T.R.
ERCIYES UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES
DEPARTMENT OF AGRICULTURAL SCIENCES AND TECHNOLOGIES

THE EFFECT OF L-ASCORBIC ACID SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY, TONIC IMMOBILITY, AND BLOOD HEMATOLOGICAL PARAMETERS IN LAYING HENS UNDER DIFFERENT STOCKING DENSITY CONDITIONS

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Master's Thesis

June 2022
KAYSERİ

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June 2022
KAYSERİ

COMPLIANCE WITH SCIENTIFIC ETHICS

I declare that all the information in this study was obtained by academic and ethical rules. I also state that, as required by these rules and behaviors, I have fully cited and referenced all materials and results that are not inherent in this study.

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Signature: _____

The Master's thesis entitled “The Effect of L-Ascorbic Acid Supplementation on Performance, Egg Quality, Tonic Immobility, and Blood Hematological Parameters in Laying Hens under Different Stocking Density Conditions" was prepared by Erciyes University Graduate Thesis Proposal and Thesis Writing Directive.

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YUMURTACI TAVUKLARDA FARKLI YERLEŞIM SIKLIĞI KOŞULLARINDA VITAMIN C İLAVESININ PERFORMANS, YUMURTA KALITESI, TONIK İMMOBILITE VE KAN HEMATOLOJIK PARAMETRELERI ÜZERINE ETKISI

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Yüksek Lisans Tezi, Haziran 2022
Danışman: Assist. Prof. Dr. Asiye YILMAZ ADKINSON

ÖZET

Yüksek yerleşim sıklığı (YS) kanatlılarda strese neden olur ve yüksek ortam sıcaklıklarında olumsuz etkisi artabilir. L-askorbik asit (AA), yumurta tavuklarında stres önleyici olarak kullanılır ve stresin azalmasına katkıda bulunabilir. Bu çalışma, L-askorbik asit ilavesinin farklı yerleşim sıklığı koşullarında yumurta tavuklarında performans, yumurta kalitesi, tonik hareketsizlik ve kan hematolojik parametreler üzerine etkisini araştırmak amacıyla yapılmıştır. Toplam 160, 58 haftalık yumurta tavuğu (Hyline W80), iki YS ve iki AA konsantrasyonuna göre dört muamele grubuna ayrılmıştır. Muamele grupları aşağıdaki gibidir: 1) Bazal diyet + 0 mg/kg AA ile 3 yumurta tavuğu/kafes (560 cm2), 2) Bazal diyet + mg/kg AA ile 5 yumurta tavuğu/kafes (366 cm2), 3) 3 bazal diyet + 150 mg/kg AA ile yumurta tavuğu/kafes ve 4) 5 bazal diyet +150 mg/kg AA ilavesiyle yumurta tavuğu/kafes. Yüksek YS, deneme sonu canlı ağırlığı ve yumurta verimini (adet) azaltmış ve yem tüketimi (YT) artırmıştır ($P<0.01$), bununla birlikte, yem dönüşüm oranı (YDO), yumurta verimi (%) ağırlığı, kütlesi ve yumurta kabuğu özellikleri ve tonik hareketsizlik (TI) önemli derecede etkilememiştir. Yüksek YS, yumurtaların Haugh biriminde (HU) azalmaya ve solunum hızı, tüy skoru ($P<0.05$) ve kan trigliserit, aspartat aminotransferaz (AST) ve kortizol konsantrasyonunun ($P<0.05$) artmasına neden olmuştur. Rasyona AA ilavesi YT, yumurta sarısı Roche renk skalası (RRS) ve kırmızılık (a*) değerlerinde artışa neden olurken, yumurta sarısı parlaklığı (L*) değerlerinde azalmaya neden olmuştur. YS*AA kombinasyonları performans, yumurta özellikleri, solunum hızı, tüy skorunu, TI ve kan bileşenlerini etkilememiştir. Kan heterofil, lenfosit, monositler, eozinofil, bazofiller ve H/L oranı YS, AA'dan etkilenmedi. Denemedeki YS*AA kombinasyonları, deneme boyunca ölçülen

parametrelerde önemli bir farklılığa neden olmamıştır. Sonuç olarak, yüksek YS'nin vücut ağırlığı, YT, yumurta üretimi, tüy skoru ve solunum hızı üzerinde olumsuz etkileri vardır. AA'nın diyete dahil edilmesi, RRS ve a* renk parametrelerini olumlu yönde etkiler. Bununla birlikte, yumurta tavuğu yemlerine AA eklenmesi, yüksek çevre koşullarında YS'nin olumsuz etkilerini iyileştirememiştir.

Anahtar Kelimeler: Yerleşim sıklığı, Yumurta tavuğu, Askorbik asit, Yumurta kalitesi, Performans.

THE EFFECT OF L-ASCORBIC ACID SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY, TONIC IMMOBILITY, AND BLOOD HEMATOLOGICAL PARAMETERS IN LAYING HENS UNDER DIFFERENT STOCKING DENSITY CONDITIONS

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ABSTRACT

High stocking density (SD) causes stress in poultry and its negative effects may be increased at high environmental temperatures. L-ascorbic acid (AA) is used as an anti-stressor in laying hens and it may contribute to stress reduction. This study was conducted to investigate the effect of L-ascorbic acid supplementation on performance, egg quality, tonic immobility and blood hematological parameters of laying hens under different stocking density conditions. A total of 160, 58-week-old laying hens (Hyline W80) were divided into four treatment groups (two SD and two AA concentrations). The treatment groups were as follows: 1) 3 laying hens/cage (560 cm^2) with basal diet + 0 mg/kg AA; 2) 5 laying hens/cage (366 cm^2) with basal diet + 0 mg/kg AA; 3) 3 laying hens/cage with basal diet + 150 mg/kg AA; 4) 5 laying hens/cage with basal diet +150 mg/kg AA. The SD decreased final body weight and increased feed intake (FI) ($P<0.01$), decreased egg production ($P<0.01$), however, there was no significant effect on feed conversion ratio (FCR), egg yield, weight, mass and eggshell traits and tonic immobility (TI). High SD caused a decrease in Haugh unit (HU) of eggs and increased respiratory rate, feather score ($P<0.05$) and blood triglyceride, aspartate aminotransferase (AST) and cortisol concentration ($P<0.05$) levels. The AA supplementation into laying hen diets caused an increase in FI, egg yolk Roche color score (RRS) and redness (a*), however, a decrease in yolk lightness (L*) values. The SD*AA combinations didn't affect performance, egg traits, respiratory rate, feather score, TI and blood components. Blood heterophil, lymphocyte, monocytes, eosinophil, basophils and H/L ratios were not influenced by the SD and AA treatments. The SD*AA combinations did not cause a significant difference in the measured parameters in overall period. In conclusion, high SD had negative effects on body weight, FI, egg production, feather score and respiratory rate. Inclusion of AA

into diet positively affected RRS and a* color parameters. However, supplementation of AA into laying hen diet didn’t improve negative effects of SD at high environmental conditions.

Keywords: Stocking density, Laying hens, L-Ascorbic Acid (AA), Egg quality, Performance.

TABLE OF CONTENTS

THE EFFECT OF L-ASCORBIC ACID SUPPLEMENTATION ON PERFORMANCE, EGG QUALITY, TONIC IMMOBILITY, AND BLOOD HEMATOLOGICAL PARAMETERS IN LAYING HENS UNDER DIFFERENT STOCKING DENSITY CONDITIONS

COMPLIANCE WITH SCIENTIFIC ETHICS ii
APPROVAL iv
ACKNOWLEDGMENT v
ÖZET vi
ABSTRACT viii
TABLE OF CONTENTS x
ABBREVIATIONS xiii
LIST OF TABLES xiv
LIST OF FIGURES xvi
INTRODUCTION 1

CHAPTER 1

GENERAL INFORMATION

1.1. Effect of stocking density on layer hens 3
1.2. Effects of L-ascorbic acid (AA) on laying hens 6

CHAPTER 2

METHOD AND MATERIAL

2.1. Animals, experimental design and diets 11
2.2. Animal Grouping 11
2.3. Ingredients and nutritional composition of basal diet 12
2.4. Care and feeding of animals 12
2.5. Calculation of live weight, feed consumption and feed efficiency 14
2.6. Evaluation of egg yield, egg weight and internal quality traits 14

2.7. Eggshell properties 15
2.8. Characteristics of feed materials and additives 15
2.9. Determination of tonic immobility, respiration rate, and feather value 16
2.10. Blood components and leukocyte cell counts 16
2.11. Statistical analyses 17

CHAPTER 3

RESULTS

3.1. Effect of treatments on body weight (BW).....18
3.2. Effect of treatments on feed intake (FI) 20
3.3. Effect of treatments on the feed conversion ratio (FCR).....22
3.4. Effect of treatments on egg production, number (EP).....24
3.5. Effect of experimental treatments on egg yield percentage, % (EY%).....26
3.6. Effect of experimental treatments on egg weight (EW) 28
3.7. Effect of experimental treatments on egg mass (EM) 30
3.8. Effect of experimental treatments on Haugh unit (HU) 32
3.9. Effect of experimental treatments on eggshell weight (ESW).....34
3.10. Effect of experimental treatments on eggshell ratio (ESR) 36
3.11. Effect of experimental treatments on eggshell thickness (EST).....38
3.12. Effect of experimental treatments on egg yolk height (HMM) 40
3.13. Effect of experimental treatments on Roche color scale values (RRS).....42
3.14. Effect of experimental treatments on egg yolk brightness (L*) 44
3.15. Effect of experimental treatments on egg yolk redness (a*).....46
3.16. Effect of experimental treatments on egg yolk yellowness (b*) 48
3.17. Effect of experimental treatments on blood parameters (BP).....50
3.18. Effect of experimental treatments on respiratory count (RC), tonic immobility (TI) and feather scoring (FS) 53

CHAPTER 4

DISCUSSION - Conclusion and recommendations

4.1. Effect of stocking density on performance traits.....56
4.2. Effect of stocking density on egg yield and egg quality traits.....57

