

## Nutrient digestibility, performance, and egg quality traits of quails raised in different stocking densities and ascorbic acid supplementation in a hot, tropical environment

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**Abstract:** This study investigated the effects of stocking density (SD) and ascorbic acid (AA) supplementation on nutrient digestibility and laying performance of quails in a tropical environment. Four hundred and eight Japanese quails were designated in a 3 × 2 factorial arrangement with four replicates. The birds were distributed to three SDs, namely 40 (SD-1), 45 (SD-2), and 50 birds per m<sup>2</sup> (SD-3) by allotting 15, 17, and 19 birds per cage, respectively (cage size: 3750 cm<sup>2</sup>). Each SD was supplemented with AA (0 and 250 mg/kg). Interaction was identified between SD and AA on egg production ( $P < 0.01$ ), while the highest value was yielded in SD-2 supplemented with AA (83.6%). SD-3 achieved lower ether extract (EE) and crude fiber (CF) digestibility ( $P < 0.05$ ) and eggshell thickness ( $P < 0.01$ ) than SD-1 or SD-2. AA supplementation enhanced dry matter (DM), EE, CF, crude ash (CA), and calcium (Ca) digestibility ( $P < 0.05$ ). AA improved egg weight, feed conversion, and protein and energy efficiency ratios as well as eggshell weight and thickness ( $P < 0.05$ ). The SD of 45 birds per m<sup>2</sup>, combined with AA supplementation at 250 mg/kg, can be recommended for quails housed in high-temperature environments.

**Key words:** Ascorbic acid, environmental temperature, heat stress, stocking density, quail

### 1. Introduction

Quails are commonly raised in multitier cages during both growing and laying periods. Providing an optimal stocking density (SD) for quails will yield optimal laying performance [1]. An optimal SD provides adequate ventilation, airflow, as well as space for birds' movement and access to feed and water [2]. Thus, SD is a serious issue in poultry production because it depends on the country and its production system [3].

Researchers are growingly interested in increasing the SD due to its potential for reducing production costs [4]. However, a high SD can be stressful and can negatively influence quails' laying performance and immune system [3]. The deleterious effects of a high SD on quails' laying performance can be indicated by decreased feed intake, egg production rate, egg weight or egg mass, and feed efficiency [2,5,6]. These effects may be attributed to the reduced airflow, enhanced ammonia concentration, and reduced opportunity to access feed and water [7]. Furthermore, enhanced heat accumulation inside the cages—particularly in a hot environment that thus leads to heat stress—contributes to lowered laying performance at a high SD [5,8]. Khan et al. [9] suggested that the optimum temperature for laying poultry ranges between 18 and 22 °C.

One of the possible approaches that may be employed to overcome physiological stressors induced by a high SD or high ambient temperature is the use of ascorbic acid (AA). For this purpose, AA is included in a quail's diet due to its antistress effects [10,11]. In stressful conditions, the AA's concentration is reduced, and its requirement may thus exceed its synthesizing ability [9,12]. Beneficial effects of dietary AA on poultry kept in high-temperature environments is associated with decreasing corticosteroid synthesis [13]. AA is also required for mineral and amino acid metabolism, as well as the production of white blood cells required for birds' immune status [9]. The positive effects of AA supplementation have been documented in breeder or laying hens [12,14,15] and quails [16,17], although those effects indicated variable results.

Several studies have addressed the SD's effect on performance in laying hens, although few studies have focused on laying quails, and the results are variable [2,16,18]. Moreover, there is a scarcity of information on the effects of SD and AA supplementation by emphasizing the nutrient digestibility, performance, and egg quality of quails raised in high-temperature environments. Therefore, the objective of this study was to investigate the effects of SD and AA supplementation on the nutrient

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digestibility, laying performance, and egg quality traits of quails raised in a tropical environment.

