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Growth rate, either through genetics or diet, mainly determines the outcome concerning broiler welfare

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A B S T R A C T

There is a trend towards broiler production systems with higher welfare requirements, which often use a combination of factors to improve broiler welfare. This makes it difficult to entangle whether improvements are due to housing conditions, diet, genetics or a combination of these factors. Therefore, it remains unknown to what extent differences in welfare can be attributed to breed (i.e., genetics), growth rate (i.e., diet) or the interaction between the two. We compared fast- (Ross 308, **R**), medium- (Ranger Classic, **RC**) and slower-growing broilers (Hubbard JA757, **H**) receiving diets differing in balanced protein (**BP**) content (i.e., 80, 90 and 100%). We identified effects on behaviour, responses to behavioural tests and litter quality at three target body weights (**TBW**s, 0.2, 1.2 and 2.4 kg), and welfare scores and litter DM content at TBW 2.6 kg. The experiment had a 3 × 3 factorial design with four replicates (pens) per treatment (a total of 36 pens). We hypothesised that reducing the growth rate will improve the welfare of all breeds and that breeds will not differ in welfare if they have a comparable growth rate. Indeed, reducing the growth rate improved hock burn scores in all breeds. R broilers also had improved cleanliness scores and RC broilers improved gait scores. Reducing the growth rate increased the number of R broilers approaching a human and novel object and showing running behaviour in the free-space test, while it reduced the number of RC broilers approaching a human and showing comfort behaviour and did not affect behaviour in H broilers. In addition, litter quality was improved for R broilers (wetness and DM), while reduced for H broilers (DM) with reduced growth rate, and no effects of growth rate on litter quality were found for RC broilers. Thus, reducing growth rate (i.e., reducing BP in diets) improves welfare in all breeds, but breeds did respond differently concerning behaviour and litter quality, with more beneficial effects for fast-growing broilers compared to medium- and slower-growing broilers. Hardly any differences in behaviour, welfare scores and litter quality were found between breeds when they had a comparable growth rate (R80 vs RC100 and RC80 vs H100), except for R80 having better scores for cleanliness and gait scores (only males) and higher litter DM content compared to RC100. These findings suggest that growth rate, either through genetics or diet, is mainly determining the outcome concerning broiler welfare.

Implications

There is a trend towards broiler production systems with higher welfare requirements, which often use a combination of factors to improve broiler welfare. Here, we show that reducing the growth rate through the diet benefits broiler welfare, although the size of effects depends on the type of breed used (i.e., fast-, medium- or slower-growing breed). Furthermore, breeds hardly differed concerning welfare when having a comparable growth rate. This suggests that growth rate, either through genetics or diet, is mainly determining the outcome concerning broiler welfare.

Introduction

Increased pressure from non-governmental organisations has caused a trend towards broiler production systems with higher welfare requirements, mainly in North-West Europe (Vissers et al., 2019). These 'higher-welfare' systems often use a combination of factors to improve broiler welfare, such as slower-growing breeds, lower stocking density, daylight and/or environmental enrichment

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(Bracke et al., 2019). In the Netherlands, higher welfare production systems with slower-growing breeds often also use a different dietary composition (Mostert et al., 2022). This makes it difficult to entangle whether improvements in broiler welfare are due to housing conditions, diet, genetics or a combination of these factors.

When comparing fast- to slower-growing breeds (i.e., genetics), housed under similar conditions and receiving the same diet, many studies report differences in behaviour and other welfare indicators. Here, we define fast-growing broilers as having a growth rate of ≥ 60 g/day and slower-growing broilers as having a growth rate of ≤ 50 g/day. In general, slower-growing broilers show more walking, standing, foraging, comfort and aggressive behaviour, and less eating, sitting and inactive behaviour compared to fast-growing broilers (van der Eijk et al., 2022a, 2022b; Dawson et al., 2021; de Jong et al., 2021; Dixon, 2020). However, one study reported no differences in behaviour (Baxter et al., 2021, comparing 53 vs 65 g/day). Concerning fearfulness and play behaviour, results are less consistent. Slower-growing broilers were more difficult to approach (Wilhelmsson et al., 2019, comparing 45 vs 55 g/day) and showed more avoidance behaviour (Baxter et al., 2021) indicating that they are more fearful. Yet, slower-growing broilers approached a human or novel object more (van der Eijk et al., 2022b), indicating that they are less fearful. No differences between breeds have also been reported, e.g. in response to a novel object test (Baxter et al., 2021) and in latency to approach a human or novel object (van der Eijk et al., 2022b) Slower-growing broilers showed more play behaviour (frolicking and sparring), although they did not differ in food running (Baxter et al., 2021). Yet, another study reported breeds did not differ in play behaviour (sparring, frolicking, wing flapping and running) (van der Eijk et al., 2022b). Thus, slower-growing broilers differ from fast-growing broilers concerning their behaviour, and they may also differ in fear and play behaviour. However, findings are not always consistent, which may depend on differences in testing at the same age or BW and different breeds being tested.

Concerning welfare, slower-growing broilers are often reported to have better welfare scores, such as gait, footpad dermatitis, hock burns, skin lesions and cleanliness (see Nicol et al., 2024 for review). However, differences are not always found for gait (de Jong et al., 2021), footpad dermatitis (Santos et al., 2022; de Jong et al., 2021; Dixon, 2020), hock burns (van der Eijk et al., 2023; de Jong et al., 2021) and cleanliness (van der Eijk et al., 2023). Similarly, litter quality is often better when housing slower- compared to fast-growing broilers under similar conditions (van der Eijk et al., 2022b, 2023; Santos et al., 2022), although here too differences are not always found (Baxter et al., 2021; Wilhelmsson et al., 2019).