4.3 Effect of SD on blood parameters, feather score and tonic immobility 59
4.4. Effect of L-ascorbic acid supplementation into diets on performance traits 60
4.5. Effect of L-ascorbic acid treatments on egg yield and quality traits 61
4.6. Effect of AA on blood parameters, feather score and tonic immobility 62
4.7. Conclusion and recommendations 62
REFERENCES 63
CV 76

ABBREVIATIONS

a*:	Egg yolk redness value
b*:	Egg yolk yellowness value
L*:	Egg yolk brightness value
HU:	Haugh unit
Hmm	Albumin height
CP:	Crude protein
AA:	L-Ascorbic Acid
VC:	Vitamin C
FI:	Feed intake
SD:	Stocking density
FCR:	Feed conversion ratio
RRS:	Roche color scale values
EM:	Egg Mass
EST:	Eggshell thickness
LDL:	low-density lipoprotein
HDL:	High-density lipoprotein
SE:	Standard error
P:	Level of significance

LIST OF TABLES

Table 2.1. Animal grouping 11
Table 3.1. Effects of stocking density, L-Ascorbic Acid and their interactions on body weight (BW) 19
Table 3.2. Effects of stocking density, L-Ascorbic Acid and their interactions on feed intake (FI) 21
Table 3.3. Effects of stocking density, L-Ascorbic Acid, and their interactions on feed conversion ratio (FCR) 23
Table 3.4. Effects of stocking density, L-Ascorbic Acid and their interactions on egg production (number) 25
Table 3.5. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yield % (EY%) 27
Table 3.6. Effects of stocking density, L-Ascorbic Acid and their interactions on egg weight (EW) 29
Table 3.7. Effects of stocking density, L-Ascorbic Acid and their interactions on egg mass (EM) 31
Table 3.8. Effects of stocking density, L-Ascorbic Acid and their interactions on Haugh unit (HU) 33
Table 3.9. Effects of stocking density, L-Ascorbic Acid and their interactions on eggshell weight (ESW) 35
Table 3.10. Effects of stocking density, L-Ascorbic Acid and their interactions on eggshell ratio (ESR) 37
Table 3.11. Effects of stocking density, L-Ascorbic Acid and their interactions on eggshell thickness (EST) 39
Table 3.12. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk height (HMM) 41
Table 3.13. Effects of stocking density, L-Ascorbic Acid and their interactions on Roche color scale values (RRS) 43
Table 3.14. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk brightness (L*) 45

Table 3.15. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk redness (a*).....47
Table 3.16. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk yellowness (b*).....49
Table 3.17. Effects of stocking density, L-Ascorbic Acid and their interactions on blood parameters (BP).....51
Table 3.18. Effects of stocking density, AA and their interactions on blood parameters (BP).....52
Table 3.19. Effects of stocking density, L-Ascorbic Acid and their interactions on respiratory rate, Tonic immobility (TI) and Feather score (FS).....53

LIST OF FIGURES

Figure *1.* Chemical structure of L-ascorbic acid 7
Figure 2.1. Determination of egg quality traits.....15
Figure 2.2. Animal experimental house.....16
Figure 2.3. Blood samples under microscope.....17
Figure 3.1. Effects of SD and AA on body weight in the overall periods.....20
Figure 3.2. Effects of experimental treatments on feed intake in the overall periods ..22
Figure 3.3. Effects of experimental treatments on feed conversion ratio in the overall periods 24
Figure 3.4. Effects of experimental treatments on egg production in the overall periods 26
Figure 3.5. Effects of experimental treatments on egg yield % in the overall periods 28
Figure 3.6. Effects of experimental treatments on egg weight in the overall periods ..30
Figure 3.7. Effects of experimental treatments on egg mass in the overall periods 32
Figure 3.8. Effects of experimental treatments on Haugh unit in the overall periods 34
Figure 3.9. Effects of experimental treatments on eggshell weight in the overall periods 36
Figure 3.10. Effects of experimental treatments on eggshell ratio in the overall periods 38
Figure 3.11. Effects of experimental treatments on eggshell thickness in the overall periods 40
Figure 3.12. Effects of experimental treatments on egg yolk height in the overall periods 42
Figure 3.13. Effects of experimental treatments on Roche color scale values in the overall periods 44
Figure 3.14. Effects of experimental treatments on egg yolk brightness in the overall periods 46
Figure 3.15. Effects of experimental treatments on egg yolk redness in the overall periods 48

Figure 3.16. Effects of experimental treatments on egg yolk yellowness in the overall periods 50
Figure 3.17. Effects of experimental treatments on respiratory rate in the overall periods 54
Figure 3.18. Effects of experimental treatments on tonic immobility in the overall periods 54
Figure 3.19. Effects of experimental treatments on feather score in the overall periods 55

INTRODUCTION

Poultry industry is a fast-growing sector worldwide. In 2020, global meat production included 40% of poultry meat and egg production increased by 150% during the last three decades (FAO, 2021). World population is expected to be around 10 billion by the year 2050. Just because of growing world population and changing lifestyles, both the quantity and quality of animal-originated products need to be improved. Products of animal origin are very important sources of essential nutrients. To meet the requirements of growing population, poultry meat and egg production should be increased. Since 1990, worldwide annual egg production has achieved on average a 2.8% increase, reaching 76.8 million metric tons in 2018 (Gonzalez-Mora et al., 2022). In order to increase animal production, it is necessary to increase the number of animals available, as well as their productivity.

Increase of productivity may cause stress and negatively affect welfare of birds. To assess the well-being of birds, it is important to recognize stress and its impacts on animal performance, physiological and behavioral functions (Panda and Cherian, 2013). In order to increase the profitability in animal production per unit area, it is necessary to create optimum breeding conditions. Stocking density (SD) is described as the number of chicks or body weight per unit of available space (Estevez, 2007; Europe Council Directive, 2007). Overstocking density is one of the important stress factors that affect the health and production of birds (Houshmand et al. 2012). High stocking density may maximize farm profitability per area but exerts serious threats on animal health and welfare (Heckert et al. (2002). On the other hand, animal right defenders have been pushing for good animal welfare and governments preparing new legislations on behalf of animal benefits. The European Union (EU) Council Directive (1999/74/EC) identifies a space allowance for conventional unfurnished cages per laying hens as 550 cm^2. Alternative housing systems require nearly double space (1,111 cm^2) per hen. In EU after 1 January 2012, conventional cage system has been banned and higher floor space (750 cm^2) was allowed per hen. In the USA, cage space for each laying hen is between 432 -

555 cm^2 (UEP, 2016). High stocking density could contribute to reduced laying hens performance with high ambient temperatures and lack of adequate ventilation of the birds (Feddes et al., 2002). Moreover, increased heat accumulation inside the cages at a high stocking density especially in a hot season is directly related to poorer laying performance and heat stress (Abbas et al., 2022).

Heat stress plays an important role in the performance of laying hens and decreases body weight, feed intake, egg production (Lara and Rostagno, 2013; Puthpongsiriporn et al., 2001), egg quality and eggshell traits (Mahmoud et al., 1996). Also, heat stress often leads to respiratory problems, poor meat quality, low reproductive rate, immune deficiency and high mortality (Marai and Haeeb, 2010). High temperatures and humidity can increase water intake while decreasing feed intake of laying hens (Gutierrez, Min and Chang, 2009). Vitamin C is a powerful biological antioxidant and many animal research have shown that vitamin C supplementation was successful in reducing oxidative stress in animals raised under stressful conditions (Yu et al., 2021). Vitamin C is one of the essential nutritional elements for poultry to improve performance, metabolism, product quality and immune system of laying hens (Devaki et al., 2017; Attia et al., 2009; Khan, 2012; Gowe et al., 2008). In addition, vitamin C increases the number of good bacteria that aid in protection against bad bacteria and health problems (Carr and Maggini, 2017). Vitamin C can be associated with its function as a regulator of body temperature and immune and antioxidant activity in poultry (Attia et al., 2009).

The main objective of this thesis was to investigate the effect of L-Ascorbic acid supplementation on performance, egg quality, tonic immobility and blood hematological parameters of laying hens under different stocking density and hot summer conditions.

CHAPTER 1

GENERAL INFORMATION

Management practices, stocking density and flock size can have an important impact on the welfare of laying hens. Stocking density affects both product performance and the health status of laying hens, especially when laying hens are raised under heat stress during the hot summer season. When laying hens are kept at a high stocking density, several problems may occur in the flock, including feather pecking, cannibalism, feed competition and an increase in the number of broken eggs (Zimmerman et al., 2006).

1.1. Effect of stocking density on layer hens

Stocking density is one of the important stress factors that affect the health and production of birds. Kang et al. (2016) determined that feed intake, egg mass and daily egg production significantly increased at lower stocking density (5, 6, 7 birds per m^2) as compared to higher stocking density (10 birds per m^2). Also, eggshell strength, number of broken egg and production rate were higher at lower stocking density than higher stocking density group. Furthermore, researchers did not find significant variation in leukocyte (L) density, however, heterophil (H), H/L ratio and corticosterone were greatly enhanced at lower stocking density as compared to higher stocking density.

Saki et al. (2012) investigated the effects of stocking density (2,000, 1,000, 667 and 500 cm^2 and 1, 2, 3 and 4 hens per cage). The findings revealed that the body weight, feed consumption, daily egg production, egg weight, egg mass, per unit eggshell weight, egg surface area, yolk color, plasma calcium and plasma uric acid were significantly lower in 4 hens/per cage than 1 hen/ per cage. But, the 4 hens per cage offered significant improvement in feed conversion ratio (FCR) and had greater eggshell ratio, plasma glucose, magnesium and iron than 1 hen per cage.

Benyi et al. (2006) investigated the effects of stocking density (2, 3 and 4 hens per cage and 1100, 733 or 550cm^2 per hen) of two different poultry houses on the performance of

white and brown Hyline laying hens. Researchers reported that hens housed 2 or 3 per cage had greater body weight gain, higher egg production, better feed conversion and lower mortality than those housed 4 per cage.

