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The effect of feeder space allocation on productivity and physiology of Hy-Line W-36 hens housed in conventional cages

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ABSTRACT Insufficient feeder space for laying hens could increase competition at the feed trough, leading to disrupted feeding, inadequate nutrient intake, stress, and reduced productivity. The effects of feeder space allocation (FSA) on physiology and productivity were evaluated in beak-trimmed Hy-Line W-36 hens ($n = 480$). They were obtained at 16.5 wk of age and housed on 4 tiers of shallow conventional cages. Five pullets/cage were housed at a stocking density of 434 cm²/hen and a feeder space of 12.2 cm/hen. After 1.5 wk of acclimation, baseline measurements were taken for feed utilization, bone mineralization, and heterophil:lymphocyte ratios. At 20 wk of age, pullets were given 5.8, 7.1, 8.4, 9.7, 10.9, or 12.2 cm of feeder space/bird (16 cages/treatment). Physiological and production measures were calculated monthly or twice a month for 12 mo. The heart, spleen, and right adrenal gland were collected from each hen at the end of the study. Data were analyzed using a repeated measures GLM incorporat-

ing cage, tier, FSA, and hen age. There were no effects of FSA on total egg production, bone mineral density, bone mineral content, heterophil:lymphocyte ratios, or organ weights. Hens with reduced FSA utilized more feed ($P < 0.001$), had poorer feed conversion ($P < 0.001$), and laid eggs with slightly thicker and heavier shells ($P < 0.001$). There were effects of FSA on total egg weight ($P < 0.001$) and hen-day egg production ($P < 0.001$), but they were of low magnitude and not linear ($P > 0.05$). Because BW was similar among FSA treatments, the results suggest that reduced feeder space did not limit feed intake. In addition, reduced FSA did not lower bone mineralization or cause physiological stress in W-36 hens housed in shallow cages, suggesting that it did not impair hen welfare. However, it did result in poorer feed efficiency, possibly related to greater feed wastage, predictive of an adverse economic effect from reducing feeder space.

Key words: laying hen, feeder space, production, physiology, welfare

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INTRODUCTION

Housing space and access to resources such as feed are critical to the well-being of laying hens (Appleby et al., 2004). However, data are currently lacking to determine the amount of feeder space needed to ensure that caged hens have adequate access to feed. If individual feeder space allocation (FSA) is too low, then competition for access to the feeder may induce aggression, thereby disrupting feeding and ultimately leading to poor welfare, reduced productivity, and even mortality. Without adequate feeder space, low-ranking hens

in particular may be prevented from feeding with their cage mates and thus suffer adverse effects (Hughes, 1983).

Existing literature comparing shallow to deep cages suggests that feeder space influences the productivity of laying hens. More than 70% of the studies summarized by Hughes (1983) found that hens in shallow cages (i.e., with more feeder space) had increased egg numbers, feed intake, and BW compared with those in deep cages. Greater FSA led to a decrease in mortality in 30% of the studies and an increase in egg size in 56% of the studies. Adams and Craig (1985) performed a meta-analysis of previous studies and found a similar increase in egg production for hens in shallow as compared with deep cages.

Poor reproductive performance in deep cages with limited feeder space could be due to chronic stress. Chronic stress not only impairs reproduction (Siegel,

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1995) but also affects the immune system (Moberg, 2000), causing the number of lymphocytes to decrease and the number of heterophils to increase (Gross and Siegel, 1983). Gross organ changes that lead to immunosuppression, including adrenal hypertrophy and reduced spleen weight, are also indicators of chronic stress (Siegel, 1995; Puvadolpirod and Thaxton, 2000). Moreover, if an animal is stressed and has an increased heart rate for an extended period of time, the heart muscle has to work harder and the heart becomes enlarged. For instance, Cunningham et al. (1988) found that heart weight increased for low-ranking hens and for hens housed at a greater density.

