Growth performance, well-being, and gut microbial population of broilers raised in cages and floor pens under the hot and humid tropical climate

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ABSTRACT

The study was carried out to compare the growth performance, gut microbiota, incidence of footpad dermatitis (FPD), leg weakness, physiological, and immunological responses in broilers kept in cage (CS) and floor pen (FS) rearing systems under a tropical environment. One-day-old male Cobb 500 chicks were allocated to either three-tiered wire-floored battery cages or floor pens with wood shavings. The body weight on day 42 and feed intake from days 1 to 42 of caged chickens were lower than those of chickens reared in floor pens. However, the caged chickens had significantly better overall (days 1–42) feed conversion ratios than those on floor pens. The FS system was detrimental to the incidence of FPD. The FS birds showed higher and lower caecal counts of \textit{Escherichia coli} and \textit{Salmonellae}, respectively than the CS birds. The FS birds had longer durations of latency-to-lie than their caged counterparts, which suggested that the former had a lower incidence of leg weakness. Higher serum basal concentrations of corticosterone, ovotransferrin and ceruloplasmin were noted in the FS chickens compared to the CS group. Antibody titre against Newcastle disease vaccinations was not affected by the rearing system. In conclusion, the cage rearing system appeared to benefit performance, the incidence of FPD, physiological response, fear reactions, and intestinal population of \textit{E. coli} in broiler chickens under the hot and humid tropical conditions. However, raising broilers on floor pens improves body weight and leg strength, and reduces the caecal \textit{Salmonellae} population compared to caged broilers.

HIGHLIGHTS

- Caged broilers have better feed efficiency and lower caecal \textit{Escherichia coli} population and incidence of food-pad dermatitis than those on floor pens.
- Raising broilers on floor pens improves body weight and leg strength, and reduces the caecal \textit{Salmonellae} population compared to caged broilers.
- Broilers on floor pens are more physiologically distressed than their caged counterparts.

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Introduction

The hot and humid climate is a major constraint for optimum poultry production in the tropics (Oloyo 2018). If temperatures are high, sensible heat loss is minimized and almost all heat will have to be lost as insensible (latent) heat. Latent heat loss is the heat lost from the body through the elimination of respiratory moisture and its efficiency is impaired by high humidity. Hence, the combination of high temperatures and increased humidity is detrimental to poultry and may depress growth performance, immune response, disease resistance, and survivability (Syafwan et al. 2011; Saeed et al. 2019; Abioja et al. 2020).

Litter floor pens and cages are conventional rearing systems for commercial broiler chicken production in the tropical and sub-tropical regions (Prabakaran 2003). Both floor pen and cage rearing systems may have beneficial and adverse impacts on growth performance, health, and welfare of broiler chickens (Wang et al. 2015). For example, floor pens with a poor litter quality may lead to a higher incidence of pododermatitis and hock burn in broilers (Berg 2004). On the contrary, broilers raised in cages had increased mortalities and leg disorders when compared to those on litter floors (Fouad et al. 2008; Sogunle et al. 2008; Shields and Greger 2013). Rearing chickens in cages...
may also prevent the expression of natural behaviours such as ground scratching and dustbathing (Shields and Greger 2013). However, separating broilers from their faeces, as in a cage system, provides a better hygienic condition compared to the litter rearing system (Willis et al. 2002) and may reduce the occurrence of diseases (Al-Bahouh et al. 2012), and thus have welfare benefits (Duncan 2001). Because the rearing system may have a profound economic impact, it is crucial to determine the relationships among the rearing system, welfare, health, and growth performance. Accordingly, decisions can be made on which rearing system is acceptable for commercial production. Although the effects of floor pen system (FS) and cage system (CS) on welfare and growth performance of poultry are well documented, the findings are inconsistent as some studies recommended rearing broilers in litter-based housing system (Fouad et al. 2008; Özhan et al. 2016) while others recommended cages (Kim et al. 2014) or did not observe obvious welfare differences between floor pens and cages (Fortomaris et al. 2007). These discrepancies could be attributed to variations in the stocking densities practiced for different rearing systems. Unlike earlier work, in the present study, both FS and CS broilers were stocked at a similar density and this is important to ensure that stocking density is not a confounding factor in the comparison between the two rearing systems. Furthermore, earlier work comparing both rearing systems were mainly conducted under temperate conditions. In a hot tropical environment, chickens tend to drink more to alleviate heat stress (Bruno et al. 2011; Sayed and Downing 2011) and consequently more water will be excreted through droppings, which in turn will result in wet litter problem. Wet litter may increase obnoxious odour coming from ammonia and bacterial action in the droppings and this may compromise the welfare of chickens through irritation of the mucous membrane, distress, susceptibility to respiratory diseases, contact dermatitis, and leg weakness (de Jong et al. 2014).

