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Floor space and betaine supplementation alter the nutrient digestibility and performance of Japanese quail in a tropical environment

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ABSTRACT

This study investigated the effects of floor space and betaine supplementation on the nutrient digestibility and performance of laying quail, at an advanced stage of production, in a tropical environment. In total, 408 laying quail (23 weeks old) were distributed in 3 × 2 factorial arrangements with four replicates. The first factor was floor space (250 cm^2 [FS-1], 221 cm^2 [FS-2], and 200 cm^2 per bird [FS-3]) by allocating 15, 17, and 19 birds per cage (cage size: 3750 cm^2). The second factor was betaine supplementation, administered at levels of 0 (Control) and 0.12% (Betaine). The birds were housed under a natural, tropical environment that was outside the predominant thermoneutral zone, indicating heat stress conditions. Interactions were found between floor space and betaine on crude fiber digestibility, egg production, and eggshell thickness, with the highest values being yielded in FS-2 group supplemented with betaine. Reducing the floor space to 200 cm^2 negatively affected nutrient digestibility and performance. The birds kept in FS-3 had lower ($P < 0.05$) dry matter, crude protein, crude fat, and calcium digestibility values than those kept in FS-1 and FS-2. Birds housed in FS-2 exhibited higher egg weight than those in FS-3 ($P < 0.05$), although they did not differ from those in FS-1. Furthermore, a lower eggshell weight in FS-3, compared with FS-1 and FS-2, was observed ($P < 0.01$). Betaine supplementation enhanced the dry matter, crude protein, crude fat, crude ash, and calcium digestibility values ($P < 0.05$). Accordingly, betaine improved feed intake, egg weight, feed conversion, and protein and energy efficiency ratios ($P < 0.01$). Betaine also resulted in higher albumen, yolk, and eggshell weights ($P < 0.01$). In conclusion, a floor space of 221 cm^2, combined with betaine supplementation at 0.12%, can be applied for raising quail under high environmental temperatures.

1. Introduction

Providing optimal floor space for quail, supported by adequate ventilation and appropriate temperature and humidity, will result in optimal performance (Ayoola et al., 2014). It is common practice to reduce floor space by increasing the number of laying birds to intensify poultry production and reduce production costs. In the cage population, floor space per bird decreases as the number of birds increases (Saki et al., 2012). However, reduced floor space generally results in decreased laying performance due to insufficient feed intake and the negative effects of induced stress (Mousavi et al., 2016). High stocking density enhances heat accumulation inside the cages (Abudabos et al., 2013; Adebiyi and Adu, 2011), and this is associated with increased humidity due to the birds' respiration processes (Ahmad et al., 2006; Baziz et al., 2010). This is further exacerbated by high ambient temperatures, which lead to heat stress by hampering the birds' ability to dissipate extra heat to the surrounding environment (Faitarone et al., 2005; Farag and Alagawany, 2018). According to Vercese et al. (2012), quail exposed to 27 °C already exhibit signs of heat stress.

Many physiological responses to high environmental temperatures have been reported, such as a depressed immune response, as well as changes in the electrolyte balance and osmotic pressure of body cells (Farghly et al., 2018; Lara and Rostagno, 2013). High temperatures also reduce blood flow to the digestive tract, which decreases nutrient absorption (Latipudin and Mushawwir, 2011), and to the ovaries, which alters ovarian function and decreases laying performance (Rozenboim et al., 2007). Previous studies have revealed that reducing the floor space per bird also decreases feed intake (Faitarone et al., 2005; Seker et al., 2009), which lowers egg production, egg weight, and feed efficiency (Faitarone et al., 2005; Özbey et al., 2004; Seker et al., 2009).