Limited feed intake associated with difficulty in gaining access to the feeder could also result in a Ca shortage, ultimately leading to osteoporosis—a disease that is widespread in commercial laying hens and that contributes to approximately 35% of mortalities during the egg production cycle of caged hens (McCoy et al., 1996). One way to determine if hens are at risk for osteoporosis is to measure skeletal integrity noninvasively using dual-energy x-ray absorptiometry (DEXA). The bone mineral density (BMD) and bone mineral content (BMC) determined from DEXA scans of live White Leghorn hens are positively correlated with bone-breaking force and bone ash weight (Schreiweis et al., 2003). In addition, as tibial BMD and BMC decrease, the incidence of bone breakage increases (Mazzucco and Hester, 2005).

The effect of FSA on these indicators of physiological stress has not been addressed in previous studies. Therefore, the goal of the current study was to determine if hens given limited FSA experienced stress and lower productivity. It was predicted that hens with reduced feeder space would have increased heterophil:lymphocyte ratios and adrenal and heart weights, as well as decreased spleen weight and bone mineralization. Additionally, it was predicted that hens with limited FSA would show poorer productivity, feed efficiency, and egg quality.

MATERIALS AND METHODS

At 16.5 wk of age, 480 beak-trimmed Hy-Line W-36 pullets were obtained from a commercial integrator and housed in 96 cages (61.0 cm wide × 35.6 cm deep) on 4 tiers in 1 room of the Layer Research Unit at the Purdue University Poultry Research Farm. There were 5 pullets per cage, providing 434.3 cm² of floor space and 12.2 cm of feeder space (baseline value) per pullet. Before housing, each pullet was banded with Swiftack tags (Heartland Animal Health Inc., Fair Play, MO). Ambient temperature was maintained between 21 and 24°C during the winter, fall, and early spring through use of thermostatically controlled heaters. During hot weather (late spring and summer), attempts were made to maintain room temperature within the same range through the use of evaporative cooling. All procedures

were approved by the Institutional Animal Care and Use Committee of Purdue University.

Pullets were acclimated for 1.5 wk before collection of baseline data, which began at 18 wk of age and continued for 2 wk. At 20 wk of age, feeder space in each cage was altered from 12.2 cm of feeder space/hen to 5.8, 7.1, 8.4, 9.7, 10.9, or 12.2 cm of feeder space/hen by blocking access to a portion of the feeder using corrugated plastic (see Figure 1 in Thogerson et al., 2009). It was anticipated that all 5 hens would be able to eat at the same time with 10.9 and 12.2 cm of feeder space, with fewer numbers at lower FSA. Only every other cage along the row was populated to prevent hens from feeding at adjacent cage feeders. A balanced, factorial randomized block design was used. Treatments were balanced by tier, distance from the door, and mean SD of BW collected at 16.5 wk of age. Hens had access to feed and water to allow ad libitum consumption throughout the study. Three drip nipples were provided per cage. Hens were hand-fed twice daily with the feed trough filled to 1/3 capacity to minimize feed wastage. Feed troughs were mounted outside of the cage at the front with only 1 side of the trough available for feeding. The inside dimensions of the feed trough were 7.1 cm wide by 10.2 cm deep. A prelay diet consisting of 15.5% CP, 2.25% Ca, and 0.35% available P was fed to the hens for the first 4.5 wk of the study (16.5 to 21 wk of age). Beginning at 21 wk of age and until termination of the study at 68 wk of age, hens were fed a laying hen diet formulated to contain 17% CP, 3.8% Ca, and 0.3% available P. Hens were exposed to an incremental light cycle beginning with 13L:11D when they were first placed in laying cages at 16.5 wk of age. Day length was increased by 30 min/wk starting at 17 wk of age to 16L:8D by 22 wk of age. Mean light intensity, measured over the feed trough, was 20 lx. Data collection during the treatment period was initiated when hens were 22 wk of age and continued every 4 or 8 wk until the conclusion of the study when the hens were 68 wk of age. Data collection took 2 wk to complete at each time period.