Behaviour as a measurement of welfare in farm animals has generated much discussion and controversy, but it is important in providing information about animals’ needs, preferences, and internal state (Mench 2004). There has been substantial work on the effect of the cage system on the behaviour of both broilers and laying hens (Fortomaris et al. 2007; Fouad et al. 2008; Chen et al. 2019). Hence, the current experiment aimed to use physiological stress response, immune response, fearfulness, the incidence of leg weakness and footpad dermatitis (FPD), caecal bacterial count, and growth performance to assess the well-being of broiler chickens in floor pen and cage rearing systems while maintaining an identical floor space/stocking density.

Elevation in circulating concentrations of corticosterone (CORT) is a hallmark of stress in avian species (Scanes 2016). Recently, Zulkifli et al. (2014) showed that exogenous administration of CORT modified serum concentrations of acute phase proteins (APP): ovotransferrin (OVT), ceruloplasmin (CPN), and alpha-1 acid glycoprotein (AGP) in broiler chickens. Stressors such as overcrowding (Shakeri et al. 2014; Najafi et al. 2015), high temperature (Najafi et al. 2015; Olubodun et al. 2015), and feed deprivation (Najafi et al. 2016) may modify serum APP concentration. The APP have a pivotal role in maintaining the homeostasis in animals with respect to psychophysical (non-inflammatory) stressors (Murata et al. 2004). Footpad dermatitis and leg weakness were included in the present study because both welfare problems are closely associated with rearing system (Shields and Greger 2013). Jones (1997) considered fear as an undesirable state of suffering and as a powerful and damaging stressor. Housing birds in different rearing systems have been found to induce different levels of underlying fearfulness (Campo et al. 2008; Al-Aqil and Zulkifli 2009). Moreover, free-ranged hens showed less fearfulness level compared to their caged counterparts (Scott et al. 1998). It is possible, therefore, to hypothesise that housing chickens in an environment with more complicity can dampen underlying fearfulness. The rearing environment is considered an important factor in determining gut microflora in poultry (Gabriel et al. 2006). Santos et al. (2008) reported that chickens raised on litter floor pens had lower intestinal Salmonella colonisation than those in cages. The authors suggested that the threat of Salmonella was reduced when chickens had access to faecal matter, which served as a seeding agent for competitive exclusion microorganisms. However, Willis et al. (2002) noted that chickens raised on litter floor pens had a higher probability of infection and excretion of Campylobacter jejuni than their caged counterparts.