The addition of betaine is expected to overcome the disturbance induced by restricted floor space, since it is the most effective organic osmolyte for controlling osmotic disturbance (Attia et al., 2018; Chand et al., 2017; Hammer and Baltz, 2002), and it has been shown to limit

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the adverse effects of heat stress in poultry (Attia et al., 2009; Egbuniwe et al., 2018; Ratriyanto and Mosenthin, 2018). The accumulation of organic osmolytes in cells, and in cell organelles, exposed to osmotic disturbances can replace inorganic ions. It can also protect enzymes and cell membranes from being inactivated by inorganic ions, thereby allowing cells to survive (Honarbakhsh et al., 2007; Petronini et al., 1992). Betaine stabilizes the intestinal cell structure and enables optimal nutrient digestion and absorption processes (Attia et al., 2016; Eklund et al., 2005). Previous studies revealed that betaine improved nutrient digestibility in laying hens (Attia et al., 2016; Ezzat et al., 2011) and quail housed under high environmental temperatures (Ratriyanto et al., 2017). Accordingly, the beneficial effects of betaine on laying performance and egg quality have been observed in quail (Haryadi et al., 2015; Ratriyanto et al., 2017), laying hens (Attia et al., 2016; Ryu et al., 2002), and ducks (Awad et al., 2014).

However, few studies have focused on how floor space affects the performance of laying quail (Akram et al., 2000; El-Shafei et al., 2012; Özbey et al., 2004), and the results have been conflicting. Furthermore, no study has addressed the effects of floor space and betaine supplementation on the nutrient digestibility, laying performance, and egg quality of quail housed under high temperatures in tropical climates. Therefore, the objective of this study was to fill this gap by investigate the effects of floor space and betaine supplementation on nutrient digestibility, performance, and egg quality of quail, at an advanced stage of production, housed in a tropical environment.

2. Materials and methods

2.1. Experimental site and microclimate

The research protocol was approved by the Institute for Research and Community Service of Sebelas Maret University, Surakarta, Indonesia. The quail (*Coturnix coturnix japonica*) were housed on the experimental farm of Sebelas Maret University, located in Jatikuwung, Karanganyar, Indonesia (latitude: 7°31′09.5”S, longitude: 110°50′42.4”E; altitude: 150 m above sea level). The experiment was conducted from August to October during the area's hot-dry season. The birds were kept under natural, tropical conditions, with average, ambient temperatures in the morning (06.00 h), midday (12.00 h), and evening (18.00 h) of 26.6 °C, 33.4 °C, and 29.9 °C and average relative humidity levels of 78.2%, 58.2%, and 72.1%, respectively (Fig. 1). The lighting schedule was 16 h light (5am-9pm) and 8 h dark (9pm-5am).

2.2. Experimental design and diets

In total, 408 female Japanese quail (*Coturnix coturnix japonica*; 23 weeks old), with an average body weight of 154.56 ± 4.99 g, were used in this study. The birds were distributed in a 3 × 2, completely randomized experimental design. The first factor was the floor space (three levels), while the second factor was betaine supplementation (two levels), with four replicates, for a total of 24 cages. Each cage was considered an experimental unit. The birds were allotted three floor spaces, namely 250 cm^2 (FS-1), 221 cm^2 (FS-2), and 200 cm^2 per bird (FS-3), corresponding to 15, 17, and 19 quail per cage, respectively, with a cage size of 3750 cm^2 (75 × 50 cm). These floor spaces were equal to stocking densities of 40 birds/m^2, 45 birds/m^2, and 50 birds/m^2. Meanwhile, the cages used for digestibility measurement had three sizes: 15 × 16.7 cm (FS-1), 15 × 14.7 cm (FS-2), and 15 × 13.3 cm (FS-3).

The basal diet consisted of maize, rice bran, soybean meal, and fish meal, and it was formulated to contain 18% crude protein and 2800 kcal/kg of metabolizable energy (Control; Table 1). The assay diet (Betaine) was obtained by supplementing 0.12% betaine (0.125%

Table 1
Composition (%) and calculated analysis nutrient content of experimental diets.

Ingredients	Control (%)	Betaine (%)
Maize	45.750	45.750
Rice bran	18.020	17.895
Soybean meal (46%)	20.200	20.200
Fish meal (52%)	6.700	6.700
Coconut oil	1.300	1.300
DL- methionine	0.090	0.090
Choline chloride	0.100	0.100
Dicalcium phosphate	0.830	0.830
Limestone	6.310	6.310
Premix[a]	0.350	0.350
NaCl	0.350	0.350
Anhydrous betaine	–	0.125
Total percentage	100	100
Nutrient content		
Metabolizable energy (kcal/kg)	2800.00	2797.00
Crude protein (%)	18.00	17.99
Crude fiber (%)	4.25	4.23
Crude fat (%)	4.86	4.85
Lysine (%)	1.04	1.04
Choline (%)	0.19	0.19
Methionine (%)	0.45	0.45
Calcium (%)	3.40	3.40
Available phosphorus (%)	0.50	0.50
Betaine	–	0.12

