Keel fracture changed the behavior and reduced the welfare, production performance, and egg quality in laying hens housed individually in furnished cages

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ABSTRACT Keel fracture has adverse effects on welfare, behavior, health, production performance, and egg quality of laying hens. To investigate this, 90 healthy Lohmann white laying hens with normal keel bones at 17 wk of age (**WOA**) were used in this study and housed individually in furnished cages. All hens were marked with fractured keel (**FK**) or normal keel (**NK**) based on the keel bone status through palpation at 5 time-points (22, 27, 32, 37, and 42 WOA). After the palpation, the behavior was observed for 2 consecutive days at each time-point, and the total number of eggs produced, dirty eggs, broken eggs, and feed intake of FK and NK laying hens were recorded at 27–32, 32–37, and 37–42 WOA, respectively. After each behavioral observation, 10 fresh FK hens and 10 NK hens were randomly selected to determinate the welfare and egg quality. The results showed that the incidences of keel fracture increased with the age of laying hens. Compared with NK hens, the sitting and standing behaviors significantly increased ($P < 0.05$) while feeding, walking, perching, and jumping behaviors significantly decreased ($P < 0.05$) in FK hens. There were no significant changes in drinking, preening, comforting, cage pecking, and nesting behaviors between NK and FK hens ($P > 0.05$). During the experiment period, the egg production rate, body weight, daily feed intake, and eggshell strength, thickness, and weight decreased ($P < 0.05$) and duration of tonic immobility increased ($P < 0.05$) in FK hens compared with those in NK hens. At 27–32 WOA, FK hens had significantly elevated broken egg rate ($P < 0.05$). There were no significant differences in the dirty egg rate, egg shape index, protein height, Haugh unit, feather cover score, and toe and foot pad health score ($P > 0.05$). Therefore, keel fracture in laying hens caused changes in behavior and reduced the welfare, production performance, feed intake, and eggshell quality.

Key words: laying hen, keel fracture, behavior, welfare, performance

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INTRODUCTION

In modern intensive production systems for laying hens, furnished cages and noncage systems have become common because of the increasing attention given to hen welfare (Stratmann et al., 2015; Weeks et al., 2016) and the ban imposed by the European Union on the traditional cage-raising system from 2012. Noncage systems allow more space for hens to practise natural behaviors (Stratmann et al., 2015); however, it evidentially increases the production and management cost (Lay et al., 2011) and the incidences of mortality and keel fracture (Wilkins et al., 2004; Rodenburg et al., 2008; Käppeli et al., 2011; Jung et al., 2019).

Furnished cages (also called enriched cages) with perches, nests, litter, and other facilities allow the native behavior and improve the welfare of hens compared with conventional cages (Leyendecker et al., 2005; Sherwin et al., 2010; Lay et al., 2011). However, their usage can lead to some welfare and health problems such as keel damage (including keel fracture and deviation) (Rørvang et al., 2019). Some studies have shown that keel fractures cause pain and stress response (Riber et al., 2018; Wei et al., 2019). The incidences of keel fractures increase with the hen's age, with a peak

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record during laying period, which gradually decrease after 42 wk of age (**WOA**) (Gebhardt-Henrich and Fröhlich, 2015; Stratmann et al., 2015). Heerkens et al. (2015) found that incidences of keel fractures were positively correlated with foot pad disorders. In addition, keel fractures influenced the performance and egg quality in laying hens. Nasr et al. (2012b, 2013) and Candelotto et al. (2017) found that in laying hens with keel fractures, the egg production, its weight, and eggshell surface area were lower than those in hens without keel fractures. Candelotto et al. (2017) further investigated the relationships between susceptibility to keel fracture and egg quality. The results indicated that the higher the susceptibility of laying hens to keel fracture, the thicker the eggshell and the lower the broken strength.

The keel is an important structural bone in birds that helps flying and breathing (Claessens, 2009). Keel fracture changes behavior and resting locations, reduces motility, and affects the utilization of facilities for laying hens (Nasr et al., 2012a, b). Casey-Trott and Widowski (2016) studied the changes in behavior of laying hens with and without keel fractures in small and large furnished cages. They found that fractured keel (**FK**) hens spent more time resting on the perch than standing on floor compared with the hens without FK. However, Nasr et al. (2012a) found that FK hens spent more time sleeping on the floor and rarely used the perch that was 100 cm above the ground. In addition, nesting time of FK hens was longer than that of normal keel (**NK**) laying hens (Gebhardt-Henrich and Fröhlich, 2015). However, most of the behaviors, such as walking, jumping, sitting, preening, and comforting, have not been systematically studied. Therefore, to further understand the effect of keel fractures on behavior, welfare, production performance, and egg quality in laying hens, healthy Lohmann white laying hens were housed individually in furnished cages, and their status of keel bone was assessed regularly.