## 2. Materials and methods

### 2.1. Experimental site and microclimate

The research protocol was approved by the Animal Ethics Committee of Sebelas Maret University, Surakarta, Indonesia. The experiment was conducted under natural tropical conditions in the Animal Experimental Unit of Sebelas Maret University, which is located in Karanganyar, Indonesia (7°31'09.5"S, 110°50'42.4"E). The research was performed from July to October during the hot and dry season. During this experiment, the average ambient temperature in the morning (06:00 hours) was 26.6 °C, at midday (12:00 hours) was 33.4 °C, and in the evening (18:00 hours) was 29.9 °C. Furthermore, the relative humidity in the morning, midday, and evening was 78.2%, 58.2%, and 72.1%, respectively. These humidity and temperatures are predominantly outside the thermoneutral zone for laying quails.

### 2.2. Animals, experimental design, and diets

Four hundred and eight 23-week-old Japanese quails (*Coturnix coturnix japonica*) with an average body weight of  $154.92 \pm 3.77$  g were randomly allotted in a  $3 \times 2$  factorial arrangement. The first factor was the three levels of SD, while the second factor was two levels of AA supplementation. There were four replicates for each experimental unit. The three SDs were 40 (SD-1), 45 (SD-2), and 50 birds per m<sup>2</sup> (SD-3), equivalent to 15, 17, and

19 quails per cage, respectively. The cage size was 3750 cm<sup>2</sup> (75 × 50 cm). These SDs were equal to floor spaces of 250, 221, and 200 cm<sup>2</sup> per bird. The cages sizes used for determining the nutrient digestibility were 16.7 × 15 cm (SD-1), 14.7 × 15 cm (SD-2), and 13.3 × 15 cm (SD-3).

The two dietary treatments were the basal diet (Table 1) and basal diet supplemented with AA at a level of 250 mg/kg. The supplementation of AA was performed by supplementing per kg basal diet with 710 mg of AA product with 35.3% purity (ascorbic acid, Nutriad, Belgium) at the expense of rice bran following the procedure of Ratriyanto et al. [19]. In addition, all experimental groups received the same management practices.

### 2.3. Data collection

The birds were administered the basal diet during the four-week preexperimental period, during which time the birds had free access to water and feed. The SD and AA supplementation treatments lasted for two periods of 28 days each. The data of feed intake as well as egg production and weight were collected daily and averaged for each experimental unit. The feed conversion ratio (FCR) was obtained by dividing the feed intake by egg mass [20]. The energy efficiency ratio (EER) was calculated as grams of egg mass per 100 kcal of metabolizable energy intake, while protein efficiency ratio (PER) was calculated as grams of egg mass per gram of protein intake [21]. The physical egg quality consisted of the yolk, albumen, and eggshell weight; the yolk and albumen index; and eggshell thickness, and it was measured on the last three days of

**Table 1.** Ingredients and nutritional composition of the basal diet.<sup>1</sup>

Ingredient	Proportion (g/kg)	Nutrient composition	Content
Yellow corn	457.50	Metabolizable energy (kcal/kg)	2800.0
Rice bran	180.20	Crude protein (g/kg)	180.0
Soybean meal	202.00	Crude fiber (g/kg)	42.5
Fish meal	67.00	Crude fat (g/kg)	48.6
Coconut oil	13.00	Crude ash (g/kg)	53.4
DL-methionine	0.90	Lysine (g/kg)	10.4
Choline chloride	1.00	Methionine (g/kg)	4.5
Dicalcium phosphate	8.30	Calcium (g/kg)	34.0
Limestone	63.10	Available phosphorus (g/kg)	5.0
Vitamin-mineral premix <sup>2</sup>	3.50		
NaCl	3.50		

<sup>1</sup>Other experimental diet was supplemented with 250 mg/kg ascorbic acid.

