Commercial layer hybrids kept under organic conditions: a comparison of range use, welfare, and egg production in two layer strains

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ABSTRACT Outdoor range areas provide laying hens with improved opportunities to perform natural behaviors and increase the available space per bird, however, birds are also exposed to potentially stressful factors including weather and predators. Ability to cope with challenging environments varies between different strains and must be considered to ensure good welfare. The aim of this study was to determine how suitable 2 hybrids, the Dekalb White (**DW**) and the Bovans Brown (**BB**), are for organic production with special emphasis on ranging behavior. A total of 1,200 hens were housed according to organic regulations across 12 flocks of 100 birds. Range and shelter use, effect of weather, vegetation cover, egg production and quality, and mortality were assessed in addition to a range of clinical welfare indicators. Initially a greater proportion of DW hens accessed the range. However, after approximately 2 mo, a greater proportion of BB were using the range and venturing further from the house. DW hens were more likely to use the shelters than BB hens ($P < 0.001$). Vegetation was also worn away to a greater extent in the BB ranges. Weather affected the proportion of hens that went outside, the distance ranged from the popholes, and shelter use. BB hens were found to have better plumage condition ($P < 0.001$), fewer footpad lesions ($P < 0.001$), fewer comb wounds ($P < 0.001$), and lower mortality rates ($P = 0.013$). Both hybrids experienced keel bone fractures, though DW hens had more at the cranial portion ($P < 0.001$) and BB at the caudal portion ($P < 0.001$). DW hens had an earlier onset of lay and higher egg production than BB hens ($P < 0.001$), though BB hens laid heavier eggs ($P < 0.001$) with thicker shells ($P = 0.001$). Overall, BB hens seemed to perform superiorly or equivalently to the DW hens for all variables apart from egg production. These results demonstrate the importance of considering the strain of bird selected for organic production systems in order for the birds to reap the potential benefits that are offered by outdoor access.

Key words: genotype, laying hen, outdoor access, range use, welfare

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INTRODUCTION

Modern commercial strains of laying hens have been selected predominately for individual performance traits such as egg production and feed efficiency (Rodenburg et al., 2008), with little regard to behavioral traits as hens have been traditionally housed in cages where behavioral expression is limited (Widowski et al., 2016). As consumer demand for more humanely raised food grows, producers are transitioning away from caged housing systems towards alternative cage-free systems, such as organic poultry production (Rondoni et al., 2020). In addition to being cage-free, organic hens are also provided with outdoor access with the presumption being that outdoor access equates to improved hen welfare (Bennett et al., 2016; Pettersson et al., 2016). In reality, outdoor access can cause poor welfare if hens are unable to adapt to novel and sometimes challenging environments, a trait that varies amongst individuals and between strains of laying hens (Jones et al., 1995; Hocking et al., 2004; Campbell et al., 2016, 2020a).

Outdoor access provides hens with additional space and improved opportunities to perform highly motivated behaviors such as dustbathing and foraging.

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Studies have shown that birds with outdoor access have lower incidences of feather pecking, improved plumage condition (Mahboub et al., 2004; Rodriguez-Aurrekoetxea and Estevez, 2016; Bari et al., 2020; Sibanda et al., 2020), and reduced prevalence of keel bone deformities (Regmi et al., 2016) and footpad dermatitis (Rodriguez-Aurrekoetxea and Estevez, 2016). However, the benefits that hens gain from outdoor access depend greatly on whether or not the birds choose to utilize the outdoor area as well as their ability to cope with stressful situations. Through the use of RFID technology it has been shown that a wide amount of variation exists between individuals and how frequently they exit the house, how long they stay on the range, and how far they choose to venture from the house (Campbell et al., 2017, 2018, 2020b; Larsen et al., 2017). Within a flock, there may also be small subpopulations that never or rarely leave the house (Campbell et al., 2017; Larsen et al., 2017).

It is believed that selection of modern commercial strains for production traits lead to associated changes in birds' fearfulness and ability to cope in novel environments, affecting how they utilize outdoor areas (Rodenburg et al., 2008; Campbell et al., 2016; Kolakshyapati et al., 2020). Outdoor environments expose birds to potentially stressful situations including weather (rain, wind, temperature extremes), parasites, disease, and predation (Lay Jr et al., 2011; Sossidou et al., 2011). More fearful birds may choose to remain inside the protected house or may remain in close proximity to the house if they do choose to go outdoors. When comparing behavioral traits of various strains of laying hens, there appears to be a trend with white strains being more fearful (Odén et al., 2002; De Haas et al., 2013) and displaying longer periods of tonic immobility (Albentosa et al., 2003; Mahboub et al., 2004) than brown strains of birds.

For birds to be successful in organic production systems, they must be able to cope with novel and dynamic environments while maintaining adequate production values. The aim of this study was to determine how suitable 2 popular laying hen hybrids, the Dekalb White and the Bovans Brown, are for organic egg production, with special emphasis on their ranging behavior. Among the variables investigated were also prevalence of a range of clinical welfare indicators, egg production, and egg quality traits.

MATERIALS AND METHODS

Animals and Housing

The study took place from May 3 to September 27, 2018 at the poultry experimental facilities at Aarhus University, Foulum. We used non-beak trimmed laying hens from 2 hybrid lines (N = 1200), Dekalb White (**DW**, n = 600) and Bovans Brown (**BB**, n = 600). Egg color was the same as the hen color. Hens from both hybrids were acquired from the same rearing company (TopÆg A/S, Viborg, Denmark) and were reared under the same organic conditions which consisted of multitier housing, provision of roughage, and outdoor access when weather permitted. All birds were tagged with a leg band using unique colors for each pen. The birds were 17 wk old when housed at the start of the study and 38 wk old at the end of the experimental period, where the majority were adopted by private persons, and the remaining birds were euthanized by CO_2 gas. A total of 120 birds (10 birds per pen) were examined postmortem (reported in Wurtz et al., in prep.).

The birds were housed according to the organic regulations (Landbrugsstyrelsen, 2020). They were kept in 12 flocks of 100 birds, that is, 6 flocks of each hybrid, in pens measuring 4 m × 4.5 m with a useable net area of 17.46 m^2, hence a stocking density of 5.7 hens per m^2. Indoors, the birds had ad libitum access to feed (2 circular feeders, 4.52 cm feeder space/hen) and water (14 nipples/100 hens). A layer of wood shaving litter (4−5 cm) was provided at the start of the experiment. In each pen, the birds had access to wooden perches (H × W: 3.8 cm × 5.7 cm) placed 60 and 100 cm above the floor (18.4 cm/hen) and 14 roll-away nest boxes with a total nest box area of 132.6 cm^2/hen. Some birds managed to fly into neighboring pens and to prevent this, these specific birds (approximately 25) had the flight feathers trimmed on one wing before being placed back in their original pens.

The light program was 12L:12D at 17 wk (lights on 7:00−19:00) and increased weekly by 1 h of light until it reached 16L:8D at 21 wk of age (lights on 5:00−21:00). Dusk and dawn periods (20 min each) were contained in the light period by gradually decreasing/increasing light intensity. Natural light came in through transparent sections/panels in the roof and the upper part of the outer walls.

From each pen, the hens could access a 4.5 m × 90 m (4.05 m^2 per hen) outdoor area through a pophole (80 cm wide and 40 cm high), facing south-west. The popholes were opened every day, beginning from the day they arrived, at 8:00, and closed again 45 min after sunset, starting at 21:50 (early May) and increasing gradually to 23:00 (midsummer) from where closing time gradually decreased to 20:10 (late September). Each outdoor area was covered with vegetation/grass and had four shaded areas, each measuring 2 m × 10 m. The shaded areas were spaced 10 m apart with the first being placed 5 m from the pophole. Shade was provided by gray green-house shade cloth, blocking 85% of the sun light, mounted on poles at a height of approximately 2 m. Along the fence, signs were placed every 5 m to indicate the distance from the pophole for observation purposes.