The influence of stocking density on laying hen performance and blood parameters was investigated by Abudabos et al. (2013) and three stocking densities were experimented as ; low (28.0 kg/m^2), medium (37.0 kg/m^2) and high (40.0 kg/m^2). It was observed that feed consumption and body weight gain were significantly affected by the stocking density. However, there was no impact on layer hen's mortality rate and feed conversion ratio. Body weight gain and feed consumption were improved in medium and low-density rates as compared to high-density of layer hens.

Onbaşilar and Aksoy (2005) examined the impact of cage and floor density (1, 3 or 5 hens per cage and 1968, 656 and 393.8 cm^2 floor space per hen) on stress parameters of laying hens. The same number of cages with different cage densities were systematically assigned to three battery floors (first floor=top, second=middle, third=bottom). It was observed that egg weights on the first floor were higher than those on the other floors. The group with 5 hens per cage had significantly lower body weight, egg production, egg weight and plumage score than the other groups and significantly higher average of egg albumen index, Haugh unit, serum glucose and H–L ratio. Serum cholesterol rates were greater and plasma corticosterone values were lower in cages with one hen than the cages with five hens.

Kahraman et al. (2020) investigated the effects of different stocking densities (412.5 and 495 cm^2/hen) and feeding programs (4 feeding time 6:00 am., 9:30 am, 6:00 am to 6:30 pm, and 9:30 am to 1:30 h) on laying hen's performance. The lowest feed consumption and FCR values and the greatest egg production were observed at one time feeding at 09:30h and 495 cm^2 /chicken density. The highest feed consumption and the lowest egg production were observed at feeding at 09:30-13:30 h and 412.5 ^{cm2} /hen stocking density. Also, feeding one time (09:30h) decreased the feed consumption, but feeding two times (06:00h and 06:30h) increased feed consumption.

In another study, Menezes et al. *(*2009) explained the effect of economic conditions on laying hen's efficiency of different stocking densities. A total of 264 laying hens were allocated in 4x4 factorial design, 4 different cage densities with 4 groups of 8, 10, 12 and 14 animal /cage (625, 500, 416.6 and 357.14 cm^2 /animals). Cage density affected extra-

large and medium type eggs and the daily production. In the production phase, cages with housing densities of 625, 500, 416.6 and 357, 14 cm^2/animal had no effect on the quality parameters of DeKalb White hens.

Altan et al. *(*2002) investigated the impacts of cage density on the efficiency of a brown and white hybrid laying hens under high-temperature conditions. White layers were 68 weeks of age and housed 3, 4 or 5 hens/cage (respectively 640, 480 and 384 cm2/hen); brown layers at 3 or 4 hens/cage (respectively 640 and 480 cm^2/hen stocking density). It was found that houses with 3 or 4 hens per cage had no influence on egg production and egg quality. In white layers, increasing cage density to 5 hens/cage reduced egg production and Haugh Units (HU). High cage density had no significant effect on shell quality or egg weight.

Cook et al. (2005) investigated the effect of stocking density (54, 60, 66 and 72 inc^2) on production performance of laying hens and reported no significant differences in any of the feeding behavior parameters of these 4 treatment groups. Among the groups, there was no significant difference in daily feed intake, but simultaneous and diurnal feeding patterns significantly changed with stocking densities.

Mtileni et al. (2007) investigated the effect of stocking density on body weight, egg weight and feed intake of Ross broiler breeder hens (n = 120) during the late medium egg production period (from 50 to 54 wk of age). Birds were assigned randomly to six pens at densities of 15, 20 and 25 birds per pen, resulting in floor space allowances of 5, 6.67 and 8.33 birds/m^2. Stocking density, time (in days) and their interactions all had a major impact on body weight, egg weight and feed intake. Birds with a density of 6.67 per m^2 had lighter body weight, but their eggs were heavier than the birds with a density of 5 per m^2. Birds with 8.33/ per m^2 had similar body weight and egg weight with the other groups. It was also found that average feed intake differed between the groups, with a decrease in feed intake as density increase.

In another study (Son et al., 2020), a total of 500 Hyline brown laying hens at 28 weeks of age were randomly placed in 2 stocking densities of 750 and 500 cm^2 /bird. The laying period was improved by the stocking density of 500 cm^2 /bird, but feather damage was increased when compared with stocking density of 750 cm^2 bird. On the other hand, stocking density did not affect egg quality.

In a previous study (El-Tarabany, 2016), Japanese quail were divided into three groups and the floor spaces (200 (S1), 167 (S2) and 143 (S3) cm^2/bird). There was a significant increase in egg mass, immunity parameters, egg weight, egg production, yolk height and Haugh unit at low stocking density (S1). Japanese quail's total leucocyte count in the S3 group was lower and but the highest H/L ratio was observed in S3 group. Corticosterone quantities were less in S1 group than in the greater stocking density groups.

Astaneh et al. (2018) investigated the effect of stocking density (12 and 18 chicks per cage and cage size of 1.2x1.2 m^2). The data were evaluated over three time points of 0-24, 25-42, and 0-42 day. Stocking density had no effect on feed intake or body weight gain from 0 to 24 days of age, but FCR decreased significantly in the 12 chick per cage group. Feed conversion ratio, body weight gain and feed intake were considerably higher in 12 chicks per cage group than in 18 chicks per cage group for 25-42 and 0-42 durations. At 0-42 days, the production per m^2 was substantially higher in the 18-chick density group. Lower stocking density of chicks per m^2 improved the heterophil count while reducing the lymphocyte count and increasing the heterophil:lymphocyte ratio.

The other study, Turkyilmaz et al. (2008), reported that stocking density had no significant impact on the blood corticosterone concentration, immune reaction, the heterophil:lymphocyte ratio (H: L) of broiler chickens housed at 15, 20 and 25 broilers/m^2 stocking densities during the summer period.

1.2. Effects of L-ascorbic acid (AA) on laying hens

L-Ascorbic acid (Figure 1), also known as vitamin C, is one of the basic vitamins (Davey et al., 2000). It has a wide range of medical applications against a variety of diseases. L-ascorbic acid is a small carbohydrate molecule that is essential to all living organisms. Most of the plants and animals are synthesizing their own vitamin C. This vitamin is found in a variety of plant and animal-derived products, especially those of plant sources, in amounts that are structurally multiple orders of magnitude greater than those of the other vitamins (Tripathi et al., 2009).

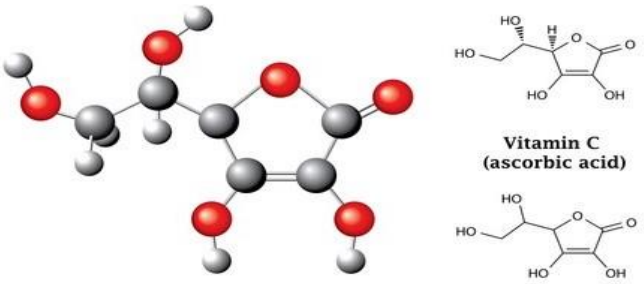


Figure *1*. Chemical structure of L-ascorbic acid

In an experiment, effects of control, supplementation of 150 mg of vitamin C, 150 mg of -dl-tocopherol acetate/kg of diet (Vit. E group) and their combination (150 mg of l-ascorbic acid/kg of diet plus 150 mg of -dl-tocopherol) on live weight and egg quality of layer hens were investigated. When compared to the control groups, vitamin C, vitamin E and vitamin C + E groups caused an increase in final live weight, egg, egg albumen weight, egg yolk and eggshell weights (Ajakaiye et al., 2011).

In a similar study (Rajabi and Torki, 2021), it was found that vitamin C supplementation improved egg quality properties and blood parameters of laying hens under cold stress. In the trial, a total of 144 Lohmann-LSL Lite laying hens (65 weeks old) were supplemented with two doses of vitamin C (0, 240 mg/kg). There were no significant effects of vitamin C supplementation on final body weight, feed intake, FCR, egg weight, egg mass and the other egg quality parameters. However, vitamin C supplementation did not affect blood properties such as glucose, triglycerides, cholesterol and H/L.

Mirfendereski and Jahanian (2015) investigated the effects of 2 cage densities (5 and 7 hens per cage), 3 Cr levels (0, 500, and 1,000 ppb as CrMet) and 2 dietary vitamin C (VC) levels (0 and 500 ppm as L-ascorbic acid). The findings revealed that hens in cages with a higher stocking density had lower daily egg production, egg mass and feed intake than those in cages with a lower stocking density. In terms of egg production and feed conversion efficiency, there were considerable CrMet x VC interactions. Dietary treatments did not affect plasma triglyceride or high-density lipoprotein concentrations, but additional CrMet lowered plasma cholesterol levels. Plasma insulin and glucose amounts were considerably higher in hens kept at a density of 7 hens/cage than in hens

kept at a normal cage density and dietary CrMet supplementation significantly lowered insulin and glucose plasma concentrations.

Asli et al. (2007) investigated the effects of dietary probiotics, yeast, vitamin E and vitamin C supplementation on performance, serum and yolk cholesterol and immune response of heat stressed laying hens. In the trial, experimental groups were; 1) control, 2) basal diet plus 50 mg multi strains probiotic, 3) basal diet plus 1 g yeast of Saccharomyces cerevisiae, 4) basal diet plus 200 mg vitamin C and 5) basal diet plus 200 mg vitamin E/kg diet. Hen performance, egg quality (shell thickness, shell resistance, shell percent and Haugh unit) and serum and yolk cholesterol concentrations were not significantly influenced by the treatments. But, in the vitamin E group, the highest yolk percent was found. Immune response increased with vitamin E and C supplementation to diet, although differences were not significant as compared to the other groups.

Seven (2008) reported that 250 mg/kg Vitamin C supplementation into the diet caused a significant increase in feed intake, FCR, egg weight and daily egg production when the birds were stressed with heat. The control group had a greater death rate than the vitamin C treatments groups. Vitamin C supplementation improved organic matter and crude protein content digestibility. Under heat stress, the eggshell weight and thickness increased in vitamin C treatments. However, the percentage shape index, yolk index or Haugh unit and albumen index did not change in response to present treatments.

