Effect of age, stocking density, genotype, and cage tier on feather score of layer pure lines

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Abstract
The objective of the study was to investigate the effect of genotype, age, stocking density, and cage tier on the feather score of egg-laying pure lines. The trial was carried out with five white (BLACK, BLUE, MARON, BROWN, and D229) and six brown (RIR1, RIR2, BAR1, BAR2, COL, and LINE54) eggshell lines as classified by the Ankara poultry research institute. In the experiment, 162 chicks were randomly selected from each of the 11 lines thus, a total of 1782 chicks (810 and 972 white and brown layer pure lines respectively). At 17 weeks of age, pullets were chosen at random and placed in 3- tiered battery type cages. Each tier housed 99 chickens (11 lines; 3 different stocking densities; 3 replications). The stocking density was either 5 birds, 6 birds, and 7 birds per cage cell that corresponded to 720
cm², 600 cm², and 514.28 cm² of floor space per bird, respectively. Feeding was ad libitum during the growth and egg production periods. Feather scores of the head, neck, breast, back, wings, and tail regions of the chickens were taken at the 30th, 40th, 50th, and 60th week of age. The results indicated that age, stocking density, cage tiers, and genotypes have a significant effect on the feather score of the head, neck, breast, back, wings, and tail; feather score significantly decreased at an increasing age and stocking density (P < 0.01). It was observed that the feather score of the chickens on the top tier was significantly increased (P < 0.01). It was found that RIR2, BLACK, and COL pure lines had the best feather score. These findings suggest that chickens placed in top cage tiers with low stocking density (5 chickens/ cage cell) and RIR2, BLACK and COL pure line genotypes improve feather score.

Keywords: Layer, feather score, genotype, stocking density, cage tier

1. Introduction
The rapid growth of the world's population has resulted in ensuring maximum efficiency from the unit production area in agricultural activities as one of the most important objectives [1]. Compared to other animal species, poultry (largely chickens) has advantages such as the utilization of small agricultural areas, high production rate due to the application of all kinds of automation and mechanization in production, short-term capital transformation, and continuous income throughout the year [2]. During the first half of the twentieth century in industrialized societies, chicken production gained an intensive structure that ensured important developments in genetics, breeding, care-feeding techniques, and improvements in preventive medicine. Due to this structure, production is done with completely high-yielding commercial hybrid materials [3,4,5].
In modern layer production, different egg-laying hybrids are housed in multi-tier cages until the end of the production period. The adaptation of egg-laying hybrids reared in different cage tiers is also different [6,7,8].

Although the increase in the number of cage tiers in the cage system sets additional space for more chicken and egg yields, it also brings with it some flaws. Several studies have confirmed the negative effects of stocking density in cages on feed consumption, body weight, egg production and egg quality, and plumage condition [9,10,11]. Depending on the stocking density, genotypes are also reported to respond differently [10,12,13]. It is suggested that there is a very close relationship between stocking density and mortality rate, and the higher the number of animals stocked per unit area, the higher the mortality rate [10,14].

Today, most of the egg-laying hens are housed in conventional cages excluding the European Union block and some developed states [15]. It was revealed that there is a significant difference between cage tiers and cage positions, and some production features [8]. In layer production, it is necessary to keep the animals away from stress factors such as those resulting from the cage systems for the health of the animals, ensuring animal welfare, and avoiding production losses [16,17,18].

Feather loss in layer production adversely affects animal health, increases feed consumption and has effects on egg production. It is known that the feather quality of the chickens deteriorates as they age. At the end of the oviposition period, some are almost completely naked. If the chickens lose their feathers largely due to feather pulling, feather molting, or other reasons, there will be deterioration in natural thermal insulation and an increase in heat loss in the chicken body [19]. Chickens compensate for this heat loss by increased feed intake since there is a high correlation between feather score and feed consumption [20]. In chickens housed under commercial conditions, severe feather pecking (SFP) continues to be a serious welfare problem and significantly increases economic loss due to increased feather
damage and loss, increased mortality rates, increased feed consumption, and decreased egg
production [21,22,23]. Feather damage is largely influenced by genetic and environmental
factors [24]. Furthermore, behavioral traits such as feather pecking are considered in the
recent layer breeding programs during trait selection due to their welfare implications (e.g.,
feather loss and damage) [25,26].