[a] The premix supplied the following per kilogram diets: 42,000 IU vitamin A; 7000 IU vitamin D_3; 28 mg vitamin E; 7 mg vitamin K; 7 mg vitamin B1; 18 mg vitamin B2; 2 mg vitamin B6; 42 mg vitamin B12; 88 mg vitamin C; 21 mg calcium *D*-pantothenate; 140 mg niacin; 35 mg choline chloride; 420 mg manganese; 70 mg iron; 0.7 mg iodine; 350 mg zinc; 0.7 mg cobalt; 14 mg copper, 35 mg sanoquin (antioxidant).

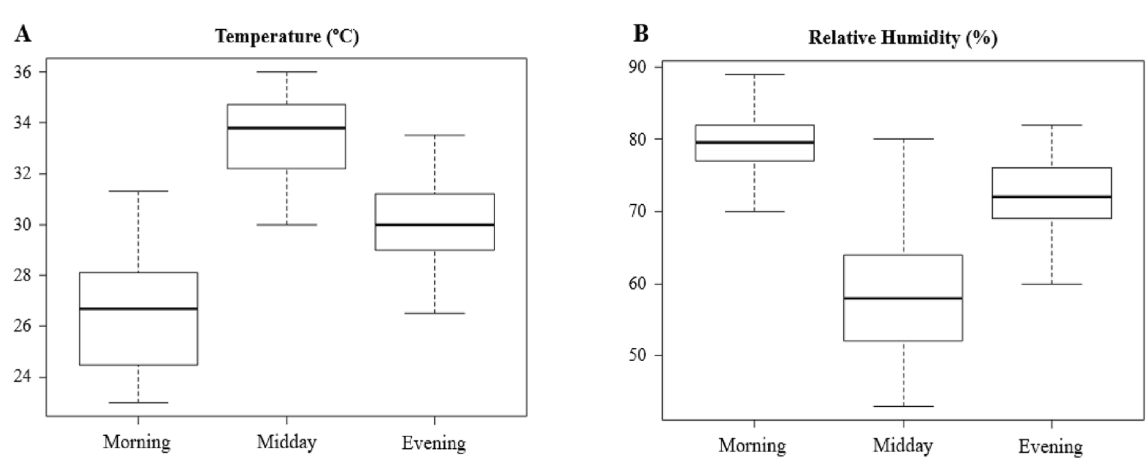

Fig. 1. Boxplot showing the temperature (A) and relative humidity (B) in the morning, midday, and evening inside the poultry cage during the study period.

Table 2
Nutrient digestibility (%) for different floor spaces and betaine supplementation.

Treatments		Dry Matter	Crude Protein	Crude Fat	Crude Fiber	Crude Ash	Nitrogen Free Extract	Calcium
Interaction floor space × betaine								
FS-1	Control	76.59	76.90	89.15	36.76[b]	59.52	91.76	78.42
FS-1	Betaine	79.13	79.83	90.96	39.61[ab]	70.61	92.72	79.81
FS-2	Control	75.98	76.68	89.22	27.84[c]	57.75	92.14	76.36
FS-2	Betaine	80.40	80.08	90.52	48.64[a]	71.83	93.36	81.87
FS-3	Control	74.65	74.92	88.80	27.65[c]	55.32	91.61	74.85
FS-3	Betaine	78.29	75.85	88.94	36.56[b]	71.14	92.60	77.29
SEM		1.39	2.05	0.55	4.85	4.69	0.80	1.84
P value		0.69	0.81	0.12	0.01	0.79	0.98	0.43
Effect of floor space								
FS-1		77.86[ab]	78.36[a]	90.06[a]	38.18	65.06	92.24	79.12[a]
FS-2		78.20[a]	78.38[a]	89.87[a]	38.24	64.79	92.75	79.12[a]
FS-3		76.47[b]	75.39[b]	88.87[b]	32.10	63.23	92.10	76.07[b]
SEM		0.98	1.45	0.39	3.43	3.32	0.57	1.30
P value		0.04	0.04	0.01	0.17	0.85	0.72	0.02
Effect of betaine								
Control		75.74[b]	76.17[b]	89.06[b]	30.75[b]	57.53[b]	91.84	76.54[b]
Betaine		79.28[a]	78.59[a]	90.14[a]	41.60[a]	71.19[a]	92.89	79.66[a]
SEM		0.80	1.18	0.31	2.80	2.71	0.46	1.06
P value		0.00092	0.16	0.00307	0.00671	0.00013	0.14	0.03