Torki et al. (2014) reported that vitamin C (250 mg/kg) supplementation into poultry diets did not affect body weight, egg yield, egg mass and feed efficiency, but reduced eggshell weight and eggshell thickness.

Delos Reyes et al. (2021) noted that dietary supplementation of vitamin C (250, 500, 1000, 2000 or 3000 mg/kg) under normal (20.3°C) temperature conditions increased hen-day egg production and egg mass and decreased FCR, ratio of cracked number of eggs and no-shell eggs. Addition of VC into the diet did not affect egg quality, tibia traits and antioxidant status of liver.

Poultry diets were supplemented with vitamin C (200, 300, and 400 g/ton) and egg production rate was higher in the control group than 300 g and 400 g vitamin C supplemented groups. Egg weight and egg mass were significantly higher in 300 g/ton vitamin C-supplemented group than the control and the other vitamin supplemented groups. Dietary vitamin C supplementation significantly improved feed consumption and

conversion ratio. Also, shell quality was improved by vitamin C supplementation (El-Gendi et al., 1999).

Wang et al. (2011) reported that vitamin C supplementation (VC, 0 and 500 mg/kg) led to a considerable improvement in egg production throughout 4-6 weeks. White blood cell (WBC), total protein and albumin concentrations were not impacted by vitamin C supplementations. Aminolaevulinic acid (ALA) and the interactive impact of ALA x vitamin C were observed to improve the Hemoglobin, serum and red blood cell (RBC) iron concentrations. Furthermore, the supplementation of ALA x VC increased the percentage of blood lymphocytes in hens that were not supplemented with ALA. From 3 to 6 weeks, a single dose of ALA or vitamin C increased the yolk color unit and albumin height. Also, ALA improved the eggshell color. The supplementation of vitamin C led to a significant increase in the Haugh unit. However, neither ALA nor Vitamin C had an impact on egg weight, eggshell breaking strength or eggshell thickness. The findings showed that dietary ALA and VC supplementation could improve laying hen blood iron status and egg quality.

A group of 8 birds at the average ages from 143 to 311 days were fed with vitamin C-supplemented diets (0, 50, 100, 200 and 400 ppm) for 6 periods of 28 days. It was observed that the egg production and egg weights were not affected by dietary treatments except for the fifth 28-day period when egg production was slightly higher for the control group and then for the vitamin C groups. Feed consumption was generally higher in the control group and FCR values were generally better with 50 and 100 ppm vitamin C. Egg specific gravity was dramatically affected by diet in 4 of the 6 periods with eggs from the highest vitamin C treatments (200 and 400 ppm) (Bell et al., 1990).

The effect of vitamin C supplementation at 0 or 200 mg/kg and Selenium (Se) at 0.3 mg/kg in the form of sodium selenite or selenized yeast were investigated. It was seen that Se supplementation of the basal diet massively increased laying effectiveness, although vitamin C supplementation considerably lowered feed intake and egg production. Selenite and Se-enriched yeast both enhanced the vitamin E and Se concentration levels in the yolk and albumen. Vitamin C supplementation into the diet increased the oxidative stability of yolk lipids. A basal diet with vitamin C dramatically intensified the oxidative stability of yolk lipids, showing that vitamin C functioned as a pro-oxidant and egg yield (Skrivan et al., 2013).

Jahanian and Mirfendereski (2015) investigated the effects of chromium-methionine (CrMet) and vitamin C supplementations on performance, egg quality measurements and plasma antioxidant capacity of laying hens housed at high stocking density conditions. The treatments were 5 or 7 hens/cage (40×45cm2) densities and two supplemental vitamin C (0 and 500mg/kg) and 3 Cr levels (0, 500, and 1000µg/kg as CrMet). It was determined that high stocking density had no effect on egg yield between 28-33wk of age, however, decreased 33-38wk of age, and significantly lowered egg mass. Supplemental vitamin C improved FCR at high stocking density. In high stocking density, shell thickness was improved. High stocking density caused a decrease in blood vitamin C level, however, vitamin C addition increased blood plasma vitamin C. High stocking density caused an increase in blood plasma and yolk malondialdehyde (MDA) concentrations and addition of vitamin C caused a decrease in plasma and yolk MDA contents of laying hens.

It was observed that vitamin E (200 mg/kg), vitamin C (125 mg/kg) and vitamin E+C supplementation did not affect feed consumption as compared to the control group. The control group had the highest mortality rate. In contrast to the controls or Vit E, final body weight, feed efficiency and daily egg production were considerably higher in Vit E + Vit C group. Furthermore, egg weight increased substantially in all of the groups that were given the supplemented treatments. Supplementing with vitamin E or vitamin C had no impact on the percentage of albumen, eggshell or Haugh units. In comparison to control birds, the egg specific gravity and shell thickness appeared to be significantly higher in Vit E and Vit C groups. The percentage of egg yolk in Vit E and Vit E + Vit C groups was substantially higher than in the control and Vit C groups (Çiftçi et al., 2005).

Büyükkılıç Beyzi et al. (2020) investigated the effects of VC and thyme essential oil (TEO) supplementations and their combination on performance and egg quality traits of laying hens under heat stress (HS) conditions. It was found that animal body weight, feed intake, egg weight and egg quality were not influenced by the VC and TEO addition.

CHAPTER 2

METHOD AND MATERIAL

2.1. Animals, experimental design and diets

The Erciyes University Institutional Animal Care and Use Committee (protocol #FYL-2021-11010) reviewed and approved the protocol for animal use in this investigation which was performed at the Erciyes University Agricultural Research and Application Center (ERÜTAM). This research was performed during the summer season of 2021. The hens were separated according to their body weight, egg production and egg weight. All laying hens were raised under the same environmental conditions and were fed with the same starter diets for 21 days before the initiation of the experiments. The experiment was conducted in a completely randomized design with 2×2 factorial arrangements, with two different stocking density (SD, 560 and 366 cm^2) and two different dietary L-ascorbic acid (AA, 0, and 150 mg/kg) treatments. A total of 160, 58-week-old laying hens (Hyline W80) were allocated to 1 of the 4 treatments with 10 replicates. The treatment groups are as follows: 1) 3 laying hens/cage (560 cm^2) with basal diet + 0 mg/kg AA; 2) 5 laying hens/cage (366 cm^2) with basal diet + 0 mg/kg AA; 3) 3 laying hens/cage with basal diet + 150 mg/kg AA; 4) 5 laying hens/cage with basal diet +150 mg/kg AA addition. The basal diet ingredients and nutritional composition are provided in Table 1. Each SD was achieved by raising different numbers of hens per cage with a size of 42x40x46cm^2. Diets and water were provided *ad libitum*. In the experiment, an automatic lighting equipment was set daily as 16 h light and 8 h dark (light period from 05:00 am to 9:00 pm).

2.2. Animal Grouping

Animals were grouped into four different groups according to their stocking density and L-Ascorbic Acid (AA) supplementation ratio (Table 2.1).

Table 2.1. Animal grouping

Groups	Feed supplemented	Cages /animal	Total number of animals
Group 1	Control feed + AA0	10 cages/3 laying hens	30
Group 2	Control feed + AA0	10 cages/5 laying hens	50
Group 3	Control feed + AA150g/kg	10 cages/3 laying hens	30
Group 4	Control feed + AA150g/kg	10 cages/5 laying hens	50
Total			160

2.3. Ingredients and nutritional composition of basal diet

A commercial feed which produced from the same batch with a same nutrient composition for laying hens was used in the experiment. The feed composition is provided in Table 2.2. Feeds were sealed with plastic caps and stored in a shaded area with the lid closed. Every 14 days, L-ascorbic acid were mixed by a feed mixer.

2.4. Care and feeding of animals

The hens were housed in (42x40x46 cm) in conventional laying hen wire cages with 3 floors and 40 cages. The hens were allocated 3 to 5 hens per cage. Treatment groups were divided into cage compartments and housed in different parts and floors of the cages. To prevent birds from eating feed from the adjacent cages, partitions were placed between the feeders. Birds were separated according to their body weight, egg production and egg weight. All laying hens were raised under the same environmental conditions.

There were 8 data collection periods and each was 14 days. Duration of the experiment was 16 weeks (112 days). The first period was 0-14 days and the last period was 98-112 days. Internal and external quality characteristics of 160 eggs were examined. Feed consumption was determined in cage bases and divided by number of animals and calculated individually. Cages furnished with two nipple drinkers in each cage.

Table 2.2. Composition of experimental diets

Feed materials	**Quantity, %**
Corn	51.62
Sunflower meal (36% CP)	18
Soy bean meal (46% CP)	9.8
Calcium carbonate	7.95
DDGS	4.19
Meat bone meal	3.41
Animal Fat	2.24
Molasses 8.5	2
Salt	0.25
Vitamin -mineral premix*	0.2
Phytase enzyme	0.1
D-L-Methionine	0.07
L-Lysine	0.07
Sodium Bicarbonate	0.05
Mycotoxin Binder	0.05
Total, kg	100
Chemical composition**	
Dry matter, %	89.28
Crude protein, %	18.10 %
Crude fat, %	4.70 %
Crude fiber	4.98 %
Ash	12.24 %
Methionine***	0.40
Lysine***	0.80
Calcium***	3.80
Available Phosphorus***	0.66
Metabolic Energy (kcal ME/kg)****	2,780,000 Kcal/kg

*Vitamin-mineral premix per kilogram of the diet, Vitamin A, 15,000 IU; Vitamin D3 2000 IU; Vitamin E, 40.0 mg; Vitamin K, 5.0 mg; Vitamin B1 (thiamine), 3.0 mg; Vitamin B2 (riboflavin), 6.0 mg; Vitamin B6, 5.0 mg; Vitamin B12, 0.03 mg; Niacin, 30.0 mg; Biotin, 0.1 mg; Calcium D-pantothenate, 12 mg; Folic acid, 1.0 mg, Choline chloride, 400 mg, Manganese, 80.0 mg; Iron, 35.0 mg; Zinc, 50.0 mg; Copper, 5.0 mg; Iodine 2.0 mg; Cobalt, 0.4 mg; Selenium, 0.15 mg assures. **Dry matter, crude protein, and crude ash were analyzed with NIR (Perten DA 7200, Perkin Elmer Co., USA) system. ***Compositions are calculated according to NRC feedstuff tables (1994). ****ME values calculated according to TSE (1994).