From these findings, it is clear that slower growth may contribute to improved broiler welfare. However, there are differences between slower- and fast-growing broilers in a breed's specific growth rate and genetic background (Dawson et al., 2021). Previous studies have indicated improved broiler welfare when reducing protein content in the diet, e.g., improved footpad dermatitis scores and litter quality (Lambert et al., 2023; Brink et al., 2022; Van Harn et al., 2019). When feeding a fast- and slower-growing breed a low- or high-protein diet, no interactions were found between breed and diet on behaviour (panting, huddling, touch test), welfare (cleanliness, footpad dermatitis, lameness, hock burns) and litter quality, and diet type was therefore suggested to have minor effects on broiler welfare (Wilhelmsson et al., 2019). Yet, it remains unknown to what extent differences in welfare can be attributed to breed, growth rate or the interaction between the two.

Therefore, this study aimed to identify how breeds (i.e., a fast-, medium- and slow-growing breed) differ concerning their behaviour and welfare when they have comparable growth rates due to diets with different protein content (i.e. 80, 90 or 100% balanced protein content). To unravel the question of whether welfare is related to breed, growth rate or both, a medium-growing breed was included to ensure overlap in growth rates between the different breeds when feeding different balanced protein diet programmes. We hypothesised that reducing the growth rate will improve the welfare of all breeds and that breeds will not differ in welfare if they have a comparable growth rate.

Material and methods

Experimental design

The experiment had a 3 × 3 factorial design with three broiler breeds, Ross 308 (**R**), Ranger Classic (**RC**) and Hubbard JA757 (**H**) that received three diet programmes (100, 90 and 80% balanced protein (**BP**) diet). These BP levels were intended to reach an overlap in growth rate between the different breeds. Fast-growing broilers had a growth rate of ≥ 55 g/day, medium-growing broilers had a growth rate of 50–55 g/day and slower-growing broilers had a growth rate of 45–50 g/day. The experiment was carried out at Laverdonk, the experimental farm of Agrifirm (Heeswijk-Dinther, The Netherlands).

Animals and housing

Day-old broiler chicks, originating from a parent stock of 45 weeks of age (R and H) or 42 weeks of age (RC), were obtained from a commercial hatchery (Probroed & Sloot, Meppel, The Netherlands). A total of 2 280 broilers per breed were randomly allocated to the three diet programmes, resulting in nine experimental groups (R100, R90, R80, RC100, RC90, RC80, H100, H90 and H80). Each experimental group was replicated 4 times, resulting in a total of 36 pens. The room consisted of 3 rows with 12 pens per row and a block consisted of three pens per row with four blocks in total. Experimental groups were pseudo-randomly assigned to a specific pen so that each breed appeared 4 times per row and each experimental group appeared once per block. Broilers were housed in groups of 190 (14 birds/m^2) with an exact 50/50 male/female distribution (i.e., straight run). Depopulation was targeted at an approximate weight of 2.8 kg, resulting in depopulation at 43 (R100 and 90), 48 (R80), 51 (RC100), 55 (RC90), 62 (RC80, H100 and 90) and 63 days of age (H80) (target stocking density of 39.2 kg/m^2). See Table 1 for achieved growth rates per experimental group.

The room was mechanically ventilated, and the temperature at arrival of the day-old broilers was 33 °C and was gradually reduced to a constant temperature of 20 °C at 40 days of age. The lighting programme used was 24L:0D at arrival, 20L:4D from day 1 to 6 and 18L:6D from day 7 onwards. Light intensity at chick height (± 25 cm) was measured at five points in each pen and was on average 26.5 lux and ranged between 10.6–50.5 lux within pens, with an average range between 19.6–30.0 lux between pens. Floor pens (13.5 m^2, length 4.45, width 3.14 and height 0.75 m) had wood shavings as bedding material (1 bale/pen ~1.25 kg/m^2) and further included five pan feeders and 30 nipple drinkers without

Table 1
Actual growth rate (g/day) for each broiler breed (R = Ross 308, RC = Ranger Classic and H = Hubbard JA757) receiving diets differing in percentage of balanced protein (BP) (100, 90 or 80%).

Breed	100% BP	90% BP	80% BP
R	64.7	61.3	55.3
RC	54.4	52.8	49.8
H	49.9	48.8	45.8

cups. Pens included a net up to 1.3 m high to avoid (slower-growing) broilers from escaping to other pens. Broilers had ad libitum access to feed and water. A four-phase feeding programme was applied where broilers received approximately the same amount of starter diet (300 g), grower diet 1 (1 kg), grower diet 2 (1.2 kg), and the amount of finisher diet differed per experimental group due to differences in feed efficiency and length of the fattening period. All breeds were fed the same diet to distinguish the effects of different diet compositions from those of breed. 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). All diets were produced and delivered by ABZ Diervoeding (Leusden, The Netherlands; see Supplementary Table S1 for diet compositions, calculated and analysed nutrient values). Chicks were vaccinated against Infectious Bronchitis (Poulvac IB QX, Zoetis, The Netherlands) at the hatchery and at 10 days of age via spray. Chicks were further vaccinated for Newcastle Disease (Avishield ND, Genera Inc., Croatia) at 10 days of age via spray and for Gumboro (Cevac IBD L, CEVA Sante Animale B.V., The Netherland) at 21 days of age via the drinking water.

Behaviour observations

Behaviour was observed live at pen level using instantaneous scan sampling, at 7 (R and RC100), 9 (RC90 and 80, H), 23 (R100 and 90), 27 (R80), 29 (RC 100 and 90), 31 (RC80, H100 and 90), 34 (H80), 36 (R100 and 90), 42 (R80), 45 (RC100), 48 (RC90), 52 (RC80 and H100) and 55 (H90 and 80) days of age, with a total of three times per experimental group (pen). These ages were chosen based on comparable target body weights (**TBW**s) of experimental groups (0.2, 1.2 and 2.4 kg) to ensure early, mid and late observations throughout the experimental period. Actual weights during observations slightly differed from TBWs (Table 2).

On each observation day, specific pens were observed once in the morning (0830–1230 h) and once in the afternoon (1330–1600 h). Each observation consisted of scanning a fixed area (± 3 m^2) 5 times after a 5–min habituation period. The area was predetermined and included feeders and drinkers. Per scan, the behaviour of all broilers in the area was scored according to the ethogram (Supplementary Table S2), and scans were performed immediately after one another with scan duration being approximately 1 min. Behavioural observations were carried out by four observers. Reliability between the four observers (inter-observer agreement) was high (index of concordance: average of 0.77 and range 0.72–0.90).