The hens were fed organic layer feed (DLG, Fredericia, Denmark); starter layer feed (Natur Æg Start) from placement to 24 wk of age, and phase 1 feed (Natur Æg Fase 1) from 25 to 38 wk of age. Diets met or exceeded the nutritional requirements stated in NRC (1994). Roughage was provided outdoors on Tuesdays, Thursdays and Saturdays, and in the first 2 mo it was maize silage, whereas carrot silage was offered in the last three months. Remnants of roughage were removed every Tuesday before fresh roughage was added. The hens additionally had ad libitum access to mussel shells.

Data Collection

Range and Shelter Use Throughout the experimental period, range use was recorded on Thursdays by live observation. All outdoor areas were scanned eight times between 8:30 and 15:30 in a fixed order (outdoor area 1 −12). The number of hens in each 5-m section of the outdoor area was counted, as were the number of hens under the shade. Proportion of range use was calculated as the total number of hens in the outdoor area divided by the total number of hens in the pen (indoors and outdoors). Proportion of shelter use was calculated as the total count of hens under the shaded areas divided by the total number of hens outdoors. The maximum distance from the popholes at which at least one hen was observed was recorded and the maximum distance at which 90% or fewer of the birds were observed was calculated.

Weather Data Weather data were obtained from a weather station managed by the Danish Meteorological Institute (**DMI**), positioned 1.6 km north-west of the experimental facility. Throughout the experimental period data were collected on an hourly basis from 08:00 to 16:00 for temperature (average,°C), relative humidity (average, %), precipitation (total, mm), wind speed (average, m/s) and wind direction (average, degrees). In addition, the minimum and maximum daily temperatures were obtained.

Vegetation Type, Cover, and Height When the experiment began, the outdoor areas were covered with vegetation, mainly grass and clover. At the end of study, both vegetation type and cover were evaluated 25, 55, and 85 m from the popholes. Vegetation type was scored as either grass, clover, "other vegetation", or "bare ground", and the percentage of each type was determined by placing a 1 m × 1 m frame with a 5 cm × 5 cm grid in the middle of each outdoor area at each of the three distances from the pophole.

The height of the vegetation was also measured at the same date, using the same 1 m × 1 m frame described above, however, only four of the 25 grid-squares of the frame were used (grid-squares used (row, column): 2, 2; 2, 4; 4, 2; 4, 4). The height was determined in the middle of each range at six distances from the popholes (10, 25, 40, 55, 70, and 85 m). At the closest distance (10 m), the area was completely covered by water on the day of sampling for pens 5, 6, 7, 9, and 10 and thus, no assessments were done for these at this distance. Nevertheless, none of the other pens had any vegetation at the 10 m distance.

Welfare Assessment The welfare of all hens was assessed on the day of arrival (17 wk) and again on the last day of the experimental period (38 wk). To minimize handling, the birds were taken directly from the transport crates upon arrival at 17 wk, and at 38 wk, the birds were placed in crates after assessment for transport/killing to take place immediately. Four trained and experienced observers each assessed 25 birds within each pen. A training session involving the full protocol and approximately 30 birds was arranged prior to arrival of the experimental birds. Furthermore, the first 25 birds of the experiment (both at 17 and 38 wk of age) were assessed by the observers together for calibration purposes. The assessment included an evaluation of the plumage condition of 9 specific body parts (Table 1). The condition of tail and flight feathers was also assessed but on separate scale (Table 1). The number of skin injuries for each of the same 9 body parts and on the comb were counted (Table 1), and comb color was recorded (normal or abnormal [blue or pale]). Presence of keel bone damage was assessed using the palpation method, including all fractures regardless of whether they were old or fresh as well as deviations of the keel bone from a straight line (Table 1). Both keel bone deviations and damages were scored separately for three equal-sized parts of the keel bone (A: the cranial part; B: the medial part; C: the caudal part). Feet condition was scored for presence of bumble foot, hyperkeratosis, and missing toes/nails, whereas footpad lesions were scored as none, mild, or severe (Table 1). Finally, the hens were weighed.

Mortality The pen number and date were recorded for dead or culled hens, and a postmortem examination was performed to determine the cause of death or reason for the necessity of culling.

Egg Variables Eggs were collected and counted on Mondays, Wednesdays, and Fridays, and the weekly egg production per pen was calculated. Apart from the total number of eggs, the eggs were categorized into eggs found in the nest boxes, floor eggs, and eggs found in the range area. This categorization was regardless of the condition of the eggs (intact and non-shelled eggs and eggs with cracked shell or peck holes). Furthermore, the total number of intact eggs found in the nest boxes was determined.

In wk 22, 27, 32, and 37, a number of eggs were picked at random, and examined according to a range of egg quality traits. Twenty eggs were sampled from each pen in wk 22, and 15 eggs were sampled during the other weeks. The chosen quality variables were divided into external appearance of the eggs (deformed eggs, shell calcification, blood on surface of the shell), physical traits (egg weight (g), egg length and width (mm)), and eggshell traits (eggshell thickness at top, middle, and bottom (mm), egg shell weight (g)) following the protocol described in Nasr et al. (2012). An Egg Shape Index and the Shell Percentage were calculated as described in the Supplementary Materials and Methods.

Ethics Statement

The experiment was carried out according to the guidelines of the Danish Animal Experiments Inspectorate with respect to animal experimentation and care of animals under study.

Table 1. Welfare indicators used for welfare assessment when the hens were 17 and 38 wk of age.

	Score					
Condition	0	1	2	3	4	5
Plumage condition, body*[,1]	Intact feathers	Some feathers scruffy, up to 3 missing feathers	More damaged feathers, > 3 feathers missing	Bald patch, < 5 cm diameter or < 50% of area	Bald patch, > 5 cm diameter or > 50% of area	Completely denuded area
Plumage condition on: tail and flight feathers	Intact feathers	Few feathers separated, but none broken or missing	A lot of feathers separated, and/or few broken or missing	All feathers separated, a lot of broken or missing feathers	Most of the feathers missing or broken	Almost all feathers missing
Skin injuries, body*	No wounds, < 3 pecks	Wounds < 2cm, or > 3 pecks	Wounds ≥ 2 cm	-	-	-
Comb color	Normal: Red	Abnormal: Blue/pale	-	-	-	-
Keel bone, fracture**[,2]	No: No callus/pieces of fractured bone palpable	Yes: callus/pieces of fractured bone palpable	-	-	-	-
Keel bone deviation**[2]	No deviation: Straight or deviation ≤ 0.5 cm	Deviation: deviation > 0.5 cm	-	-	-	-
Feet, Footpad lesions[2]	None: No lesion	Mild: Small lesion ≤ 0.2 cm	Severe: Larger lesion > 0.2 cm	-	-	-
Feet, Bumble foot[2]	No: No dorsal swelling of footpad	Yes: Dorsal swelling of footpad	-	-	-	-
Feet, hyperkeratosis[2]	No: Smooth skin of foot and toepads	Yes: Excessive growth and thickening of the outer layer of epidermis of foot and toepads	-	-	-	-
Feet, missing toes/nails[2]	No	Yes	-	-	-	-

*Nine body parts were assessed: head, neck, back, rump, coverts, underneck, breast, legs, and belly.
**Measured separately for three parts of the keel bone: A: the cranial part; B: the medial part C: the caudal part.
[1]According to Bilčík and Keeling (1999).
[2]Modified from the HealthyHens protocol (Jung et al., 2019).