MATERIAL AND METHODS

Ethics Statement

All experiments were approved by and conducted according to the guidelines of the Institutional Animal Care and Use Committee of Northeast Agriculture University (IACUCNEAU20150616).

Experimental Animals and Management

A total of 90 healthy Lohmann white laying hens of 17 WOA were used in this study and individually housed in furnished cages, each of size 50 cm length × 70 cm width × 70 cm height, having 2 wooden square perches (20 cm and 40 cm above the wire-mesh floor, and 45 cm and 25 cm away from front wire-mesh sidewall, respectively). The horizontal distance between 2 perches was 20 cm, and there was 1 closed nest box (35 cm length × 20 cm width × 25 cm height, installed on the left and rear of the sidewalls), 1 nipple drinker, and 1 rectangular feeder (installed outside of the front sidewall). The laying hens house was semi-enclosed with a combination scheme of natural light and artificial light. Artificial light was programed for 16 h (5:00–21:00 h), followed by 8 h of dark, and light intensity was 18–22 lux. The house had natural ventilation. During the entire experiment period (17–42 WOA), all laying hens had free access to feed and water. The temperature of laying hens house was 20–28°C, and the relative humidity was 45–70% throughout the experiment. Laying hens were provided with a standard commercial layer diet of 2,800 kcal/kg metabolic energy and 16.08% crude protein.

Assessment of Keel Fracture

Assessment of keel damages for all laying hens was performed by one worker according to the palpated method (Scholz et al., 2008b; Casey-Trott et al., 2015). Palpations were performed by running the thumb and index fingers down the spine or ventral and lateral surface edge of keel bone, feeling for alterations such as S-derivations, bumps or depressions, and other indicators of keel damage. Keel status can be classified as NK, deviated keel (**DK**), and FK. DK is indicated with the deviation of the keel bone from a 2-dimensional straight plane in either the transverse or median sagittal plane, including bending, S-shaped, twisted, or curved keels (Casey-Trott et al., 2015). FK is indicated with the presence of sharp bends, shearing, fragmented sections, callus materials, and clear bumps on the ventral and lateral surfaces of keel bone (Casey-Trott et al., 2015). If a hen had both a fractured and DK, then it was categorized as FK because the fracture was more likely to be associated with pain than deviations (Scholz et al., 2008b). In our experiment, keel status of all laying hens was assessed by palpation starting at 17 WOA and then palpated every 5 wk thereafter. According to the objective of this study, FK and NK hens were marked at each palpating examination according to their keel bone status, and the incidence of keel damage at each time-point was calculated according to palpated assessment, except 42 WOA. At the end of the experiment (42 WOA), all hens were euthanized and dissected to validate the accuracy of palpation, and the prevalence of keel damage was calculated based on the results of visual observation at this time-point.

Behavioral Observation

After palpation of FK and NK hens at each time-point, that is, 22, 27, 32, 37, and 42 WOA, behavioral observations were conducted for 2 consecutive days using a video recording system (Hikvision DS-IT5; Hikvision, Hangzhou, China). On each behavioral observation day, the behaviors of the laying hens with NK and fresh FK (n = 6 each) were observed for 7 h. This included 2 h each, in morning (08:30–10:30 h), afternoon (12:30–14:30 h), and evening (16:00–18:00 h), as well as

30 min before and after artificial lighting was turned off (20:30–21:30 h). The behaviors were classified as "state" and "event" behaviors. The state behaviors were standing, feeding, walking, sitting, perching, and nesting, and these data were collected using the Focal Animal Sampling method at 5-s interval over 7 h of observation period, with each behavior being counted as the percentage of the total observation times. The event behaviors were drinking, cage pecking, preening, comforting, and jumping. Continuous recording and one-zero sampling method were used to sample each event behavior over 7 h of observation period, and each event behavior was expressed as the total numbers sampled. The results were calculated by the mean of each behavior expression over all observation time. The definition of all the behaviors is shown in Table 1.