2.5. Calculation of live weight, feed consumption and feed efficiency

Body weight of animals was determined at the beginning and end of the study individually with the help of a precise balance (±1 g). Feed consumption of laying hens was determined for each cage (3 and 5 animals according to their stocking density) at two-week intervals and calculated by difference of given and removed feeds. The average daily feed consumption per animal was obtained by dividing the amount of feed consumed between two weighing by the number of days (14 days) of feeding and the number of animals belonging to that group. Feed conversion ratio was calculated as the ratio of consumed feed to egg mass per period. Every two weeks, the FCR was calculated. The egg mass was calculated by multiplying the period egg weights with the % yield.

2.6. Evaluation of egg yield, egg weight and internal quality traits

Egg yield was recorded daily and egg yields were calculated as both number and percentage (%) in every 14 days. Egg yield percentage was calculated as follows: number of eggs produced / total number of eggs to be produced in every 14 days of each sub-group x 100. Six eggs collected in the last three days of each period (14^{th}, 28^{th}, 42^{nd}, 56^{th}, 70^{th}, 84^{th}, 98^{th} and 112^{th} days). In those periods of the study, egg were selected randomly selected (160 eggs in total) and weighed with a precise scale (± 0.1 g). Internal quality traits (albumin height, Haugh unit (HU), Roche color scale) were determined using the Egg Analyzer®. Color parameters of egg yolk (L* - brightness, a* - redness and b* - yellowness) were evaluated with a Minolta CR-400 (Minolta Co, USA) color-meter in every 14 days (Figure 2.1).



Figure *2.1.* Determination of egg quality traits

2.7. Eggshell properties

Shells of the broken eggs (g) were washed with tap water and dried at 75 ºC for 24 h in an oven. The dried eggshells were weighed on a precise scale (±0.1 g). Eggshell percentage was determined through dividing the shell weights by the egg weight and multiplying by 100. Shell thickness (µm) was measured with a digital micrometer (±0.01 mm) from the sharp, blunt and middle parts of the broken eggshells and shell thickness value was determined by taking the arithmetic mean of these three measurements.

2.8. Characteristics of feed materials and additives

Feed dry matter (DM), crude protein (CP), crude ash (CA), crude oil (CO) and crude fiber (CF) analyses were performed with the use of the NIR instrument (Model DA7000 Perten Instruments, Inc. Springfield Illinois, USA). The feed additives used in this study were manufactured by Farmavet International Feed and Water Additives Specialist Company (Istanbul, Turkiye).

2.9. Determination of tonic immobility, respiration rate, and feather value

The duration of tonic immobility of laying hens was determined by sampling one laying hen from each sub-group. The birds were placed in a quiet and stable location to measure how long the laying hens could remain motionless before becoming restless. Time was measured with a stopwatch and each laying hen was kept asleep for between 10 seconds and 600 sec (10 minutes) (Campo and Davila, 2002). Breathing rate in 2 minutes was measured for one animal from each sub-groups using a stopwatch.

Feather loss score was calculated according to AssureWel system (NFACC, 2017) including 6 body parts (neck, breast, cloaca/vent, back, wings and tail) and pecking damage to the skin of the hind body and comb and bumble foot lesions (Figure 2.2).



Figure *2.2.* Animal experimental house

2.10. Blood components and leukocyte cell counts

At the end of the experiment, blood samples (10 ml) was collected from the wing vein of randomly selected one laying hen from each sub-group. Blood samples were centrifuged

(5 min, 2500 rpm) and serum was separated and stored in Eppendorf tubes at -80 ° C until analysis day. The serums were taken out from the freezer on the day of analysis, thawed and serum glucose, total protein, triglyceride cholesterol, high-density lipoprotein (HDL), alanine aspartate aminotransferase (AST), alanine aminotransferase (ALT) were analyzed. Concentrations were determined in the autoanalyzer by using kits.

To determine blood leukocyte cell counts, blood samples of hens were collected at 16th week of experiment. A total of 40 birds (each of 10 hens) were used to determine blood cell count. For this process, two drops of blood from wing vein were smeared on the glass slides. Then smears were stabilized by using methyl alcohol fixation. Blood smears were stained by using Wright stains (Figure 2.3). With the use of a microscope with 100×, heterophils (H), lymphocyte (L) basophiles, eosinophiles and monocytes were counted and H:L ratio were calculated (Gross and Siegel, 1986).

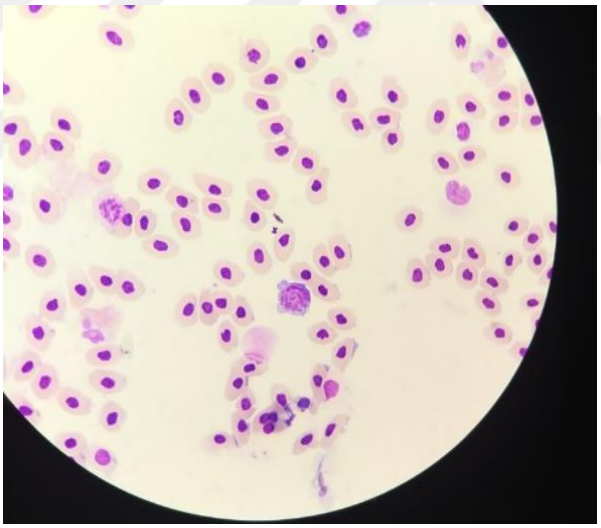


Figure *2.3.* Blood samples under microscope

2.11. Statistical analyses

A completely randomized design with a 2×2 factorial analysis of variance with a two-way ANOVA using the multivariate procedure in the SPSS statistics program Version 25 was applied to data related to SD and L-ascorbic acid and their interactions. Duncan tests were used to determine differences between averages. The P <0.05 level was taken into consideration for the level of significance. All data were expressed as Means± standard errors.

CHAPTER 3

RESULTS

This experiment was carried out to determine the effects of SD and AA supplementation on reduction of SD stress in laying hens under summer conditions. The data were obtained and summarized below.

3.1. Effect of treatments on body weight (BW)

The effect of AA supplementation on body weight and body weight change (%) are given in Table 3.1 and Figure 3.1. According to the table in terms of SD, there were significant differences in the final body weight and body weight change ($P<0.01$). High SD caused a decrease in BW and change (%). However, AA supplementation and SD*AA interaction did not affect body weight and change (%) of laying hens.

Table 3.1. Effects of stocking density, L-Ascorbic Acid and their interactions on body weight (BW)

Treatments	Initial weight	Final weight	Change, %
Stocking density, SD			
SD3	1482.30±39.26	1576.00±38.67^{a}	6.00±0.40^{a}
SD5	1449.35±39.26	1408.35±38.67^{b}	4.28±0.40^{b}
L-Ascorbic Acid, AA			
AA0	1397.70±39.26	1467.50±38.67^{b}	4.72±0.40^{b}
AA150	1433.95±39.26	1516.85±38.67^{a}	5.56±0.40
Interactions			
SD3* AA 0	1434.40±55.52	1521.90±54.69^{b}	5.74±0.56^{b}
SD3*AA 150	1361.00±55.52	1413.10±54.69^{c}	3.71±0.56^{d}
SD5* AA0	1530.20±55.52	1630.10±54.69^{a}	6.27±0.56^{a}
SD5* AA 150	1337.70±55.52	1403.60±54.69^{c}	4.86±0.56^{c}
P-SD	0.822	0.004**	0.004**
P- AA	0.518	0.373	0.146
P-SD* AA	0.291	0.289	0.585

^{a,b,c}: Values with different superscript in a column differ significantly, **:$p<0.01$. P: probability, SD: stocking density, AA: L-Ascorbic Acid, BW: bodyweight.

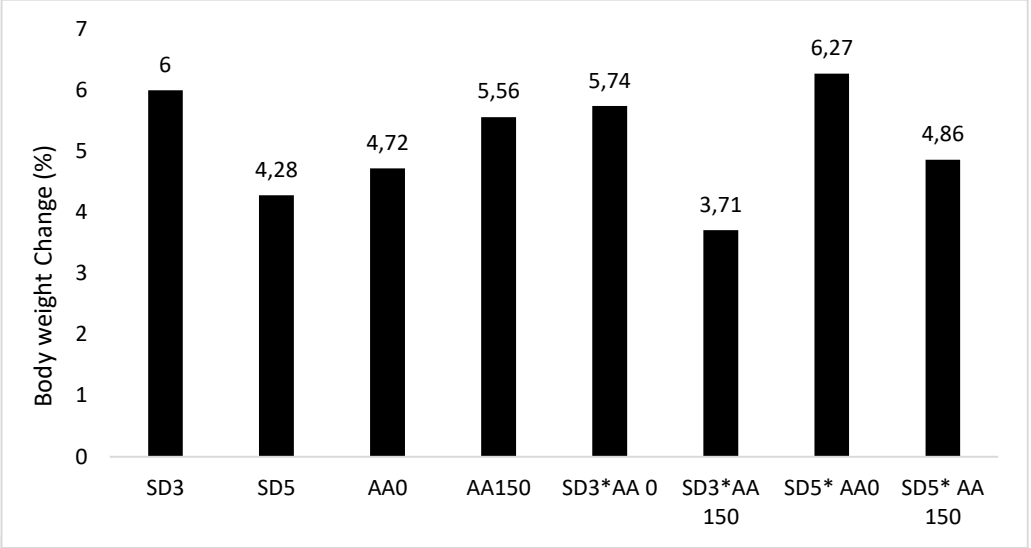


Figure *3.1.* Effects of SD and AA on body weight in the overall periods

3.2. Effect of treatments on feed intake (FI)

Effects of SD, AA supplementations into laying hen rations and their interaction on feed intake are shown in Table 3.2 and Figure 3.2. The high SD increased feed intake of laying hens in all periods, except for 7^{th} period ($P<0.05$ and $P<0.01$). L-ascorbic acid supplementation caused an increase in feed intake only in the 6^{th} period and overall period ($P<0.05$). There was no significant difference in feed intake of SD*AA interactions during the experiment ($P > 0.05$).