Statistical Analysis

Statistical methods are only described very briefly here but a thorough description is given in the Supplementary Materials and Methods. In general, nonlinear and (generalized) linear mixed effects models were applied with pen or observer and pen nested in observer as random effect(s) to account for non-independence. Some analyses were, however, done by generalized least squares and in a few cases simpler contingency table tests were used. All models included hybrid line (DW/BB) and significant interactions with this main factor. Hen age was generally included in models as a continuous variable after centering at the age of 18 wk (first week of observations) to give intercepts a relevant interpretation. Correlation among observations over weeks from the same pen was handled by a first order autoregressive (**AR1**) structure. For some analyses only 2 ages (17 and 38 wk) were considered and then age was included as a categorical variable. In the analyses of range use, significant weather variables and two-way interactions among these were also included as explanatory variables. Analyses of vegetation included a categorical variable for the three distances at which measurements were obtained. Variance heterogeneity in hybrid, age (categorical) and distance was examined and included in models if the assumption of homogeneity would be violated. Statistical analyses were carried out in R version 4.1.2 (R Core Team, 2021) on a significance level of 0.05 and with adjustment for multiple comparison of P-values and 95% confidence intervals (**CI**) when relevant.

RESULTS

Range and Shelter Use

Range Use The proportion of hens observed in the outdoor range changed over weeks and thus by age in a nonlinear fashion described by a fifth degree polynomial that differed between hybrids (simultaneous test for interaction between hybrid and powers of age up to order four: $\chi^2_4 = 49.8$, $P < 0.001$; Figure 1A).

A larger proportion of DW hens compared to BB hens went outside in the first third of the experimental period. Throughout the study the daily high temperature ranged from 9.4 to 31°C and the average daily precipitation was 1.5 mm. Wind speed ranged from 1.1 to 6.9 m/s. The weather conditions on observation days affected the proportion of hens that went outside (simultaneous test for all weather variables, $\chi^2_4 = 101.1$, $P <$ 0.001), but no interactions between weather variables

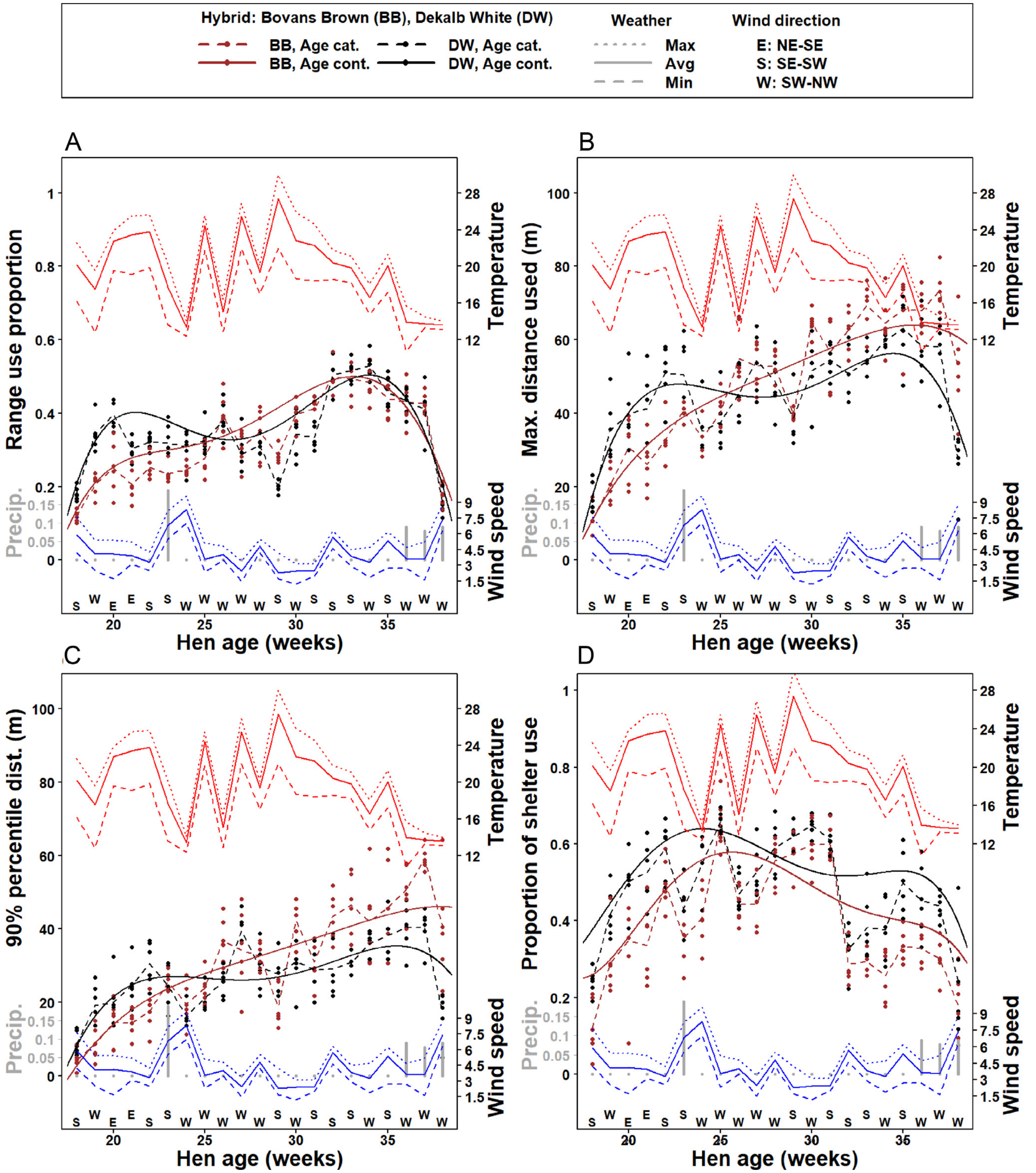

Figure 1. Range use of the two hybrids during the weekly observations reported as (A) proportion of hens observed anywhere in the range, (B) the hens' average maximum distance from the pophole while in the range, (C) the distance from the popholes where 90% or fewer of the hens were observed on each observation day, and (D) the proportion of hens using the shelters while on the range. Black lines and dots = DW, brown lines and dots = BB. Red lines = mean outdoor temperature (min, average, max; °C), blue lines = mean wind speed (min, average, max; m/s), gray lines (vertical) = average hourly precipitation (mm).

and hybrid were found. However, there was a complex interplay between the different weather variables, as could be expected. These interacting effects could not be separated, however, if there was no wind blowing, fewer birds would go outside as the temperature rose. If temperature was artificially set to zero, there was a negative relationship between wind and number of birds outdoors. As wind and temperature simultaneously rose, more birds were observed outside. Additionally, a larger proportion of birds accessed the range when winds were coming from the west. For additional details refer to Table S1.

The hens' average maximum distance from the pophole was described by fourth order polynomials depending on hybrid (simultaneous interaction test: $\chi^2_4 = 65.9$, $P < 0.001$; Figure 1B). In the first third of the period, some DW hens were observed further away from the entrance than BB hens, and in the last two thirds it was the other way around. Significant interactions between weather variables were found (simultaneous test for all weather variables and their interactions: $\chi^2_5 = 75.0$, $P < 0.001$; for more details see Table S2).

Finally, we analyzed the average 90th percentile distance, that is, the average distance from the popholes where 90% or fewer of the hens in outdoor areas were observed on each observation day (Figure 1C). Again, evolvement in age was described by a fourth order polynomial with hybrid dependent parameters (simultaneous test of interactions: $\chi^2_4 = 42.2$, $P < 0.001$). DW hens had the longest ranging distances in the first weeks, whereas in the last part of the experimental period, more BB hens went further away from the popholes. The average 90th percentile distance for DW hens changed from 8.8 m at 18 weeks of age to 17.2 m at wk 38, whereas for BB hens it changed from 4.4 m to 40.7 m, respectively. Weather conditions also affected ranging distance (simultaneous test for all weather variables: $\chi^2_5 = 79.6$, $P < 0.001$; see Table S3 for details) and a significant interaction between hybrid and temperature was present ($P < 0.001$).