<sup>2</sup>Vitamin-mineral premix supplied per kg diet: 42000 IU vitamin A, 7000 IU vitamin D<sub>3</sub>, 28 mg vitamin E, 7 mg vitamin K, 7 mg vitamin B1, 18 mg vitamin B2, 2 mg vitamin B6, 42 mg vitamin B12, 21 mg Ca D-pantothenate, 140 mg niacin, 35 mg choline chloride, 420 mg Mn, 70 mg Fe, 0.7 mg I, 350 mg Zn, 0.7 mg Co, and 14 mg Cu.

each period (days 26–28) according to Stadelman and Cotterill [22]. Nine eggs per replicate (216 eggs per period) were collected to determine the egg quality traits. The eggs were individually weighed and cracked, and the yolk and eggshell were weighed. The albumen weight was calculated by subtracting the yolk and eggshell weight from egg weight. The yolk and albumen diameters as well as their heights were measured with a digital micrometer (0–150 × 0.001 mm, Digital Caliper 1108-150, INSIZE, China). The eggshell thickness was measured using a digital micrometer (0–25 × 0.001 mm, Digital Outside Micrometer 3109-25A, INSIZE, China).

#### 2.4. Nutrient digestibility measurement

After two periods of feeding treatment, two birds per replicate (48 birds in total) were picked to assess nutrient digestibility over a five-day collection period [19]. The excreta were repeatedly sprayed using 0.1 M sulfuric acid to avoid further microbial fermentation. The collected excreta were pooled in each replicate, homogenized, and sun-dried thereafter. The excreta were ground through a 0.5-mm mesh screen prior to their analyses. The crude protein (CP) determination was performed according to the Kjeldahl method, while other proximate fractions

were determined following the procedure outlined by the Association of Official Analytical Chemists [23]. Ca was determined by atomic absorption spectrophotometry [23]. The nutrient digestibility was calculated according to Emamzadeh and Yaghobfar [24] using the following equation:

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

#### 2.5. Data analysis

All data were analyzed with a two-way analysis of variance according to the following model:  $y_{ijk} = \mu + \alpha_i + \beta_j + \alpha\beta_{ij} + \epsilon_{ijk}$ , where  $\mu$  = general mean,  $\alpha_i$  = effect of SD,  $\beta_j$  = effect of AA,  $\alpha\beta_{ij}$  = interaction effect of SD and AA, and  $\epsilon_{ijk}$  = experimental error. The statistically different means were compared using Duncan's multiple-range test at  $P < 0.05$ . All statistical analyses were conducted using R version 3.5.3 [25].

### 3. Results

#### 3.1. Nutrient digestibility

No interaction appeared between SD and supplementation of AA on nutrient digestibility ( $P > 0.05$ ; Table 2), which indicates that the nutrient digestibility values did not differ

**Table 2.** Nutrient digestibility of quails (%) raised in different stocking densities and supplemented with ascorbic acid.

Treatments	DM	CP	EE	CF	CA	Ca	
Interaction SD × AA							
40	Control	77.4 ± 2.3	77.7 ± 2.9	90.1 ± 0.9	34.9 ± 1.9	51.8 ± 7.5	79.2 ± 2.6
40	AA	79.5 ± 2.2	72.5 ± 8.0	89.9 ± 0.9	37.1 ± 2.7	73.5 ± 5.0	80.6 ± 3.7
45	Control	76.6 ± 0.2	77.5 ± 1.0	90.1 ± 0.6	30.6 ± 2.4	49.1 ± 5.3	77.1 ± 2.7
45	AA	80.8 ± 0.4	77.5 ± 2.0	91.1 ± 0.8	34.9 ± 1.0	76.9 ± 3.8	80.3 ± 1.4
50	Control	75.4 ± 2.9	75.7 ± 4.7	88.9 ± 0.7	27.9 ± 1.5	45.9 ± 5.9	75.6 ± 2.6
50	AA	78.6 ± 2.5	69.7 ± 9.8	88.9 ± 0.8	31.9 ± 1.9	72.1 ± 2.9	79.1 ± 4.1
P-value		0.19	0.53	0.19	0.35	0.73	0.74
Effect of SD							
40		78.4 ± 2.4	75.1 ± 6.3	89.9 ± 0.8 <sup>ab</sup>	36.1 ± 2.5 <sup>a</sup>	62.4 ± 13.2	79.9 ± 3.1
45		78.8 ± 2.2	77.5 ± 1.5	90.6 ± 0.9 <sup>a</sup>	32.7 ± 2.9 <sup>ab</sup>	63.0 ± 15.5	78.7 ± 2.6
50		77.0 ± 3.0	72.7 ± 7.8	88.9 ± 0.7 <sup>b</sup>	29.9 ± 2.6 <sup>b</sup>	58.9 ± 14.6	77.4 ± 3.7
P-value		0.78	0.27	0.04	0.04	0.52	0.27
Effect of AA							
Control		76.8 ± 2.1 <sup>b</sup>	76.6 ± 3.1	89.7 ± 0.9	31.5 ± 3.5 <sup>b</sup>	51.3 ± 6.1 <sup>b</sup>	77.6 ± 2.8 <sup>b</sup>
AA		79.4 ± 1.9 <sup>a</sup>	73.5 ± 7.5	89.9 ± 1.2	34.4 ± 2.9 <sup>a</sup>	72.4 ± 4.2 <sup>a</sup>	79.8 ± 3.0 <sup>a</sup>
P-value		0.02	0.12	0.78	0.03	<0.01	0.04