Production Performance and Egg Quality

Measurement of Production Performance To compare the performance of the NK and FK hens, the hens from 3 different age groups (ie, 27–32, 32–37, and 37–42 WOA) were used as the focal birds. First, the number of eggs laid, dirty eggs, broken eggs, and feed intake from all hens (except FK hens) were noted at 27 WOA. These laying hens were then palpated at 32 WOA to determine NK or FK. The performance data and feed intake of the NK and FK hen groups at 27–32 WOA were measured based on the assessment of keel status at 32 WOA. Namely, if a hen had NK at 32 WOA, the performance data and feed intake of this bird at 27–32 WOA period were categorized into the NK group. At 32 WOA, if a hen had FK, its corresponding data were categorized into the FK group. Similarly, hens had NK or FK at 37 and 42 WOA, and their performance data and feed intake at 32–37 and 37–42 WOA were also classified into the FK or NK group, respectively.

Measurement of Egg Quality Following the day after behavioral observation at each time-point, 10 eggs were collected from other NK and FK hens (n = 10, each) to analyze its quality in terms of egg weight (g), eggshell weight (g), yolk weight (g), and eggshell thickness (mm). Thickness was measured using an electronic digital caliper at both ends and middle, and eggshell strength (kg/cm^3) was determined using an egg force reader (ORKA-ESTG-1; ORKA Technology Ltd, Ramat Asharon, Israel). Albumen height (mm) was determined using an egg-quality gauge (EMT-5200, Japan), and egg-shape index was calculated as egg width/egg length × 100% (Das et al., 2010). Haugh unit was calculated using the following formula: 100log ($H-1.7\ W^{0.37} + 7.6$), where H is albumen height (mm); W is egg weight (g); and 100, 0.37, and 7.6 are constants. Egg surface area (cm^2) was calculated using the following formula: $3.9782\ W^{0.7056}$, where W is egg weight (g) and 3.9782 and 0.7056 are constants.

Table 1. Behavioral categories and definitions.

Behaviors	Definitions[1]
State behaviors	
Standing	Laying hen standing on feet, legs extended, no movement of the body but with eyes open. Head is erect or in relaxed posture
Feeding	Laying hen directing its head in feed trough and carrying out pecking, ingesting, or eating feed, once or repeatedly. Including standing breaks of ≤5s followed by resumption of behavior
Walking	Moving more than 3 paces in one direction, and head erect
Sitting	Laying hen sitting with abdomen touching on the ground, wings closed, eyes opened, and without any extra activity
Perching	Laying hen with 2 feet on a perch for more than 3s, including standing, sitting, and walking
Nesting	Including standing, sitting, laying, preening in the nest box
Event behaviors	
Drinking	Laying hen directing its head to nipple drinker, pecking and swallowing water. Including standing breaks of ≤5s followed by resumption of behavior
Preening	Laying hen using its beak to gently peck, nibble, comb, preen feathers, or using claw to scratch its wings or head
Comforting	Including body shaking, tail shaking, wing raising or stretching, leg stretching, simultaneous wing and leg stretching, and sham dustbathing
Cage-pecking	Pecking or scratching perch, nest box, or floor of cage
Jumping	Laying hen jumping up or down from the perch

[1]Behavioral definitions from Webster and Hurnik, 1990; Zhao et al., (2014); Casey-Trott and Widowski, (2016).

Assessment of Welfare

After each palpation at 27, 32, 37, and 42 WOA, new hens from NK and FK groups (n = 10 except, in KF group at 42 WOA, where n = 6) were randomly selected to assess welfare parameters, including body weight, feather cover, foot pad and toe health, and tonic immobility test (**TI**).

Tonic Immobility Test The selected NK and FK hens were brought into a quiet room and placed lying on their backs in a U-shaped wooden groove. To induce TI, a tester gently pressed the hen's head for 15s with one hand and their chest with another. To ensure the success of TI, the testing hens had to stay still for at least 10 seconds. If each trial was successful, the TI duration of the bird kept in the lying position until it turned over and stood up was recorded. Each time, the bird was induced 3 times. If the hens were stationary for 20 min, a TI duration of 20 min was recorded (Alm et al., 2016). The mean TI duration of all the NK and FK hens at each time-point was used in the data analysis.

Feather Cover After TI was taken, the feather cover score was taken on 8 parts of the body: head, neck, back, tail, wings, chest, cloaca, and legs. The conditions of feather cover were scored from 0 to 3 (Wechsler and Huber-Eicher, 1998): 0, best feather condition with no damage; 1, having slight damage but skin well covered; 2, feather with moderate damage and area skin of <1 cm × 1 cm exposed; 3, feather having severe damage and area skin of >1 cm × 1 cm exposed. Total feather cover scores for all parts were calculated for each bird; the lowest (best feather) score was 0 and the highest (worst feather) score was 24. The mean feather cover score from all the hens at each time-point was used for the data analysis.

