Effects of indoor stocking density on performance, egg quality, and welfare status of a native chicken during 22 to 38 weeks

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ABSTRACT This study investigated the effects of indoor stocking density on performance, egg quality, and welfare status of a native chicken, Beijing You Chicken (BYC), during 22 to 38 wk. A total of 1,040 19-wk-old BYC pullets were randomly allocated to 4 groups (2 replicates each) and reared in 8 individual floor pens with separate covered shed and uncovered outdoor areas. The indoor stocking densities were 5, 6, 7, and 8 hens/m^2, and the birds were fed corn-soybean based diets. The performance was calculated for 22 to 30, 30 to 38, and 22 to 38 wk, and egg quality indices were measured at the end of weeks 26, 29, and 36. The feather cover and gait score of the birds were assessed at the end of weeks 29 and 36. The results showed that average feed intake (AFI) and mortality rate of BYC in the 8 hens/m^2 group were higher than other groups during 22 to 30 wk ($P = 0.001$ and $P = 0.005$); the egg mass and egg-laying rate were higher in groups with lower stocking density, in contrast to the feed egg ratio during 30 to 38 wk. The AFI, mortality rate, and 38-wk body weight were affected by stocking density during 22 to 38 wk ($P < 0.05$). Most of egg quality indices were not changed by stocking density ($P > 0.05$), except for individual indicators, such as Haugh unit at week 26 ($P = 0.012$) and egg grade at week 29 ($P = 0.026$). The feather cover and gait scores of birds were affected by indoor stocking density at 36 wk of age, with the 8 hens/m^2 group having lower scores than the 5, 6, and 7 hens/m^2 groups ($P = 0.042$ and $P = 0.039$), whereas the 7 hens/m^2 groups having no difference with the 5 and 6 hens/m^2 groups ($P > 0.05$). Overall, the results suggested that the performance and egg quality of BYC were not significantly affected by equal to or less than 7 hens/m^2 under this system ($P > 0.05$), but 8 hens/m^2 had an adverse effect on the performance and welfare status of the native chicken during 22 to 38 wk.

Key words: indoor stocking density, performance, egg quality, welfare status

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INTRODUCTION

With the development of modern intensive animal husbandry, stocking density becomes one of the most important environmental and management factors. To pursue for the maximum economic benefits, the stocking density is often set very high in poultry farms. High stocking density has been reported to bring a number of negative effects, such as affecting broiler's feed intake (Heckert et al., 2002; Tang et al., 2012), decreasing body weight and average weight gain (Huang et al., 2009; Yanai et al., 2018; Zhang et al., 2018), lowering the performance and egg mass (**EM**) per hen per day (Anderson et al., 2004), resulting in worse feed egg conversion and higher mortality rate (Benyi et al., 2006), affecting the egg quality (Jahanian and Mirfenderski, 2015; Kang et al., 2018), and having adverse effects on the health and welfare of chicken (Dawkins et al., 2004; Robins and Phillips, 2011; Li et al., 2017; Feng et al., 2018). In recent decades, animal friendly production systems are gaining popularity in Europe and many countries (Magdelaine and Mirabito, 2001), the consumers began to pay more and more attention to the food quality (Grunert, 2005) and animal welfare, and also have willing to pay for the animal welfare products (Fredrik et al., 2007; Yang, 2018), whereas the high stocking density becomes one of the obstacles (Kunzmann, 2011) which require the poultry producers to make comprehensive trade-offs on the stocking density.

Previous research about stocking density in laying hens has mainly been conducted in indoor systems, such as cage system and aviary system (Guo et al., 2012; Saki et al. 2012; Jahanian and Mirfenderski, 2015; Meng et al., 2015; Wang et al., 2016; Kang et al., 2018). Generally high stocking density will affect the hen's performance, but exactly how high the stocking density is different for different breeds and production system. Beijing You Chicken (**BYC**), a dual-purpose native chicken in Beijing, is well known for its special appearance (DB11/T 404-2006), slow growth rate, low performance, but high-quality meat and eggs (Liu and Xu,

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Table 1. Experimental design and treatments.

Group	Group size (hen)	Indoor stocking density (hens/m^2)	Actual stocking density (m^2/hen)	Indoor feeding space (cm/hen)
1	100	5	0.2	7.2
2	120	6	0.167	6
3	140	7	0.143	5.2
4	160	8	0.125	4.5

2001), such as the yolk color (**YC**) of BYC is deeper than Lomnman Pink (6.37 vs. 4.70), the yolk percentage of BYC is higher than Lohmann Pink (27.76% vs. 19.85%); the dry matter, crude fat content, cephalin and lecithin content, and free amino acid content of BYC are higher than those in Lohmann Pink ($P < 0.05$), whereas the egg weight (**EW**), eggshell color (**ESC**), albumen height (**AH**), Haugh unit (**HU**), and relative eggshell weight were lower in BYC than those in Lohmann Pink ($P < 0.05$) (Zhang et al., 2009). Due to these characteristics and potential market value, many farms and companies in many districts have started raising BYC under free range systems during the last decade, but owing to the different housing conditions, feed and management, and different indoor or outdoor stocking density, the birds' performance, egg quality, and welfare status were different.