DM: Dry matter, CP: crude protein, EE: ether extract, CF: crude fiber, CA: crude ash, Ca: calcium, SD: stocking density, AA: ascorbic acid.

<sup>ab</sup>Means in the same column and treatment with no common superscript differ significantly at  $P < 0.05$ .

for all SDs with or without AA supplementation. The birds kept in SD-2 showed higher ( $P < 0.05$ ) ether extract (EE) digestibility than those housed in SD-3, although the results did not differ between those housed in SD-2 and SD-1. The birds housed in SD-1 had higher ( $P < 0.05$ ) crude fiber (CF) digestibility than those housed in SD-3, although the results also did not differ between those of SD-1 and SD-2. These results indicate that the highest SD generated the lowest EE and CF digestibility values. However, SD did not influence the dry matter (DM), CP, or crude ash (CA) digestibility values. The birds fed a diet supplemented with AA exhibited higher DM digestibility than those with a nonsupplemented diet ( $P < 0.02$ ). The enhancement in DM digestibility following AA supplementation was in line with improvements in the digestibility values of CA, CF, and Ca ( $P < 0.05$ ).

### 3.2. Performance traits

There was an interaction identified between SD and AA supplementation on egg production, wherein AA produced more eggs ( $P < 0.01$ ) in all three SDs (Table 3). The birds raised in SD-2 that received AA supplementation generated the highest egg production (83.62%) compared to the other groups. The birds housed in SD-2 generated more eggs compared to those in SD-3 ( $P < 0.01$ ), although

the results did not differ between those housed in SD-2 and SD-1. Furthermore, increasing the SD to 50 birds per  $m^2$  tended to increase the FCR ( $P = 0.09$ ) as well as decrease the EER ( $P = 0.08$ ) and PER ( $P = 0.08$ ). The supplementation of AA's enhancement of the egg weight compared to that of a nonsupplemented diet ( $P = 0.01$ ) concurs with the improvement in the FCR ( $P = 0.03$ ), EER ( $P = 0.03$ ), and PER ( $P = 0.03$ ) following AA supplementation (Table 3). In addition, SD and AA did not affect the birds' feed intake.

### 3.3. Egg quality traits

No interaction occurred between SD and AA supplementation on egg quality ( $P > 0.05$ ; Table 4), thus indicating that the egg quality indicators did not differ for all SDs with or without AA supplementation. The birds housed in SD-3 yielded lower eggshell thickness compared with those housed in SD-1 and SD-2 ( $P = 0.01$ ). However, different SDs did not alter the other egg quality parameters. The supplementation of AA significantly enhanced the eggshell weight ( $P = 0.02$ ) and eggshell thickness ( $P < 0.01$ ) compared to those of a nonsupplemented diet (Table 4), all of which correspond with the improvement of egg weight identified in this experiment (Table 3). However, AA supplementation did not influence the albumen and yolk weight as well as albumen and yolk index.