Foot Pad and Toe Health Foot pad condition and toe health were assessed for hyperkeratosis, dermatitis, and bumble foot (Heerkens et al., 2015). Hyperkeratosis of both foot pad and toe was scored as 0 (absent) or 1 (present). Foot pad and toe dermatitis were scored as 0–2: 0, perfect foot pad and toe, with no damage; 1, slight dermatitis on foot pad and toe with a small epithelium lesion (<0.2 cm); 2, severe dermatitis on foot pad and toe with a large epithelium lesion (>0.2 cm). Bumble foot was scored as absent (0) or present (1). The mean score of foot pad and toe health was obtained from all hens at each time-point for the data analysis. All hens were weighted after welfare assessment.

Statistical Analysis

A statistical analysis was performed using the SPSS 22 software (SPSS Inc., Chicago, IL). All the data were tested for normal distribution using the Kolmogorov-Smirnov test. The differences in behaviors, egg quality, and welfare from NK and FK laying hens at each time-point were analyzed using one-way ANONA with Duncan's multiple comparison. Production performance and feed intake data were analyzed by a repeated-measures analysis. As production performance data followed the Mauchly's Test of Sphericity, one-way ANONA was used to examine the difference in production performance with Levene's test for quality of variances and Duncan's multiple comparison. Owing to feed intake data not meeting the Mauchly's Test of Sphericity criteria, a multivariate ANOVA was used under a general linear model with repeated measurements to analyze the difference in feed intake using the tests of between-subjects factor (NK and FK groups) and with-subjects factor (27–32, 32–37, and 37–42 WOA). The results were expressed as mean ± SEM, and differences of $P \leq 0.05$ were considered statistically significant.

RESULTS

Assessment of Keel Bone Fracture

At 42 WOA, the results of palpation showed that there were 46 (51.1%) FK hens, 21 (23.3%) DK hens, and 23 (25.6%) NK hens among the total. On sacrifice, the visual observation results indicated that there were 49 (54.4%) FK hens, 22 (24.4%) DK hens, and 19 (21.2%) NK hens. Therefore, compared with visual observation results, the palpated accuracy of FK and DK was reduced by 3.3% and 1.1%, respectively, and that of NK was increased by 4.4%.

As shown in Figure 1, the keel bones of all laying hens were normal at the initiation of the study. However, at

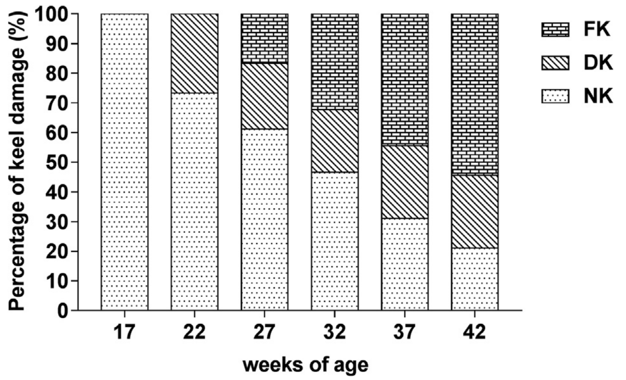

Figure 1. Percentages of laying hens with normal keel (NK), deviated keel (DK), and fractured keel (FK) bones at 6 time-points.

22 WOA, 26.7% of laying hens had DK bones, but no FK. At 27 to 42 WOA, DK remained generally stable at 21.1–24.4%, while FK consistently increased from 16.7% to 54.4%, and NK consistently decreased from 61.1% to 21.2%. Therefore, with increase of age, the keel fracture also increased from 16.7% at 27 WOA up to 54.4% at 42 WOA.

Behavioral Observation

Behaviors of NK and FK laying hens are shown in Table 2. Compared with NK hens, the standing and sitting significantly increased ($P < 0.05$) while feeding, walking, perching, and jumping significantly decreased in FK hens ($P < 0.05$). However, there were no significant changes observed in nesting, drinking, preening, comforting, and cage-pecking behaviors between NK and FK laying hens ($P > 0.05$).

Measurement of Production Performance

The production performance of NK and FK laying hens at 3 experimental periods (27–32, 32–37, and 37–43 WOA) is shown in Table 3. During these periods, the mean egg production and daily feed intake of FK hens significantly decreased compared with those of NK hens ($P < 0.05$). The percentage of broken egg from FK hens was significantly increased at 27–32

Table 2. Behaviors of normal keel (KN) and fractured keel (FK) laying hens.