It is regulated that the stocking density for free range laying hens was less than 9 hens/m^2 indoors and 4 m^2/hen outdoors in European countries (Directive EU, 1999), and the stocking density recommended for free range BYC above 19 wk of age was less than 6 hens/m^2 indoors, and 4 m^2/hen outdoors according to the local standard (DB11/T 1378-2016), whereas current stocking density is usually more than 6 birds/m^2 indoors, and far below 4 m^2/hen outdoors for commercial free range BYC; therefore, the present study aims to compare the effects of 4 mostly used indoor stocking density (5, 6, 7, and 8 hens/m^2) on performance, egg quality, and welfare status of the native chicken, and provide some theoretical references for the birds' production in free range system.

MATERIALS AND METHODS

Experimental Design and Birds

This experiment was conducted at BYC Breeding Farm, Daxing District, Beijing. A total of 1,040 19-wk-old BYC pullets (1.72 ± 0.14 kg/hen) were randomly allocated to 4 groups (2 replicates each) and reared in 8 individual floor pens with separate covered plastic shed and uncovered outdoor areas. The indoor stocking densities were 5, 6, 7, and 8 hens/m^2 floor areas (100, 120, 140, and 160 birds per pen, respectively, the available indoor floor area is 20 m^2), and the related treatments are summarized in Table 1.

Each pen is 3 m width and 8 m length, and the aisle is 1.2 m width. The feed troughs were suspended in layers outside the wire mesh near the aisle, and 2 Plasson drinking fountains were used in each pen. Nest boxes, perches, and rice hulls were provided in each pen. The uncovered outdoor area (3 m width and 15 m length) was fully enclosed with wire mesh, having 1 or 2 walnut trees in the middle. A covered plastic shed (3 m width and 2.5 m length) connected the pen and the uncovered outdoor area (see Figure 1). In the covered shed, there was no hardening of the ground so that the birds could scratch the soil and have dust baths. The shed also served as a temperature transition zone for the chicken entering and leaving the uncovered outdoor area. The birds could enter the shed via a pop hole set under the south wall of the pen. The chicken keeper used a hand crank to control the rise and fall of the rolling curtain on the outside of the covered shed every morning and evening, so as to control the entry and exit of the chicken. During the daytime, the chicken were free to stay in the pen, the covered shed, and the uncovered outdoor area. After the birds returning to the pen at dusk, the pop hole was closed and the curtain was put down. The birds were fed twice a day (6:30 in the morning and 14:00 in the afternoon) and kept under the same management, natural ventilation, and lighting program. The adaptation period was from 19 to 21 wk of age, and the experiment was from 22 to 38 wk of age. Another behavior observation experiment was conducted at the same time: the ranging behavior of the birds and the use of the covered shed and uncovered outdoor area were observed and recorded for consecutive 3 d at 22, 26, 30, 34, and 38 wk of age (the results were summarized in other report).

The birds were fed commercial corn-soybean-based diets formulated according to breed requirement with 15.07% crude protein (**CP**), 11.20 MJ/kg metabolizable energy (**ME**), and 2.03% calcium during 19 to 21 wk; 15.51% CP, 11.08 MJ/kg ME, and 2.75% calcium during 22 to 38 wk in mash form, as shown in Table 2. Light was provided by energy-saving lamps 2 m off the ground, and average light intensity was 10 lux. The light controller was set to provide 16 h of light for each pen. The temperature and humidity were maintained at appropriate conditions. Under severe weather conditions, the birds were confined inside the pen to reduce the stress.

The study was performed in accordance with local ethical guidelines and met the requirements of the Institutional Animal Care and Use Committee.

Measurement and Methods

Feed intake, egg numbers, and EW in each pen were recorded every day, and weekly average feed intake (**AFI**), egg-laying rate, EM, feed egg ratio (**FER**), and mortality rate for each replicate group were calculated for 22 to 30, 30 to 38, and 22 to 38 wk. All of the birds were individually weighed at the end of 38 wk.