In parallel with the prohibition of the conventional cage system, many countries are putting a
ban on beak trimming and this will cause serious problems in the egg industry. In the light of
all of these, determining the feather scores of egg-laying genotypes will become even more
important. Therefore, there is a need for a known method for the identification of the feather
score of the egg-laying chickens and to introduce measures to be taken during the production
period [27]. Since one of the physical methods used to determine animal welfare is the
feather condition of chickens, in this respect, it is also important to score feathers. Feather
scoring at regular intervals is also a useful tool for evaluating the health status of animals in
the flock [28].

Egg chicken production systems have become a sustainable industry today as a result of
significant improvements in genotype, nutrition, health protection, and treatment. Within this
sector, the development of genotypes housed in the conventional cage system gained
importance, and the countries that achieved this, have taken the lead in the layer breeder
chickens sector. It is established that in Turkey, developments started in the 1960s, and
efforts to develop egg-laying parent lines gained momentum in the 1990s [29]. As a result of
these studies, many egg-laying parent lines have been developed. Moreover, studies are
continuously carried out on the performance characteristics of these lines. However, basic
features such as feather scores for selection and breeding the lines are also needed. Therefore,
this study was designed to evaluate the effect of age, genotype, stocking density, and cage tier
on the feather score of the various layer pure lines bred in Turkey.
2. Materials and methods

2.1. Animal materials

The current research was carried out with five white and six brown egg shelled laying pure lines. BLACK, BLUE, MARON, BROWN, and D229 were the white egg lines, and RIR1, RIR2, BAR1, BAR2, COL, and LINE54 brown egg lines as classified by Ankara Poultry Research Institute. A total of 1782 day old chicks (810 white and 972 brown layers) and, 162 chicks from each of the 11 lines were used in this experiment. These were taken from the Ankara Poultry Research Institute Directorate of the Ministry of Agriculture and Forestry.

2.2. Chicken housing

The experiment was overseen at the Directorate of Agricultural Research Institute of the Central Black Sea Gate Belt of the Ministry of Agriculture and Forestry. Day-old chicks were raised in a fully controlled environment brooder pen until the age of 17 weeks. In the brooder pen, the starting temperature was at 33 °C on the first day, gradually lowered to 19 °C for 30 days and then fixed at 19 °C, with an average of 26 °C. The average relative humidity was 60% with the lowest and highest being 50% and 70% respectively. On the first day, a continuous lighting program of 23 hours of light, and 1 hour of darkness (23L: 1D) was provided to the chicks. In the following days, the light was restricted for 1 hour. From 3 (after 14 days) to 17 weeks, a lighting program of 10 hours light and 14 hours of darkness was maintained.

In the rearing pen, lighting was provided by a fluorescent lamp that gave white light. Ventilation was ensured with 4 fans at the back of the pen and there were light refractors on the fans that prevented sunlight from outside. 2 fans and pads were provided on the sidewalls of the pen for the cooling process. Heating was by LNG-powered air blowers. Ventilation,
lighting, humidity, and heating were automatically regulated by the control panel in the pen.

Grower or rearing pen cages were 65 cm × 120 cm × 40 cm. Each cage cell contained 4
nipple drinkers and a feeder of 120 cm long. Chicken manure was automatically removed by
the moving tape underneath them. Free feeding was carried out during the growth period.

Animals were transferred to the production pen at the age of 17 weeks. Pullets were randomly
selected from each line (BLACK, BLUE, MARON, BROWN, and D229 white egg layers;
RIR1, RIR2, BAR1, BAR2, COL, and LINE54 brown egg layers). The stocking density was
either; 5 birds, 6 birds, and 7 birds per cage cell that was matched to 720 cm², 600 cm², and
514.28 cm² floor space per bird respectively, and in cage tiers that were coded as 1, 2, and 3
from the bottom to top. Ventilation was ensured with 8 fans at the back of the poultry pens.