Shelter Use Of the hens using the outdoor range, a larger proportion of DW hens used the shelters compared to BB hens (intercept difference: χ^2_1=5.69, $P = 0.017$; Figure 1D). As for range use, changes in shelter use was modelled by a fifth order polynomial with interaction with hybrid up to the fourth power of age. The simultaneous test of the 4 interactions was actually not significant ($\chi^2_4 = 8.06$, $P = 0.089$) but the interaction for the fourth order parameter was ($\chi^2_1 = 4.85$, $P = 0.028$). The weather conditions significantly affected shelter use (simultaneously test for all 6 parameters: $\chi^2_6 = 104.8$, $P < 0.001$; see further model details in Table S4).

Vegetation Type, Cover, and Height At the end of the experimental period, a larger proportion of bare ground was recorded in outdoor ranges of BB hens compared to DW hens, see Table S5. The difference varied among distances (hybrid-distance interaction: $\chi^2_2 = 8.16$, $P = 0.017$) and was only significant in the recordings 55 m from the popholes (means BB vs. DW: 48.0 vs. 3.7%; $\chi^2_1 = 12.4$, $P_{adj} = 0.003$; Table S5 and Table S6). Grass cover was more predominant the further away from the popholes, but in the BB hens' outdoor area the proportion of grass cover was smaller, see Table S5. There was a tendency of interaction between distance (as factor) and hybrid ($\chi^2_2 = 5.41$, $P = 0.067$; pairwise comparisons in Table S6) and in the additive model there were differences both between hybrids ($\chi^2_1 = 5.61$, $P = 0.018$) and among distances ($\chi^2_2 = 20.6$, $P < 0.001$). Clover and other vegetation were only observed 55 m and 85 m from the popholes, and no differences between hybrids or distances were found, see Table S7.

The vegetation height was affected differently for DW and BB, with a difference depending on the distance from the popholes (interaction $\chi^2_4 = 18.5$, $P = 0.001$). The vegetation was generally higher in outdoor ranges of DW hens and increased with distance from popholes (more details in Table S8).

Welfare Variables

Plumage Condition There was no interaction between hybrid and age. The plumage condition worsened during the experimental period at both ages for both DW and BB hens ($\chi^2_1 = 2929.6$, $P < 0.001$), with a rate ratio of an increased plumage score of 4.2 (95% CI [4.0, 4.5]) for wk 38 compared to wk 17, and DW hens had a higher plumage score (i.e., worse feather condition) compared to BB hens (rate ratio = 1.4; $\chi^2_1 = 50.8$, $P < 0.001$, 95% CI [1.3, 1.4]).

Differences in the total plumage score were mainly due to the subscores for tail, wings and breast. When the scores from these body parts were analyzed separately, using the dichotomized plumage score (0: Good, 1: Moderate/poor), we found an interaction for the tail score between age and hybrid ($\chi^2_1 = 4.24$, $P = 0.039$; Table 2). For the wing scores, no significant interaction between age and hybrid were found. The odds ratio for DW having a "moderate/poor plumage score" of the wings compared to BB was 2.7 ($\chi^2_1 = 28.1$, $P < 0.001$, 95% CI [2.0,3.8]). The scores were worse at 38 than 17 wk of age with an odds ratio of 6.2 ($\chi^2_1 = 235.1$, $P < 0.001$, 95% CI [4.8,8.0]). Practically no plumage damage was seen on the breast in wk 17, but in wk 38, the odds ratio for having a "moderate/poor plumage score" on the breast was 4.8 for DW compared to BB hens ($\chi^2_1 = 20.4$; $P < 0.001$, 95% CI [2.6, 9.6]). For more details regarding plumage condition see Table S9.

Keel Bone Damage Prevalence of keel bone fractures in section A were affected by an interaction between age and hybrid ($\chi^2_1 = 16.1$, $P < 0.001$; Table 3). At 17 wk no difference was found between hybrids, but fractures in

Table 2. Dichotomized plumage scores for tails at 17 and 38 wk of age in Dekalb White (DW) and Bovans Brown (BB) hens.

Hybrid/Age	Comparison	Prevalence of moderate/ poor scores (2−5)[1]	Odds ratio[2]	SE	95% CI[3]	z	P[3]
BB	38 vs. 17	0.26 vs. 0.11	3.1	0.51	2.0−4.6	6.75	<0.001
DW	38 vs. 17	0.53 vs. 0.20	4.8	0.66	3.4−6.7	11.4	<0.001
17 wk	DW vs. BB	0.20 vs. 0.11	2.3	0.53	1.3−4.0	3.61	0.001
38 wk	DW vs. BB	0.53 vs. 0.26	3.6	0.72	2.2−5.9	6.34	<0.001

[1]Raw data.
[2]The statistical values (SE, CI, z, and P) are for the odds ratios but test statistics from log-odds difference.
[3]CI and P-values are adjusted for multiple comparisons.

Table 3. Odds ratios for keel bone fractures in section A and deviations in section B at 17 and 38 wk of age in Dekalb White and Bovans Brown hens.

Fractures in section A							
Hybrid/Age	Comparison	Prevalence[1]	Odds ratio[2]	SE	CI[3]	z	P^3
BB	38 vs. 17	0.06 vs. 0.10	0.62	0.13	0.36−1.1	−2.20	0.093
DW	38 vs. 17	0.20 vs. 0.12	1.8	0.30	1.2−2.7	3.67	<0.001
17	DW vs. BB	0.12 vs. 0.10	1.2	0.23	0.77−2.0	1.08	0.647
38	DW vs. BB	0.20 vs. 0.06	3.6	0.73	2.2−5.9	6.36	<0.001
Deviations in section B							
Hybrid/Age	Comparison	Prevalence[1]	Odds ratio[2]	SE	CI[3]	z	P^3
BB	38 vs. 17	0.13 vs. 0.04	4.1	0.99	2.2−7.4	5.75	<0.001
DW	38 vs. 17	0.16 vs. 0.01	15.1	5.61	6.1−37.4	7.29	<0.001
17	BB vs. DW	0.01 vs. 0.04	3.1	1.29	1.1−8.6	2.66	0.028
38	BB vs. DW	0.16 vs. 0.13	0.82	0.15	0.52−1.3	−1.04	0.675

[1]Raw data.
[2]The statistical values (SE, CI, z, and P) are for the odds ratios but test statistics from log-odds difference.
[3]95% CI and P-values are adjusted for multiple comparisons.

section A were more likely for DW hens at 38 compared to 17 wk of age, and at 38 wk, the odds were 3.6 times higher for DW compared to BB hens. The risk of fractures in section B was dependent on age (38 vs. 17 odds ratio = 4.4, $\chi^2_1 = 106.2$, $P < 0.001$, 95% CI [3.2, 5.9]), but neither hybrid nor the interaction between hybrid and age. Keel bone fractures in section C were only observed in one BB hen at 17 wk of age, and therefore, fractures were not analyzed at this age. At 38 wk, the proportion of BB and DW hens with a fracture in section C was 0.20 and 0.10, respectively (BB vs. DW odds ratio = 2.1, $\chi^2_1 = 14.7$, $P < 0.001$, 95% CI [1.5, 3.1])).

At 17 wk of age, less than 2% of the hens had deviations in keel bone sections A and C and precluded valid modeling with interaction between age and hybrid. The odds were higher at 38 wk of age (section A: 38 vs. 17 odds ratio = 7.1, $\chi^2_1 = 64.9$, $P < 0.001$, 95% CI [4.1, 12.4]; section C: odds ratio = 12.2, $\chi^2_1 = 60.2$, $P < 0.001$, 95% CI [5.3, 28.2]). There were no significant differences between hybrids for deviations in section A and C. For deviations in keel bone section B there was an interaction between age and hybrid ($\chi^2_1 = 9.57$, $P = 0.002$; Table 3). In both hybrids the odds of having a deviation were higher at 38 wk of age compared to 17 wk (BB: odds ratio = 4.1, $P_{adj} < 0.001$, 95% CI [2.2, 7.4]; DW: odds ratio = 15.1, $P < 0.001$, 95% CI [6.1, 37.4]), and more BB hens had a deviation in week 17 compared to DW (odds ratio = 3.1, $P_{adj} = 0.028$, 95% CI [1.1, 8.6]).