Materials and methods

Chickens, management and housing

Two hundred Cobb 500 male chicks (1-day-old) were procured from a commercial hatchery. Upon arrival (day 1), the chicks were wing-banded for identification, and their individual body weight was recorded
An equal number of chicks were randomly assigned to either battery cages with wire floors or litter floor pens (each with 10 replicates; 10 birds per replicate). Both rearing systems were in a conventional naturally ventilated house. The profile of environmental temperature and relative humidity during the experimental period is shown in Table 1. The floor space allowed for both rearing systems was 1044 cm² per bird. The CS broilers were placed in stainless steel three-tier-cages with wire floors. Each cage measured 116 (width) × 90 (length) × 50 (height) cm³. The cage’s mesh was constructed of 7 mm diameter wire and contained 6.25 cm square orifices. Feeders (trough type) were placed outside the cages. The FS chicks were placed on a concrete floor using wood shavings as litter material (the depth was 8 cm). The width and length of each floor pen were similar to the battery cage, but the height was 70 cm. Each pen was provided one plastic hanging tube feeder. Each cage and pen were provided with two bottle drinkers. The feeding and drinking spaces allowed for each bird in both systems were 9 cm and 4.2 cm, respectively. The lighting durations provided for both CS and FS birds were continuous for the first two days, and from day 3 onwards, lighting was 18 h per day. Birds were fed commercial broiler starter (crumble form) (12.56 MJ/kg ME and 21.0% CP) and finisher (pellet form) (12.98 MJ/kg ME and 19.0% CP) from day 1 to 21, and day 22 to 42, respectively. The chicks were vaccinated against Newcastle disease intraocularly (Sunvac ND Clone, Sunzen Biotech, Shah Alam, Selangor, Malaysia) at 7 and 21 days of age.

### Growth performance

Prior to feeding, individual body weight was recorded on days 1, 21, and 42. Feed intake from days 1 to 21 and 22 to 42 was recorded, and feed conversion ratios (FCR) (feed/gain) were calculated.

### Tonic immobility test

At 42 days of age, three chickens per pen and cage were randomly selected for a tonic immobility (TI) test. Each bird was caught gently using the two hands, held in an inverted manner, and transferred to a different room (without having visual contact with their peers) for the TI test. The TI was measured using the procedure of Benoff and Siegel (1976) with some modifications. The induction for TI started as soon as the chicken reached the TI room by restraining the chicken on its right side/wing for 15 s. After the induction, the experimenter retreated about 1 m but remained within sight of the chicken, making neither noise nor unnecessary movement. During the TI induction, there was no direct eye contact between the experimenter and the chicken to avoid the prolonged duration of TI (Jones 1986). A stopwatch was used to record latencies until the chicken righted itself. If the chicken righted itself in less than 10 s, the TI induction procedure was repeated. If TI was not induced after three attempts, 0 s was considered as the duration of TI. A total of 600 s was allowed as the maximum TI duration. Upon TI measurement, the chicken was spray-painted on its back for identification and returned to its cage or floor pen. It is assumed that neither the procedure of catching nor the returning of chickens disturbed the other flock members (Lagadic et al. 1990).

### Latency-to-lie test

The latency-to-lie (LTL) was conducted following the methods described by Weeks et al. (2002) and Berg and Sanotra (2003). On day 42, three birds (those that were not tested for TI test) from each cage and pen were selected at random and removed one by one with minimal disturbance to other members of the flock. The birds were placed individually in a container (150 × 90 × 50 cm³) filled with water (3 cm) with no visual contact with other chickens, and the time spent standing before making the first attempt to sit down was recorded (maximum = 600 s). All the birds were placed in the containers by the same experimenter, and the three birds from the same pen/cage were tested individually at one time.

### Incidence of footpad dermatitis

Footpad lesions were scored in each bird on day 41 through the visual scoring system (Table 2), as described by Nagaraj et al. (2007). All birds were scored by the same experimenter.

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**Table 1.** The profile of mean environmental temperatures and humidity during the experimental period.

| Experimental period | Temperature, °C | Relative humidity, % |
|---------------------|----------------|----------------------|
|                     | Minimum | Maximum | Minimum | Maximum |
| Week 1              | 25      | 33      | 56      | 94      |
| Week 2              | 28      | 35      | 49      | 89      |
| Week 3              | 23      | 31      | 70      | 100     |
| Week 4              | 25      | 31      | 66      | 94      |
| Week 5              | 25      | 33      | 59      | 94      |
| Week 6              | 28      | 34      | 59      | 89      |
Blood parameters