Egg quality indices at the end of weeks 26, 29, and 36 were measured and calculated. A total of 20 fresh

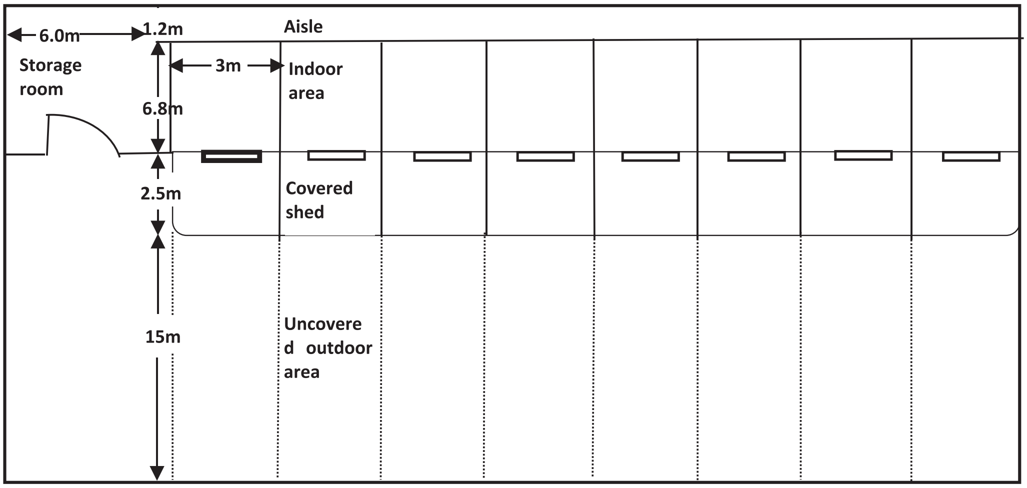

Figure 1. The drawing picture of the pen facility.

Table 2. Composition and nutrient levels of the basal diet.

Ingredients,%	19 to 21 wk	22 to 38 wk
Corn	65.5	64.0
Soybean meal	21.5	23.2
Wheat bran	5.0	3.8
Limestone	4	5
Layer premix[1]	4	4
Total	100	100
Calculated nutrient level[2]		
ME/(MJ/kg)	11.20	11.08
Crude protein/%	15.07	15.51
Calcium/%	2.03	2.75
Total phosphorus/%	0.51	0.51
Available phosphorus/%	0.29	0.29

[1]Layer premix provided per kilogram of diet: vitamin A, 100 to 250 KIU; vitamin D3, 60 to 80 KIU; vitamin E, 0.5 KIU; vitamin K3, 80 mg; vitamin B1, 45 mg; vitamin B2, 180 mg; vitamin B6, 100 mg; vitamin B12, 0.5 mg; D-calcium-pantothenate, 220 mg; nicotinamide, 720 mg; folic acid, 20 mg; biotin,2 mg; copper, 0.2 to 0.8 g, ferrous iron, 1.5 to 5 g; zinc, 0.8 to 2.4 g; manganese,1.5 to 3 g; iodine, 10 to 30 mg; selenium, 2 to 6 mg.

[2]Calculated using NRC (1994) values.

eggs were randomly selected from each replicate pen, giving a total of 40 eggs for each group. Egg weight, HU, AH, YC, egg grade (**EG**), eggshell strength (**ESS**), eggshell thickness (**EST**), ESC, egg shape index, relative yolk weight, relative eggshell weight, and relative albumen weight were measured and calculated within 24 h. The measuring method and apparatus were the same as those described by Geng et al. (2018).

At the end of 29 and 36 wk, the feather cover and gait score of the birds were assessed in the morning when all the birds stayed in the pen. Five sites were selected in each pen according to the "Z" type, and 3 hens were randomly selected at each site for observation and feather cover scoring, after which they were placed on the ground for gait observation and scoring (at least 30 s, assessed by a specially trained staff), then immediately returned to the pen. The feather cover scoring was conducted according to Tauson et al. (2005) and Heerkens et al. (2015). Briefly, the body was divided into 5 parts (neck, back, wings, tail, and anus). For the wings, the side with worse feather coverage was chosen. Each part was scored using a 4-point scale, where 1 was worst, 2 was bad, 3 was general, and 4 was best. Each bird's feather score was the sum of the score of 5 parts, with the minimum value being 5 and the highest 20 and a greater score indicating a better overall condition of the feathers. Gait score referred to the method adopted by the authors (Geng et al., 2007), mainly focusing on the walking balance of chicken. The original 0 to 2 point system was revised to 1 to 4 point system, 1 being the worst and 4 being the best. See Table 3 for the specific scoring criteria.

Table 3. The scoring criteria for feather cover and gait.