Skin Injuries During the examination of the 11 body parts in wk 17 and 38, we only found 2 skin injuries in total; one in wk 17 on the neck of a DW hen and one in wk 38 on the coverts of a BB hen.

Feet Footpad lesions were near absent in wk 17 in both hybrids, with no severe lesions observed and only 1 (0.2%) mild lesion in BB and 5 (0.8%) hens with mild lesions in DW. In wk 38, the prevalence of severe and mild lesions were 15 and 16%, respectively in BB, and 19 and 40% in DW. Analyzed separately in wk 38, the odds of being in a worse footpad lesions category was higher for DW than BB (odds ratio = 3.5; $\chi^2_1 = 30.9$, $P < 0.001$, 95% CI [2.5, 4.9]). Prevalence of hyperkeratosis was associated with an interaction between age and hybrid ($\chi^2_1 = 15.3$, $P < 0.001$). The odds increased with age in both hybrids, but more for BB hens, and at 38 wk, BB hens were more likely to have hyperkeratosis (Table 4). Bumble foot was not observed at 17 wk of age, and at the end of the experimental period 2% of DW and 3% of BB hens were observed with bumble foot, but this difference was not statistically significant. Very few hens were observed to have missing toes or nails; 2 BB and 3 DW hens at 17 wk, and one BB and 3 DW hens at 38 wk.

Comb Color and Wounds Comb color differed between the ages; at 17 w, 22% of the BB and 18% of the DW hens had an abnormal comb color, whereas at 38 w this was seen in only 2% and 1% of BB and DW hens, respectively (wk 17 vs. 38: odds ratio = 38.4; $\chi^2_1 = 324.6$, $P < 0.001$, 95% CI [22.4, 65.7]). Hybrid did not significantly affect comb color and no interaction between age and hybrid was detected.

In contrast, the prevalence of comb wounds was higher in wk 38 than in wk 17 (wk 38 vs. 17: odds ratio = 15.5; $\chi^2_1 = 255.3$, $P < 0.001$, 95% CI [10.3, 23.4]) with 7% BB and 32% DW having comb wounds in wk 38, whereas the corresponding numbers were 1% and 5%

Table 4. Odds ratios for hyperkeratosis at 17 and 38 wk of age in Dekalb White and Bovans Brown.

Hybrid/Age		Prevalence[1]	OddsRatio[2]	SE	CI[3]	z	P^3
BB	38 vs. 17	0.21 vs. 0.04	6.7	1.56	3.8−11.9	8.18	<0.001
DW	38 vs. 17	0.10 vs. 0.06	1.9	0.42	1.1−3.3	2.91	0.013
17 wk	BB vs. DW	0.04 vs. 0.06	0.71	0.22	0.33−1.5	−1.11	0.635
38 wk	BB vs. DW	0.21 vs. 0.10	2.5	0.58	1.4−4.4	3.90	<0.001

[1]Raw data.
[2]The statistical values (SE, CI, z, and P) are for the odds ratios but test statistics from log-odds difference.
[3]CI and P-values are adjusted for multiple comparisons.

in wk 17, and DW hens were more likely to have wounds on the comb (odds ratio = 8.4; $\chi^2_1 = 49.7$, $P < 0.001$, 95% CI [5.3, 13.2]), but we detected no interaction between age and hybrid.

Body Weight As expected, the interaction between strain and age affected the weight of the hens ($\chi^2_1 = 75.2$, $P < 0.001$). BB weighed more than DW hens at 17 wk of age (1,355 g vs. 1,206 g; z = 23.3, $P < 0.001$, 95% CIs [1,343, 1,367] and [1,195, 1,216], respectively) and 38 wk of age (1,969 g vs. 1,733 g; z = 24.7, $P < 0.001$, 95% CIs [1,951, 1,987] and [1,718, 1,748], respectively). Furthermore, BB had a higher weight gain over time as well (38 vs. 17 wk: diff = 614.1, SE = 7.77, 95% CI [595.0, 633.1]) compared to the DW hens (38 vs. 17 weeks: diff = 527.0, SE = 6.21, 95% CI [511.8, 542.3]).

Mortality Out of the 1,200 hens that entered the study, 9 were found dead and 4 were culled during the experimental period. DW had a higher mortality compared to BB hens (11 vs. 2; $\chi^2_1 = 6.23$, $P = 0.013$). The most common cause of death was egg yolk peritonitis (n = 7), and the other causes of death were cloacal cannibalism (n = 1), beak deformation (n = 1), crop impaction (n = 1), strangulation (n = 1), acute internal bleeding (n = 1), and unknown (n = 1). The 13 hens died throughout the experimental period (3 in May, 5 in June, 1 in July, 1 in August, and 3 in September). The dead/culled hens did not differ regarding body condition (9 normal (0.69) vs. 4 skinny (0.31); $\chi^2_1 = 1.92$, $P = 0.166$) and we found no association between cause of death (found dead or culled) and body condition (Fisher's exact test, $P = 0.530$).

Production and Egg Quality Variables

Egg Production The onset and progress of the egg production in the experimental period are shown in Figure 2A. The onset of laying was earlier for DW than BB hens, illustrated both by the larger laying percentage in wk 17 (1.4 ± 0.14% vs. 0.07 ± 0.030%; z = 9.71, $P_{adj} < 0.001$, 95% CIs [1.0, 1.7] and [0.0, 0.1], respectively) and the time difference between the inflection points, where the curves for egg production start leveling out (z = −11.5, $P_{adj} < 0.001$), which was at 19.9 wk and 20.5 wk for DW and BB hens (95% CIs [19.7, 20.0] and [20.43, 20.6], respectively). The estimated maximum egg production for BB hens was 94.8 ± 0.48% (95% CI [93.5, 96.1] compared to 93.6 ± 0.53% (95% CI [92.2,95.0]) for DW hens, but the difference was not significant (P_{adj} = 0.092 Figure 2A). The area under the curve, which expresses the total number of eggs laid in the experimental period, was higher for DW hens (1,604 ± 4.0 vs. 1,561 ± 4.6; z = 7.08, $P_{adj} < 0.001$, 95% CIs [1,594, 1,615] and [1,548, 1,573], respectively).

The percentage of eggs found on the floor increased with age for both hybrids (slope for BB: 0.3 ± 0.07%; DW: 1.6 ± 0.09%, 95% CIs [0.17, 0.44] and [1.37, 1.74], respectively; Figure 2B), and the slope was steeper for DW compared to BB hens ($\chi^2_1 = 52.8$, $P < 0.001$). A total of 152 eggs were found in the outdoor range (DW: 39, BB: 113), and no difference between hybrids was detected ($\chi^2_1 = 2.84$, $P = 0.092$).