Cooling was provided by 3 fans and pads that were found on the front and sidewalls of the
poultry house. Lighting was done by a fluorescent lamp that gave 36 watts of white light.

Ventilation, lighting, humidity, and heating were automatically regulated by the control panel
in the poultry pen. The chickens were housed in 3-tiered battery type cages measuring 60 cm
× 60 cm × 45 cm. The cage tiers were equal to each other, the sides of the cages were
galvanized sheet metal and the back was knitted wire mesh. The front side was made suitable
for the chickens to remove their heads, but there was a wire mesh that prevented them from
escaping. There was plastic tape on the 1st and 2nd cage tier and knitted wire mesh on the 3rd
tier. The front and the back height of the cage were 45 cm and 40 cm respectively. 2 nipple
drinkers were provided in each cage cell. Chicken feces was mechanically removed by the
underlying moving bands or tapes. Eggs were mechanically collected with cloth tape or band
under the feeder. Feeder length was ensured at 60 cm for each cage cell.

In this experiment, the stocking plan for the distribution of animals to cages was randomly
carried out. For each tier, 99 (11 lines, 3 different stocking densities, and 3 replications), a
total of 297 cage units were used. In the laying pen, the lowest and highest temperature was
19 °C and 25 °C respectively with an average of 22 °C. The average humidity was 55% with
the lowest being 40% and the highest 70%. The temperature in the poultry pen was measured
with 6 temperature sensors (3 on each pen sidewalls) placed at an interval of 13 meters and a
height of 2 m. On the other hand, a relative humidity sensor was placed at a height of 2 m in
the center of the poultry pen. Lighting was provided with a fluorescent lamp that gave 36
watts of white light at a height of 3 m from the ground and a distance of 120 cm to the cages.
In the rearing or growth pens, chickens were stimulated to oviposition by increasing the
lighting time for 30 minutes on the day they were moved to the laying pen. In the following
period, 30 minutes of lighting time was increased each week, and when the photoperiod
reached 16 hours of light and 8 hours dark, the photoperiod was maintained until the end of
the experiment.
Light intensity in the poultry house was taken at the beginning and end of the experiment
with the help of a light meter (Luxmeter; Digital light meter TT T-ECHNI-C VC1010D)
positioned in front and the middle of the cage (Table 1).
Research materials were vaccinated according to the vaccination program (Table 2).
The rules of biosecurity were meticulously observed. No signs of disease were found during
the trial and no drug application was performed.

2.3. Feeds and feeding
Standards feeds were obtained from a private feed factory in Ankara. Chicks were fed layer
breeder chick starter feed for the first 3 weeks during the growth period (20% Crude protein;
2900 Kcal/kg Metabolic Energy (ME); 6% Crude fiber; 1% Calcium; 0.5% Phosphorus),
layer breeder grower feed in 4-10 weeks (18% Crude protein; 2800 Kcal/kg ME; 5% Crude
fiber; 1% Calcium; 0.5% Phosphorus), layer breeder developer feed in 11-17 weeks (15.5%
Crude protein; 2600 Kcal/kg ME; 6% Crude fiber; 1% Calcium; 0.5% Phosphorus), pre layer
breeder feed in 17-19 weeks (17.5% Crude protein; 2700 Kcal/kg ME; 7% Crude fiber; 2% Calcium; 0.5% Phosphorus), layer breeder feed after 20 weeks (18% Crude protein; 2800 Kcal/kg ME; 6.5% Crude fiber; 3.8% Calcium; 0.45% Phosphorus). Free feeding was done during periods of growth and oviposition.