Physiology, Health, and Stress Measures

At the start of the experiment, 96 hens, distributed across every other cage, were selected as focal hens for bone and blood measurements. Given that hens with a higher BW tend to have a higher dominance rank in their group (Cloutier and Newberry, 2000), the heaviest and the lightest hens in the cage were selected as focal hens to ensure that both higher and lower ranking hens were represented. During initial baseline data collection and every 8 wk thereafter (totaling 7 time periods), the left humerus and tibia of the focal hens were scanned for BMD and BMC using a DEXA scanner (Norland Medical Systems, Fort Atkinson, WI) following the procedure of Schreiweis et al. (2003). The humerus, which comprises cancellous, cortical, and, in some cases, med-

ullary bone (Fleming et al., 1998), was chosen for scanning because it is representative of the structural bones usually affected by osteoporosis and is one of the most frequently fractured bones in caged hens (Gregory and Wilkins, 1989). For comparison, the tibia, containing medullary bone, was scanned (Hester et al., 2004). The live, unanesthetized hens were restrained on their backs in a foam holding device and secured with fastening straps (Schreiweis et al., 2003; Hester et al., 2004). The scanning took an average of 10 min/bone, and the orientation of the respective bone was the same for each scan. It took 5 d to scan all 96 hens.

To determine heterophil:lymphocyte ratios, blood samples were taken from the wing veins of the same focal hens every 8 wk (totaling 7 time periods), beginning with baseline data collection at 18 to 20 wk of age. Following methods for determination of heterophil:lymphocyte ratios described by Gross and Siegel (1983), 1 drop of blood was placed on a slide and then spun with a DiffSpin2 Slide Spinner (model 701-22, Stat Spin Inc., Norwood, MA). Slides were stained using Protocol Hema 3 Stain Set (Fisher Scientific Company LLC, Kalamazoo, MI) and heterophils and lymphocytes were counted until a total of 100 leukocytes were identified. The heterophil:lymphocyte ratio was calculated.

At the conclusion of the experiment when hens were 68 wk of age, the hens were killed by CO₂. The heart, spleen, and the right adrenal gland were collected from each hen in the study, trimmed of connective tissue and fat, and weighed to the nearest thousandth of a gram.

Productivity of Hens

Records of egg production and feed utilization/cage were maintained for the duration of the study (for BW data, see Thogerson et al., 2009). Feed utilization included feed eaten and wasted. When a hen died, the entire cage was removed from the analysis (8 cages removed from the study). Because mortality was so low (Thogerson et al., 2009), hen-housed productivity was not analyzed.

Feed utilization per hen (including during the baseline period), feed efficiency (kilograms of feed used per dozen eggs), and hen-day egg production were calculated for each 4-wk period (totaling 12 time periods for measures involving egg production; 13 for feed utilization). Egg weight and shell quality (shell weight and thickness) of 10 eggs per cage were determined every 8 wk (totaling 7 time periods). Eggshells were broken by hand and the egg contents were discarded. Shells were rinsed with tap water, dried overnight at 60°C, cooled to room temperature, and weighed. Shell weights and shell thickness included shell membranes. Shell thickness was measured with a caliper (B. C. Ames Co., Waltham, MA) to the nearest thousandth of an inch and converted to millimeters. Each shell was measured at 8 different points (2 at the blunt end, 2 at the pointed end, and 4 around the equator) and averaged.

