object (indicating less fear) compared to fast-growing broilers. Previous studies are inconsistent with regard to differences in fear between slower- and fast-growing broilers, with some studies indicating slower-growing broilers show less fear in an NO test (Lindholm et al., 2016) and others reporting more fear in HA and TI test (Baxter et al., 2021; Lindholm et al., 2016; Wilhelmsson et al., 2019), or no difference in NO test (Baxter et al., 2021). Results may depend on the type of slower-growing breed and test used. Responses to the novel object and human approach test may not only be related to fear, as fear-related responses are complex and it is unlikely that a particular behaviour is only related to fear (Forkman et al., 2007). Several other factors could have influenced birds' responses, such as activity (incl. walking ability), exploration and social motivation (Forkman et al., 2007; Jones, 1996). Slower- and fast-growing broilers did not differ with regard to play behaviour. Previously, slower-growing broilers were reported to show more play behaviour compared to fast-growing broilers (Baxter et al., 2021; Rayner et al., 2020). Discrepancies might be caused by different types of breeds being used and the amount of studies identifying play behaviour is limited, making it difficult to draw conclusions.

Slower-growing broilers made better use of enrichments by sitting on the bale, compared to fast-growing broilers. This is in line with previous studies where slower-growing broilers made more use of elevated structures compared to fast-growing broilers (de Jong et al., 2021; Güz et al., 2021; Malchow et al., 2019; Malchow and Schrader, 2021; van der Eijk et al., 2022; Wallenbeck et al., 2016). Differences may be explained by a higher activity level of slower-growing broilers (Bokkers and Koene, 2003; de Jong et al., 2021; Dixon, 2020; van der Eijk et al., 2022; Wallenbeck et al., 2016) or by a different body conformation (de Jong and Gunnink, 2019; Norring et al., 2016), resulting in fewer problems with maintaining their balance or in a better ability to fly or climb on a bale.

Overall, slower-growing broilers showed less ingestion, more locomotor, comfort and foraging behaviours, made better use of enrichments and seem to have lower fear compared to fast-growing broilers, although breeds did not differ with regard to play behaviour. Differences between breeds are likely caused by their genetic background or ontogeny, and might further be related to better leg health and litter quality increasing the performance of locomotor, comfort and foraging behaviours. These findings suggest improved welfare of slower-growing broilers. However, it should be noted that not all breeds identified as slower-growing may behave differently from fast-growing breeds, and not all slower-growing breeds necessarily behave similarly to each other as was indicated by Dawson et al. (2021). Improved broiler welfare may therefore be related to a breed's specific growth rate, next to breed-specific behaviour, i.e. genetic differences. For further research it would be interesting to identify differences in behaviour between breeds growing at a similar growth rate.

5. Conclusions

In conclusion, fast- and slower-growing broilers responded similarly to reducing stocking density. Performance of comfort, foraging and play behaviours were positively affected by a reduction in stocking density, indicating that reducing stocking density improved welfare of both fast- and slower-growing broilers. Performance of locomotive, comfort and foraging behaviours, fear and enrichment use were positively affected by using slower-growing broilers, suggesting improved welfare compared to fast-growing broilers. Thus, reducing stocking density and using slower-growing broilers would benefit broiler welfare, where combining both would further improve broiler welfare with regard to their behaviour.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.applanim.2022.105754.

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