2.4. Feather score

Feather score was performed by an experienced person on the 30, 40, 50, and 60th week of the trial. Chickens with damaged feathers were not tested at the beginning of the trial and those with a total feather score of 4 full points were also included in the experiment. Feather score was carried out according to the loss and damage of the feathers on the head, neck, chest, back, wings, and tail. A feather score scale of 1-4 was used; 1- no feathers, 2- half feather loss, 3- 1/3 feather loss, and 4- full feather coverage. Feather scores for each body area were obtained separately. A total of 6 points from 6 body parts of chickens showed that they lost all of their feathers, and a total of 24 points indicated that they had all of their feathers maintained [30,31].

2.5. Statistical analyzes

The experiment was a completely randomized block design. The significant level was considered at P < 0.05. SPSS statistical package program was used in the analysis of the data. Duncan's test was used for multiple range tests to determine the difference between averages [32].

3. Results

3.1. Effect of age on feather score
The results obtained from all pure lines of different ages are given in Table 3. Considering
the head region, the head feather score decreased as the flock age increased. However, while
the statistical difference between the 30th, 40th, and 50th weeks was not significant (P >
0.05), the head feather score at the 60th week was found to be statistically lower than the
other age groups (P < 0.01). Neck area feather score decreased together with age and the
statistical difference between each measured age was very significant (P < 0.01). The feather
score of the back part decreased with age leading to a statistical difference (P < 0.01). The
effect of age on the tail feather score was found to be significant (P < 0.01) and the feather
score decreased as the age advanced. The wing feather score decreased with age and resulted
in a statistical difference (P < 0.01) and there was no difference between wing feather score
on the 30th and 40th weeks. However, the differences were significant in the 40th and 50th
weeks, 50th, and 60th weeks. Generally, the total feather scores of the 30, 40, 50, and 60th
week were 23.59; 23.19; 22.53, and 22.09 respectively. As seen, total feather scores
decreased with an increase in flock age. This decrease caused the statistical difference
between ages (P < 0.01).

3.2. Effect of genotype on feather score
The results of egg-laying genotypes and their effect on feather scores are indicated in Table 3.
In the study, D229, BLUE, MARON, BROWN, BLACK, RIR1, RIR2, COL, and LINE54
lines were found to have a superior head feather score, while BAR2 and RIR1 were in the
middle group and BAR1 had the lowest feather score. The effect of the genotype on the head
feather scores was found to be statistically significant (P < 0.01). About neck feather scores,
the formed arrangement was, BLACK, BROWN, MARON, COL, LINE54, BLUE in the 1st
group; BLUE, MARON, RIR2, COL, and LINE54 in the 2nd group; D229, BLUE, and RIR2
in the 3rd group; BAR1 in the 4th group; BAR2 in the 5th group; RIR1 in the 6th group.
Neck feather scores indicated a reducing trend from the 1st towards the 6th group leading to a statistical difference (P < 0.01). As to back feather scores, the observed order was, BROWN, BLACK, RIR2, and COL lines in the 1st group; MARON line in the 2nd group; D229 and BLUE line in the 3rd group; D229, RIR1, and LINE54 lines in the 4th group; Bar1 and BAR2 line in the 5th group (P < 0.01). Therefore, the best lines in terms of back feather scores are BROWN, BLACK, RIR2, and COL, while the lines with the lowest are BAR1 and BAR2. The order of the lines as far as tail feather score was concerned, RIR2 and COL line in the 1st group; COL and BLACK line in the 2nd group; BLACK and BLUE line in the 3rd group; BLUE, D229, BROWN and MARON lines in the 4th group; RIR1 line in the 5th; LINE54 line in the 6th; BAR1 and BAR2 line in the 7th group. It was noted that the tail feather scores followed a diminishing trend from the 1st towards the 7th group and hence, a statistical difference (P < 0.01). Regarding wing feather scores, Blue, MARON, BROWN, BLACK, RIR1, RIR2, LINE54, and COL lines had the best score, while the D229, BLUE, MARON, RIR1, COL, and LINE54 lines had medium and BAR1 and BAR2 lines had the lowest tail feather scores (P < 0.01). Evaluating the breast feather scores found that RIR2, COL, LINE54, BLUE, MARON, BROWN, and BLACK lines had the highest, RIR1, BAR2, BLUE, and MARON lines had medium, D229, BAR1, BAR2, and RIR1 lines had the lowest breast feather scores (P < 0.01). In regards to overall total feather scores, the order of lines appeared as, BROWN, BLACK, RIR2, and COL lines in the 1st group; MARON and BROWN lines in the 2nd group; BLUE and MARON lines in the 3rd group; D229 line in the 4th group; LINE54 line in the 5th; RIR1 line in the 6th group; BAR1 and BAR2 line in the 7th group. The overall total feather scores as mentioned above revealed a decreasing trend from the 1st group towards the 7th group, which caused a statistical difference (P < 0.01). Genotypes with the best feather score were those in the 1st group while those in the 7th group had the lowest feather score.
3.3. Effect of stocking density on feather score