FS-1 = floor space 250 cm^2; FS-2 = floor space 221 cm^2; FS-3 = floor space 200 cm^2; SEM = standard error of the mean.
[a,b,c] Means in the same column and treatment with no common superscript differ significantly ($P < 0.05$).

anhydrous betaine with 96% purity; Betafin, Danisco, Finland) to the basal diet at the expense of rice bran (Ratriyanto et al., 2009a). Choline chloride was included in the diet to eliminate the function of betaine as a methyl group donor (Attia et al., 2005; Hassan et al., 2005; Honarbakhsh et al., 2007).

2.3. Data collection

During the pre-experimental period (four weeks), the birds were fed with the basal diet. Water and feed were provided *ad libitum*. The floor space and betaine supplementation treatments lasted for two periods of 28 days each (2 × 28 days). The feed intake, egg production, and egg weight were recorded daily. The feed conversion ratio was calculated by measuring the ratio of feed intake to egg mass (Card and Nesheim, 1976). The protein efficiency ratio was calculated as grams of product (egg mass) per gram of protein intake, whereas the energy efficiency ratio was calculated as grams of product: (egg mass) × 100/total energy intake (Cheng et al., 1997; Suprijatna et al., 2009). Measurement of the physical egg quality—including the yolk, albumen, and eggshell weight; yolk and albumen index; and eggshell thickness—was performed during the last three days of each period (days 26–28), following Stadelman and Cotteril (1995). In total, 216 eggs from each period (nine eggs per replicate) were randomly collected for assessing the egg quality parameters. The eggs were weighed and cracked, and the egg components were weighed thereafter. The albumen and yolk height and their diameters were measured with a digital caliper (0–150 × 0.001 mm, Digital Caliper 1108-150, *INSIZE*, China). The shell thickness was measured using a digital micrometer (0–25 × 0.001 mm, Digital Outside Micrometer 3109-25A, *INSIZE*, China).

2.4. Nutrient digestibility measurement

At the end of the experiment, 48 birds (two per replicate) were randomly picked to observe *in vivo* nutrient digestibility. The digestibility trial was performed over a five-day collection period, following the procedures of Ratriyanto et al. (2014a). To avoid bacterial fermentation during collection, the excreta were periodically sprayed using 0.2 N H_2SO_4. The collected excreta were pooled, homogenized in each replicate, and sun dried until they were completely dry and could be ground. The excreta samples were milled through a 0.5-mm mesh screen before analysis. Crude protein was determined via the Kjeldahl method, while other, proximate analyses were performed as outlined by the Association of Official Analytical Chemists (AOAC, 2001). Calcium analysis was performed by atomic absorption spectrophotometry (AAS), according to the AOAC (2001) method. Calculation of nutrient digestibility coefficients was performed according to Emamzadeh and Yaghobfar (2009), as follows:

$$\text{Nutrient digestibility (\%)} = \frac{\text{Nutrient intake (g)} - \text{nutrient excreted (g)}}{\text{Nutrient intake (g)}} \times 100\%.$$

2.5. Data analysis

The data were submitted to an analysis of variance using two-way ANOVA. The following model was applied: $y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \varepsilon_{ijk}$, where μ = the general mean; α_i = the effect of floor space; β_j = the effect of betaine supplementation; $\alpha\beta_{ij}$ = interaction effect of floor space and betaine supplementation; and ε_{ijk} = experimental error. The statistically different means were compared using Duncan's multiple-range test at $P < 0.05$. Statistical analyses were performed using R (R Core Team, 2015).