At 42 days of age, two chickens from each cage and pen were selected at random and removed (on an individual basis) with minimal disturbance effect on the other members of the flock. The birds were immediately slaughtered following the halal procedure (Farouk et al. 2014), blood samples were obtained, and serum samples were harvested and kept at \(-80\) °C until further analysis. The CORT was measured by using a commercial EIA kit (AC-15F1, IDS, Boldon, UK) following the instructions of the manufacturer. This kit had intra-assay and inter-assay variability of < 6.7% and < 7.8%, respectively, with 27 ng/mL limit of detection. The CPN was measured by the rate of formation of a coloured product from the substrate, 1,4-phenylenediamine dihydrochloride, and CPN (Zulkifli et al. 2014). The modified radial immunodiffusion method (Mancini et al. 1965) was used to measure OVT. Antibody response to live Newcastle disease (ND) vaccine was used to evaluate the humoral immunity of the birds. Antibody titre to ND vaccines was measured by ELISA using commercial kits (IDEXX Laboratories, USA) in accordance with the recommended protocols of the manufacturer.

Caecal microflora quantification

Following blood collection, the caecal content was collected, rapidly frozen in liquid nitrogen, and stored at \(-80\) °C. A quantitative real-time PCR method was used for the determination of Salmonellae, Campylobacters, Clostridia, and Escherichia coli populations (Bello et al. 2018). The DNA extraction from the caecal content samples was done by QIAamp® DNA Stool Mini kits following the manufacturer’s instruction. After the PCR products being purified using the MEGA quick-spin™ (Intron Biotechnology, Inc), a Nanodrop ND-1000 spectrophotometer was used for measuring the purity and concentration of the extracted DNA samples. The number of copies of a template DNA per mL of elution buffer was calculated accordingly. Standard curves were formed using a serial dilution of PCR products from pure cultures. The primers used for the determination of bacterial quantifications are presented in Table 3. The q-PCR was performed with the BioRad CFX96 Touch (BioRad, USA) using optical grade plates. The PCR reaction was carried out in 25 μL total volume using the iQTM SYBR Green Supermix (BioRad, USA). Each reaction consisted of 12.5 μL SYBR Green Supermix, 1 μL of each Primer, 1 μL of DNA samples, and 9.5 μL nuclease-free H2O. The reaction conditions for amplification of DNA started with an initial denaturation (1 min at 94 °C), followed by 35 amplification cycles (30 s at 90 °C, 30 s at 54 °C, 60 s at 72 °C), and the final extension (8 min at 72 °C) for Salmonellae, 1 cycle (6 min at 94 °C) for denaturation, followed by 35 cycles (1 min at 94 °C, 1 min at 57 °C and 1 min at 72 °C) and a final extension of (7 min at 72 °C) for Campylobacters, 94 °C for 2 min, 30 cycles (94 °C for 30 s, 56 °C for 30 s and 72 °C for 30 s) and extension at (72 °C for 10 min) for E. coli or 60 °C (Clostridia) for 30 s, and 72 °C for 20 s. To confirm the specificity of amplification, a melting curve analysis was carried out after the last cycle of each amplification. The DNA copy numbers of each template per mL of elution buffer were calculated using the formula available online (http://cels.uri.edu/gsc/cndna.html) and then converted to cells/g of digesta.

### Statistical analysis

Data were subjected to ANOVA using the GLM procedure of SAS (SAS 2005). All data were analysed using the rearing system as the main effect. Cage level was also included as a main effect in the analysis of TI duration and number of inductions to elicit TI in CS birds. Individual chicken samples were used in the analysis of body weight, physiological, TI, LTL, FPD and gut bacterial population data. The feed intake and FCR data were analysed with cage/pen samples used in the analysis. Due to heterogeneity of variances, prior to analyses, the tonic immobility duration and antibody titre against ND vaccination data were transformed to common logarithms (log 10) Untransformed means are presented in the figures. Chi-square was