Statistics

Data were analyzed using GLM in Minitab 15 (Montgomery, 2005). Because the FSA was applied to the cage, all data were averaged by cage. For measures taken over time, the blocking factor, cage, was included as a fixed effect to accommodate the repeated measures design. Tier, distance from the door, and side of the room were included as blocking factors in analyses without cage, and in analyses with cage, cage was nested within tier and distance from the door. Including these factors controlled for room position effects. In analyses of measures taken over time, age was included, and nested within period (baseline, when FSA was 12.2 cm, vs. treatment, the 12 mo after feeder space adjustments). To test our hypotheses, each analysis included FSA and, for measures taken over time, FSA × age and FSA × period interactions. For BMD and BMC, bone type and interactions with bone type were also included in the analysis because data were collected from both the humerus and tibia. The analyses for heart and adrenal weights also had BW in the model. However, for the spleen, we had to remove BW from the model and use spleen as a percentage of BW to obtain a linear error structure. To determine if the effects of treatment on organ weights were linear, FSA was treated as a continuous variable (see appendix for statistical models). Post hoc Tukey tests were used for mean comparisons. For significant FSA effects, trends of linearity across monthly or twice-monthly means were investigated using post hoc linear contrasts in JMP 6 (Montgomery, 2005). Due to missing data, the number of data points for each analysis differed slightly. Baseline data were taken when the hens were 18 to 20 wk of age, before regular egg laying; thus, analyses involving egg production did not include baseline data. The assumptions of GLM (linearity, homogeneity of variance, and normality of error) were confirmed post hoc, and suitable transformations were applied as necessary to meet these assumptions. Organ weights, BMC, and BMD were log-transformed. The heterophil:lymphocyte ratios were square-root transformed. However, raw, untransformed values are presented in Tables 1 and 2, along with SEM values calculated from the raw data.

RESULTS

Productivity of Hens

Data for egg quality are shown in Table 1. Hens with less feeder space produced eggs with slightly thicker shells and slightly greater shell weights. The effects were linear for both shell thickness and shell weight. There was a significant effect of FSA on average total egg weight. There was no linear trend to these data, but FSA of 7.1 and 12.2 cm differed significantly from 8.4, 9.7, and 10.9 cm, whereas 7.1 cm differed significantly from 5.8 cm.

Table 1. Effects of feeder space allocation (FSA) on production parameters

FSA	Eggs/hen per day	Egg weight (g)	Shell weight (g)	Shell thickness (mm)	Feed/dozen eggs (kg)
5.8	0.873 ^{ab}	59.1 ^{bc}	5.13 ^{ab}	0.350 ^{ab}	1.28 ^a
7.1	0.839 ^c	59.8 ^a	5.19 ^a	0.352 ^a	1.31 ^a
8.4	0.869 ^{ab}	58.8 ^{cd}	5.05 ^c	0.347 ^b	1.16 ^b
9.7	0.854 ^{bc}	59.0 ^{cd}	5.12 ^{ab}	0.351 ^a	1.18 ^b
10.9	0.862 ^{ab}	58.5 ^d	5.04 ^c	0.347 ^b	1.16 ^b
12.2	0.880 ^a	59.6 ^{ab}	5.09 ^{bc}	0.347 ^b	1.18 ^b
SEM	0.0057	0.14	0.018	0.0010	0.014
FSA main effect					
<i>F</i> -value	$F_{5,914} = 6.44$	$F_{5,500} = 11.84$	$F_{5,500} = 10.17$	$F_{5,500} = 6.20$	$F_{5,913} = 22.74$
<i>P</i> -value	<0.001	<0.001	<0.001	<0.001	<0.001
Linear effect					
<i>F</i> -value	$F_{1,914} = 2.64$	$F_{1,500} = 0.94$	$F_{1,500} = 14.51$	$F_{1,500} = 11.37$	$F_{1,913} = 61.11$
<i>P</i> -value	0.104	0.332	<0.001	<0.001	<0.001

^{a-d}Means within a column lacking a common superscript differ ($P < 0.05$).

During the baseline period, feed utilization was similar among the groups later assigned to FSA treatments (Figure 1). During the treatment period, hens with less feeder space used more food (Figure 1, FSA \times period GLM: $F_{5,1000} = 6.83$; $P < 0.001$) and required more kilograms of feed to produce a dozen eggs (Table 1). The effects for both of these variables were linear (feed usage, test for linearity $F_{1,1000} = 88.10$; $P < 0.001$; feed conversion, Table 1). There was no FSA effect on total egg production over the full laying cycle (overall total production/hen to 66 wk of age = 254 ± 1.1 ; feeder space GLM: $F_{5,64} = 1.55$; $P = 0.186$). There was an FSA effect on eggs/hen-day, but this effect was not linear (Table 1). All FSA, except 9.7 cm, significantly differed from 7.1 cm, and 9.7 cm significantly differed from 12.2 cm.