3. Results

3.1. Nutrient digestibility

There was no interaction between floor space and betaine supplementation on nutrient digestibility ($P > 0.05$), except in the case of crude fiber ($P = 0.01$; Table 2). The results indicated that, for all floor spaces, with or without betaine supplementation, most of the nutrient digestibility values did not differ. Without betaine supplementation, smaller floor spaces (FS-2 = 27.84% and FS-3 = 27.65%) generated lower ($P = 0.01$) crude fiber digestibility than the larger floor space (FS-1 = 36.76%). In contrast, under conditions of betaine supplementation with a smaller floor space, the crude fiber digestibility was maintained. The birds kept in FS-3 had lower ($P < 0.05$) dry matter, crude protein, crude fat, and calcium digestibility values than those kept in FS-1 and FS-2. However, floor space did not affect the crude fiber, crude ash, or nitrogen-free extract digestibility values.

Table 3
Productive performance of quail raised in different floor space and supplemented with betaine.

Treatments		Feed Intake (g)	Egg Production (%)	Egg Weight (g)	Feed Conversion Ratio	Protein Efficiency Ratio	Energy Efficiency Ratio
Interaction floor space × betaine							
FS-1	Control	22.84	73.96[c]	9.37	3.30	1.68	10.84
FS-1	Betaine	24.03	83.74[ab]	9.77	2.94	1.89	12.18
FS-2	Control	22.35	73.24[c]	9.49	3.22	1.73	11.12
FS-2	Betaine	24.34	86.81[a]	9.91	2.83	1.96	12.62
FS-3	Control	22.37	72.90[c]	9.26	3.31	1.68	10.78
FS-3	Betaine	23.67	82.21[b]	9.76	2.95	1.88	12.11
SEM		0.60	2.99	0.13	0.12	0.07	0.44
P value		0.68	0.04	0.74	0.17	0.14	0.13
Effect of floor space							
FS-1		23.44	78.85[ab]	9.57[ab]	3.12	1.79	11.51
FS-2		23.35	80.03[a]	9.70[a]	3.02	1.85	11.86
FS-3		23.02	77.55[b]	9.51[b]	3.13	1.78	11.45
SEM		0.42	2.12	0.10	0.09	0.05	0.31
P value		0.68	0.04	0.04	0.66	0.54	0.53
Effect of betaine							
Control		22.52[b]	73.37[b]	9.38[b]	3.28[a]	1.70[b]	10.92[b]
Betaine		24.01[a]	84.25[a]	9.82[a]	2.91[b]	1.92[a]	12.32[a]
SEM		0.34	1.73	0.08	0.07	0.04	0.25
P value		0.00170	0.00004	0.00003	0.00009	0.00010	0.00010

FS-1 = floor space 250 cm^2; FS-2 = floor space 221 cm^2; FS-3 = floor space 200 cm^2; SEM = standard error of the mean.
[a,b,c] Means in the same column and treatment with no common superscript differ significantly ($P < 0.05$).

Furthermore, betaine supplementation enhanced dry matter digestibility, compared with a non-supplemented diet (79.28% vs 75.74%; $P < 0.01$). The improvement in dry matter digestibility due to betaine supplementation corresponded with enhancements in the digestibility values of crude protein (78.59% vs 76.17%), crude fat (90.14% vs 89.06%), crude ash (71.19% vs 57.53%), and calcium (79.66% vs 76.54%; $P < 0.05$).

3.2. Productive performance indicators

An interaction was found between floor space and betaine supplementation in terms of egg production, where betaine supplementation generated higher egg production ($P = 0.04$) in all three floor spaces (Table 3). The birds housed in FS-2 and receiving betaine supplementation yielded the highest egg production compared with the other treatments. Furthermore, different floor spaces affected egg weight, but the floor space parameter did not affect other performance indicators. Birds housed in FS-2 produced heavier eggs than those in FS-3 (9.70 g vs 9.51 g; $P = 0.04$), although the results did not differ between FS-2 and FS-1. Betaine supplementation increased feed intake compared with a non-supplemented diet (24.01 g vs 22.52 g; $P < 0.01$), which concurs with the findings that egg production (84.25% vs 73.37%) and egg weight (9.82 g vs 9.38 g) were higher following betaine supplementation (Table 3). Furthermore, betaine supplementation improved the feed conversion (2.91 vs 3.28), as well as the protein efficiency ratio (1.92 vs 1.70) and the energy efficiency ratio (12.32 vs 10.92) over the non-supplemented diet.