The effect of stocking density on the feather score is shown in Table 3. The head feather scores of the group that had a stocking density of 7 chickens were statistically lower than the groups with a stocking density of 5 and 6 (P < 0.01). Furthermore, the neck and back feather scores of the animals that were stocked 5 in a group were found to be higher than in the group of 6 and 7. It was also indicated that as the stocking density increased, the neck and back feather scores decreased, which resulted in a statistical difference (P < 0.01). Similarly, as the stocking density increased, the decrease in tail and wing feather scores led to a statistical difference (P < 0.01). This same trend was observed in terms of the overall feather scores. The effect of the stocking density on breast feather scores was found to be non-significant (P > 0.05).

3.4. Effect of cage tier on feather score

Trial results on the effect of the cage tier on the feather score are in Table 3. The head and neck feather scores of the animals on the 3rd tier were found to be higher than those of the animals on the 1st and 2nd tier (P < 0.01). The tail feather scores of the animals on the 2nd tier were observed to be lower than those of the animals on the 1st and 3rd tier (P < 0.01). In terms of breast feather scores, the breast feather scores of animals on the 1st tier were lower than that of the animals on the 2nd and 3rd tier (P < 0.05). The effect of the cage tier on the back and wing feather scores showed no significant difference (P > 0.05). Generally, the overall feather scores of the hens on the 3rd tier were higher than those on the 1st and 2nd tier (P < 0.01).

4. Discussion
It is fully established that the pecking behavior of chickens is normal physiological behavior however, severe feather pecking is a very common and harmful behavioral problem in egg-laying chickens such as increased feather damage. For this reason, it is important to develop genotypes without excessive pecking behavior. In chickens, feather loss in both production and research is inevitable depending on the production system. In addition to the natural loss, it has been proven that feather loss can be caused by nutritional deficiencies, genetic structure, stress, manure scraping tape, cage material, and pecking from other chickens in the cage. The decrease in the total feather scores of the bird's body parts and statistical differences due to the age obtained from this study are in accordance with the previous results on Danish commercial farms [33]. In a study, Yamak and Sarıca [27] concluded that the 30th-week feather score would be insufficient to determine for the following weeks, and that only the 50th-week evaluation would be late in terms of yield periods, and that the 40th-week feather score would be sufficient in terms of feather score estimation in the following weeks. Furthermore, the authors reported that chickens of different ages had different feather scores. Also, previous studies have verified an age-related decrease in feather scores in different body regions of chickens [31,33,34,35,36]. A possible reason for the difference in neck feather scores in all age groups may be because the animals extend their necks out of the wire grids for feeding while the decrease in the back and tail area would be that chickens have more contact with the upper ceiling of the cage, they exhibit the pecking behavior or behavior of jumping on top of each other. The results of this study showed that the effect of genotypes on feather score is significant which is similar to previous findings in studies [27,34,37]. Also, Su et al. [38] found that the feather score of the low-intensity pecking line was high in comparison with the high-intensity pecking line. Other authors noted that white egg shelled layers have better plumage
In contrast, Dekalb white hens (white leghorn origin) were found to have a higher average feather damage score compared with ISA brown hens (Rhode Island Red origin) [41]. Also, there was no observed difference in the average feather damage at 40 weeks of age between Dekalb white and ISA crosses [42]. Decina et al. [24] emphasized that feather damage results from multiple factors and genetic and foraging behavior are the most important factors.