### Table 2. Scoring system of footpad dermatitis.

| Score | Description                                                                 |
|-------|-----------------------------------------------------------------------------|
| 0     | Footpads with no lesions, dermal ridges intact within central plantar footpad surface, with or without discolouration. |
| 1     | Footpads with mild lesions, dermal ridges not intact within a central, round to oval ulcer on the central plantar footpad surface, roughened lesion surface with small tag of crust. |
| 2     | Footpads with severe lesions, a brown crust >1.5 cm in diameter adhered to the central plantar footpad, sometimes extending up to the hock joint. |

### Table 3. Primers used in real-time PCR.

| Primer | Primer |
|--------|--------|
| Salmonellae | F-5’-TCGTACATCCATCACCTACCC-3’ |
|            | R-5’-AAAGTTGAAAGACGGAAGA-3’ |
| Campylobacters | F-5’-CTG TAT ATC CCT TAA GTG CAC CC-3’ |
|            | R-5’-AGG CAC GCC TAA ACC TAC TAT AGC T-3’ |
| Escherichia coli | F-5’-GTG TGA TAT CTA CCC GGT TCG C-3’ |
|            | R-5’-AGA ACC GTT GTG TGG TAA TCA CGA-3’ |
| Clostridia | F-5’-GAG TTT GAT CMT GGC TCA G-3’ |
|            | R-5’-CCC TTT ACA CCC AGT AA-3’ |
used for the analysis of the mortality data. The statistical significance is considered at \( p \leq .05 \).

**Results**

**Growth performance**

The growth performance and mortality rates of broilers attributed to the rearing system are shown in Table 4. The rearing system did not significantly \((p > .05)\) affect feed intake from days 1 to 21 (starter period). Overall (days 1–42), the FS birds consumed significantly \((p < .05)\) more feed than their CS counterparts. On day 21, the rearing system had a negligible effect \((p > .05)\) on body weight. However, the FS broilers showed significantly \((p < .05)\) greater body weights than those of CS on day 42. The FCR of FS and CS birds from days 1 to 21 were not significantly different \((p > .05)\). From days 1 to 42, the CS broilers had significantly \((p < .05)\) better FCR than their FS counterparts. The mortality rate was not significantly \((p > .05)\) changed by the rearing system.

**Tonic immobility test**

Both CS and FS broilers showed similar durations of TI (Figure 1(a)). However, the number of inductions to elicit TI was significantly \((p < .05)\) higher in CS birds when compared to those reared in floor pens (Figure 1(b)). Cage level had no significant \((p > .05)\) effect on TI duration and number of attempts to induce TI in the CS birds (Figure 2(a,b)).

**Latency-to-lie test**

The FS broilers exhibited significantly \((p < .001)\) longer LTL duration compared to their CS counterparts on day 42. (Figure 3).

| Item                          | Floor pen              | Cage              |
|-------------------------------|------------------------|-------------------|
| Feed intake, g/bird           |                        |                   |
| Days 1–21                     | 1341.70 ± 8.060^a      | 1321.20 ± 10.710^a|
| Days 1–42                     | 4648.70 ± 59.40^a      | 4277.20 ± 53.150^a|
| Body weight, g/bird           |                        |                   |
| Day 21                        | 1012.20 ± 9.680^a      | 991.10 ± 7.960^a  |
| Day 42                        | 2475.70 ± 37.370^a     | 2346.60 ± 22.670^a|
| Feed conversion ratio, feed/gain |              |                   |
| Days 1–21                     | 1.330 ± 0.014^a        | 1.340 ± 0.017^a   |
| Days 1–42                     | 1.880 ± 0.021^a        | 1.790 ± 0.021^b   |
| Mortality, %                  | 4.5^*                  | 5^a               |

Means within rows followed by different superscript letters are significantly different \((p < .05)\).
Footpad dermatitis score

There was no recorded incidence of FPD (Figure 4) among the CS chickens on day 41. On the contrary, the mean FPD scores of FS chickens was 1.0.