Item	NK	FK	*P* value
State behaviors			
Standing	28.60 ± 0.67	34.22 ± 1.02	0.001
Feeding	35.21 ± 0.83	31.03 ± 1.31	0.017
Walking	8.38 ± 0.45	6.05 ± 0.29	0.001
Sitting	7.63 ± 0.23	10.53 ± 1.14	0.025
Perching	13.07 ± 0.99	10.03 ± 0.46	0.015
Nesting	7.03 ± 0.60	8.07 ± 0.54	0.218
Event behaviors			
Drinking	177.25 ± 9.92	169.75 ±16.94	0.708
Preening	124.13 ± 9.85	119.88 ±11.20	0.780
Comforting	42.63 ± 3.83	32.50 ± 3.07	0.058
Cage-pecking	59.00 ± 3.52	54.63 ± 3.97	0.423
Jumping	23.88 ± 2.91	13.13 ± 1.19	0.004

Table 3. Performance and feed intake between normal keel (NK) and fractured keel (FK) laying hens at 3 periods.

Item (unit)	27–32 wk of age			32–37 wk of age			37–42 wk of age		
	NK	FK	*P* value	NK	FK	*P* value	NK	FK	*P* value
Number of birds	42	29	—	28	40	—	19	49	—
Number of eggs	1,388	931	—	949	1,318	—	651	1,645	—
Egg production (%)	94.44 ± 0.75	91.72 ± 0.88	0.023	96.84 ± 1.03	94.14 ± 0.97	0.011	97.90 ± 0.95	95.92 ± 0.86	0.041
Dirty egg rate (%)	2.32 ± 0.53	3.69 ± 0.64	0.103	2.12 ± 0.69	2.94 ± 0.58	0.364	2.00 ± 0.77	2.99 ± 0.48	0.278
Broken egg rate (%)	0.97 ± 0.31	2.42 ± 0.38	0.004	1.19 ± 0.54	1.90 ± 0.45	0.277	0.93 ± 0.62	2.31 ± 0.39	0.057
Daily feed intake (g)	116.24 ± 1.41	112.21 ± 1.14	0.031	121.76 ± 0.88	119.45 ± 0.72	0.048	126.11 ± 0.92	123.67 ± 0.74	0.045

WOA ($P < 0.05$); however, there was no significant difference observed at 32–37 and 37–42 WOA ($P > 0.05$). There was no significant difference in dirty egg rate between NK and FK hens at each experimental period ($P > 0.05$). Overall, keel fracture decreased the egg production and daily feed intake during the experimental period.

Measurement of Egg Quality

The comparisons between the quality of eggs from NK and FK laying hens at 4 timepoints (27, 32, 37, and 42 WOA) are shown in Table 4. At 27 WOA, the egg weight, eggshell thickness, eggshell strength, and egg surface area of FK hens were evidently less than those of NK hens ($P < 0.05$). At 27 WOA, the eggshell weight of FK hens tended to be lower than that of NK hens ($P = 0.051$), but the difference between the groups was not significant. Haugh unit from FK hens was lower than that of NK hens at 37 WOA ($P < 0.05$). Compared with NK hens, FK hens had lower eggshell thickness, strength, and weight at 32, 37, and 42 WOA ($P < 0.05$). Overall, keel fractures mainly affected the external, but not the internal, egg quality.

Assessment of Welfare

Welfare assessments of NK and FK hens at evaluated time-points (27, 32, 37, and 42 WOA) were as shown in Table 5. The hens with FK had an evidently higher TI value at all time-points ($P < 0.05$) and a significantly decreased body weight at 27 and 42 WOA ($P < 0.05$). However, there was no significant difference in feather cover score, foot pad and toe hyperkeratosis scores, and toe dermatitis score between NK and FK hens during the experimental periods ($P > 0.05$), except in FK laying hens at 37 WOA showing a greater score in foot pad dermatitis ($P < 0.05$). Furthermore, the incidence of bumble foot was not found in NK and FK laying hens throughout the experimental period.