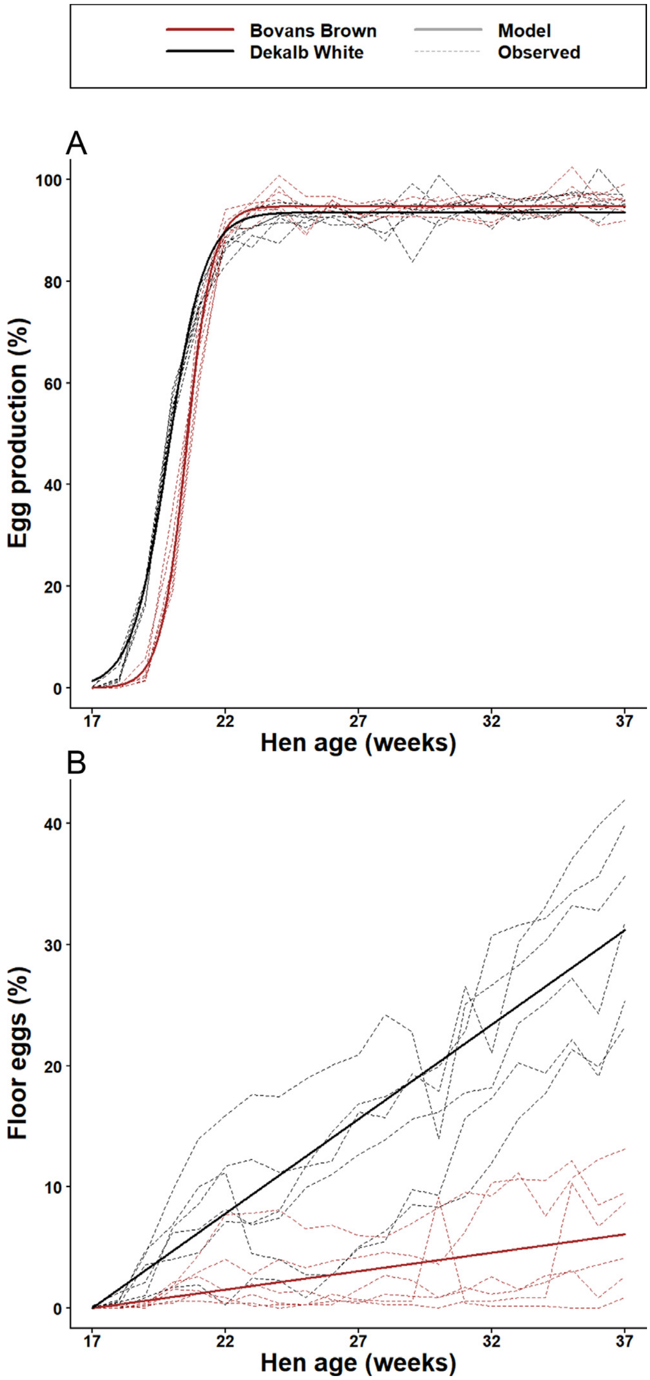

Figure 2. The onset and progress of (A) the total egg production (%) and (B) the proportion of floor eggs (%) in the experimental period of the two hybrids. Black lines = DW, brown lines = BB.

Egg Quality The analysis of egg width (N = 727) revealed an interaction between hybrid and age ($\chi^2_3 = 34.0$, $P < 0.001$; Figure 3A). Width increased from wk 22 to 27 in BB hens and from wk 22 to 32 in DW hens, where after it plateaued, and BB hens had significantly wider eggs (22 and 27 wk: $P_{adj} < 0.001$, 32 wk: $P_{adj} = 0.009$) at all ages, although this was only a tendency at wk 37 ($P_{adj} = 0.062$). The length of the eggs (N = 726) was only dependent on age ($\chi^2_3 = 270.2$, $P < 0.001$; Figure 3B). For egg shape index (N = 766),

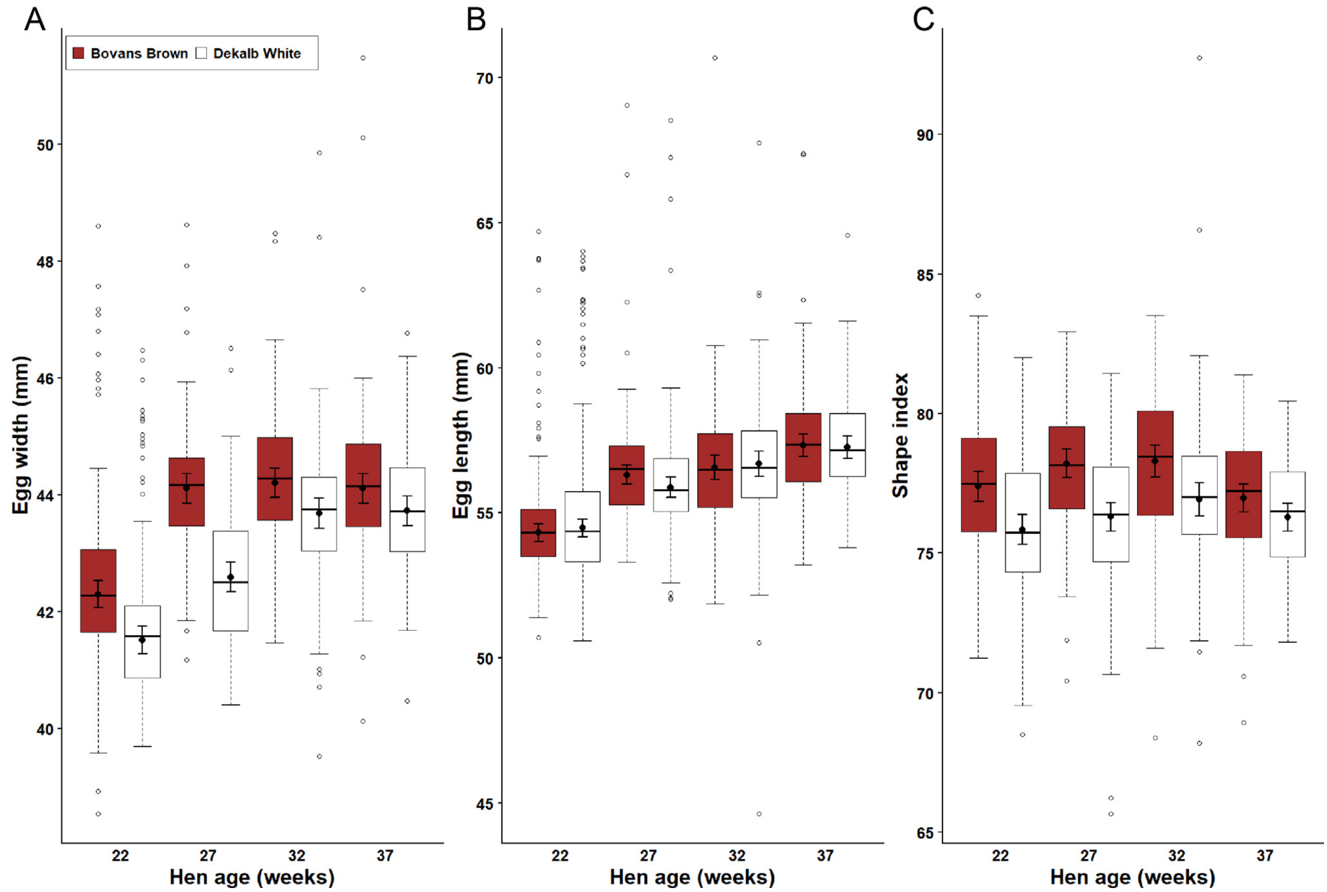

Figure 3. Egg quality variables measured at ages 22, 27, 32, and 37 wk for the 2 hybrids, that is, (A) egg width (mm), (B) egg length (mm), and (C) egg shape index. Boxplots were constructed using N = 727, N = 726, and N = 766, respectively, with outliers (780 − N) overlaid. White = DW, brown = BB.

interaction between hybrid and age was at the border of significance ($\chi^2_3 = 7.64$, $P = 0.054$; Figure 3C). BB hens had a higher shape index at all ages (22 and 27 wk: $P_{adj} < 0.001$, 32 wk: $P_{adj} = 0.001$) except for in week 37 ($P_{adj} = 0.106$), and the development over weeks differed between hybrids.