Caecal microflora quantification

Rearing system showed no effect on populations of Campylobacters and Clostridia (Figure 5(a,b)). However, CS chickens had significantly \( p < .001 \) lower E. coli population and higher Salmonellae population \( p < .01 \) compared with the FS group (Figure 5(c,d)).

Blood parameters

Raising broilers on floor pens significantly \( p < .001 \) elevated basal concentrations of CORT, OVT \( p < .001 \), and CPN \( p < .01 \) compared to those of CS (Figure 6(a–c)). However, rearing system had no significant \( p > .05 \) influence on serum levels of antibody titres against ND vaccinations (Figure 6(d)).

Discussion

The current study showed that the rearing system may influence feed intake, body weight, and FCR in broilers. The FS birds consumed more feed, which resulted in greater body weights on day 42 when compared to the CS group. These results are consistent with those of Fouad et al. (2008), who reported that broilers reared on floor pens showed higher feed intake and greater weight gains than those in cages. However, Fouad et al. (2008) allowed a bigger floor space for the broilers reared on floor pens than those in cages. Kim et al. (2014) reported that caged broilers showed better growth performance than those on the floor, but the former were stocked at a lower density. Thus, it appears that stocking density is a more important determinant of growth performance than the housing system. In the present study, both CS and FS birds were allowed a similar floor space. The higher feed intake in the FS broilers compared to their CS counterparts could be associated with an enriched environment in the former. Beattie et al. (2000) reported that pigs raised in enriched environments (straw bedded pens) consumed more feed than those kept in barren pens (slatted floor pens). Despite showing greater body weights, the FS birds had poorer overall FCR than those of CS. The noted improved FCR in CS chickens compared to their FS counterparts could be attributed to less activity in the former (Iqbal et al. 2012). The superior FCR among the CS birds in this study may also be associated with the noted lower total number of gut E. coli when compared to the FS chickens. Similarly, Li et al. (2017) indicated that broilers raised on floor pens had a higher population of caecal E. coli than caged birds. The increased population of gut E. coli in the FS birds may be associated with litter quality. Wet litter encourages the growth of bacteria and other microorganisms, which can cause subsequent infections (Wang et al. 2016; Heitmann et al. 2020). In the present study, the caged birds’ droppings were collected in stainless steel trays and cleaned manually. One of the major advantages of keeping birds in cages is that they are separated from their droppings and thus increased hygiene which could be associated with the reduced caecal E. coli counts in CS birds noted in the present study. The close relationship between feed efficiency and gut microbiota in chickens has been well documented (Stanley et al. 2012). The overgrowth of pathogenic...
microflora was associated with a decreased nutrient digestibility, osmolality, pH, and growth performance, and increased viscosity of the supernatant of the small intestine in broilers (Mathlouthi et al. 2002; Schulze 1996) suggested that the higher caecal E. coli counts of broilers on floor pens could be associated with the increased prevalence of FPD, which may serve as a gateway to the bacteria. FPD is a serious economic and welfare problem in broiler production (Berg 2004). In the present study, the FS birds exhibited a mean FPD lesion scores of 1, but none of their CS counterparts’ birds were affected. Close contact with wet, sticky and compact litter, and high ammonia concentration could lead to FPD (Nagaraj et al. 2007). The current results agree with those obtained by Santos et al. (2008), who also found that caged broilers had higher caecal Salmonellae counts than those on the floor pens. Santos et al. (2008) suggested that the rough wood components in the litter may serve as a seeding agent for competitive exclusion microorganisms and this may reduce Salmonella colonisation in the gut. The coarse components of litter have also been shown to stimulate the proventriculus to produce more hydrochloric acid and subsequently reduced gizzard pH (Santos et al. 2008). Because Salmonellae are not acid-tolerant, lower gizzard pH can play a profound role in restricting the caecal colonisation of the bacteria.