3.3. Egg quality parameters

An interaction between floor space and betaine supplementation was found for eggshell thickness ($P < 0.01$). The birds kept in FS-3 without betaine supplementation exhibited the thinnest eggshells (Table 4). Betaine supplementation increased eggshell thickness for each floor space, with the highest eggshell thicknesses observed in FS-1 and FS-2. Furthermore, there was a lower ($P < 0.01$) eggshell weight for the birds kept in FS-3 (0.765 g) compared with FS-1 (0.783 g) and FS-2 (0.791 g). Betaine supplementation in the diet increased the albumen weight (6.06 g vs 5.86 g), yolk weight (2.94 g vs 2.74 g), and eggshell weight (0.798 g vs 0.762 g) compared with a non-supplemented diet ($P < 0.01$; Table 4)—all of which corresponds with the increased egg weight observed in this study (Table 3). However, betaine supplementation generated lower albumen and yolk indices.

4. Discussion

4.1. Nutrient digestibility

The results of the present research indicated that small floor space negatively affects the birds' nutrient digestion. Reducing floor space without betaine supplementation lowered crude fiber digestibility, while betaine supplementation maintained fiber digestibility. This finding suggests that the birds required a compatible osmolyte, such as betaine, to maintain an appropriate environment for intestinal microflora. Betaine is an effective osmolyte that supports the growth of intestinal microflora, especially during suboptimal conditions, such as heat or osmotic stress (Ratriyanto and Mosenthin, 2018). In line with this study, it has been shown that betaine is useful for improving animal performance under suboptimal conditions, such as high environmental temperatures, low nutrient availability, and unhygienic surroundings (Attia et al., 2009; Ratriyanto et al., 2017; Spreeuwenberg et al., 2007).

Restricted floor space negatively affects nutrient digestion. However, birds can be placed in a smaller floor space if the environment inside the cage (temperature, humidity, and ventilation) remains appropriate (Ayoola et al., 2014). It has been hypothesized that, in a smaller floor space, birds have trouble physiologically adapting to the unfavorable effects of the environment (Mahfudz et al., 2015). A small floor space negatively affects the birds by increasing cage temperature, accumulating CO_2, reducing fresh air for respiration, and decreasing nutrient absorption (Shanaway, 1994). The negative effects of a high stocking density (small floor space) have been observed previously, where the villus height of the jejunum was lower in birds kept in a high stocking density compared to those kept in a low stocking density; and this was associated with a smaller intact gut area for absorption (Chegini et al., 2018). In addition, Saki et al. (2012) showed that increased stocking density decreased nutrient absorption in laying hens.

Several observations in quail support the findings of this study, in which betaine supplementation under high ambient temperature improved the dry matter, crude protein, crude fat, crude fiber, and crude ash digestibility values (Ratriyanto et al., 2012, 2017). Similarly, other studies revealed that betaine supplementation increased the digestibility of crude protein and crude fat in laying hens (Attia et al., 2016;

Table 4
Egg quality measures for quail raised in different floor space and supplemented with betaine.

Treatments		Albumen Weight (g)	Yolk Weight (g)	Eggshell Weight (g)	Albumen Index (%)	Yolk Index (%)	Eggshell Thickness (mm)
Interaction floor space × betaine							
FS-1	Control	5.98	2.73	0.762	16.14	49.86	0.192[b]
FS-1	Betaine	6.08	2.88	0.803	14.89	48.02	0.197[a]
FS-2	Control	5.74	2.74	0.783	16.52	49.49	0.189[bc]
FS-2	Betaine	6.07	2.99	0.800	15.39	48.63	0.197[a]
FS-3	Control	5.87	2.74	0.741	16.43	49.63	0.187[c]
FS-3	Betaine	6.03	2.94	0.790	15.28	48.13	0.192[b]
SEM		0.08	0.06	0.013	0.38	0.49	0.002
P value		0.09	0.43	0.08	0.97	0.41	0.00008
Effect of floor space							
FS-1		6.03	2.80	0.783[a]	15.51	48.94	0.194
FS-2		5.90	2.86	0.791[a]	15.96	49.06	0.193
FS-3		5.95	2.84	0.765[b]	15.85	48.88	0.190
SEM		0.05	0.05	0.009	0.27	0.35	0.002
P value		0.07	0.33	0.00488	0.19	0.88	0.70
Effect of betaine							
Control		5.86[b]	2.74[b]	0.762[b]	16.37[a]	49.66[a]	0.191
Betaine		6.06[a]	2.94[a]	0.798[a]	15.18[b]	48.26[b]	0.194
SEM		0.04	0.05	0.007	0.22	0.29	0.002
P value		0.00013	0.00007	0.00006	0.00001	0.01	0.40

FS-1 = floor space 250 cm^2; FS-2 = floor space 221 cm^2; FS-3 = floor space 200 cm^2; SEM = standard error of the mean.
[a,b,c] Means in the same column and treatment with no common superscript differ significantly ($P < 0.01$).