Behaviour tests

Behaviour tests were performed after each pen observation during the morning sessions. The following tests were performed:

Human approach and novel object test

The observer walked at a normal pace to a fixed location, turned around and immediately started the observations. The number of chickens within 0.5 m in front of the observer was recorded every 30 s for 3 min. In addition, the latency of the first chicken to enter the 0.5 m circle around the observer was recorded. The novel object test was performed directly after the human approach test (as previously described by van der Eijk et al., 2022b). The observer presented a novel object to the chickens by placing it on the litter, standing up/raising slowly, walking backwards for approximately 2 m and immediately started observations. The number of chickens within a 0.3 m radius of the object was recorded every 30 s for 3 min. In addition, the latency of the first chicken to enter the 0.3 m circle around the object was recorded. The novel object differed for each TBW in 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 plastic block wrapped in coloured tape (length 5, width 2 and height 10 cm) and plastic tube wrapped with coloured tape (length 50, diameter 3.2 cm).

Free-space test

The free-space test was adapted from Liu et al. (2020). The observer entered the pen, walked to the end of the pen, turned around and walked to the front of the pen and tried to drive as many chickens as possible away from the area between the feed and drinking line, after which the observer exited the pen. The area was video recorded for 5 min using a camera on a tripod. From the recorded videos, the observer used continuous all-occurrence sampling of specific behaviours (Supplementary Table S3) over the whole 5–min observation period (as previously described by van der Eijk et al., 2022b).

Welfare measurements

Welfare measurements were assessed once per experimental group at 41 (R100 and 90), 45 (R80), 48 (RC100), 52 (RC90), 55 (RC80 and H100) and 58 (H90 and H80) days of age, and these ages were chosen based on comparable TBWs of R, RC and H broilers (2.6 kg). Actual weights during the assessment differed slightly from TBWs (Table 2). Welfare measurements included gait score, footpad dermatitis, hock burns, cleanliness and injuries of 15 males and 15 females per pen (n = 30 per pen) according to Welfare Quality protocol (Welfare Quality®, 2009). To assess the quality of locomotion, gait scores were recorded, and each bird was assigned a score between 0 (perfect) and 5 (unable to walk). Footpad dermatitis and hock burns were assigned a score between 0 (no lesions) and 4 (severe lesions) and the worst score of both feet/hocks was noted down. Cleanliness was scored by inspection of the breast area and assigned a score between 0 (completely clean) and 3 (very dirty). Injuries were assigned a score of 0 (no scratches or wounds), 1 (single scratch or small wound ≤ 0.5 cm^2) or 2 (multiple scratches and/or large wounds > 0.5 cm^2). All welfare measurements were performed by one trained observer in the pens (as previously described by van der Eijk et al., 2023).

Litter quality

Litter quality was assessed in each pen on the same days as behavioural observations (as previously described by van der Eijk et al., 2023). Litter quality was scored by a panel of two trained assessors. Litter was scored on a scale of 1–10 for friability and wetness according to van Harn et al. (2009), where a litter score

Table 2
Actual average weight and range between brackets for each broiler breed (R = Ross 308, RC = Ranger Classic and H = Hubbard JA757) at each target BW category (0.2, 1.2, 2.4 and 2.6 kg).

Breed	0.2 kg	1.2 kg	2.4 kg	2.6 kg
R	0.18 (0.17–0.19)	1.18 (1.14–1.23)	2.28 (2.13–2.46)	2.72 (2.66–2.80)
RC	0.19 (0.16–0.21)	1.25 (1.22–1.31)	2.45 (2.42–2.50)	2.63 (2.60–2.66)
H	0.19 (0.18–0.20)	1.23 (1.19–1.27)	2.58 (2.50–2.66)	2.72 (2.59–2.80)

of 1 corresponded with low litter quality (very wet, completely caked) and a score of 10 corresponded with high litter quality (dry, completely friable).

Litter samples were taken on the same day as welfare measurements. Litter samples for DM content were taken at three locations per pen, at the feeding line, the drinking line and between the drinking line and the separation fence with the adjacent compartment. The litter samples were taken by using a bulb planter/tin can (diameter 10 cm) from top to bottom to the (concrete) floor. The three samples per pen were pooled and placed in a plastic bag. This pooled sample was then dried in a drying oven at 105 °C for 24 h. If direct processing of the (pooled) samples was not possible, they were stored in the freezer (–20 °C), after which they were later processed/dried.

Statistical analysis

SAS Software version 9.4 was used for statistical analysis (SAS Institute Inc., Cary, USA), and data were analysed at pen level. Behavioural data were aggregated over the five subsequent scans and expressed as a percentage of broilers performing a certain behavioural category divided by the total number of broilers observed: ingestion (eating and drinking), locomotion, inactive, standing, comfort (comfort and dust bathing), foraging (foraging and ground pecking). Behavioural test data were expressed as the percentage of birds performing a certain behaviour divided by the total number of birds in the pen, the percentage of time for latencies compared to the duration of tests (3 min) and the average number of birds approaching the human or novel object over the whole test period. Behavioural data and behavioural test data were analysed using linear mixed models consisting of fixed effects breed, diet, TBW, and all 2-way and 3-way interactions. A backward regression procedure was used when fixed effects (i.e., 2-way and 3-way interaction) had $P > 0.1$, but breed*diet was always included. Pen (1–36) and block (1–4) were included as separate random effects. The normality of the data was assessed based on model residuals. Behavioural data were log-transformed (except for inactive behaviour), and behavioural test data were square root transformed to obtain normality of residuals. Occurrences of aggressive and other behaviour were very low and were therefore excluded from statistical analysis. Posthoc pairwise comparisons were corrected by Tukey-Kramer adjustment. For interactions, we focused on significant differences within breed and within diet at comparable TBWs. We further focused on differences between H100 and RC80, RC100 and R80, as these experimental groups also reached comparable growth rates.