The present findings showed that the rearing system had a negligible effect on the intestinal population of Campylobacters and Clostridia in broilers. Willis et al. (2002) studied the effect of the cage and floor rearing environments on the isolation trends of Campylobacter jejuni in broilers over a year and noted significantly higher isolations in those on floor pens compared to cages. On a cautionary note, however, the authors reported a seasonal effect on bacterial isolation. Hence, the effect of the hot and humid tropical environment on the gut population of Campylobacter spp. in broilers may be different from studies conducted under temperate conditions. The present results agree with those of (Kim et al. 2014) that the housing system had a negligible effect on the intestinal population of Clostridium spp. in broilers.

In agreement with earlier work (Mench 2004), the CS broilers, as measured by LTL test, had weaker legs than those of FS. Rizk et al. (1980) reported that caged broilers were more susceptible to gait problems, leg abnormalities, and impaired walking ability than those reared on the floor. The stress or discomfort caused by wire floors may have resulted in weaker legs among the caged broilers (Fouad et al. 2008).

Figure 5. Mean (±SEM) caecal Clostridia (a), Campylobacter (b), Escherichia coli, and Salmonellae populations in broiler chickens under cage and floor pen rearing systems in a hot and humid tropical environment at 42 days of age. Means with different letters are significantly different at \( p \leq .05 \).
The present findings agree with those of Zulkifli et al. (1998), and Fraisse and Cockrem (2006) that cage level had a negligible effect on TI durations in chickens. On the contrary, Jones (1985a, 1985b) reported that hens caged individually in the top tier of a three-tier battery system showed longer TI durations than those from the middle and lower tier. Jones (1986) suggested that birds in the top tier had less external stimulation and this may have accounted for the higher underlying fearfulness. In the present study, although the caged broilers were reared in three-tier cages, the height of the top-tier did not restrict the visual field. Thus, the birds may have received similar levels of stimulation compared to those at the middle and bottom levels and consequently did not result in greater underlying fearfulness.

Fouad et al. (2008) subjected broilers to a novel object test and concluded that caged boilers were more fearful than those on floors. The authors suggested that caged birds were more fearful because of frustration resulted from the restriction of expressing normal behaviour such as foraging and dust bathing. In the present study, birds in both rearing systems had similar TI durations. However, as measured by the number of inductions to evoke TI, birds raised on floor pens were more susceptible to TI than their caged counterparts. Thus, it appears that floor pens reared birds were more fearful than their cages reared counterparts. The higher susceptibility to TI in FS than CS birds could also be associated with the measured higher basal concentrations of CORT, OVT, and CPN. The profound impact of environmental stressors on fearfulness in poultry has been well documented (Jones 1996). Campo and Carnicer (1994) reported that heat exposure prolonged TI duration in various breeds of hens. A positive relationship between levels of stress and fear has been reported (Jones et al. 1992). We noted that the FS chickens, as measured by basal CORT and APP, were more physiologically distressed than their CS counterparts. Work by Fouad et al. (2008) demonstrated that caged broilers had elevated heterophil to lymphocyte ratios than those reared in litter floor pens which suggested the former were more physiologically distressed. However, Fouad et al. (2008) subjected the caged broilers to a smaller space than those on floor pens while in the present study,
both groups of broilers were provided to similar floor space. It is well documented that overcrowding may elicit the physiological stress response in chickens (Bessei 2006). Furthermore, unlike the present study, Fouad et al. (2008) exposed their birds to 24 °C, and relative humidity of 50%, which were considered favourable to litter quality and, in turn, may dampen physiological distress in their chickens. The elevated basal CORT noted in FS birds compared to their CS counterparts could also be associated with higher activity. Koelkebeck and Cain (1984) compared plasma corticosterone and behaviour of laying hens stocked at various densities and raised on floor pens and in cages. The authors demonstrated, irrespective of floor space allowed, hens on floor pens showed significantly higher walking and object pecking activities than those on floor pens and had increased plasma CORT. It could also be attributed to the exposure to a higher level of ammonia and a higher incidence of contact dermatitis as a result of wet litter conditions (Weaver and Meijerhof 1991). The authors indicated that the high moisture content of the litter increased microbial activity, which in turn led to an increase in temperature and ammonia in broiler houses. Thus, the present findings suggested that raising broilers on litter floor pens in the hot and humid tropical environment may compromise the welfare of broilers when compared to battery cages. The present work confirmed that serum levels of APP are reliable physiological indices of stress in avian species (Shakeri et al. 2014; Najafi et al. 2015; Zulkifli et al. 2018).