DISCUSSION

It is well known that keel bone damage is a serious concern for the welfare and health of laying hens in commercial production systems (Harlander-Matauschek et al., 2015). The reports suggest a higher prevalence of keel fractures of about 63.0% and 96% in hens at 50 and 60 WOA, respectively, (Rodenburg et al., 2008; Petrik et al., 2015) in noncage housing systems. However, in furnished cages, 30% (Habig and Distl, 2013) or 53.3% of hens (Sherwin et al., 2010) have been reported to have FK bones. Our results showed that the prevalence of keel fracture in laying hens increased from 16.7% at 27 WOA to 54.4% at 42 WOA. Our results are in agreement with the aforementioned reports, indicating that the keel fractures were prevalent in furnished cages. Some researchers also suggest that the incidence of keel fracture increases with age of hens (Petrik et al., 2015; Casey-Trott et al., 2017;

Table 4. Comparation of egg quality from normal keel (NK) and fractured keel (FK) laying hens at 4 time-points.

	27 wk of age			32 wk of age			37 wk of age			42 wk of age		
Item (unit)	NK	FK	*P* value	NK	FK	*P* value	NK	FK	*P* value	NK	FK	*P* value
EW (g)	56.89 ± 0.66	54.88 ± 0.59	0.039	58.94 ± 1.81	57.98 ± 0.57	0.622	52.42 ± 0.62	51.41 ± 1.63	0.573	62.12 ± 1.18	61.27 ± 2.01	0.722
ESI (%)	77.00 ± 0.07	76.75 ± 0.09	0.835	77.46 ± 0.50	77.21 ± 1.02	0.834	77.64 ± 0.61	78.05 ± 0.61	0.639	77.38 ± 1.06	76.25 ± 2.25	0.222
EsT (mm)	0.39 ± 0.02	0.35 ± 0.02	0.001	0.41 ± 0.04	0.37 ± 0.01	0.007	0.39 ± 0.08	0.37 ± 0.05	0.044	0.41 ± 0.01	0.37 ± 0.02	0.002
EsW (g)	7.40 ± 0.11	7.02 ± 0.15	0.051	8.10 ± 0.14	7.64 ± 0.07	0.009	7.31 ± 0.17	6.78 ± 0.11	0.019	8.70 ± 0.07	8.14 ± 0.08	0.045
EsS (kg/cm^3)	4.63 ± 0.19	4.11 ± 0.11	0.031	4.59 ± 0.07	4.01 ± 0.18	0.011	4.38 ± 0.15	4.00 ± 0.09	0.040	4.86 ± 0.18	4.29 ± 0.13	0.020
ESA (cm^2)	68.86 ± 0.56	67.13 ± 0.51	0.040	69.79 ± 0.48	70.56 ± 1.52	0.637	65.00 ± 0.55	64.07 ± 1.44	0.558	73.26 ± 0.98	72.52 ± 1.68	0.708
HU	97.03 ± 1.25	94.65 ± 1.94	0.320	92.55 ± 1.83	89.87 ± 0.85	0.208	92.26 ± 0.83	90.13 ± 0.54	0.049	91.94 ± 1.18	91.01 ± 1.46	0.628
YW (g)	14.56 ± 0.49	14.17 ± 0.36	0.538	15.38 ± 0.25	14.61 ± 0.43	0.142	14.58 ± 0.42	15.33 ± 0.23	0.138	16.72 ± 0.34	16.16 ± 0.40	0.304
AH (mm)	9.35 ± 0.26	8.78 ± 0.41	0.265	7.97 ± 0.17	8.55 ± 0.41	0.205	8.08 ± 0.16	7.81 ± 0.10	0.195	8.56 ± 0.27	8.35 ± 0.32	0.625

Abbreviations: AH, albumen height; ESA, egg surface area; ESI, egg-shape index; EsT, eggshell thickness; EsS, eggshell strength; EsW, eggshell weight; EW, egg weight; HU, haugh unit; YW, yolk weight.

Table 5. Assessment of welfare between normal keel (NK) and fractured keel (FK) laying hens at 4 time-points.