Score	Feather cover	Gait	Assessment
1	Fewer feather cover, >50% scratch marks and some were picked off	Very hesitantly move, did not take a few steps to stop, even squat down	Worse
2	Few feather cover, 20 to 50% scratch marks and some were picked off	Walk irregular, small steps, very unbalanced	Bad
3	General feather cover, <20% scratch marks	Walk regularly and balanced	General
4	Good feather cover, complete and smooth, no scratch marks	Walking easily, regular gait, even striding, well balanced	Best

Statistical Analyses

The data were expressed as mean ± SD, and analyzed statistically using the SPSS 18.0 Software for Windows (SPSS Inc. Chicago, IL). One-way ANOVA was used to analyze the effects of stocking density on performance, egg quality, gait score, and feather cover score. Duncan's test was used for multiple comparisons. The percentage was arcsine transformed before analysis. $P < 0.05$ was regarded as statistically significant.

Table 4. Effects of indoor stocking density on performance of BYC during 22 to 30 wk.

Indoor stocking density (hen/m^2)	AFI/(kg)	EM/(kg)	Egg laying rate/(%)	FER/(kg:kg)	Mortality rate/(%)
5	0.67 ± 0.02^b	0.15 ± 0.09	47.50 ± 25.39	4.47	0.17 ± 0.38^b
6	0.67 ± 0.02^b	0.14 ± 0.09	46.42 ± 26.32	4.79	0.09 ± 0.27^b
7	0.68 ± 0.02^b	0.15 ± 0.08	47.93 ± 24.31	4.53	0.24 ± 0.56^b
8	0.69 ± 0.02^a	0.15 ± 0.10	44.62 ± 24.48	4.60	0.69 ± 0.75^a
P value	0.001	0.997	0.979	0.967	0.005

Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).
AFI = average feed intake; EM = egg mass; FER = feed egg ratio.

Table 5. Effects of indoor stocking density on performance of BYC during 31 to 38 wk.

Indoor stocking density(hen/m^2)	AFI/(kg)	EM/(kg)	Egg laying rate/(%)	FER/(kg:kg)	Mortality rate/(%)
5	0.72 ± 0.04^b	0.21 ± 0.03^a	64.55 ± 4.93^a	3.43^c	0.32 ± 0.49
6	0.72 ± 0.04^b	$0.20 \pm 0.02^{a,b}$	60.94 ± 5.01^b	$3.60^{b,c}$	0.16 ± 0.34
7	0.73 ± 0.04^b	$0.184 \pm 0.02^{b,c}$	57.13 ± 3.74^c	$4.06^{a,b}$	0.14 ± 0.29
8	0.76 ± 0.04^a	0.180 ± 0.02^c	56.51 ± 4.63^c	4.22^a	0.34 ± 0.43
P value	0.011	0.004	0.001	0.001	0.345

Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).
AFI = average feed intake; EM = egg mass; FER = feed egg ratio.

Table 6. Effects of indoor stocking density on performance of BYC during 22 to 38 wk.

Indoor stocking density(hen/m^2)	AFI/(kg)	EM/(kg)	Egg laying rate/(%)	FER/(kg:kg)	Mortality rate/(%)	38-wk BW/(kg)
5	0.69 ± 0.04^b	0.17 ± 0.07	55.53 ± 20.44	4.06	0.24 ± 0.44^b	2.06 ± 0.18^a
6	0.69 ± 0.04^b	0.17 ± 0.07	53.25 ± 20.55	4.06	0.12 ± 0.30^b	$2.04 \pm 0.22^{a,b}$
7	0.70 ± 0.04^b	0.16 ± 0.06	52.26 ± 18.23	4.38	0.19 ± 0.45^b	$1.99 \pm 0.19^{b,c}$
8	0.73 ± 0.05^a	0.16 ± 0.08	50.21 ± 18.40	4.56	0.52 ± 0.63^a	1.97 ± 0.21^d
P value	0.002	0.887	0.726	0.921	0.004	0.001

Values with different letter superscripts in the same column mean significant difference ($P < 0.05$).
AFI = average feed intake; EM = egg mass; FER = feed egg ratio; BW = body weight.

RESULTS

The performance of BYC at different stages is shown in Tables 4–6. Table 4 shows that performance of BYC during 22 to 30 wk was affected by stocking density ($P < 0.05$), with AFI and mortality rate in the 8 hens/m^2 group being higher than in the 5, 6, and 7 hens/m^2 groups ($P = 0.001$ and $P = 0.005$). However, the EM, egg-laying rate, and FER were not changed among the groups ($P > 0.05$). Table 5 shows that the AFI, EM, egg-laying rate, and FER of BYC were significantly affected by stocking density during 30 to 38 wk ($P < 0.05$), except for the mortality rate ($P > 0.05$). The AFI was higher in the 8 hens/m^2 group than in the 5, 6, and 7 hens/m^2 groups ($P = 0.011$), whereas there were no differences among the 5, 6, and 7 hens/m^2 groups. The EM in the 8 hens/m^2 group was lower than in the 5 and 6 hens/m^2 groups ($P = 0.004$), but had no difference in the 7 hens/m^2 group. The egg-laying rate in the 6 hens/m^2 group was lower than in the 5 hens/m^2 group, but higher than in the 7 and 8 hens/m^2 groups ($P = 0.001$). The FER in the 7 and 8 hens/m^2 groups were higher than in the 5 and 6 hens/m^2 groups ($P = 0.001$).