The DM content of the litter was statistically analysed using Genstat statistical software (Genstat™ Release 21.1, VSN International LTD, Hemel Hempstead, UK) using ANOVA with block included as a blocking factor and breed, diet and breed*diet interaction as explanatory variables. Differences between groups were analysed using Fisher's unprotected least significant difference test, in case, the effect was significant.

For welfare scores, a different statistical approach was used to assess the effects over multiple scores. Gait, footpad dermatitis, hock burns, and cleanliness were analysed with ordinal regression using R package ordinal (R version 4.3.2). Some scores were aggregated before analysis (for gait, score 1 + 2 and 4 + 5, score 0 did not occur, and for hock burns, score 4 did not occur). The fitted models include nested random intercept for block and pen and were fitted using the function clmm. For fixed effects, a full model was fitted first including diet, breed, sex, all 2-way and the 3-way interaction. Litter scores were analysed similarly with fixed effects breed, diet, breed*diet, and TBW was modelled as a continuous variable. Using backward selection, the interactions that are not significant were dropped (P-value > 0.05), except for breed*diet which was always included, and for footpad dermatitis, sex*diet was dropped (P-value 0.04) as this was not sufficiently of interest. For each welfare score, the selected model was used for further inference. R package emmeans was used to calculate the mean effect per diet/breed/sex. These estimates were centred and shown on the logit scale used when fitting the ordinal regression model. Subsequently, pairs of estimates were tested. Occurrences of skin lesions were very low; therefore, this welfare indicator was excluded from statistical analysis. Posthoc pairwise comparisons were corrected by Tukey-Kramer adjustment. Similar to the behaviour data, we focused on significant differences within breed and within diet and further between H100 and RC80, RC100 and R80 as these experimental groups also reached comparable growth rates.

Results

Behaviour observations

An interaction between breed, diet and TBW was found for ingestion ($P < 0.01$), where R100 showed more ingestion compared to H100 at 1.2 kg ($P < 0.05$) (Fig. 1A), and for inactive behaviour ($P < 0.05$), with no significant differences between experimental groups at the same TBW after correction for multiple comparisons. Further, an interaction between breed, diet and TBW was found for comfort behaviour ($P < 0.01$), where RC90 showed more comfort behaviour compared to H90, RC80 and R90 at 2.4 kg ($P < 0.05$) and RC100 showed more comfort compared to R100 at 2.4 kg ($P < 0.05$) (Fig. 1B). An interaction between breed and diet was found for locomotion behaviour ($P < 0.05$), with no significant differences between experimental groups after correction for multiple comparisons. See Supplementary Table S4 for means and SEs over TBWs of behaviours scored.

Behaviour tests

An interaction between breed, diet and TBW was found for the number of broilers approaching the human ($P < 0.01$). At 1.2 kg, more H90 broilers approached the human compared to R90 ($P < 0.001$), and more H100, RC100 and R80 broilers approached the human compared to R100 ($P < 0.05$). At 2.4 kg, more RC100 broilers approached the human compared to H100, RC80, RC90 and R100 ($P < 0.05$), and more R80 broilers approached the human compared to R100 ($P < 0.01$) (Fig. 2).

Interactions between breed and diet were found for the number of birds approaching the human ($P < 0.001$) and the novel object ($P < 0.01$). More H90 and R80 broilers approached the human compared to R90 ($P < 0.05$). More H100, RC100 and R80 broilers approached the human compared to R100 ($P < 0.01$) (Fig. 3A). More H100, RC100 and R80 approached the novel object compared to R100 ($P < 0.05$) and more H90 approached the novel object compared to R90 ($P < 0.05$) (Fig. 3B). Interactions between breed and diet were found for running ($P < 0.01$) and sparring ($P < 0.05$) during the free-space test. RC80 showed more running compared to H80 ($P < 0.05$), and H100 and R80 showed more running compared to R100 ($P < 0.01$) (Fig. 3C). RC100 showed more sparring compared to R100 ($P < 0.01$) (Fig. 3D). See Supplementary Table S5 for means and SEs over TBWs of responses in behavioural tests.

Welfare measurements

Interactions between breed and diet were found for hock burns ($P < 0.01$), for cleanliness ($P < 0.01$) and a tendency for gait ($P = 0.06$). Regarding hock burns, H80 had lower (better) scores compared to H100 ($P < 0.05$), and H90 had lower scores compared to RC90 ($P < 0.01$). Furthermore, RC80 had lower scores compared