	27 wk of age			32 wk of age			37 wk of age			42 wk of age		
Item (unit)	NK	FK	*P* value	NK	FK	*P* value	NK	FK	*P* value	NK	FK	*P* value
BW (kg)	1.56 ± 0.05	1.46 ± 0.10	0.024	1.62 ± 0.02	1.55 ± 0.03	0.081	1.65 ± 0.03	1.57 ± 0.08	0.080	1.69 ± 0.04	1.58 ± 0.04	0.040
TI (min)	5.64 ± 0.28	7.08 ± 0.50	0.025	6.35 ± 0.47	7.83 ± 0.41	0.033	5.30 ± 0.26	7.52 ± 0.27	0.001	7.30 ± 0.83	9.82 ± 0.80	0.046
FC (score)	0.75 ± 0.25	1.38 ± 0.26	0.107	1.00 ± 0.27	1.39 ± 0.32	0.387	1.38 ± 0.26	1.63 ± 0.18	0.448	1.01 ± 0.27	1.63 ± 0.38	0.196
TH (score)	0.25 ± 0.16	0.25 ± 0.16	1.000	0.25 ± 0.16	0.38 ± 0.18	0.619	0.38 ± 0.18	0.38 ± 0.18	1.000	0.38 ± 0.18	0.50 ± 0.19	0.642
TD (score)	0.25 ± 0.25	0.38 ± 0.18	0.693	0.38 ± 0.21	0.50 ± 0.18	0.705	0.38 ± 0.26	0.50 ± 0.19	0.705	0.50 ± 0.27	0.88 ± 0.29	0.362
FPH (score)	0.13 ± 0.13	0.13 ± 0.13	1.000	0.25 ± 0.16	0.25 ± 0.16	1.000	0.38 ± 0.18	0.25 ± 0.16	0.619	0.25 ± 0.16	0.38 ± 0.18	0.619
FPD (score)	0.50 ± 0.19	0.88 ± 0.35	0.362	0.88 ± 0.23	1.25 ± 0.25	0.285	0.75 ± 0.31	1.63 ± 0.26	0.050	1.25 ± 0.31	1.75 ± 0.25	0.233

Abbreviations: BW, body weight; FC, feather cover; FPD, foot pad dermatitis; FPH, foot pad hyperkeratosis; TD, toe dermatitis; TH, toe hyperkeratosis; TI, tonic immobility.

Rufener et al., 2018). Consistent with these results, we found that keel fracture did not occur at the onset of the study (17–22 WOA); however, the incidences increased to over 50% at 42 WOA. This may indicate that hens loss the calcium in their bones for eggshell mineralization at the high peak of laying (Whitehead, 2004). As the laying period continues, the reduction of calcium content in bone increases bone fragility, resulting in a higher risk of keel fractures in laying hens (Fleming et al., 2004).

During behavioral observation, we found that keel fracture impairs the mobility of laying hens. Standing and sitting behavior increased, and the feeding, walking, perching, and jumping behavior decreased in FK hens. This finding was consistent with the report of Nasr et al. (2012b), indicating that FK hens spent more time sitting on the floor and rarely used perches installed 100 cm and 150 cm high. This suggested that keel fracture causes pain and reduces the activity, thus resulting in increased sitting and decreased perching (Nasr et al., 2012a; Casey-Trott and Widowski, 2016). Sandilands et al. (2009) showed that keel fracture reduced the strength of flapping wings, thus decreasing the ability of the laying hens to sleep or rest on the perch. Therefore, the decrease observed in jumping and walking behaviors of FK hens suggests that keel fracture suppressed the mobility. We also speculated that this may be due to the pain caused by the pressure of abdominal muscles on FK bone involved in the processes of flighting or jumping between the ground and the perch.

Feeding behavior is one of the preferentially performed behaviors in hens. Nasr et al. (2013) found that the FK hens ate more than NK hens, while Casey-Trott and Widowski (2016) showed that there was no difference in feeding of laying hens with and without FK. Contrarily, we found that the feeding behavior and feed intake of FK hens was lower than those of NK hens. We speculated that this decrease in feeding may be related to the impaired mobility in hens and stress induced by keel fractures. Some researchers have reported that negative emotions, heat, and oxidative stress downregulate the expression of avian orexin and orexin receptors genes, which may inhibite the feeding motivation (Greene et al., 2016; Nguyen et al., 2017). Likewise, our previous study has found that keel fracture causes stress response and inhibits the gene expression of orexin and its receptors (Wei et al., 2019). Therefore, the decreased feeding behavior seen in FK hens may be attributed to the inhibited orexin system, induced by the stress from keel fracture.

In this study, NK hens laid more eggs than FK hens. This could to be related to the physiological stress induced by fracture, as it is known to reduce the production performance of laying hens (Odihambo et al., 2006). The difference in egg production between NK and FK laying hens was also noted by Thiruvenkadan et al. (2010), who indicated that keel fracture causes pain and stress, disrupts the hormones required for ovulation, and decreases the responses of granulosa cells to luteinizing hormone. In addition, FK hens had lower egg production was found by Rufener et al. (2018), who speculated that it might be due to the enhancement of bone metabolism and the redistribution of available resources to fracture healing. Likewise, a major reason for the decrease in egg production of FK hens may be the reduction of feed behavior and intake as seen in our study.