Physiology, Health, and Stress Measures of Hens

Baseline values for heterophil:lymphocyte ratios, BMD, and BMC were similar among the groups lat-

er assigned to FSA treatments (data not presented). There were no significant effects of feeder space on postmortem organ weights, heterophil:lymphocyte ratios, BMD, or BMC (Table 2).

DISCUSSION

The results of the current study were contrary to the predictions. It was expected that relatively low-ranking hens would be excluded from the feeder by larger more dominant hens, especially at lower FSA, thus limiting their feed intake and resulting in physiological stress and nutrient deprivation. However, the hens did not demonstrate aggression and therefore dominance relationships could not be established (Thogerson et al., 2009). Furthermore, there were few effects of FSA on production parameters and no significant effects of FSA on physiological data.

The BMD and the BMC of the tibia and humerus (Table 2), and feed utilization data (Figure 1), showed that hens at lower FSA were not deprived of Ca because bone mineralization was similar among hens sub-

Table 2. Effects of feeder space allocation (FSA) on organ weights, heterophil:lymphocyte (H:L) ratios, bone mineral density (BMD), and bone mineral content (BMC)

FSA	Heart weight ¹ (g)	Spleen weight ¹ (g)	Right adrenal weight ¹ (g)	H:L ratio ²	BMD ¹ (g/cm ²)	BMC ¹ (g)
5.8	5.3	1.5	0.081	0.63	0.183	2.03
7.1	5.1	1.4	0.080	0.67	0.184	2.00
8.4	5.2	1.5	0.080	0.67	0.181	1.98
9.7	5.2	1.4	0.081	0.62	0.187	2.06
10.9	5.2	1.5	0.082	0.62	0.176	1.94
12.2	5.3	1.4	0.082	0.72	0.187	2.09
SEM	0.08	0.05	0.0023	0.036	0.0032	0.060
FSA main effect						
<i>F</i> -value	$F_{1,82} = 0.58$	$F_{1,83} = 0.43$	$F_{1,82} = 1.03$			
<i>P</i> -value	0.449	0.513	0.313			
FSA \times period interaction						
<i>F</i> -value				$F_{5,263} = 1.11$	$F_{5,516} = 0.35$	$F_{5,516} = 0.84$
<i>P</i> -value				0.358	0.884	0.523
FSA \times period \times bone interaction						
<i>F</i> -value					$F_{5,516} = 0.42$	$F_{5,516} = 0.58$
<i>P</i> -value					0.833	0.718

¹Untransformed means presented; statistics based on analysis of log-transformed data.

²Untransformed means presented; statistics based on analysis of square-root-transformed data.

jected to varying FSA treatments. The overall mean BMC and BMD values obtained in the current study are similar to those reported for hens at 67 wk of age (2.13 g and 0.196 g/cm², respectively) in Schreiweis et al. (2005).

The organ weights were similar to those previously reported in the literature and indicated that hens did not show physiological signs of long-term stress. The heart weights in the current study were slightly lower than those of 7.4 to 8.2 g observed by Mumma et al. (2006) in laying hens. The spleen weights in the current study were similar to the 1.2 to 1.5 g reported for broilers in Puvadolpirod and Thaxton (2000) and 1.7 g reported for laying hens by Bunchasak et al. (2005). Adrenal weights in the current study fell in between those reported in previous studies (0.13 g, Lay and Wilson, 2002; 0.04 g, Hetland et al., 2003).

Although there were no differences among FSA for heterophil: lymphocyte ratios, the values, ranging from 0.62 to 0.72 for the hens in the current study, are within the ranges of those reported in other studies. Gross and Siegel (1986) reported heterophil:lymphocyte ratio values of 0.33 for nonstressed pullets and 1.08 for fasted pullets. McFarlane and Curtis (1989) reported that heterophil: lymphocyte ratios increased linearly from 0.53 to 0.86 in chicks as number of concurrent stressors increased from 0 to 6. Nevertheless, caution is needed in comparing results across studies due to differences in strains, ages, and environmental conditions.