Table 3.2. Effects of stocking density, L-Ascorbic Acid and their interactions on feed intake (FI)

Treatments	FI1	FI2	FI3	FI4	FI5	FI6	FI7	FI8	Overall
Stocking density, SD									
SD 3	98.40±0.74[b]	97.60±0.74[b]	98.75±0.52[b]	97.50±0.54[b]	99.65±0.62[b]	101.55±0.92[b]	109.10±1.75	106.45±1.84[b]	101.13±0.43[b]
SD 5	114.30±0.74[a]	115.10±0.74[a]	115.50±0.52[a]	116.45±0.54[a]	117.70±0.62[a]	116.20±0.92[a]	112.15±1.75	111.80±1.84[a]	114.90±0.43[a]
L-Ascorbic Acid, AA									
AA 0	105.45±0.74	106.20±0.74	107.05±0.52	106.30±0.54	108.05±0.62	107.50±0.92[b]	109.85±1.75	106.95±1.84	107.17±0.43[b]
AA 150	107.25±0.74	106.50±0.74	107.20±0.52	107.65±0.54	109.30±0.62	110.25±0.92[a]	111.40±1.75	111.30±1.84	108.86±0.43[a]
Interactions									
SD3* AA 0	97.60±1.04	98.40±1.05	99.20±0.74	96.50±0.76	99.10±0.88	99.40±1.30	107.20±2.48	102.20±2.60	99.95±0.61
SD3*AA 150	113.30±1.04	114.00±1.05	114.90±0.74	116.10±0.76	117.00±0.88	115.60±1.30	112.50±2.48	111.70±2.60	114.39±0.61
SD5* AA0	99.20±1.04	96.80±1.05	98.30±0.74	98.50±0.76	100.20±0.88	103.70±1.30	111.00±2.48	110.70±2.60	102.30±0.61
SD5* AA 150	115.30±1.04	116.20±1.05	116.10±0.74	116.80±0.76	118.40±0.88	116.80±1.30	111.80±2.48	111.90±2.60	115.41±0.61
P-SD	0.000**	0.000**	0.000**	0.000**	0.000**	0.000**	0.226	0.047*	0.000**
P- AA	0.093	0.777	0.840	0.085	0.164	0.042*	0.536	0.103	0.009
P-SD* AA	0.849	0.080	0.163	0.400	0.866	0.242	0.370	0.119	0.288

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

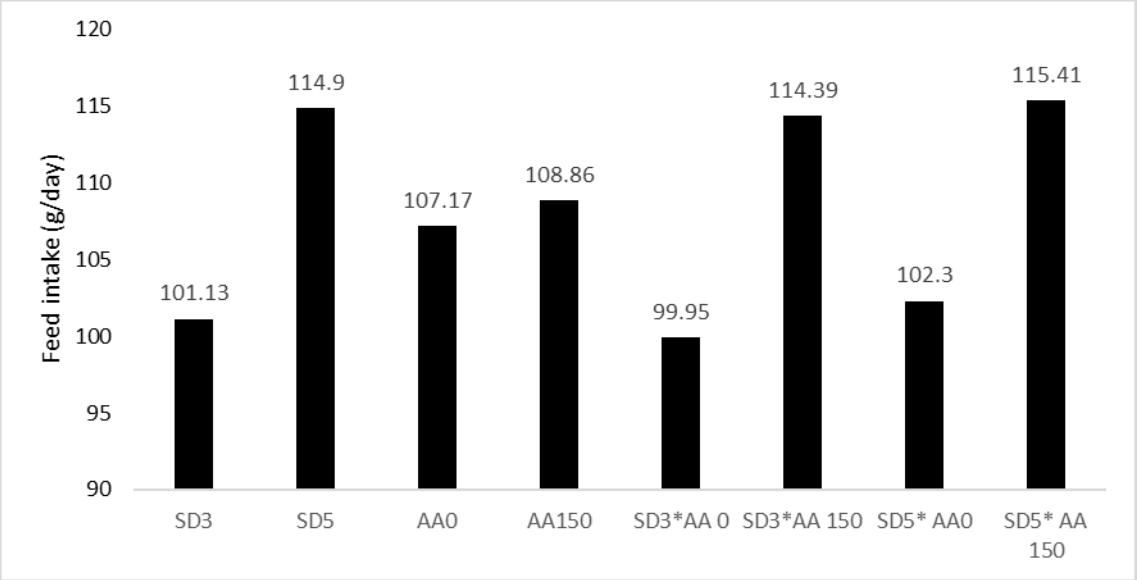


Figure *3.2.* Effects of experimental treatments on feed intake in the overall periods

3.3. Effect of treatments on the feed conversion ratio (FCR)

Effects of SD, AA supplementations and their interactions on feed conversion ratios are shown in Table 3.3 and Figure 3.3. Stocking density affected of FCR values in the 1st, 2nd, 3rd, 4th, 5th and 6th periods of the experiment ($P<0.05$). However, there was no significant differences among the groups with addition of AA and SD*AA interactions.

Table 3.3. Effects of stocking density, L-Ascorbic Acid, and their interactions on feed conversion ratio (FCR)

Treatments	FCR1	FCR2	FCR3	FCR4	FCR5	FCR6	FCR7	FCR8	Overall
Stocking density, SD									
SD 3	1.64+0.03[b]	1.55+0.05[b]	1.61+0.05[b]	1.56+0.04[b]	1.61+0.05[b]	1.53+0.04[b]	1.66+0.04	1.67+0.07[b]	1.68+0.06
SD 5	1.83+0.03[a]	1.89+0.05[a]	1.84+0.05[a]	1.82+0.04[a]	1.86+0.05[a]	1.75+0.04[a]	1.69+0.04	1.85+0.07[a]	1.82+0.06
L-Ascorbic Acid, AA									
AA 0	1.73+0.03	1.69+0.05	1.80+0.05	1.73+0.04	1.77+0.05	1.64+0.04	1.67+0.04	1.75+0.07	1.79+0.06
AA 150	1.74+0.03	1.75+0.05	1.66+0.05	1.65+0.04	1.70+0.05	1.64+0.04	1.68+0.04	1.77+0.07	1.70+0.06
Interactions									
SD3* AA 0	1.64+0.04	1.50+0.07	1.72+0.07	1.60+0.06	1.64+0.06	1.50+0.05	1.63+0.06	1.58+0.10	1.75+0.09
SD3* AA 150	1.83+0.04	1.89+0.07	1.88+0.07	1.87+0.06	1.89+0.06	1.77+0.05	1.71+0.06	1.91+0.10	1.84+0.09
SD5* AA 0	1.64+0.04	1.60+0.07	1.51+0.07	1.53+0.06	1.57+0.06	1.56+0.05	1.70+0.06	1.76+0.10	1.61+0.09
SD5* AA 150	1.84+0.04	1.90+0.07	1.80+0.07	1.77+0.06	1.84+0.06	1.73+0.05	1.66+0.06	1.79+0.10	1.79+0.09
P-SD	0.000**	0.000**	0.003**	0.000**	0.000**	0.000**	0.721	0.082	0.134
P- AA	0.838	0.449	0.057	0.184	0.347	0.933	0.845	0.780	0.301
P-SD* AA	0.838	0.541	0.379	0.770	0.896	0.371	0.340	0.151	0.641

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

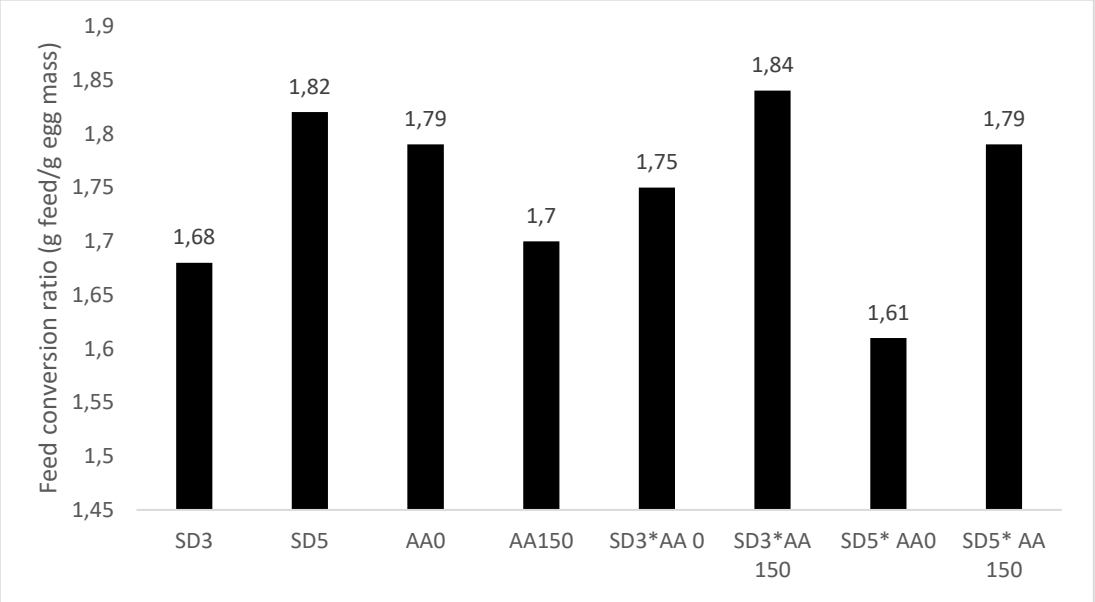


Figure *3.3.* Effects of experimental treatments on feed conversion ratio in the overall periods

3.4. Effect of treatments on egg production, number (EP)

Effects of stocking density, AA supplementation into laying hen rations and their interactions on egg production are shown in Table 3.4 and Figure 3.4. High SD caused decrease in egg production (number) during the experiment ($P<0.05$). Supplementation of AA and SD*AA interactions resulted in no significant difference between the experimental treatments ($P<0.05$).