Eggshell qualities such as thickness and strength were strongly associated with the its calcification. Candelotto et al. (2017) found that an elevated susceptibility to keel fractures was related to thinner eggshells and lower egg breaking strength in laying hens. Nasr et al. (2012b) found FK hens had reduced eggshell weight and had a tendency to lay lighter eggs. Similarly, we found that eggshell weight, thickness, and strength of FK laying hens were lower than those of NK hens. Generally, each hen needs to absorb approximately 3 g of calcium per day to synthesize normal eggshell (Roberts, 2004). However, after keel fracture, this calcium is mainly used for fracture healing and calluses formation (Scholz et al., 2008a). Therefore, eggshell thickness and strength of FK hens were decreased. Furthermore, the lower daily feed intake and feeding behavior observed in FK hens could have directly contributed to the decrease in calcium resources for eggshell formation. In other words, in this study, the keel fracture mainly affected the egg production and external aspects of egg qualities such as eggshell thickness, strength, and weight but not internal qualities, such as protein height, Haugh unit, and yolk weight.

Furthermore, keel fracture has a detrimental impact on the welfare of laying hens. The high incidences of keel fracture were positively related to foot injuries in barn and organic production systems, and the occurrences of keel fracture and hyperkeratosis were higher at 62 WOA than at 32 WOA (Riber et al., 2016). Gebhardt-Henrich and Fröhlich (2015) showed that at 65 WOA, all laying hens with bumblefoot on both feet had a keel fracture in aviary system. Therefore, foot pad and toe health of laying hens may somehow be related to the occurrences of keel fracture in group-housing systems or may be related to reduced perch use in hens with keel damage. Dermatitis is an inflammatory response of the subcutaneous tissue of foot pad that can lead to necrosis, ulceration, and even bumblefoot. Bumblefoot causes sharp pain and can seriously affect the welfare in laying hens (Lay et al., 2011). There were no differences in hyperkeratosis and dermatitis on both feet and toes in our study. This may be related to the hens being housed individually in furnished cages. In noncage and organic production systems, FK hens had severe claw injuries associated with litter quality and housing resources (Wang et al., 1998; Hocking and Wu, 2013). The percentage of claw injuries in FK hens housed in furnished cages were lower than that in the noncage systems (Lay et al., 2011; Riber et al., 2016) because the caged laying hens had limited utilization of litter resources (Rørvang et al., 2019). In the present study, we did not provide litter for laying hens in furnished cages, and there was no difference observed in the foot pad and toe injuries between NK and FK hens. Thus, the litter could be a major cause for the foot pad and toe injuries. Assessment of feather condition was also one of the important indicators of welfare state in laying hens (Rodenburg et al., 2008). In modern commercial production systems, poor feather quality is mainly caused by severe feather pecking; its occurrence is difficult to be controlled in layers of flocks (Gilani et al., 2013). This behavior leads not only to extensive feather loss and body injuries but also to cannibalism and death in laying hens (McAdie and Keeling, 2000; Gilani et al., 2013; Hartcher et al., 2015). Therefore, a greater feather quality represents a higher welfare state, and vice versa. However, there was no difference in feather quality observed in NK and FK hens in our study. This perhaps suggests that keel fracture and feather quality were not related in this study of laying hens housed individually in furnished cages.

The response of the birds to fear was used to assess their welfare. The fearfulness can be defined as a bird's avoidance of danger (Jones, 1996) and is considered to be a suffering state (Alm et al., 2016). Recording the TI duration is a common method to evaluate a bird's fearfulness. Generally, the longer the TI duration, the higher the level of fearfulness (Jones, 1996; Forkman et al., 2007). Layer strain, early life experiences, and social environment can influence the fear state in birds (Barlow, 2000; Jacobson-Pick et al., 2011; de Haas et al., 2012). In the present study, the duration of TI from FK hens was evidently longer than that from NK hens, indicating that keel fracture caused fear. We speculated that this fear may be from keel fracture–induced pain or stress, which needs to be further investigated.

CONCLUSION

In summary, our results demonstrated that keel fracture caused changes in state behaviors (ie, standing, feeding, walking, sitting, and perching), but not event behaviors (ie, drinking, preening, comforting, and cage-pocking). Keel fracture lead to a longer duration of TI in laying hens and decreased their daily feed intake, egg production, broken egg rate, and eggshell quality. Therefore, keel fracture can change parts of behavior and reduce welfare, performance, and external egg quality of laying hens.

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