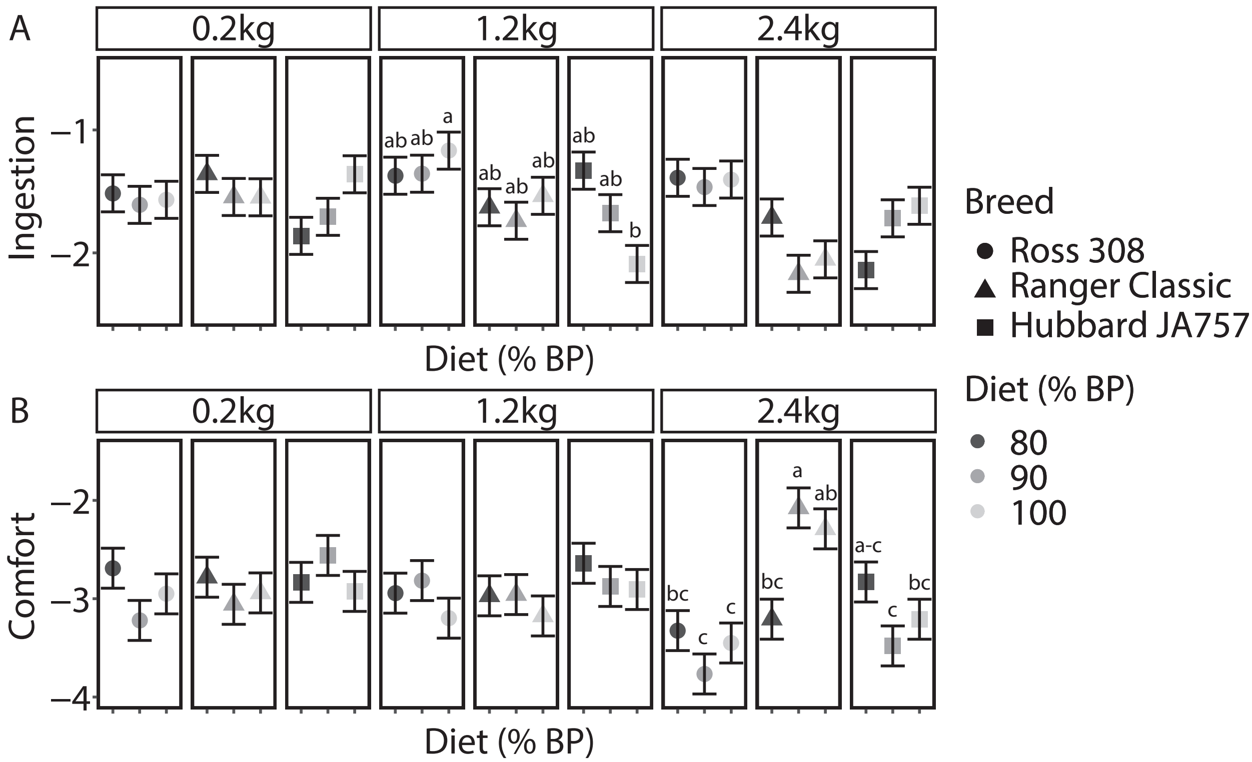

Fig. 1. Least square means of birds showing A) ingestion or B) comfort behaviour (±SE) for the fast-, medium- and slower-growing broiler breeds (R = Ross 308, RC = Ranger Classic, H = Hubbard JA757) receiving diets differing in percentage of balanced protein (BP 80, 90, 100%) for each target BW category (0.2, 1.2 and 2.4 kg). [a–c] values lacking a common superscript differ significantly ($P < 0.05$) within a specific target BW category.

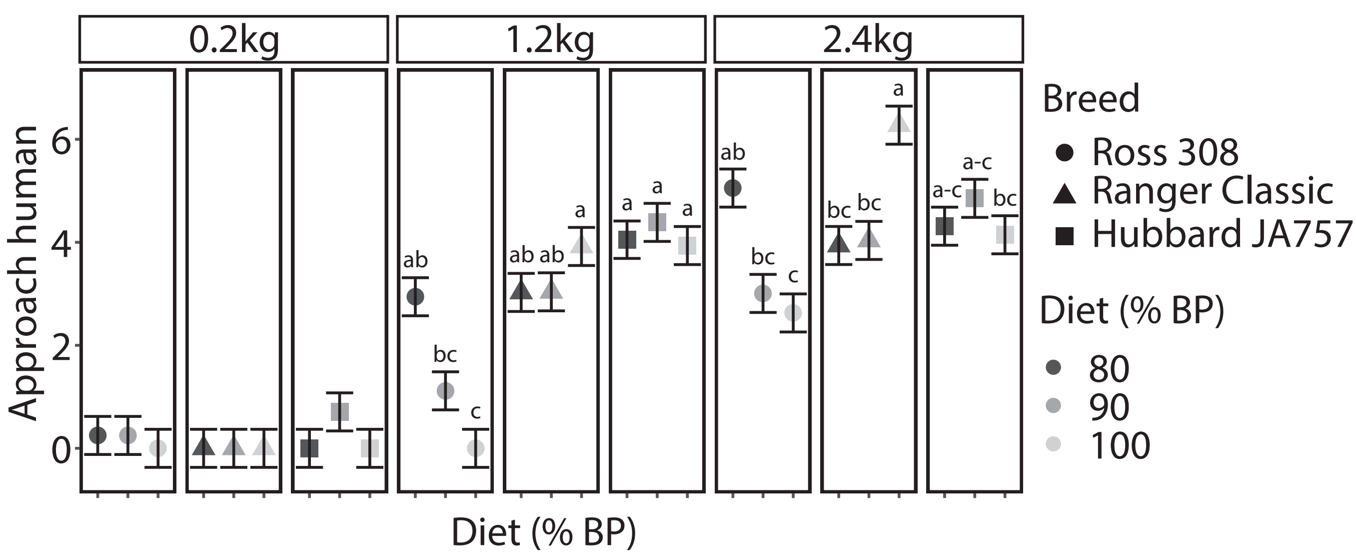

Fig. 2. Least square means of birds approaching a human (±SE) for the fast-, medium- and slower-growing broiler breeds (R = Ross 308, RC = Ranger Classic, H = Hubbard JA757) receiving diets differing in percentage of balanced protein (BP 80, 90, 100%) for each target BW category (0.2, 1.2 and 2.4 kg). [a–c] values lacking a common superscript differ significantly ($P < 0.05$) within a specific target BW category.

to RC90 and RC100 ($P < 0.01$) and RC90 and R100 had higher scores compared to R90 ($P < 0.05$) (Fig. 4A). Regarding cleanliness, R80 had lower (better) scores compared to RC80, RC100, R90 and R100 ($P < 0.01$) for both females and males. In addition, for males, R80 had lower scores compared to H80 ($P < 0.01$) (Fig. 4B). Regarding gait, RC80, RC90 and H100 had lower (better) scores compared to RC100 ($P < 0.05$) for both females and males. In addition, for females, H100 had lower scores compared to R100 ($P < 0.05$) and for males, R80 had lower scores compared to RC100 ($P < 0.01$) (Fig. 4C). For footpad dermatitis, no interaction was found between breed and diet. See Supplementary Tables S6a and S6b for means and SEs of all welfare scores. Model fits of all welfare scores are visualised in Supplementary Fig. S1.