An interaction between hybrid and age was detected both for egg weight (N = 725, $\chi^2_3 = 25.3$, $P < 0.001$) and shell weight (N = 733, $\chi^2_3 = 16.2$, $P = 0.001$; Figures 4A and 4B), and increased with age, with BB eggs being heavier (22 and 27 wk: $P_{adj} < 0.001$, 32 and 37 wk: $P_{adj} = 0.001$) and having heavier shells at all ages (22, 27, and 37 wk: $P_{adj} < 0.001$), although for shells this was only a tendency in wk 32 ($P_{adj} = 0.090$). The egg shell percentage, that is, the percentage of shell weight in proportion to the whole egg, was only dependent on age (N = 756, $\chi^2_3 = 9.39$, $P = 0.025$; Figure 4C). For egg shell thickness (N = 760) an interaction was detected between hybrid and age ($\chi^2_3 = 37.6$, $P < 0.001$; Figure 4D), and shell thickness decreased over time with BB eggs having thicker shells in wk 27 ($P_{adj} < 0.001$) and wk 37 ($P_{adj} = 0.019$) than DW hens.

Throughout the experimental period, we only recorded 2 deformed eggs (one DW in wk 27 and one DW in wk 32, both with a very small shape index), 3 eggs with shell calcification (2 BB and one DW in wk 22) and 4 eggs with blood stains on the shell (3 BB and one DW in wk 32). None of these observations were from the same egg.

DISCUSSION

In the present study, we investigated how suitable 2 popular laying hen hybrids, the Dekalb White and the Bovans Brown, are for organic egg production, with special emphasis on their ranging behavior. Several differences were found between the 2 hybrids, including differences in range use, shelter use, prevalence of different welfare issues, and production and egg quality variables. The differences are discussed below.

Range and Shelter Use

Differences between strains in regard to range access have been reported previously. For instance, Von Borell and Mahboub (2002) found that Lohmann Selected Leghorn hens accessed the range more than Lohmann Tradition hens. Kjaer and Isaksen (1998) found substantial variation in range use between ISA Brown, New Hampshire, White Leghorn, and cross of New Hampshire with White Leghorn hens. Strain differences in sociality and stress response traits have likely arisen due to selection for production traits and may impact hens' utilization of the range (Ferreira et al., 2020; Peixoto et al., 2020).

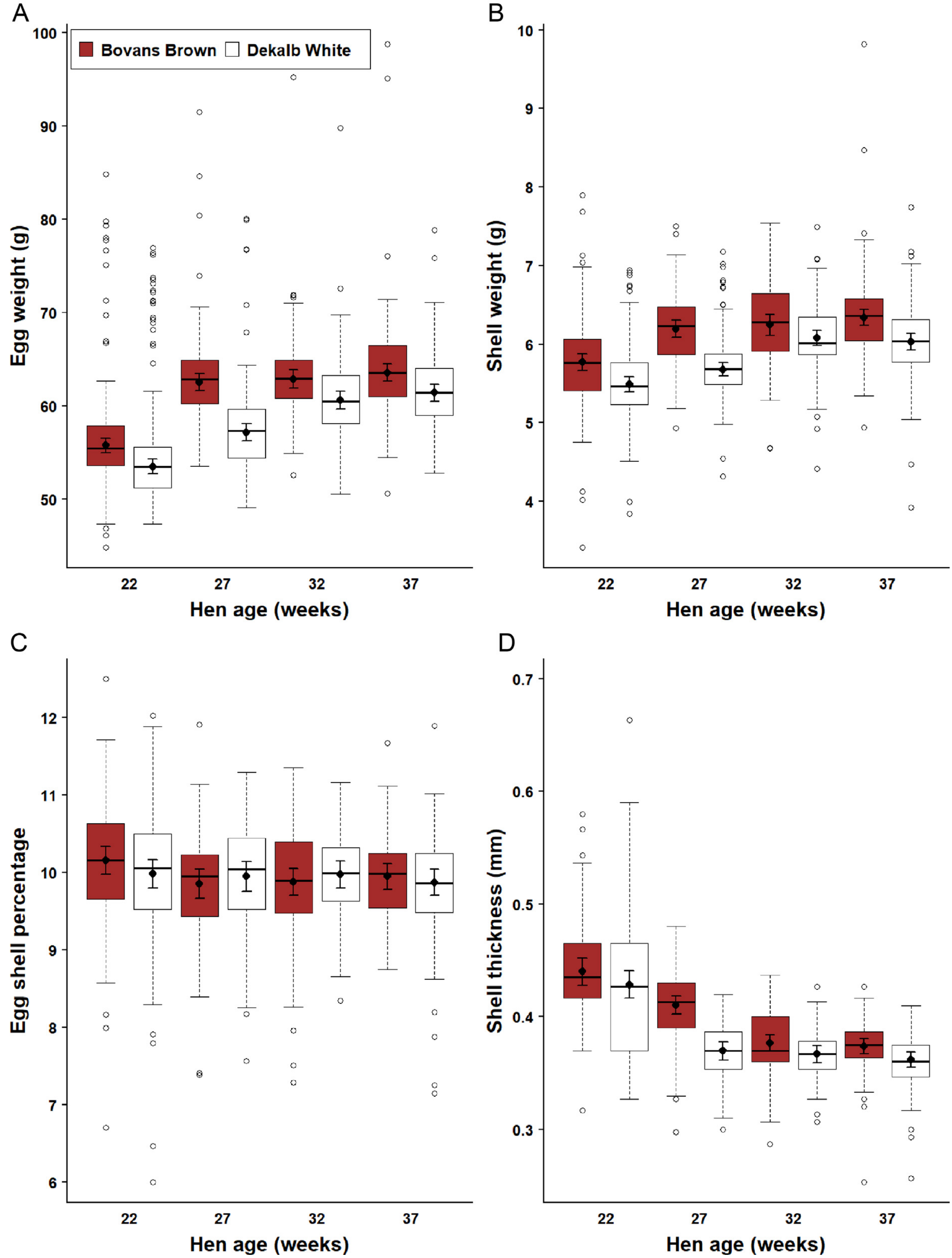

Figure 4. Egg quality variables measured at ages 22, 27, 32, and 37 wk for the two hybrids, that is, (A) egg weight (g), (B) shell weight (g), and (C) egg-shell percentage, that is, the percentage of shell weight in proportion to the whole egg, and d) shell thickness (mm). Boxplots were constructed using N = 725, N = 733, N = 756, and N = 760, respectively, with outliers (780 − N) overlaid. White = DW, brown = BB.

Strains more intensely selected for production traits have been shown to be less adaptive to heat stress (Mack et al., 2013), have higher stress responses to transport (Cheng and Jefferson, 2008), display more agonistic behaviors in group pens (Craig and Muir, 1996a; Craig and Muir, 1996b), and have altered adrenal and immune functioning (Fahey and Cheng, 2008). In our study, the greater number of DW hens accessing the range early on could be attributed to differences in sociality between the strains (Armstrong et al., 2020;

Ferreira et al., 2020). Range areas provide hens with increased space and the opportunity to distance themselves from conspecifics (Savory et al., 2006). More socially motivated hens may have been less likely to access the range where the interbird distance is increased (Chielo et al., 2016; Taylor et al., 2020).