Ezzat et al., 2011), as well as crude fiber and crude ash in laying ducks (Awad et al., 2014) kept under high ambient temperature or challenged with heat stress (Attia et al., 2009). Betaine's modes of action in improving nutrient digestibility have also been previously reported (Metzler-Zebeli et al., 2009; Ratriyanto et al., 2009b; Ratriyanto and Mosenthin, 2018). The higher nutrient digestibility due to betaine supplementation indicated betaine's osmotic support of the intestinal cells and microbes in contending with the various osmotic gradients along the digestive tract (Metzler-Zebeli et al., 2009; Ratriyanto and Mosenthin, 2018). For example, betaine stabilizes the intestinal structure of chickens by increasing the small intestine length (Ratriyanto et al., 2014b) and decreasing the crypt:villi ratio (Kettunen et al., 2001; Ratriyanto et al., 2014b), which is associated with an improvement in the intact gut area for nutrient absorption. Moreover, betaine has been shown to increase the population of Gram-positive bacteria, such as enterococci and lactobacilli, in broilers' digestive tract (Kettunen et al., 1999). Dietary betaine also modulates intestinal microbial fermentation, as indicated by increases in short-chain fatty acid contents in the ileum and caeca of broilers and ducks (Kettunen et al., 1999; Park and Kim, 2017). In addition, betaine showed a sparing effect on methionine and choline (Attia et al., 2005; Hassan et al., 2005).

4.2. Productive performance of quail

The findings of this study demonstrated that betaine was effective in enhancing quails' performance, regardless of floor space. The highest egg production was obtained in the FS-2 group supplemented with betaine, indicating the optimum conditions for birds' performance. Furthermore, laying performances in this study correlated with nutrient availability for egg production. Reducing the floor space to 200 cm^2 negatively affected the birds' performance, as indicated by the lower egg production and egg weight, which were associated with the lower nutrient digestibility (crude protein, crude fat, and calcium) of this treatment. Consequently, it is probable that this floor space resulted in the production of fewer and lighter eggs due to the lower availability of protein, fat, and calcium. Protein and fat are the major components of eggs (Ratriyanto et al., 2018), whereas calcium is required for eggshell formation (de Araujo et al., 2011).

In agreement with this study, Özbey et al. (2004) reported that reducing the floor space of Japanese quail decreased egg production, egg weight, and eggshell thickness. Similarly, breeder quail maintained in small floor space conditions produced lighter eggs compared with those in large floor space conditions (Akram et al., 2000). Other observations indicated that reducing floor space from 233 to 200 cm^2 did not influence the feed conversion ratio of laying quail, but a floor space reduction to 170 cm^2 increased the feed conversion ratio (El-Shafei et al., 2012). In growing quail, reducing the floor space from 200 to 167 cm^2 did not influence the feed conversion ratio. A similar response to floor space was observed in laying hens, in which the productive performance was shown to decline in response to decreased floor space (Kang et al., 2016). Accordingly, Mousavi et al. (2016) observed that a floor space reduction decreased egg production, although the egg weight and feed conversion ratio were not altered.