Table 6 shows that the AFI, mortality rate, and 38-wk BW were influenced by stocking density during 22 to 38 wk ($P < 0.05$). The AFI and mortality rate were higher in the 8 hens/m^2 group than in the 5, 6, and 7 hens/m^2 groups ($P = 0.002$ and $P = 0.004$), and there were no differences among the 5, 6, and 7 hens/m^2 groups. Moreover, the 38-wk BW in the 8 hens/m^2 group was lower than in the 5, 6, and 7 hens/m^2 groups ($P = 0.001$), indicating that it was higher in the lower density group and lower in the higher density group.

The egg quality of BYC at different stages is shown in Tables 7–9. Table 7 shows that stocking density had no effects on most of egg quality indices at week 26 ($P > 0.05$), except that the HU in the 7 hens/m^2 group was lower than those in 5, 6, and 8 hens/m^2 groups ($P = 0.012$). Table 8 shows that stocking density had no effects on most of egg quality indices at week 29 ($P > 0.05$), except that the EG in the 8 hens/m^2 group

Table 7. Effects of indoor stocking density on egg quality of BYC at 26 wk (n = 40).

Indoor stocking density(hen/m^2)	5	6	7	8	P value
EW/(g)	42.48 ± 3.18	43.22 ± 3.57	42.91 ± 3.17	42.76 ± 3.11	0.782
ESS/(kg/cm$^{2)}$	3.95 ± 0.96	3.99 ± 0.74	3.93 ± 1.10	4.16 ± 0.76	0.657
EST/(mm)	0.34 ± 0.03	0.34 ± 0.03	0.34 ± 0.03	0.35 ± 0.03	0.302
ESC/(%)	48.08 ± 6.41	49.98 ± 6.93	49.49 ± 5.59	48.44 ± 4.92	0.458
AH/(mm)	6.05 ± 1.32	5.96 ± 1.75	5.52 ± 1.19	6.28 ± 1.41	0.124
HU	82.68 ± 8.97^{a}	81.47 ± 11.16^{a,b}	77.91 ± 11.45^{c}	85.15 ± 6.85^{a}	0.012
EG	2.88 ± 0.40	2.78 ± 0.57	2.83 ± 0.45	2.98 ± 0.16	0.187
YC	8.33 ± 1.45	8.10 ± 0.98	8.54 ± 1.24	8.13 ± 1.38	0.388
ESI	1.33 ± 0.08	1.33 ± 0.08	1.33 ± 0.07	1.32 ± 0.06	0.801
RYW/(%)	26.78 ± 2.04	26.22 ± 2.50	26.83 ± 2.41	26.48 ± 2.25	0.610
RESW/(%)	14.54 ± 1.64	14.17 ± 1.06	14.58 ± 1.32	14.18 ± 1.43	0.370
RAW/(%)	58.68 ± 2.79	59.61 ± 2.56	58.58 ± 2.77	59.33 ± 2.99	0.276

Values with different letter superscripts in the same row mean significant difference ($P < 0.05$).
EW = egg weight; ESS = eggshell strength; EST = eggshell thickness; ESC = eggshell color; AH = albumen height; HU = Haugh unit; YC = yolk color; EG = egg grade; ESI = egg shape index; RYW = relative yolk weight; RESW = relative eggshell weight; RAW = relative albumen weight.

Table 8. Effects of indoor stocking density on egg quality of BYC at 29 wk (n = 40).