The results of the present study are in the same line with the results of the researchers who stated that the effect of cage stocking density on feather score was significant, and feather coverage for all body parts decreased with increasing stocking density [43,44]. Furthermore, Okpokho et al. [45], concluded that high stocking density accelerated aggression and feather loss, but there is no significant difference between moderate to low stocking density. However, Moinard et al. [46], showed that the feather score was not affected by the applied cage system and the stocking density.

In the current study, the best feather score between the tiers is in the third that had the highest light intensity, and also thought that there is less stress on the third tier of the cage during the time workers roam in the poultry pens. Stress rather than cage tier is thought to affect the feather score. The findings agree with a study conducted by Tünaydın and Yılmaz Dikmen [47] who reported that the mean feather scores of layers were best in the top cage tier. Also, Hartini et al. [48] noted that intensity of light during production does not affect pecking behavior that leads to feather damage and loss when they used dim light (5 lux) and bright light (60-80 lux) with Isa Brown chickens.

In conclusion, the results show that the highest feather scores at the end of the 60th week of the 11 different egg-laying pure lines that were used as animal materials were RIR2 (23.83), BLACK (23.80), and COL (23.72). The lowest feather scores were in BAR2 (20.81) and
BAR1 (20.97). Also, feather scores of all genotypes decreased with advancing age. The best feather score was 23.59 on the 30th week and the worst feather score was 22.09 by the 60th week. This regression in the feather scores may be caused by pecking, feed, genetic structure, damage to cage material, and stress. There was a decrease in feather scores of all genotypes as the stocking density increased. The best feather score was 23.22 in stocking density with 5 chickens and the worst feather score was found to be 22.54 in the 7-way stocking density. Finally, the best feather score was on the 3rd cage tier (22.99); the worst feather score was found on the 1st (22.81) and 2nd (22.75) cage tiers.

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**Table 1.** Light intensity in the cage tiers

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<tr>
<th>Tier</th>
<th>Light Intensity (Lux)</th>
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<td>1</td>
<td>30.40</td>
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<td>2</td>
<td>6.15</td>
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Table 2. Vaccination program during the growth period

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<td>1. day</td>
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<td>Subcutaneous injection</td>
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<tr>
<td>1. day</td>
<td>Infectious Bronchitis+ Newcastle (Ma5+Clone30)</td>
<td>Spraying</td>
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<tr>
<td>Week</td>
<td>Disease</td>
<td>Route of Administration</td>
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<td>----------------------------------</td>
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<td>2. week</td>
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<td>Drinking water</td>
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<td>4. week</td>
<td>Infectious Bronchitis+ Newcastle (Ma5+Clone30)</td>
<td>Drinking water</td>
</tr>
<tr>
<td>5. week</td>
<td>Gumboro</td>
<td>Drinking water</td>
</tr>
<tr>
<td>7. week</td>
<td>Newcastle (La Sota)</td>
<td>Drinking water</td>
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<td>12. week</td>
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<td>Encephalomyelitis (Encefal VAC)</td>
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<td>Wing web</td>
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<td>17. week</td>
<td>Mixture (Nob.RT+IB multi+G+ND inf)</td>
<td>Intramuscular injection</td>
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Table 3. The effect of variations of cage tier, stocking density, genotype and age on feather scores of different body parts and their interactions

<table>
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<th>Age</th>
<th>Head</th>
<th>Neck</th>
<th>Back</th>
<th>Tail</th>
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<td>3.88^a</td>
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<td>3.17^a</td>
<td>3.94^a</td>
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<td>22.09^a</td>
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a, b, c, d, e, f, g: The differences between the means shown with the different letters in the same column are significant. A: Age; G: Genotype; SD: Stocking density; CT: Cage tier; P: Significant level; *: P < 0.05 **: P < 0.01; SEM: Standard error of the mean; NS: Non significant