The increases in egg weight due to betaine supplementation were in line with the improvements in feed conversion, as well as the protein and energy efficiency ratios, indicating improvement in the nutrient utilization for egg production. Previous observations revealed an increase in performance traits due to betaine supplementation in laying poultry (Attia et al., 2016; Haryadi et al., 2015; Ratriyanto et al., 2017), supporting the results of this study. Betaine supplementation increased feed intake, egg production, and egg weight, accompanied by improvements in the feed conversion as well as protein and energy efficiency ratios in quail raised in high ambient temperatures (Haryadi et al., 2015; Ratriyanto et al., 2017). Similarly, improvements in laying performance have been observed in laying hens and laying ducks raised under high environmental temperatures (Attia et al., 2016; Awad et al., 2014). The improvement in laying performance due to betaine supplementation was in accordance with higher nutrient digestibility, leading to higher nutrient availability for egg production (Metzler-Zebeli et al., 2009). According to Attia et al. (2016), the enhancement in performance traits can also be attributed to increases in the estrogen and progesterone concentrations following betaine supplementation. These hormones regulate the ovulation rate and oviductal development (Etches, 1996). Furthermore, it was found that betaine supplementation increased ovary and oviduct size, in association with higher egg production capacity (Attia et al., 2016). Betaine has been shown to stimulate the secretion of follicle-stimulating hormone and luteinizing hormone, which promote follicle growth and ovulation, leading to enhanced egg production (Xing and Jiang, 2012; Zou and Feng, 2002). In contrast to this finding, betaine supplementation had no beneficial effect on the performance of broilers raised in hot environments (Konca et al., 2008).

4.3. Egg quality parameters of quail

A small floor space also negatively influences egg quality, especially in terms of eggshell weight and thickness. Birds kept in FS-3 without betaine supplementation generated the thinnest eggshells, while betaine improved eggshell thickness for each floor space size. Accordingly, a lower eggshell weight for the birds kept in FS-3 compared with FS-1 and FS-2 was observed, which was attributed to the lower calcium digestibility in FS-3. Calcium availability for eggshell formation has been shown to influence eggshell quality (An et al., 2016; de Araujo et al., 2011). Moreover, reducing floor space may result in heat stress, which is associated with alterations in the acid–base balance, leading to a reduction in the formation of bicarbonate ions (Alagawany et al., 2017; Cavalchini et al., 1990). Bicarbonate is required for eggshell formation in combination with calcium ions (Etches, 1996).

In line with the improvement in egg weight in this study, higher albumen, yolk, and eggshell weights were observed due to betaine supplementation. These improvements could also be attributed to the improvements in crude protein, crude fat, and calcium digestibility values, pointing to improvements in protein, fat, and calcium availability and utilization. In support of this study, previous observations under high environmental temperatures revealed that betaine improved the egg quality of quail (Ratriyanto et al., 2017) and laying hens (Attia et al., 2018, 2009; Ryu et al., 2002). Dietary betaine supplementation for quail raised under hot, tropical climate conditions has been shown to increase egg weight in association with an increase in albumen and yolk weight (Ratriyanto et al., 2017), which is attributable to the role of betaine as a methyl group donor in improving the synthesis of the yolk and albumen precursors (Ratriyanto and Mosenthin, 2018). Improvement in albumen and yolk weight due to betaine supplementation in the diet has also been observed in laying hens (Attia et al., 2009; Ezzat et al., 2011).

The improvement in eggshell weight due to betaine supplementation was in line with the enhancement in crude ash and calcium digestibility values in this study, indicating a better mineral availability and utilization; this agreed with previous observations in quail, which were fed diets containing different protein levels and supplemented with betaine (Ratriyanto et al., 2017). Studies in laying hens kept under high ambient temperatures have shown that betaine supplementation enhances eggshell weight and egg-specific gravity, demonstrating improvement in intestinal absorption and mineral utilization (Attia et al., 2009; Ezzat et al., 2011). Moreover, the observation of lower albumen and yolk indices due to betaine supplementation was in agreement with previous observations in quail (Haryanti, 2016; Ratriyanto et al., 2015). Betaine supplementation enhanced the yolk weight in association with enhanced yolk diameter, but yolk height was unaffected, resulting in a lower yolk index; this may also explain the lower albumen index (Haryanti, 2016).

5. Conclusions

The present study showed that a floor space of 221 cm^2 was the optimal space for laying quail in the hot environment of a tropical climate, while reducing the floor space to 200 cm^2 negatively influenced nutrient digestibility, subsequently decreasing egg production, egg weight, and eggshell weight. Furthermore, betaine supplementation at 0.12% improved nutrient digestibility, performance indicators, and several egg qualities in quail. Therefore, a floor space of 221 cm^2, combined with betaine supplementation, is recommended for laying quail in a tropical climate.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.05.008.

Disclosure statement

None of the authors have any conflicts of interest to declare.

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