Combined supplementation of betaine and vitamin C improves the performance of quails raised in the tropics with reduced floor space

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Abstract. This study investigated how combined betaine and vitamin C supplementation affects quails’ productive performance in reduced floor space. In total, 256 laying quails aged 23 weeks (154.6±5.0 g) were arranged in a completely randomized design of factorial 2×2 with four replicates. The birds were allocated to 16 cages with two floor spaces consisting of 250 cm², 222 cm². They were fed a diet without (Control) or with supplementation of 0.12% betaine in combination with 250 mg/kg vitamin C (BV). The collected data were analyzed by analysis of variance and continued to Duncan’s test. Interactions occurred in egg production and egg mass. The lower floor space without BV supplementation decreased egg production, but lower floor space with BV supplementation enhanced egg production (p<0.05). Supplementing BV enhanced egg mass in each floor space (p<0.05). Reducing floor space did not influence feed intake (FI), egg weight (EW), protein efficiency ratio (PER), and energy efficiency ratio (EER). BV enhances FI, egg production (EP), EW, PER, and EER and reduces the feed conversion (FCR; p<0.01). Thus, combined supplementation of betaine and vitamin C improves quails’ performance in the tropics raised with reduced floor space.

1. Introduction
Reducing floor space in quail farming by increasing the birds’ number can intensify quail production [1]. However, the high quail density due to reduced floor space increased heat accumulation and humidity inside the cages [2,3] and decreased laying performance [4]. This condition is worsened by high ambient temperatures in a tropical climate, which reduces birds’ ability to dissipate body heat [5,6]. To cope with this challenge, reducing floor space can be combined with specific feed additive inclusion.

Betaine is an organic osmolyte and has been shown to alleviate heat stress in poultry [7,8]. It accumulates in cells and cell organelles that are exposed to varying osmotic disturbances, including heat stress, and replaces inorganic ions, thus assisting cells in surviving [9,10]. Betaine assists in stabilizing the structure of intestinal cells and promoting the intestinal microbes to optimize nutrient digestion [11,12]. Several studies showed that betaine supplementation enhances the productive performance of laying poultry, including laying hens and quails [11,13]. Furthermore, vitamin C has an anti-stress effect and is particularly necessary for the diet during high temperatures since vitamin C level in the blood decreases during high temperatures. Supplementation of vitamin C also enhances the immune status of
the poultry [14]. A previous study revealed vitamin C could maintain egg production in quails raised in reduced floor space [15].

Although both betaine and vitamin C as single feed additives positively impact the laying performance of quails [7,15], the combined supplementation of these two additives has not been studied yet. Betaine and vitamin C are expected to show a synergistic effect in alleviating the osmotic disturbance due to reducing floor space in a tropical environment and improving laying performance. Based on this condition, this study determined the effect of combined supplementation of betaine and vitamin C in reduced floor space on the laying performance of quails.

2. Materials and methods
The study was performed on the experimental site of Sebelas Maret University from August to October during the hot-dry season. The average ambient temperature and relative humidity in the morning was 26.6°C and 78.2%, in the midday was 33.4°C and 58.2%, and in the evening was 29.9°C and 72.1%, respectively.

In total, 256 female laying quails of Coturnix coturnix japonica (aged 23 weeks) with an initial body weight of 154.6±5.0 g was arranged in a completely randomized design of factorial 2×2 with four replicates. The birds were allocated to 16 cages with two floor spaces consisted of 250 cm², 222 cm², analogous to 15 and 17 quails per cage. The floor size was 3,750 cm² with dimensions 75 cm in length and 50 cm in width. They were fed a diet without (Control) or with supplementation of 0.12% betaine in combination with 250 mg/kg vitamin C (BV). The basal diet was composed mainly of maize and soybean meals. It was formulated to fulfill the nutrient requirement of laying quails [16] with 18% crude protein (CP) and 2,800 kcal/kg metabolizable energy (ME), as presented in Table 1.

Table 1. Calculated nutritional composition of the layer basal diet

| Nutrient               | Content |
|------------------------|---------|
| ME (kcal/kg)           | 2,800   |
| CP (%)                 | 17.99   |
| Calcium (%)            | 3.40    |
| Non-phytate phosphorus (%) | 0.50    |

Before treatments, the quails were adapted to the floor space and basal diets for four weeks. The data collection was performed in two periods of four weeks (28 days) each. The feed intake (FI), egg production (EP), and egg weight (EW) data were determined daily. The feed conversion ratio (FCR) was obtained by dividing the FI by egg mass. Egg mass was calculated as the EP × EW, indicating egg weight resulted by a bird in a day. Furthermore, the protein efficiency ratio (PER) was determined as grams of egg mass per gram of crude protein (CP) intake. Meanwhile, the energy efficiency ratio (EER) was the grams of egg mass × 10⁰ / total metabolizable energy (ME) intake [13].

The data were submitted to analysis of variance. The statistically different means of the treatments were compared using Duncan’s test at p<0.05. The R program was applied for data analyses [17].

3. Results and discussion

3.1. Laying performances
Interactions occurred in egg production and egg mass (Table 2). The lower floor space without BV supplementation decreased egg production, but the lower floor space with BV supplementation enhanced egg production (p<0.05). Reducing the floor space to 222 cm² without BV supplementation adversely affects egg production and egg mass. Low floor space may increase the cage temperature due to birds overcrowding, lowered air quality, and increased ammonia [3,18,19]. The disadvantageous effects of insufficient floor space might be associated with modifying birds' behavior, such as resting disruptions from other birds [15,18]. A similar response to this finding, studies in laying hens revealed that reducing floor space decreased the laying performance [4,19].
Furthermore, this study revealed that BV effectively enhanced the laying performance, regardless of the floor space. The highest EP was determined in 222 cm$^2$ floor space receiving BV, illustrating the most favorable environment for laying quails. This result agrees with previous studies in quails raised with reduced floor space supplemented with betaine [7] and vitamin C [15] as single feed additives. However, when averaged between supplemented and non-supplemented groups, floor space did not influence the laying performances since the low performance in 222 cm$^2$ without BV was compensated with BV.