Table 3.4. Effects of stocking density, L-Ascorbic Acid and their interactions on egg production (number)

Treatments	EY1	EY2	EY3	EY4	EY5	EY6	EY7	EY8	Overall
Stocking density, SD									
SD 3	12.80+0.10	13.30+0.15[a]	13.50+0.15[a]	13.42+0.17[a]	13.55+0.16[a]	13.47+0.14[a]	13.52+0.14[a]	13.50+0.13[a]	13.38+0.12[a]
SD 5	12.99+0.10	12.66+0.15[b]	12.68+0.15[b]	12.57+0.17[b]	12.72+0.16[b]	12.79+0.14[b]	12.73+0.14[b]	12.73+0.13[b]	12.74+0.12[b]
L-Ascorbic Acid, AA									
AA 0	12.88+0.10	12.94+0.15	13.01+0.15	12.82+0.17	13.08+0.16	13.02+0.14	13.13+0.14	13.11+0.13	13.00+0.12
AA 150	12.91+0.10	13.02+0.15	13.17+0.15	13.16+0.17	13.19+0.16	13.23+0.14	13.12+0.14	13.12+0.13	13.12+0.12
Interactions									
SD3* AA 0	12.70+0.14	13.30+0.21	13.50+0.21	13.27+0.23	13.60+0.22	13.37+0.19	13.60+0.19	13.57+0.18	13.36+0.17
SD3* AA 150	12.90+0.14	13.30+0.21	13.50+0.21	13.57+0.23	13.50+0.22	13.57+0.19	13.43+0.19	13.43+0.18	13.40+0.17
SD5* AA 0	13.06+0.14	12.58+0.21	12.52+0.21	12.38+0.23	12.56+0.22	12.68+0.19	12.66+0.19	12.66+0.18	12.64+0.17
SD5*AA 150	12.92+0.14	12.74+0.21	12.84+0.21	12.76+0.23	12.88+0.22	12.90+0.19	12.80+0.19	12.80+0.18	12.83+0.17
P-SD	0.191	0.004**	0.000**	0.001**	0.001**	0.001**	0.000**	0.000**	0.000**
P- AA	0.826	0.710	0.457	0.153	0.625	0.278	0.942	0.987	0.502
P-SD-AA	0.236	0.703	0.457	0.868	0.349	0.961	0.429	0.443	0.653

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

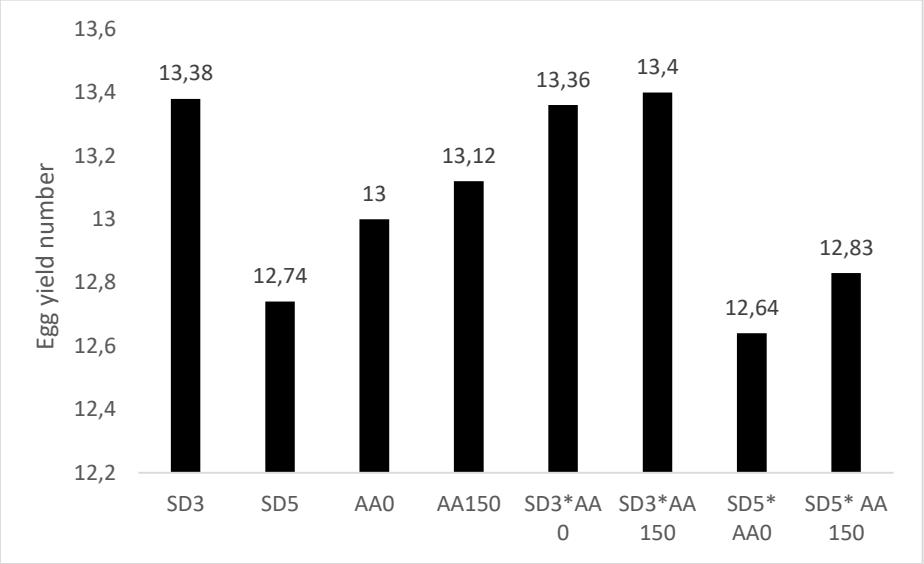


Figure *3.4.* Effects of experimental treatments on egg production in the overall periods

3.5. Effect of experimental treatments on egg yield percentage, % (EY%)

Effects of SD, AA supplementation into laying hen rations and their interactions on egg yield % are shown in Table 3.5 and Figure 3.5. The high SD increased ($P<0.01$) egg yield in Period 1, however, decreased in the 2nd period. Also, SD didn't affect egg yield (%) in the other periods and overall period. Addition of AA and SD*AA interactions did not have any significant effects on egg production percentage in different experimental periods and overall period ($P>0.05$).

Table 3.5. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yield % (EY%)

Treatments	EY%1	EY%2	EY%3	EY%4	EY%5	EY%6	EY%7	EY%8	Overall
Stocking density, SD									
SD 3	90.60+0.56[b]	95.00+1.07[a]	94.53+1.76	95.84+1.23	96.79+1.17	96.19+1.00	96.55+1.01	96.43+0.93	95.24+0.89
SD 5	92.79+0.56[a]	90.43+1.07[b]	90.57+1.76	93.81+1.23	94.92+1.17	95.45+1.00	95.00+1.01	95.00+0.93	93.50+0.89
L-Ascorbic Acid, AA									
AA 0	92.00+0.56	92.46+1.07	91.06+1.76	93.56+1.23	95.46+1.17	95.06+1.00	95.86+1.01	95.66+0.93	93.86+0.89
AA 150	91.36+0.56	93.06+1.07	94.06+1.76	96.06+1.23	96.26+1.17	96.56+1.00	95.76+1.01	95.76+0.93	94.86+0.89
Interactions									
SD3* AA 0	90.72+0.79	95.00+1.51	92.62+2.49	94.76+1.73	97.14+1.65	95.48+1.42	97.14+1.43	96.91+1.31	94.97+1.27
SD3* AA 150	93.29+0.79	89.86+1.51	89.43+2.49	92.39+1.73	93.73+1.65	94.63+1.42	94.48+1.43	94.48+1.31	92.78+1.27
SD5* AA 0	90.48+0.79	95.00+1.51	96.43+2.49	96.91+1.73	96.43+1.65	96.91+1.42	95.95+1.43	95.95+1.31	95.51+1.27
SD5*AA 150	92.29+0.79	91.00+1.51	91.72+2.49	95.22+1.73	96.12+1.65	96.27+1.42	95.52+1.43	95.52+1.31	94.21+1.27
P-SD	0.009**	0.004**	0.121	0.249	0.267	0.603	0.286	0.283	0.177
P- AA	0.439	0.707	0.228	0.160	0.616	0.287	0.960	0.972	0.444
P-SD-AA	0.632	0.707	0.761	0.843	0.354	0.941	0.441	0.452	0.727

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

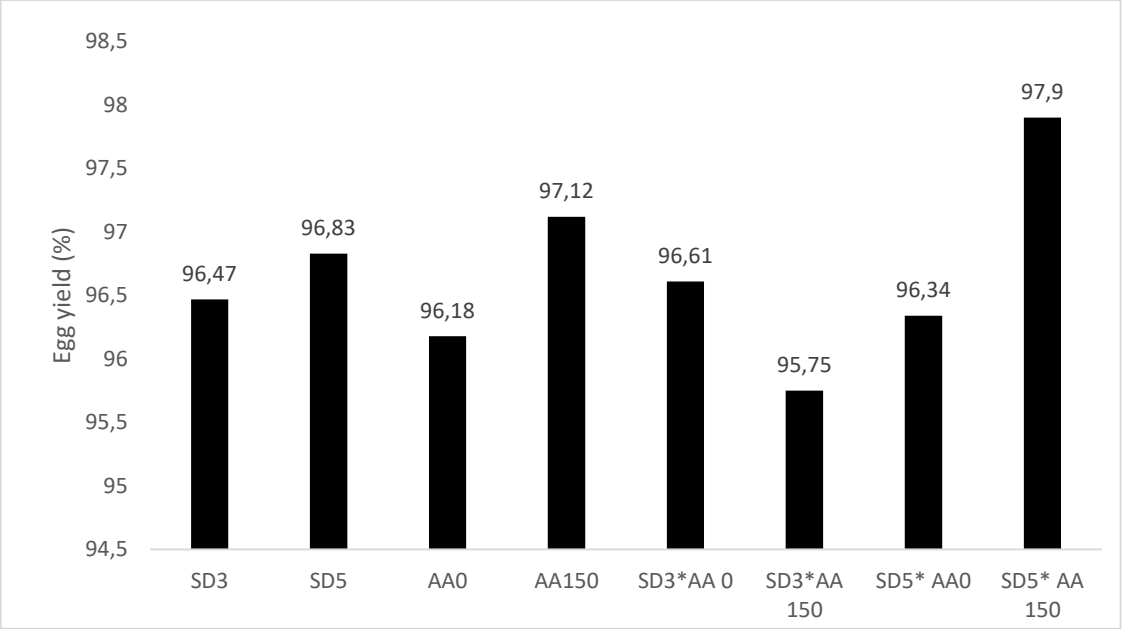


Figure *3.5.* Effects of experimental treatments on egg yield % in the overall periods

3.6. Effect of experimental treatments on egg weight (EW)

Effects of SD, AA supplementation into laying hen rations and their interactions on egg weight are shown in Table 3.6 and Figure 3.6. According to the table in terms of SD, there were significant differences in the 4th period (P<0.05). However, there was no significant difference between treatments in all the other periods (P>0.05). L-Ascorbic acid supplementation caused a significant increase in egg weight in the 3rd period (P<0.05). There was no significant difference between treatments in terms of SD*AA interactions (P>0.05).