**Table 3.** Performances of quails raised in different stocking densities and supplemented with ascorbic acid.

Treatments	FI (g)	EP (%)	EW (g)	FCR	EER	PER
Interaction SD × AA						
40 Control	23.1 ± 0.7	74.6 ± 1.1 <sup>c</sup>	9.3 ± 0.2	3.4 ± 0.1	10.7 ± 0.4	1.7 ± 0.1
40 AA	22.9 ± 0.9	79.8 ± 0.5 <sup>b</sup>	9.5 ± 0.1	3.0 ± 0.1	11.9 ± 0.4	1.8 ± 0.1
45 Control	22.6 ± 0.9	73.9 ± 1.2 <sup>c</sup>	9.3 ± 0.3	3.3 ± 0.1	10.9 ± 0.4	1.7 ± 0.1
45 AA	23.7 ± 1.9	83.6 ± 0.8 <sup>a</sup>	9.4 ± 0.3	3.0 ± 0.2	11.8 ± 0.7	1.8 ± 0.1
50 Control	22.6 ± 1.2	73.6 ± 2.2 <sup>c</sup>	9.2 ± 0.2	3.4 ± 0.2	10.7 ± 0.7	1.7 ± 0.1
50 AA	23.2 ± 1.3	78.7 ± 1.7 <sup>b</sup>	9.3 ± 0.2	3.2 ± 0.2	11.3 ± 0.7	1.8 ± 0.1
P-value	0.54	<0.01	0.52	0.65	0.62	0.57
Effect of SD						
40	23.0 ± 0.8	77.2 ± 2.9 <sup>ab</sup>	9.4 ± 0.2	3.2 ± 0.2	11.3 ± 0.7	1.8 ± 0.1
45	23.2 ± 1.5	78.8 ± 5.2 <sup>a</sup>	9.3 ± 0.3	3.2 ± 0.2	11.3 ± 0.8	1.8 ± 0.1
50	22.9 ± 1.2	76.1 ± 3.2 <sup>b</sup>	9.2 ± 0.2	3.3 ± 0.2	11.0 ± 0.7	1.7 ± 0.1
P-value	0.90	<0.01	0.13	0.09	0.08	0.08
Effect of AA						
Control	22.8 ± 0.9	74.0 ± 1.5 <sup>b</sup>	9.2 ± 0.2	3.3 ± 0.2 <sup>a</sup>	10.8 ± 0.5 <sup>b</sup>	1.7 ± 0.1 <sup>b</sup>
AA	23.2 ± 1.3	80.2 ± 2.4 <sup>a</sup>	9.4 ± 0.2	3.1 ± 0.2 <sup>b</sup>	11.6 ± 0.6 <sup>a</sup>	1.8 ± 0.1 <sup>a</sup>
P-value	0.34	<0.01	0.01	0.03	0.03	0.03

FI: Feed intake, EP: egg production, EW: egg weight, FCR: feed conversion ratio, EER: energy efficiency ratio, PER: protein efficiency ratio, SD: stocking density, AA: ascorbic acid.

<sup>a-c</sup>Means in the same column and treatment with no common superscript differ significantly at  $P < 0.05$ .