Stress may suppress the immune system (Kidd 2004). The relative weights of lymphoid organs and ND Virus hemagglutination inhibition antibody titre levels were adversely affected in chickens exposed to 26 and 52 ppm ammonia compared to the control group (0 ppm) (Wang et al. 2010). The authors concluded that high atmospheric ammonia levels lowered immunological response in broiler chickens. On the contrary, both caged and floor pens reared chickens had similar levels of ND antibody titre levels. There is no clear explanation for the phenomenon, although the discrepancies could be associated with the duration and intensity of stress (Mashaly et al. 2004).

Caged broiler chickens have been commonly associated with welfare problems such as poor bone strength due to lack of exercise, feather loss, and restriction of natural behaviour (Shields and Greger 2013). On a cautionary note, earlier studies (Fouad et al. 2008; Özhan et al. 2016) on cages and floor pens involved higher stocking densities in the former. Higher stocking densities are known to have an adverse influence on the growth performance, health, and well-being of broiler chickens (Estevez 2007; Najafi et al. 2015). Thus, the reported effects of different rearing systems on broilers may have been confounded by the discrepancies in stocking densities. The well-being of broilers on litter floor pens is not without concern particularly those raised under the hot and humid tropics. In Malaysia, the indoor temperatures of naturally-ventilated poultry houses can exceed 30 °C with relative humidity levels over 80% and such conditions may exacerbate litter quality problems (Garcês et al. 2013). The present findings show that prolonged contact with wet litter increased physiological stress response, the incidence of footpad dermatitis, and caecal population of E. coli. Within the limits of the present study, as measured by basal CORT and APP, fear reactions, FPD lesions, and intestinal E. coli population, cage production did not provide evidence that such a system can compromise the well-being of broiler chickens raised under the hot and humid tropical environment compared to floor systems with wood shaving as litter material. However, cage production reduced leg strength and increased the intestinal Salmonellae population. The decision on an optimum rearing system for broiler chickens in the tropics should consider their overall well-being. Recent work (Karcher et al. 2013; Farghly et al. 2018) on other poultry species suggested that placing plastic slats on concrete floors resulted in better welfare than the deep litter system. Thus, further studies are warranted to determine the effects of cages with plastic mesh floors on the well-being and performance of broiler chickens in the hot and humid tropical environment.

Conclusions

Based on this experimental study’s results under the hot and humid tropical climate, the rearing system significantly affects growth performance, gut microbiota, the incidence of foot-pad dermatitis, leg strength, and physiological stress response in commercial broiler chickens. While improving body weight, leg strength, and reducing the caecal Salmonellae population, floor pens could not be considered better than cages for performance and well-being. Caged broilers have better feed efficiencies and lower caecal E. coli populations and food-pad dermatitis incidence than those on floor pens. Thus, both rearing systems have advantages and disadvantages to broiler chickens. It is up to the industry to decide which advantages and disadvantages they consider the more important. If
the major criterion for broiler chicken well-being is minimal stress according to physiological measures, then assessing rearing systems is possible. From this perspective, broilers kept in cages are less distressed than their floor pen counterparts. Climatic condition is vital in determining the suitable rearing system for broiler chickens.

**Ethical approval**

The experiment was carried out following the guidelines of the Research Policy on Animal Ethics of Universiti Putra Malaysia.

**Disclosure statement**

No potential conflict of interest was reported by the author(s).

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