Dietary BV improved EP, EW, and egg mass associated with improved FI (p<0.05). Previous studies demonstrated laying performance chicken and quails kept in a high ambient temperature and given betaine supplementation in their diet [11,13]. Accordingly, many observations showed that dietary vitamin C is favorable for poultry that is kept in high temperatures [20,21]. Vitamin C acts as an antioxidant and reduces stress associated with physiological responses to improve thermotolerance [22].

### Table 2. Effect of floor space and combined supplementation of betaine and vitamin C on laying performance of quails

| Treatments               | FI (g)      | EP (%) | EW (g) | EM (g) |
|--------------------------|-------------|--------|--------|--------|
| Interaction FS × BV      |             |        |        |        |
| 250 cm$^2$ Control       | 22.82±0.78  | 74.83±5.74$^{b,c}$ | 9.40±0.05 | 6.93±0.62$^{b,c}$ |
| 250 cm$^2$ BV            | 23.77±1.05  | 79.93±4.05$^{a,b}$  | 9.62±0.18 | 7.67±0.52$^b$ |
| 222 cm$^2$ Control       | 22.30±0.99  | 70.60±4.68$^c$      | 9.46±0.12 | 6.59±0.61$^c$ |
| 222 cm$^2$ BV            | 25.31±1.50  | 86.54±4.60$^a$      | 9.90±0.15 | 8.60±0.53$^c$ |
| p value                  | 0.09        | 0.04   | 0.13   | 0.04   |
| Floor space              |             |        |        |        |
| 250 cm$^2$               | 23.30±1.00  | 77.38±5.35   | 9.51±0.17 | 7.30±0.66   |
| 222 cm$^2$               | 23.80±1.99  | 78.57±9.54   | 9.68±0.27 | 7.59±1.20   |
| p value                  | 0.37        | 0.63   | 0.17   | 0.32   |
| BV supplementation       |             |        |        |        |
| Control                  | 22.56±0.87$^b$ | 72.72±5.35$^b$ | 9.43±0.09$^b$ | 6.76±0.60$^b$ |
| BV                       | 24.54±1.46$^a$ | 83.23±5.35$^a$ | 9.76±0.21$^a$ | 8.14±0.70$^a$ |
| p value                  | 6.72×10$^{-4}$ | 1.50×10$^{-3}$ | 2.48×10$^{-3}$ | 8.59×10$^{-4}$ |

FS: floor space; BV: betaine and vitamin C, FI: feed intake, EP: egg production, EW: egg weight, EM: egg mass

Supplementation of BV reduced FCR and enhanced PER and EER (p<0.05; Table 3), which agreed with paying performance improvement in this study (Table 2). This finding demonstrated the positive impacts of BV on nutrient efficiency. Previous observation revealed that enhancement in laying performance of quails raised in high ambient temperature receiving betaine supplementation was accompanied by an increase in the FCR, PER, and EER [13,24]. Accordingly, vitamin C has also demonstrated a positive effect on these variables, expressing the enhancement of nutrient utilization [7,13]. In addition, improvement in feed efficiency might be attributed to better nutrient digestibility in laying poultry fed diet supplemented with betaine [11] or vitamin C [25,26].
Table 3. Effect of floor space and combined supplementation of betaine and vitamin C on nutrient efficiency of quails

| Treatments          | FCR      | PER       | EER       |
|---------------------|----------|-----------|-----------|
| Interaction FS × BV |          |           |           |
| 250 cm² Control     | 3.40±0.32| 1.69±0.14 | 10.85±0.91|
| 250 cm² BV          | 3.26±0.32| 1.79±0.06 | 11.52±0.40|
| 222 cm² Control     | 3.51±0.23| 1.64±0.09 | 10.53±0.55|
| 222 cm² BV          | 2.97±0.15| 1.89±0.10 | 12.15±0.62|
| p value             | 0.16     | 0.15      | 0.17      |

Effect of FS

| Treatments | FCR      | PER       | EER       |
|------------|----------|-----------|-----------|
| 250 cm²    | 3.33±0.31| 1.74±0.12 | 11.18±0.74|
| 222 cm²    | 3.24±0.34| 1.76±0.16 | 11.34±1.02|
| p value    | 0.50     | 0.62      | 0.63      |

Effect of BV

| Treatments  | FCR      | PER       | EER       |
|-------------|----------|-----------|-----------|
| Control     | 3.46±0.27| 1.66±0.11 | 10.69±0.72|
| BV          | 3.12±0.28| 1.84±0.09 | 11.83±0.59|
| p value     | 0.03     | 3.45x10⁻³ | 3.90x10⁻³ |

FS: floor space; BV: betaine and vitamin C; FCR: feed conversion; PER: protein efficiency ratio; EER: energy efficiency ratio

Means in the same column and treatment with no common superscript differ significantly at p<0.05.

4. Conclusion
A floor space of 222 cm² was optimal for raising the laying quails in a tropical climate, provided the birds received combined supplementation of betaine and vitamin C. The lower floor space without combined supplementation of betaine and vitamin C showed the lowest egg production and egg mass. Still, the low floor space combined with betaine and vitamin C supplementation showed the highest egg production and egg mass. Combined supplementation of betaine and vitamin C effectively enhanced quails' laying performance, disregarding the floor space.

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