**Table 4.** Egg quality of quails raised in different stocking densities and supplemented with ascorbic acid.

Treatments	AI (%)	AW (g)	YI (%)	YW (g)	ESW (g)	ST (mm)	
Interaction SD × AA							
40	Control	15.6 ± 0.4	6.2 ± 0.1	49.1 ± 0.7	2.9 ± 0.1	0.84 ± 0.02	0.20 ± 0.00
40	AA	15.0 ± 0.5	6.2 ± 0.1	49.5 ± 1.2	2.9 ± 0.0	0.85 ± 0.02	0.21 ± 0.01
45	Control	15.8 ± 0.3	6.0 ± 0.1	48.7 ± 0.6	2.9 ± 0.1	0.86 ± 0.01	0.20 ± 0.00
45	AA	15.2 ± 0.4	6.1 ± 0.3	48.8 ± 1.1	2.9 ± 0.1	0.88 ± 0.03	0.21 ± 0.00
50	Control	15.9 ± 0.6	6.1 ± 0.3	48.9 ± 0.9	2.8 ± 0.1	0.81 ± 0.02	0.20 ± 0.00
50	AA	15.0 ± 0.7	6.1 ± 0.2	50.2 ± 0.8	2.8 ± 0.1	0.87 ± 0.05	0.21 ± 0.00
P-value	0.74	0.57	0.48	0.36	0.35	0.27	
Effect of SD							
40		15.3 ± 0.6	6.2 ± 0.1	49.3 ± 0.9	2.9 ± 0.0	0.85 ± 0.02	0.21 ± 0.01 <sup>a</sup>
45		15.5 ± 0.5	6.0 ± 0.1	48.8 ± 0.8	2.9 ± 0.1	0.87 ± 0.02	0.21 ± 0.00 <sup>a</sup>
50		15.5 ± 0.8	6.1 ± 0.3	49.5 ± 1.1	2.8 ± 0.1	0.84 ± 0.04	0.20 ± 0.01 <sup>b</sup>
P-value	0.76	0.24	0.24	0.29	0.11	0.01	
Effect of AA							
Control		15.8 ± 0.5	6.1 ± 0.2	48.9 ± 0.7	2.8 ± 0.1	0.83 ± 0.02 <sup>b</sup>	0.20 ± 0.00 <sup>b</sup>
AA		15.1 ± 0.5	6.1 ± 0.2	49.5 ± 1.1	2.9 ± 0.1	0.86 ± 0.03 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>
P-value	0.11	0.32	0.13	0.17	0.02	<0.01	

AI: Albumen index, AW: albumen weight, YI: yolk index, YW: yolk weight, ESW: eggshell weight, ST: shell thickness, SD: stocking density, AA: ascorbic acid.

<sup>a,b</sup>Means in the same column and treatment with no common superscript differ significantly at P < 0.05.

## 4. Discussion

### 4.1. Nutrient digestibility

A high SD negatively affects birds' nutrient digestion, as indicated by lowered EE and CF digestibility. A previous observation revealed that a high SD negatively influenced nutrient digestion [26]. Birds can be housed under high-SD conditions if the ventilation, temperature, and humidity inside the cages remain appropriate [1]. At a higher SD, birds suffer from the environment's unfavorable effects, such as increased cage temperature, accumulation of carbon dioxide and ammonia, reduced fresh air for respiration, and decreased nutrient absorption [8,27]. The negative effects of a high SD have been previously observed, such as decreased villus height of the jejunum, which is correlated with less area for nutrient absorption [3]. In line with this study, reducing floor space decreased the DM, CP, EE, and Ca digestibility values in quails [26]. Accordingly, increasing the SD decreased nutrient digestibility values in laying hens [4].

Several observations in quails raised in high temperatures support this study's findings, where supplementation of AA improved the nutrient digestibility values [14,28], thus indicating that AA alleviates the unfavorable responses of heat stress [11,12]. Furthermore,

AA possesses a protective role against oxidative stress for pancreatic tissues and maintains the optimal functioning of pancreas (i.e. the secretion of digestive enzymes), thus improving nutrient digestibility [17,29]. For example, AA improved the DM, CP, and/or EE digestibility in quails [11] and laying hens [14,30] housed in a high-temperature environment. However, in the present study, the CP and EE digestibility values were not affected by AA supplementation. Moreover, dietary AA yielded greater ash, nitrogen, calcium, and phosphorus retention in laying hens [28] and Japanese quails [17].