Indoor stocking density(hen/m^2)	5	6	7	8	P value
EW/(g)	46.34 ± 3.12	46.22 ± 2.65	45.51 ± 3.87	45.03 ± 3.15	0.227
ESS/(kg/cm$^{2)}$	4.44 ± 0.83	4.29 ± 0.69	4.05 ± 1.07	4.09 ± 0.64	0.129
EST/(mm)	0.36 ± 0.02	0.35 ± 0.03	0.35 ± 0.03	0.36 ± 0.10	0.804
ESC/(%)	50.39 ± 5.56	49.66 ± 6.11	51.39 ± 23.88	44.41 ± 5.31	0.076
AH/(mm)	6.34 ± 0.08	6.14 ± 1.25	6.19 ± 1.23	6.20 ± 0.88	0.865
HU	83.79 ± 5.49	82.07 ± 7.44	82.66 ± 8.00	83.34 ± 5.47	0.675
EG	3.00 ± 0.00^{a}	3.00 ± 0.00^{a}	3.00 ± 0.00^{a}	2.93 ± 0.26^{b}	0.026
YC	8.01 ± 1.36	7.49 ± 1.45	8.16 ± 1.28	7.74 ± 1.68	0.177
ESI	1.31 ± 0.05	1.32 ± 0.07	1.29 ± 0.05	1.30 ± 0.09	0.353
RYW/(%)	27.91 ± 2.63	27.72 ± 1.61	28.42 ± 3.19	28.64 ± 2.69	0.352
RESW/(%)	14.24 ± 1.04	13.96 ± 0.96	14.01 ± 1.35	14.29 ± 1.58	0.565
RAW/(%)	57.85 ± 3.33	58.31 ± 1.88	57.57 ± 4.20	57.06 ± 3.82	0.425

Values with different letter superscripts in the same row mean significant difference ($P < 0.05$).
EW = egg weight; ESS = eggshell strength; EST = eggshell thickness; ESC = eggshell color; AH = albumen height; HU = Haugh unit; YC = yolk color; EG = egg grade; ESI = egg shape index; RYW = relative yolk weight; RESW = relative eggshell weight; RAW = relative albumen weight.

was lower than those in 5, 6, and 7 hens/m^2 groups ($P = 0.026$). Table 9 shows that stocking density had no effects on all of egg quality indices at week 36 ($P > 0.05$), except that the EST in the 8 hens/m^2 group was almost lower than in the 5 hens/m^2 group ($P = 0.054$).

The welfare status of BYC at different stages is shown in Table 10, which showed that there was a numerical difference in feather cover score by stocking density at 29 wk of age, but was not significant ($P > 0.05$). However, there was a significant difference in gait score at 29 wk of age, among which the gait score of the 8 hens/m^2 group was lower than the other 3 groups ($P = 0.046$). At 36 wk of age, the feather cover and gait scores of birds were affected by indoor stocking density, with the 8 hens/m^2 group having lower scores than those in the 5, 6, and 7 hens/m^2 groups ($P = 0.042$ and $P = 0.039$), whereas the 7 hens/m^2 groups having no difference in the 5 and 6 hens/m^2 groups ($P > 0.05$). These findings indicate that the 8 hens/m^2 group influenced the feather cover score of the hens when kept in a relatively longer period (36 vs. 29 wk).

DISCUSSION

There have had numerous studies about the effects of stocking density on performance of poultry, but the results have been inconsistent because of different breeds and rearing condition. For the broiler chickens, some believe that high stocking density will reduce the body weight, feed intake, and feed conversion ratio (Feddes et al., 2002; Dozier et al., 2005; Houshmand et al., 2012; Sun, 2013), whereas others believe that stocking density has no impact on performance of broilers (Nordquist et al., 2017). For the layers, Saki et al. (2012) compared 4 cage densities (2,000, 1,000, 667, and 500 cm^2/hen) for White Leghorn hens and found that hens in the highest stocking density had lower hen-day egg production although they did have a higher feed conversion ratio. Guo et al. (2012) showed that laying rate did not differ between hens housed in a conventional cage system and hens housed in furnished cages at small (21 hens) or large (48 hens) flock size, both at lower stocking densities than the conventional cage.

Table 9. Effects of indoor stocking density on egg quality of BYC at 36 wk (n = 40).

Indoor stocking density(hen/m^2)	5	6	7	8	P value
EW/(g)	48.76 ± 3.97	47.89 ± 2.93	47.47 ± 3.16	48.06 ± 3.56	0.403
ESS/(kg/cm$^{2)}$	3.94 ± 0.60	3.89 ± 0.75	3.96 ± 1.01	3.89 ± 1.01	0.982
EST/(mm)	0.32 ± 0.03	0.33 ± 0.03	0.34 ± 0.03	0.36 ± 0.03	0.054
ESC/(%)	50.99 ± 6.38	51.64 ± 7.19	49.46 ± 6.02	48.38 ± 5.97	0.100
AH/(mm)	5.67 ± 1.81	5.38 ± 1.09	5.40 ± 1.03	4.99 ± 1.09	0.148
HU	77.01 ± 10.24	75.99 ± 7.19	76.54 ± 7.93	73.12 ± 9.00	0.187
EG	2.75 ± 0.49	2.69 ± 0.47	2.70 ± 0.56	2.55 ± 0.68	0.417
YC	7.57 ± 1.27	8.14 ± 1.04	8.11 ± 1.14	8.05 ± 1.51	0.140
ESI	1.31 ± 0.06	1.31 ± 0.06	1.32 ± 0.07	1.31 ± 0.06	0.737
RYW/(%)	30.59 ± 4.03	29.94 ± 2.51	30.28 ± 2.33	30.29 ± 2.89	0.815
RESW/(%)	13.76 ± 1.69	13.64 ± 1.26	13.48 ± 1.29	13.91 ± 1.48	0.593
RAW/(%)	55.64 ± 5.22	56.42 ± 3.25	56.24 ± 3.10	55.79 ± 3.46	0.782

Values with different letter superscripts in the same row mean significant difference ($P < 0.05$).