Results of evaluation of the behavior, feather condition, and BW of these same hens (Thogerson et al., 2009) demonstrated a lack of either aggression or exclu-

sion of subordinate hens from the feeder. Instead, hens with restricted feeder space modified their behavior, reducing synchronization of feeding behavior by eating at different times throughout the day, resulting in no effect of FSA on plumage condition, BW, or BW uniformity. When combined with the results presented in the current study, it appears that hens of the Hy-Line W-36 strain housed in shallow cages did not experience chronic stress as a consequence of FSA, at least within the FSA range used in this study. Although there was an acclimation period of 1.5 wk, the heterophil:lymphocyte ratios were not elevated during baseline (18 wk of age) or immediately after the FSA treatments were initiated (20 wk of age), suggesting the hens did not experience short-term stress and adapted easily to house relocation and the new FSA, respectively.

Although there were no FSA effects on total egg production over the laying cycle and values were within the normal range for egg production (Hy-Line, 2003–2005), hens with less feeder space used significantly more feed (more g/hen-day). These findings are consistent with the report from Ramos et al. (1986), which showed that hens in deep cages (lower feeder space) had higher feed utilization than hens in shallow cages. Given that feed wastage was not measured in the current study, it is not known whether the increase in feed use with reduced FSA resulted from increased feed consumption or increased feed wastage. Reduced feeder space did result in slight increases in shell thickness and shell weight, which could have resulted from greater feed consumption providing more Ca for shell formation, but the increase in feed consumption, if it did occur, did not lead to increased BW (Thogerson et al., 2009), total eggs produced, or improved bone mineralization. It is well known that bone mineralization is sensitive to Ca consumption. For example, the BMD and BMC of the humerus and tibia of White Leghorn hens, as measured by DEXA, increased linearly as hens consumed increasing levels of dietary Ca (Schreiweis et al., 2003). The differences in shell thickness, shell weight, and egg weight were slight and, in the case of shell thickness, of the same order of magnitude as the precision of the caliper. Furthermore, egg weights and hen-day egg production did not change linearly with changes in feeder space, suggesting that these effects on egg weight and hen-day production could have been false positives due to the high statistical power of the study. Therefore, it is suggested that the increased feed utilization at lower FSA was likely due to increased feed wastage. There was a clear FSA effect on feed conversion. On average, the hens given the 2 lowest FSA required more than 100 g of additional feed to produce a dozen eggs, suggesting that provision of an FSA less than 7.1 cm would result in higher production costs.

From a hen welfare perspective, a clear cut-off point for FSA, below which hen welfare would be compromised, was not evident under the conditions of this study. This may be due to the use of the Hy-Line W-36 strain,

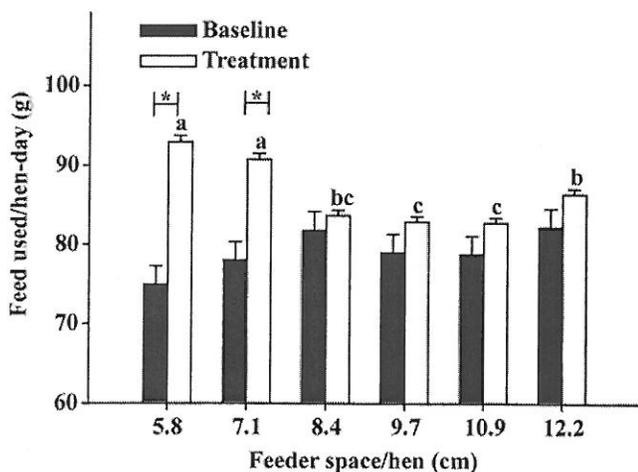


Figure 1. The effect of feeder space allocation on feed utilization (measured as average grams of feed/hen-day) during the baseline (18 to 20 wk of age) and treatment (20 to 68 wk of age) periods. Feed utilization included feed eaten and wasted. Each value represents the least squares means \pm SE. Values for the treatment period were averaged. A significant difference ($P < 0.05$) between pretreatment baseline and treatment is noted with an asterisk (*). ^{a-c}Treatment means lacking a common letter differ ($P < 0.05$). There was no linear trend to the data at baseline, but there was a significant linear trend ($P < 0.001$) for the data during the treatment stage.