Litter quality

An interaction between breed, diet and TBW was found for wetness ($P < 0.01$), with R100 being more likely to have a lower (worse) score for higher TBWs compared to H100 and R80 ($P < 0.05$) (Fig. 5). No interaction effects were found on friability,

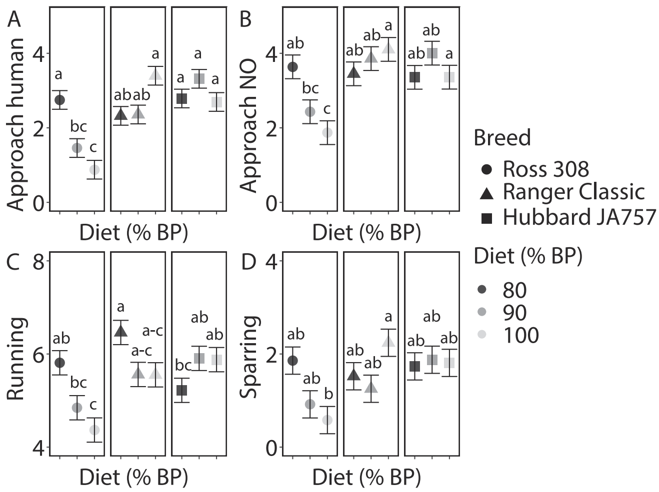

Fig. 3. Least square means of birds A) approaching a human, B) approach a novel object (NO), C) showing running or D) sparring behaviour (±SE) for fast-, medium- and slower-growing broiler breeds (R = Ross 308, RC = Ranger Classic, H = Hubbard JA757) receiving diets differing in percentage of balanced protein (BP 80, 90, 100%) over target BW categories. [a–c] values lacking a common superscript differ significantly ($P < 0.05$).

but an interaction between breed and diet was found for DM content ($P < 0.05$), with R80 having higher % compared to R90, RC100 and H80. RC80 also had higher % compared to H80, and H100 had higher % compared to RC100, H90 and H80 (Table 3). See Supplementary Tables S7a and S7b for means and SEs over TBWs of wetness and friability scores. Model fits of wetness and friability scores are visualised in Supplementary Fig. S2.

Discussion

This study aimed to identify how breeds, (i.e., a fast-, medium- and slower-growing breed) differ concerning their behaviour and welfare when they have comparable growth rates because of diets with different balanced protein content (i.e., 80, 90 or 100%). We hypothesised that reducing the growth rate will improve welfare in all breeds and that breeds will not differ in welfare when they have a comparable growth rate. However, concerning behaviour, breed-specific effects might still occur regardless of comparable growth rates (Dawson et al., 2021). Indeed, reducing the growth rate improved welfare scores in all breeds, but breeds did respond differently, especially concerning behaviour and litter quality. Furthermore, hardly any differences in behaviour, welfare scores and litter quality were found between breeds when having a comparable growth rate. These findings suggest that growth rate, either by breed (i.e., genetics) or diet, is mainly determining the outcome concerning broiler welfare.

Reducing the growth rate within breed

Overall, reducing growth rate through the diet (i.e., balanced protein content) improved welfare scores in all breeds, with all having improved hock burn scores, R broilers also having improved cleanliness scores and RC broilers tended to have improved gait scores. Furthermore, litter quality was improved for R broilers (wetness and DM) but reduced for H broilers (DM), while for RC broilers, no effects of diet were found on litter quality. Previous studies have indicated improved broiler welfare when reducing protein content in the diet, e.g., improved footpad dermatitis scores and litter quality (Lambert et al., 2023; Brink et al., 2022; Van Harn et al., 2019). However, reducing protein content also

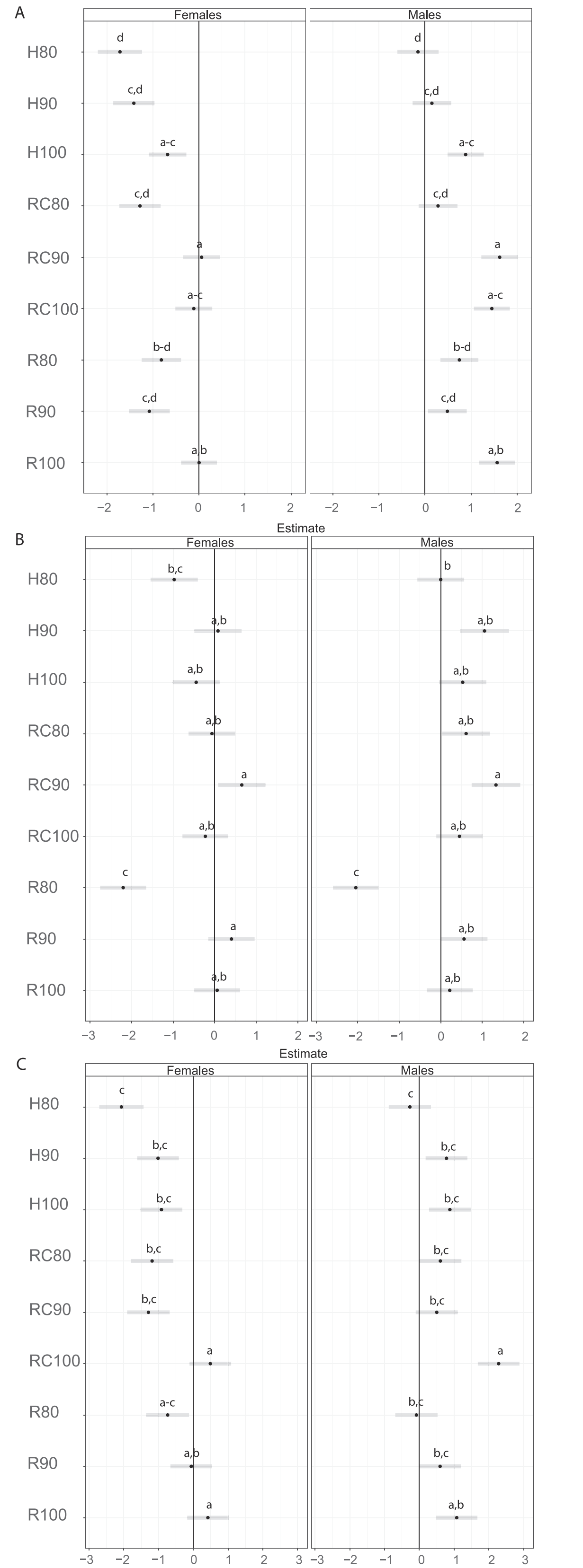

Fig. 4. Estimates of the ordinal regression model (black dot) for A) hock burn, B) cleanliness, or C) gait score with 95% confidence interval (grey area) for the fast-, medium- and slower-growing broiler breeds (R = Ross 308, RC = Ranger Classic, H = Hubbard JA757) receiving diets differing in percentage of balanced protein (100, 90 or 80%). Estimates are centred on zero per model. These estimates are on a log odds scale, higher values indicate higher odds of having a higher (ordinal) score. [a–d] values lacking a common superscript differ significantly ($P < 0.05$).