EW = egg weight; ESS = eggshell strength; EST = eggshell thickness; ESC = eggshell color; AH = albumen height; HU = Haugh unit; YC = yolk color; EG = egg grade; ESI = egg shape index; RYW = relative yolk weight; RESW = relative eggshell weight; RAW = relative albumen weight.

Table 10. Effects of indoor stocking density on feather cover and gait score.

Indoor stocking density(hen/m^2)	5	6	7	8	P value
29 wk					
Feather cover score	14.10 ± 0.34	13.93 ± 0.53	13.87 ± 0.55	13.80 ± 0.56	0.075
Gait score	3.97 ± 0.48^a	3.83 ± 0.59^{a,b}	3.70 ± 0.59^b	3.43 ± 0.42^c	0.046
36 wk					
Feather cover score	13.86 ± 0.55^a	13.77 ± 0.56^a	13.70 ± 0.40^a	13.47 ± 0.29^b	0.042
Gait score	3.83 ± 0.62^a	3.70 ± 0.59^{a,b}	3.53 ± 0.64^b	3.23 ± 0.53^c	0.039

Values with different letter superscripts in the same row mean significant difference ($P < 0.05$).

Wang (2015) compared the effects of different stocking densities on Hyline Grey laying hens under a laminated cage system and found that egg number decreased with increased stocking density, whereas the mortality rate increased. Jahanian and Mirfenderski (2015) compared the performance of Hyline W-36 layers under 2 cage densities (360 and 257 cm^2/hen) and found that the egg-laying rate was significantly decreased by 257 cm^2/hen during 33 to 38 wk (P = 0.031). Wang et al. (2016) studied the effects of 3 cage densities (900, 540, and 380 cm^2/hen) on performance of Hyline Grey laying hens during 11 to 20 wk and found that the body weight was significantly higher in the low-density group (900 cm^2/hen) than those in the medium- and high-density group at 20 wk of age ($P < 0.05$).

The present study was conducted in free range system with an indoor pen, covered shed, and uncovered outdoor area; the hens can choose to stay freely in these areas at daytime, which may provide the hens more opportunities for activities or to exhibit natural behaviors than in the cage system. The AFI and mortality rate of BYC were found to be higher in the 8 hens/m^2 group than the other 3 groups during 22 to 30 wk ($P = 0.001$ and $P = 0.005$), but the egg-laying rate and FER were not changed ($P > 0.05$). The more feeds consumed by the birds in high density group were not used for gaining weight or laying eggs, but probably used for more maintenance requirements from the congestion and more movements. Additionally, the 8 hens/m^2 group had the lowest 38-wk BW ($P = 0.001$), indicating an association between a higher stocking density and a lower body weight.

Stocking density was reported to affect egg quality in varying degrees. Sari et al. (2012) compared 4 cage densities (2,000, 1,000, 667, and 500 cm^2/hen) for White Leghorn hens and found that hens in the highest stocking density had lower EW and EM. Jahanian and Mirfenderski (2015) compared 2 cage densities (360 and 257 cm^2/hen) for Hyline W-36 hens and found that the EW in 257 cm^2/hen group decreased significantly during 33 to 38 wk ($P < 0.05$). Meng et al. (2015) compared Hy-line Brown hens in conventional cages (highest stocking density), large or small furnished cages (both the same stocking density but lower than conventional), and showed that EW was the same among all cage types, but eggs from the conventional cages had lower HU and AH was the lowest in the large furnished cages. Kang et al. (2018) compared 4 stocking densities (13, 15, 17, and 19 birds/m^2) for Hyline Brown hens in an aviary system and found that ESS and EM were lower ($P < 0.05$) for 19 birds/m^2 than for other groups. All the above studies were done in cage systems, and the present study indicated that most of the egg quality indicators were not affected by the indoor stocking density in the free range system ($P > 0.05$), except for individual indicators, such as HU at week 26 ($P = 0.012$) and EG at week 29 ($P = 0.026$).