Growth and bodyweight have been shown to be genetically associated with tonic immobility durations, suggesting that selection for production may have inadvertently lead to selection for more fearful birds (Schütz et al., 2004). Game fowl, which had been selected for aggressiveness, were used in early crosses of what ultimately became our modern-day brown laying strains. This could additionally account for the more bold personalities often observed in brown strains (Lyimo et al., 2014; Dudde et al., 2018). Hy-Line brown strains and Hy-Line white strains displayed different coping strategies when presented with acute and chronic stressors (Pusch et al., 2018). In behavioral tests, Dekalb White hens have been shown to be more fearful of humans and to have lower serotonin levels than ISA Brown hens (De Haas et al., 2013). Handling induced higher stress responses in White Leghorn hens compared to Brown Hy-Line strains (Fraisse and Cockrem, 2006). In our study, fearfulness could have impacted the distance birds were willing to travel from the popholes and the extent to which the range was utilized (Campbell et al., 2016, 2019; Hartcher et al., 2016; Larsen et al., 2018). Increased fear experienced by the DW hens could account for the shorter distance traveled in the range, the lower pasture utilization observed, and the increased use of the shelters. White egg laying and brown egg laying hens are phylogenetically distant and have been subjected to distinct selection pressures (Dudde et al., 2018). In general, white egg laying strains have been heavily selected for egg production traits whereas brown laying strains were traditionally selected as dual-purpose birds. An additional possible explanation for the lower distances traveled and less vegetative impact caused by the DW hens is the adoption of less energy demanding behavioral strategies as a trade-off for higher production (Dudde et al., 2018). Studies have demonstrated that high producing modern strains of laying hens may modify foraging behavior and display lower motivation to explore for less energy dense food sources than the ancestral red junglefowl (Schütz et al., 2001; Schütz and Jensen, 2001; Lindqvist and Jensen, 2009).

The study period took place over the summer when temperatures were high and rain was scarce, which could account for more birds going outside when temperature was lower or when wind speed and temperature increased concurrently. At the end of the study when the average temperature dropped substantially and when there was the most rain, both strains accessed the pasture less and remained closer to the popholes when the temperature decreased, and wind speeds increased. This is consistent with previous work showing that strong winds reduce the number of hens observed on the range (de Koning et al., 2018) and that fewer hens access the outdoor range as temperatures decrease (Hegelund et al., 2005; Richards et al., 2011; Chielo et al., 2016).

Welfare Variables

Over the course of the experiment, plumage condition worsened for both strains, however, DW hens had worse plumage scores at both the start and the end of the study compared to BB hens. The decreased vegetation height and increased areas of bare ground in the BB ranges could suggest that BB hens were utilizing the range for foraging, dustbathing, and as a supplemental feed source to a greater extent than the DW hens. This increased exploitation of behavioral opportunities may have led to reduced frustration and stress, possibly leading to the reduction or prevention of damaging behaviors such as feather pecking (Bestman and Wagenaar, 2003; Lambton et al., 2010; Sherwin et al., 2010). Genetic differences between strains in regard to propensity of developing feather pecking could further explain the observed differences (Jensen et al., 2005).

The most recent studies on keel bone fractures suggest that especially the fractures located at the caudal tip occur as a result of internal forces (Thøfner et al., 2020). Hens with smaller bodies, an earlier onset of lay, larger egg size at onset of lay are thought to be at an increased risk of developing fractures in the caudal tip of the keel bone (Thøfner et al., 2021). In our study, DW hens had an earlier onset of lay and smaller body size and experienced a greater number of fractures of the cranial section of the keel bone over the course of the study. BB hens, on the other hand, experienced a greater prevalence of keel fractures to the caudal tip of the keel bone which could be explained by the fact that BB eggs were wider and heavier. Hen body size was confounded with hybrid effect in our model, so effects of egg size relative to hen body size and the corresponding impact on keel bone damage could not be elucidated.

In the present study, DW hens were more likely to have footpad lesions at the end of the study period, while BB had a higher prevalence of hyperkeratosis. Weitzenbürger et al. (2006) found similar differences between strains, with Lohmann Brown hens having increased prevalence of hyperkeratosis while Lohmann Selected Leghorn were more likely to present with lesions. Heerkens et al. (2016) similarly observed more lesions in Dekalb White birds and more hyperkeratosis in ISA Brown hens. Additionally, Mahboub et al. (2004) found higher prevalence of footpad inflammation in white hens (Lohmann Selected Leghom) than brown (Lohmann Traditional). In our study, BB hens tended to range further from the house where substrate quality may have been better (dryer and less manure buildup), thus at least partly explaining the reduced prevalence of more severe footpad conditions.

The greater mortality by egg yolk peritonitis observed in the DW hens may be in part explained by the differences in range use, specifically distance traveled from the popholes, observed in our study. Egg yolk peritonitis occurs when the yolk from a developing egg is deposited within the body cavity. It is believed that complications resulting from secondary infections, such as by *E. coli*, are responsible for cases that result in mortality (Srinivasan et al., 2013). Because DW hens tended to remain closer to the house than BB hens, they may have had increased exposure to *E. coli* through manure build-up on the part of the range closest to the house. Alternatively, the observed differences could simply be due to differences in production rate or genetic predispositions between the strains.

Production and Egg Quality Variables

The observed differences in egg production between the 2 strains are likely due to differences in their genetic capacity. Our results were in line with those expected in the breeder guidelines. Similar to our results, Riczu et al. (2004) found that a brown egg laying strain (Shaver 579) produced heavier eggs with thicker eggshells, while a white strain (Shaver 2000) produced more marketable eggs overall. When comparing production of four strains of hens, Singh et al. (2009) found that H&N White hens and Lohmann White produced more eggs overall than Lohmann Brown hens and a noncommercial cross (Rhode Island Red × Barred Plymouth Rock). Furthermore, they found that Lohmann White and Lohmann Brown had similar shell weights, which were heavier than those from H&N White hens and the noncommercial cross. An additional study by Scott and Silversides (2000) reported heavier eggs and greater shell weight from ISA Brown hens than ISA White hens.

When floor eggs started appearing, they were collected daily. However, nest eggs were only collected every 3 d throughout the course of this study due to budget constraints which could account for the substantial proportion of eggs laid outside the nests. The steeper increase in floor eggs laid by the DW hens over time could be due to genetic differences in sociality or fearfulness, though these relationships warrant further exploration (Tahamtani et al., 2018). Nest use has been shown to have a genetic component, with heritability estimates ranging from 0.39 to 0.44 (Settar et al., 2006). In studies comparing nest use of various hybrids, brown strains tended to lay more outside the nests than white strains (Singh et al., 2009; Villanueva et al., 2017) though in one study, the brown strain (Hy-Line Brown) hens eventually ceased laying floor eggs as the study progressed, suggesting they were able to better adapt to using nests over time (Jones and Anderson, 2013). In our study, the lower slope of floor eggs laid by brown hens could suggest a similar phenomenon.

The anatomical structure of the hens affects the egg shape with eggs from brown hens generally having greater egg widths, which was observed in our study and length, though we only found length to be related to age (Onbaşılar et al., 2018; Sokołowicz et al., 2018). Studies have shown that shape index decreases with age as a result of changes in the anatomical structure of the pelvic bone, which was also observed in our study (Petričević et al., 2017).

CONCLUSIONS

In our study, BB hens performed superiorly or equivalently to the DW hens in all measured variables, apart from egg production. While a greater proportion of DW hens accessed the range early in the study, BB hens ventured further from the house and wore down the vegetation to a greater extent than the DW hens. When comparing health and welfare measures, BB hens were found to have improved plumage condition, improved footpad health, fewer comb wounds, and lower mortality rates. The two strains appeared to differ in where on the keel bone fractures occurred. Onset of lay was earlier and egg production was higher for the DW hens, however, the BB hens had improved egg quality measures and demonstrated fewer floor eggs.

When selecting a genetic strain for organic production the behavioral attributes must be taken into consideration to ensure good welfare. Hens with increased fearfulness may be less likely to leave the house when range area is provided, thus missing out on the potential benefits that a range can offer, such as a more varied diet, improved bone strength, and improved health and affective states resulting from the ability to perform natural behaviors. If DW strains are selected for use in organic systems, the environment and management practices must account for their behavioral specificities. For instance, provision of shelters seemed to be more utilized by the DW hens and may aid in encouraging these birds to move outdoors.

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DISCLOSURES

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

SUPPLEMENTARY MATERIALS

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.psj.2022.102005.

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