### 4.2. Quails' performance traits

Increasing the SD without AA supplementation did not affect the quails' egg production, while AA supplementation increased their egg production. The findings indicate that AA was effective in improving egg production disregarding the SD. The highest egg production was yielded in the SD-2 group receiving AA supplementation, thus revealing optimal conditions for the birds' laying performance. Increasing the SD to 50 birds per m<sup>2</sup> negatively affected their egg production, which is associated with this treatment's lower nutrient digestibility (EE and CF). Furthermore, a high SD may result in a higher cage temperature due to overcrowding, reduced air



quality due to inadequate airflow, and increased ammonia [7,8,27]. In addition, the unfavorable effects of a high SD might be attributed to the alteration of resting behavior due to disruptions from other birds [7]. Our findings agree with those of previous studies in that a high SD reduced Japanese quail egg production and weight as well as eggshell thickness [2,26]. Another observation in these birds demonstrates that increasing the SD from 38 to 47 birds per m<sup>2</sup> did not affect their egg production, although increasing the SD to more than 56 birds per m<sup>2</sup> decreased their egg production [5]. Similarly, reducing floor space adversely affected the laying hens' egg production without affecting the egg weight and FCR [20,27].

Many researchers have determined the beneficial effects of AA supplementation in poultry that are housed in high-temperature environments. AA is a primary antioxidant in biological systems [31]. In stressful conditions, the synthesis of AA is inadequate, which leads to increased cytotoxic free radicals deteriorating cells and cell membranes, stimulated protein catabolism, and lowered protein biosynthesis [17,31]. Thus, AA supplementation is beneficial for alleviating some stress related to physiological responses and for improving thermotolerance through the antioxidant effects [17]. A previous study in quails housed in a high-temperature environment revealed that dietary AA supplementation improved the antioxidant status, laying performances, and egg quality parameters [11]. Similarly, AA supplementation to the diet at 240 mg/kg increased the production rate and egg weight of quails housed at an ambient temperature of 33 °C [31]. The AA supplementation to the quails' diet at 150 and 250 mg/kg did not affect their egg production at a high environmental temperature (34 °C), while such supplementation enhanced egg production at 500 mg/kg [32]. Bardakçioğlu et al. [32] also did not observe any influence of AA on quails' feed intake, egg weight, or FCR. Furthermore, AA supplementation increased the FCR as well as EER and PER, thus indicating enhancement in the birds' utilization of nutrients [21,26].

#### 4.3. Quails' egg quality traits

A high SD adversely affects quails' egg quality, particularly the eggshell thickness. The birds housed in SD-3 produced the thinnest eggshells compared to the other SD conditions. Increasing the SD may stimulate heat stress, which is

related to the acid–base balance alteration and leads to lowered bicarbonate ion production [33]. Bicarbonate, together with Ca ions, is utilized to form the eggshell [33]. In accordance with this finding, quails housed in a high SD produced thinner eggshells than those housed in a low SD did [18,26]. Increasing the SD has also been observed to decrease eggshell strength [27] and density [18], both of which are associated with a greater broken egg rate [27].

A greater eggshell weight and thickness due to AA supplementation agreed with the improvement in CA and Ca digestibility in this study and point toward more beneficial mineral availability and utilization. Deteriorated eggshell quality in a high SD, particularly at a high ambient temperature, may be associated with a decrease in Ca uptake by the small intestine [9]. Furthermore, it has been postulated that AA is involved in eggshell formation by stimulating 1,25 dihydroxy-cholecalciferol synthesis and thus leading to an enhancement in the mobilization of Ca from the bones [11]. This finding suggests that AA enhances the serum Ca concentration needed to form the eggshell [13]. Consistent with this finding, AA supplementation enhanced eggshell weight and thickness in quails in the study of Sahin et al. [11]. Similarly, improvement in eggshell quality was observed in breeder hens that received AA supplementation [12]. Different from this finding, a previous study revealed that AA supplementation did not improve quails' eggshell weight or thickness [32].

This study demonstrates that SD of 45 birds per m<sup>2</sup> is optimal for laying quails raised in the high temperatures of a tropical climate. Increasing the SD to 50 birds per m<sup>2</sup> negatively affected their nutrient digestibility, which subsequently decreased their egg production and eggshell quality. Furthermore, AA supplementation to the quails' diet at 250 mg/kg improved their nutrient digestibility, performance, and egg quality traits, particularly their eggshell quality. Therefore, the SD of 45 birds per m<sup>2</sup>, combined with AA supplementation, is recommended to be applied to laying quails living in a tropical climate.

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