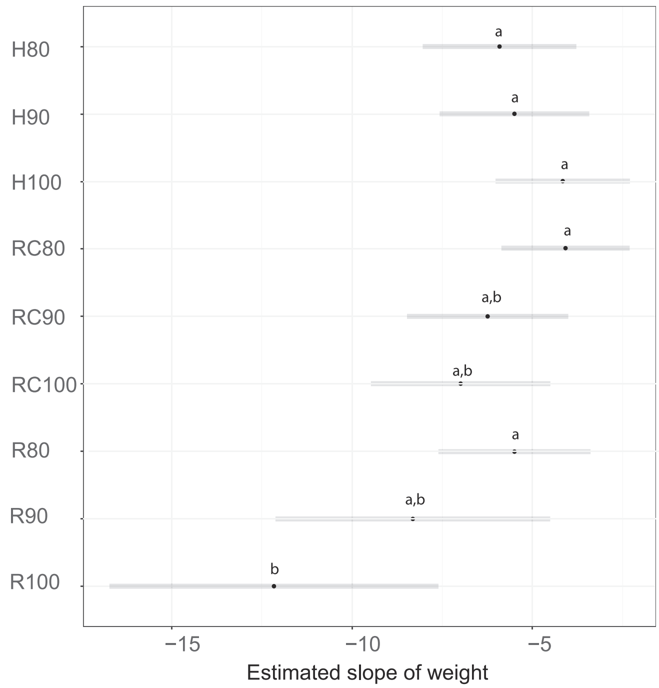

Fig. 5. Estimates (black dot) for litter wetness score with 95% confidence interval (grey area) for fast-, medium- and slower-growing broiler breeds (R = Ross 308, RC = Ranger Classic, H = Hubbard JA757) receiving diets differing in percentage of balanced protein (100, 90 or 80%). The estimated slope of weight (x-axis) quantifies the change in log odds per category of target BW (0.2, 1.2 and 2.4 kg). A negative slope implies lower ordinal scores as weight increases. [a–b] values lacking a common superscript differ significantly ($P < 0.05$).

Table 3
Mean DM content and LSD for fast-, medium- and slower-growing broiler breeds (R = Ross 308, RC = Ranger Classic, H = Hubbard JA757) receiving diets differing in percentage of balanced protein (100, 90 or 80%).

Item	Value
DM content (%)	
Breed	
R	44.3[a]
RC	42.0[b]
H	41.6[b]
Diet	
100	43.5[a]
90	41.0[b]
80	43.5[a]
Breed*Diet	
R100	44.5[a–c]
R90	41.7[b–e]
R80	46.8[a]
RC100	41.1[c–e]
RC90	40.8[d,e]
RC80	44.2[a–d]
H100	44.9[a,b]
H90	40.5[e]
H80	39.4[e]
Statistics	
LSD	
Breed	0.73
Diet	0.73
Breed*Diet	1.26
P-values	
Breed	< 0.05
Diet	< 0.05
Breed*Diet	< 0.05

[a–e] values lacking a common superscript differ significantly ($P < 0.05$).

had limited effects on cleanliness and hock burn scores, and reduced litter quality and worsened gait and footpad dermatitis scores in broilers (Wilhelmsson et al., 2019). Reducing protein content in the diet has previously been suggested to reduce water intake, as there is a reduced need to excrete surplus nitrogen (Van Harn et al., 2019). Indeed, we found that water consumption was reduced when reducing protein content in the diet (de Jong et al., in prep 2025). A lower water intake might reduce the risk of wet litter, and thereby the risk of impaired welfare, as litter quality is the main reason for the development of contact dermatitis (Bradshaw et al., 2002). Furthermore, better litter quality could lead to improved cleanliness (Louton et al., 2018; Saraiva et al., 2016), as seen for R broilers. RC broilers tended to have better gait scores and had better hock burn scores when reducing growth rate, although litter quality was not affected. This finding might be explained by the interaction between contact dermatitis and walking ability. A better walking ability may result in less contact with the litter due to sitting or lying, reducing the risk of contact dermatitis (Bessei, 2006) and contact dermatitis in turn can cause poorer walking ability (Bradshaw et al., 2002). However, it should be noted that reducing the growth rate did not affect locomotion behaviour of RC broilers. For H broilers, hock burn scores were improved while DM content was reduced when reducing the growth rate. This discrepancy might be explained by litter quality remaining similar for a long period of time, as no differences in litter wetness or friability scores were found at 2.4 kg for H broilers, and only deteriorated in the final weeks of rearing at 2.6 kg. Thus, reducing the growth rate improves welfare scores and influences litter quality, but the effects depend on the type of breed used.