which has been directly selected both for temperament and for group, rather than individual egg production (N. O'Sullivan, Hy-Line, West Des Moines, IA; personal communication). In addition, the hens used were beak-trimmed, and these results should not be extrapolated to hens of individually selected strains, especially if not-beak trimmed, because they could display intense competition at low FSA. Furthermore, the lower FSA treatments could rapidly become unacceptable from an animal welfare perspective if the hens were housed in deep cages, larger groups, or had less cage space—all of which would make it more difficult for them to move from the back to the front of the cage when other hens are in the way. The effect of FSA on hen welfare could also be greater under conditions in which hens need to have a greater feed intake and hence spend more time at the feeder (e.g., hens of larger strains, colder temperatures, less nutrient dense feed) or when feed is provided in a manner that requires either more time to ingest or greater feeding synchrony (e.g., smaller feed particle size, shallower depth of feed in the trough, lower frequency in running of automatic feeders).

The current study did have limitations. Although the feeder was blocked with corrugated plastic, hens could stand in front of the blocked part of the feeder and reach across to feed—this means that access to the feeder was greater than that implied by the FSA values (probably by a factor of 20%, or 1 extra hen feeding out of 5). Additionally, although attempts were made to emulate industry conditions as closely as possible, the current study took place in 1 room of a research facility where shallow cages were employed and where, unlike industry, feeding and egg collections were done by hand. Therefore, the results of this study need to be complemented by epidemiological data collected across multiple strains and multiple commercial production houses.

In conclusion, reduced feeder space caused poorer feed efficiency, thereby potentially increasing the cost of production, but did not limit feed intake, lower bone mineralization, or cause increased physiological stress in Hy-Line W-36 hens housed in shallow conventional cages, suggesting that it did not impair hen welfare.

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APPENDIX

The statistical models are listed below, as the GLM formulae used in the Minitab analyses. The same formulae would be used in SAS or other statistical programs.

Feed Consumption

$$Y = \text{tier} + \text{treatment} + \text{cage} (\text{tier treatment}) \\ + \text{time period} (\text{stage}) + \text{stage} + \text{tier} \times \text{treatment} \\ + \text{treatment} \times \text{stage} + \text{treatment} \\ \times \text{time period} (\text{stage}) + \text{tier} \times \text{stage} + \text{tier} \\ \times \text{time period} (\text{stage}).$$

Feed Conversion

$$Y = \text{tier} + \text{treatment} + \text{cage} (\text{tier treatment}) \\ + \text{time period} + \text{tier} \times \text{treatment} + \text{treatment} \\ \times \text{time period} + \text{tier} \times \text{time period}.$$

Egg Quality and Egg Production

$$Y = \text{tier} + \text{treatment} + \text{cage} (\text{tier treatment}) \\ + \text{time period} + \text{tier} \times \text{treatment} + \text{treatment} \\ \times \text{time period} + \text{tier} \times \text{time period}.$$

Blood

$$Y = \text{treatment} + \text{cage} (\text{treatment}) + \text{stage} \\ + \text{time period} (\text{stage}) + \text{treatment} \\ \times \text{time period} (\text{stage}).$$

Bones

$$Y = \text{cage} (\text{treatment}) + \text{time period} (\text{stage}) \\ + \text{bone} + \text{treatment} \times \text{stage} + \text{treatment} \\ \times \text{time period} (\text{stage}) + \text{treatment} \times \text{bone} + \text{stage} \\ \times \text{bone} + \text{bone} \times \text{time period} (\text{stage}) + \text{treatment} \\ \times \text{stage} \times \text{bone} + \text{treatment} \times \text{bone} \\ \times \text{time period} (\text{stage}).$$

Heart and Adrenals

$$Y = \text{BW} + \text{treatment} + \text{tier} \\ + \text{distance from door} + \text{side}.$$

Spleen

$$Y = \text{treatment} + \text{tier} + \text{distance from door} + \text{side}.$$