Usually the physical egg quality includes the internal egg quality and external shell quality. Most studies about the egg quality used different indices, methods, and instruments, measured some or all of the above indicators (Campbell et al. 2017; Al-Ajeeli et al. 2018; Geng et al. 2018; Ruhnke et al. 2018). But till now it has not been clear which indicators are more sensitive for evaluation of the egg quality. Our group studied the effects of different production systems, light conditions, and nutritional levels on egg quality (Geng et al., 2011a, b, 2015), and found that many of the indicators are often different or even contradictory, especially at different stages, such as the EW, ESC, YC, and yolk percentage in the late-laying period (63 wk of age) were significantly higher than those in the pre-laying period (50% egg-laying rate, 25 wk of age) ($P < 0.05$); the EW, YC, and yolk percentage were higher in the mid-laying period (43 wk of age) than those in the pre-laying period, but lower than in the late-laying period ($P < 0.05$) (Zhang et al., 2010). It seems to be more accurate to use a combination of several indicators rather than a single indicator to judge the egg quality. In this present study, only a single indicator was different at 26 and 29 wk of age, it is not enough to claim that the indoor stocking density had significant effects on the egg quality of BYC. This result is also in agreement with Wang (2015), who reported that stocking density had no significant effect on egg quality at different periods.

Feather cover and gait score can partly reflect the welfare status of poultry. Wang (2015) compared the impact of 5 cage densities (900, 675, 540, 450, and 380 cm^2/bird) on the welfare of Hyline Grey laying hens during 11 to 50 wk and found that a higher stocking density was associated with worse feather cover and a lower feather score. Hu et al. (2013) compared the effects of different stocking density on caged Arbor Acres chicken during 4 to 10 wk of age and found that the number of feather pecking significantly increased as stocking density increased, which affected the feather score of chickens. Wang and Wei (2017) compared the effects of 4 cage densities (30, 40, 50, and 60 birds/m^2) on 4-wk-old Hyline White layers and found that the total score of feather injury of the birds increased as the stocking density increased. The above research was done in cage condition and the birds have very limited space to use, whereas the birds in this study had a covered shed and uncovered outdoor area, which could reduce indoor stocking density at daytime, thus possibly decreasing the motivation for feather pecking (Sherwin et al., 2010). But we still found that a higher indoor stocking density was associated with a worse feather cover score when kept for a longer period (36 vs. 29 wk), indicating that there may exist a time effect of stocking density on welfare status, but exactly how long it will take for high stocking densities to affect the welfare of chickens is unclear. The related reason may be related with the lower range use of the birds in high stocking density group. It was reported that the free-range ISA brown hens used the range for longer in the lowest outdoor stocking density, and the least in the highest density (Campbell et al., 2017), Other studies in commercial free-range farms, with flock sizes from approx. 100 to 16,000 individuals housed at indoor stocking densities from 4 to 12 bird/m^2, showed on average 12% of the flock was seen on the range simultaneously, with fewer birds ranging as flock size and indoor stocking density increased (Gilani et al., 2014). In this present study, we observed most of time there was a smaller percentage of birds stayed in covered shed and uncovered outdoor area in the 8 hens/m^2 group, which may cause congestion and feather pecking of the birds in the pens.

Steenfeldt and Nielsen (2015) compared the effects of 6, 9, and 12 birds/m^2 on behavior and welfare under a rearing system with multilayer perches in a veranda and found that welfare of birds was the worst in the highest 12 birds/m^2 group, possibly because of decreased feeding and drinking space in the system, which may be also the reason of the worse feather cover in the 8 hens/m^2 group.

Stocking density affects welfare of commercial turkeys, such as behavior and leg health (Erasmus, 2017). Gait score is an indicator that can directly reflect the leg health of chicken (Garner et al., 2002), but it would generally decrease with increased stocking density (Geng et al., 2007; Li et al., 2017). In the present study, we found that gait score of the 8 hens/m^2 group was lower than those of 5, 6, and 7 hens/m^2 groups at 29 and 36 wk of age ($P = 0.046$ and $P = 0.039$), indicating that 8 hens/m^2 group adversely influenced the walking ability of the hens, as well as their welfare status even in free range system.

Limited by the present condition, the number of birds in this study was relatively small, and the effects of indoor stocking density in commercial chicken flock need to be further validated in future.

CONCLUSION

The present study indicated that indoor stocking density had significant effects on performance of the native chicken during 22 to 38 wk, especially the AFI, mortality, and 38-wk BW ($P < 0.05$). A lower stocking density was associated with a higher BW, and a higher stocking density was associated with a lower gait score and feather cover score when kept for a longer period. Overall, the results suggested that the performance and egg quality of BYC were not significantly affected by equal to or less than 7 hens/m^2 under this system ($P > 0.05$), but 8 hens/m^2 had an adverse effect on the performance and welfare status of the native chicken during 22 to 38 wk.

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