With regard to behaviour, reducing growth rate had a positive effect on fear responses (more broilers approach a human and novel object) and play behaviour (more running) in R broilers, while it had a negative effect on fear responses (less broilers approach a human) and comfort behaviour in RC broilers, and for H broilers, no effects were found on behaviour responses to the tests. Previously, reducing protein content in the diet did not affect responses to a touch test (Wilhelmsson et al., 2019). Differences in responses to our fear and play tests might not necessarily indicate changes in fear or play due to reduced growth rate alone. Other factors may have influenced birds' responses to our tests, such as walking ability, exploration motivation or age (Rasmussen et al., 2022; Forkman et al., 2007; Vestergaard and Sanotra, 1999). In R broilers, walking ability was not affected by reducing growth rate, but the differences might be explained by differences in age as we measured fear and play at comparable TBW. Previous studies have observed a reduction of fear and/or an increase in exploratory behaviour with age (Giersberg et al., 2020; Albentosa et al., 2003; Hocking et al., 2001) and similarly play behaviour increased with age (Baxter et al., 2019), although others showed play declines with age (Liu et al., 2020; Vasdal et al., 2019). For RC broilers, reducing growth rate tended to improve walking ability, likely resulting in broilers being better able to move away from the human, suggesting increased fear. Indeed, previous studies have indicated that walking ability influences broiler fear responses, especially in fear tests that use approach or avoidance behaviour (Rasmussen et al., 2022; Vasdal et al., 2018). However, it should be noted that reducing the growth rate did not affect locomotion behaviour for RC broilers. Furthermore, reducing the growth rate did not affect litter quality but comfort behaviour was reduced in RC broilers. This might suggest discomfort, as comfort behaviour is pleasurable and performed after the fulfilment of basic needs and when birds are free from suffering (Lawrence et al., 2019; Bracke and Hopster, 2006). However, for both fear and comfort behaviour, effects were only found at 2.4 kg, suggesting effects were limited. For H broilers, no effects were found on behaviour, fear or play, which might indicate they are less responsive to a reduction in growth rate. Thus, reducing the growth rate improves fear and play behaviour of a fast-growing breed, but effects are limited or not found for medium- and slower-growing breeds.

Thus, within breeds, it appears that reducing growth rate is most beneficial for R broilers, and less so for RC and H broilers, especially with regard to behaviour. R broilers had the highest growth rate, which has previously resulted in lower welfare scores and limitations in behaviour compared to medium- and slower-growing breeds like RC and H broilers (van der Eijk et al., 2022a, 2022b, 2023; de Jong et al., 2021). In the present study, R broilers' growth rate differed almost two-fold between 80, 90 and 100% BP compared to that of RC and H broilers. R broilers may therefore be more responsive to a reduction in protein content. Selection for efficient growth might have altered their response to protein levels more than that of RC and H broilers, as medium- and slower-growing breeds seem to be less sensitive to a reduction in protein content. Furthermore, reducing the growth rate of medium- and slower-growing breeds through the diet might be of less added value, as they already show improved welfare compared to fast-growing breeds when receiving the same diet (van der Eijk et al., 2022a, 2022b, 2023; de Jong et al., 2021).

Breeds with a comparable growth rate

Hardly any differences between breeds were found when having a comparable growth rate, suggesting breeds do not differ in welfare, behaviour and litter quality when having a comparable growth rate, except for R80 broilers which had improved welfare compared to RC100 broilers, with better scores for cleanliness and a tendency for better gait scores (only males) and higher litter DM content. As mentioned previously, improved litter quality likely results in improved cleanliness (Louton et al., 2018; Saraiva et al., 2016) and can indirectly result in improved gait score through less contact dermatitis (Bessei, 2006), although R80 and RC100 broilers did not differ for contact dermatitis. Age may have influenced our results as breeds were sampled 3 days apart, but this is unlikely. No differences were found between RC80 and H100 broilers, which were sampled at the same age. Overall, these findings suggest that growth rate affects welfare and behaviour more than breed. Thus, differences between breeds found in previous studies are likely due to the genetic growth rate when the growth rate is optimised via nutritional factors. Furthermore, this seems to indicate no major differences in breed-specific behaviour, at least not for the breeds tested here. However, it should be noted that experimental groups with differing growth rates did not always differ in behaviour and welfare scores. For example, R80 did not differ from H100 concerning behaviour, responses to behavioural tests, litter quality, hock burns and gait scores, whereas the growth rate of R80 was 5.4 g/day higher compared to H100.

In the current study, we used three breeds: Ross 308, Ranger Classic and Hubbard JA757. We compared R80 vs RC100 and RC80 vs H100 at comparable TBWs and having comparable growth rates. Responses regarding behaviour, welfare and litter quality might be different for other breeds. For further research, it might be valuable to compare breeds from different breeding companies when inducing a comparable growth rate. Furthermore, we compared groups at comparable BWs, suggesting differences in behaviour and welfare are more likely related to growth rate, genetic background or ontogeny (development, i.e., age) than to BW. However, it should be noted that groups differed in actual BW, especially for measurements at 2.4 and 2.6 kg. Therefore, we cannot exclude that differences in BW may have influenced our results. Further research should focus on identifying differences at the same ages and BWs. In addition, certain limitations should be taken into account when interpreting findings, any possible effects on event behaviours (such as aggression) might have been overlooked due to the scan sampling method, we did not examine for effects on hunger specifically, we did not include perches or platforms and the number of replicates included in this study was limited. Further research could focus on more detailed behaviour observations (including hunger), including perches or platforms and more replicates.

Conclusion

Breeds hardly differ in behaviour and welfare if they have a comparable growth rate (R80 vs RC100 and RC80 vs H100). This seems to indicate behaviour and welfare are influenced more by growth rate than by breed. Reducing balanced protein in the diet (i.e., growth rate) improves welfare in all breeds, with more beneficial effects for fast-growing broilers, likely due to a two-fold change in growth rate compared to medium- and slower-growing broilers. Overall, findings suggest that growth rate, either through genetics or diet, is mainly determining the outcome concerning broiler welfare.

Supplementary material

Supplementary Material for this article (https://doi.org/10.1016/j.animal.2025.101431) can be found at the foot of the online page, in the Appendix section.

Ethics approval

Experiment procedures were checked with the national legislation on animal experiments by the institutional Animal Welfare Body. As 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 (24 August 2021, Lelystad, The Netherlands).

Data and model availability statement

None of the data were deposited in an official repository. The datasets generated and/or analysed during the current study are available from the corresponding author upon reasonable request.

Declaration of Generative AI and AI-assisted technologies in the writing process

During the preparation of this work the author(s) did not use any AI and AI-assisted technologies.

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Declaration of interest

The authors declare no conflicts of interest.

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