Table 3.6. Effects of stocking density, L-Ascorbic Acid and their interactions on egg weight (EW)

Treatments	EW1	EW2	EW3	EW4	EW5	EW6	EW7	EW8	Overall
Stocking density, SD									
SD 3	66.25+0.58	66.60+0.91	65.53+0.56	64.83+0.93[b]	64.52+0.87	67.39+0.66	65.78+0.96	66.16+2.39	65.74+0.38
SD 5	67.31+0.58	66.37+0.91	66.87+0.56	67.51+0.93[a]	65.28+0.87	66.85+0.66	65.78+0.95	63.40+2.39	66.14+0.38
L-Ascorbic Acid, AA									
AA 0	66.15+0.58	66.29+0.91	64.81+0.56[b]	68.48+0.95	65.79+0.87	67.31+0.66	66.03+0.95	67.57+2.39	65.50+0.38
AA 150	67.41+0.58	65.68+0.91	67.59+0.56[a]	65.86+0.92	64.01+0.87	66.93+0.66	65.52+0.95	61.99+2.39	66.39+0.38
Interactions									
SD3* AA 0	65.54+0.82	66.42+129	63.49+0.79	65.58+1.34	66.03+1.23	67.55+0.94	65.94+1.34	68.08+3.37	65.31+0.53
SD3* AA 150	66.77+0.82	66.16+1.29	66.13+0.79	67.38+1.34	65.55+1.23	67.07+0.94	66.12+1.34	67.06+3.37	65.69+0.53
SD5* AA 0	66.96+0.82	64.78+1.29	67.56+0.79	64.08+1.30	63.01+1.23	67.22+0.94	65.61+1.36	64.23+3.37	66.17+0.53
SD5* AA 150	67.85+0.82	66.57+1.29	67.61+0.79	67.64+1.30	65.00+1.23	66.64+0.94	65.44+1.34	59.74+3.37	66.60+0.53
P-SD	0.196	0.553	0.093	0.045*	0.539	0.569	0.991	0.419	0.457
P- AA	0.128	0.636	0.001**	0.641	0.149	0.684	0.708	0.106	0.106
P-SD* AA	0.836	0.430	0.105	0.507	0.316	0.956	0.894	0.610	0.959

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05

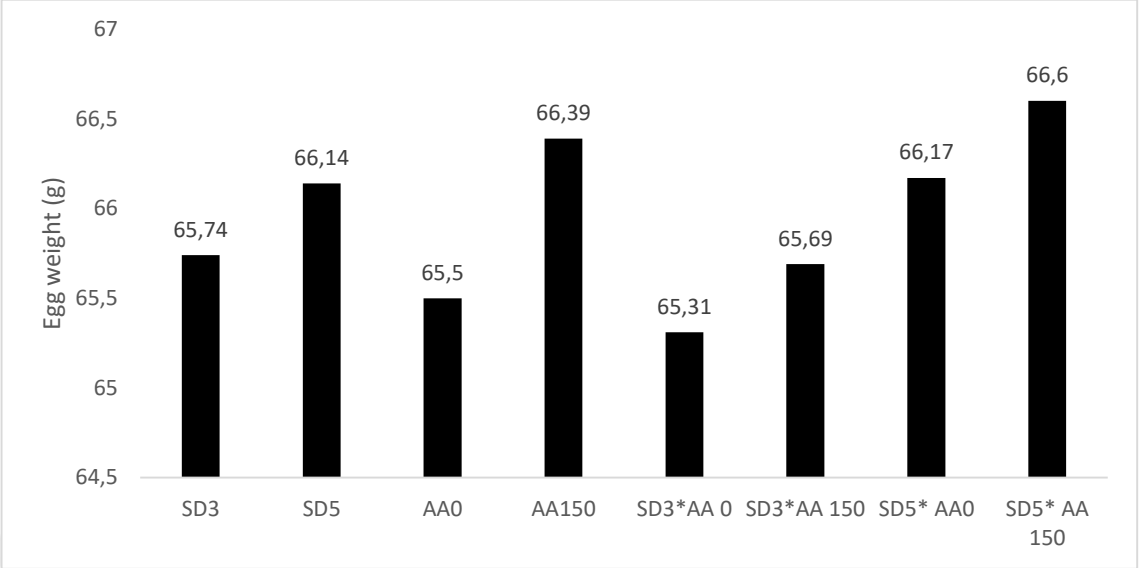


Figure *3.6.* Effects of experimental treatments on egg weight in the overall periods

3.7. Effect of experimental treatments on egg mass (EM)

Effects of SD, AA supplementation and their interactions on egg mass are shown in Table 3.7 and Figure 3.7. High SD increased egg mass in the 1st period ($P<0.05$) and AA addition increased egg mass in the 3rd period ($P<0.05$). There was no significant difference in egg mass of SD*AA interactions during the experimental period.

Table 3.7. Effects of stocking density, L-Ascorbic Acid and their interactions on egg mass (EM)

Treatments	EM 1	EM 2	EM 3	EM 4	EM 5	EM 6	EM7	EM8	Overall
Stocking density, SD									
SD 3	60.08+0.85[b]	64.45+1.91	62.38+1.42	63.12+1.41	62.59+1.29	66.59+1.15	65.70+1.17	63.97+1.71	63.29+0.96
SD 5	62.70+0.85[a]	61.34+1.91	63.26+1.42	64.73+1.41	63.83+1.29	67.12+1.15	67.22+1.17	62.38+1.71	64.07+0.96
L-Ascorbic Acid (AA)									
AA 0	60.99+0.85	64.23+1.91	60.53+1.42[b]	62.30+1.41	62.03+1.29	66.29+1.15	66.35+1.17	62.65+1.71	62.85+0.96
AA 150	61.79+0.85	61.56+1.91	65.11+1.42[a]	65.55+1.41	64.39+1.29	67.42+1.15	66.58+1.17	63.70+1.71	64.51+0.96
Interactions									
SD3* AA 0	59.57+1.21	67.34+2.70	59.45+2.01	61.74+2.00	61.34+1.83	66.45+1.63	65.96+1.65	64.56+2.42	62.66+1.36
SD3* AA 150	62.41+1.21	61.12+2.70	61.60+2.01	62.85+2.00	62.72+1.83	66.13+1.63	66.73+1.65	60.73+2.42	63.04+1.36
SD5* AA 0	60.58+1.21	61.56+2.70	65.32+2.01	64.49+2.00	63.83+1.83	66.73+1.63	65.45+1.65	63.38+2.42	63.92+1.36
SD5* AA 150	62.99+1.21	61.56+2.70	64.91+2.01	66.62+2.00	64.94+1.83	68.11+1.63	67.71+1.65	64.02+2.42	65.11+1.36
P-SD	0.037*	0.256	0.667	0.424	0.500	0.747	0.363	0.514	0.570
P- AA	0.515	0.329	0.029*	0.112	0.204	0.493	0.888	0.666	0.231
P-SD* AA	0.861	0.256	0.528	0.801	0.942	0.605	0.653	0.362	0.767

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, **: P<0.01, *: P<0.05.

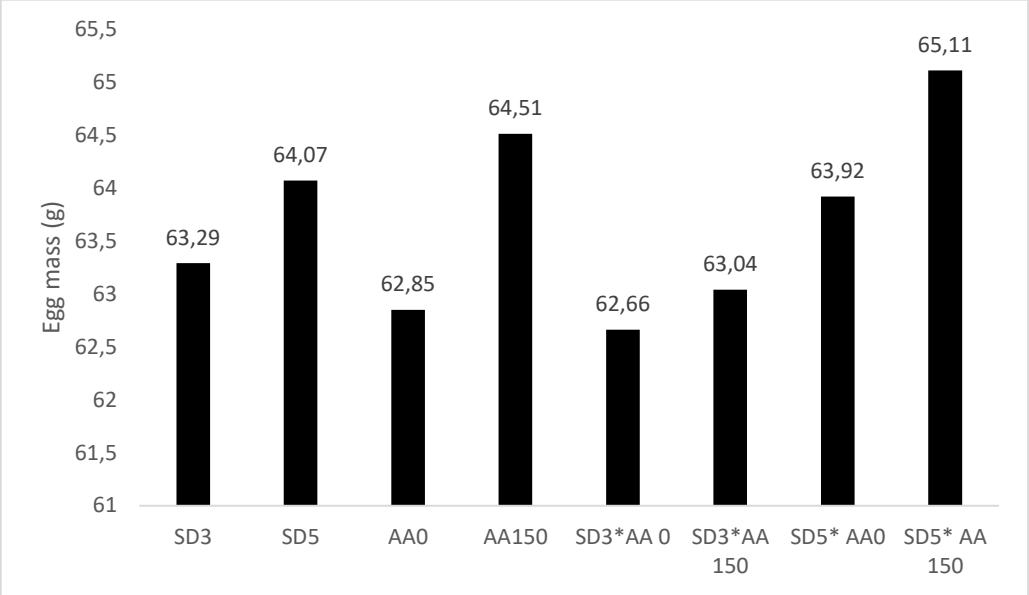


Figure *3.7.* Effects of experimental treatments on egg mass in the overall periods

3.8. Effect of experimental treatments on Haugh unit (HU)

Effects of SD, AA supplementation into laying hen rations and their interactions on Haugh unit (HU) are shown in Table 3.8 and Figure 3.8. High SD decreased HU in the 1st, 3rd, 4th, 5th, 6th and overall periods, however, it increased HU in the 2nd period (P< 0.05). Supplementation of AA significantly increased HU in the 4th period, however, AA decreased HU in the 6th and 7th periods (P<0.05). SD*AA interactions significantly affected HU values in the 1st, 4th and 7th periods (P<0.05). Supplementation of AA and SD*AA interactions did not affect HU values in overall period.

Table 3.8. Effects of stocking density, L-Ascorbic Acid and their interactions on Haugh unit (HU)

Treatments	HU1	HU2	HU3	HU4	HU5	HU6	HU7	HU8	Overall
Stocking density, SD									
SD 3	65.17+1.88[a]	57.95+1.75[b]	61.01+1.98[a]	64.23+1.62	63.26+1.74[a]	69.96+1.73[a]	64.38+1.58	69.39+2.31	65.12+1.17[a]
SD 5	55.72+1.88[b]	64.85+1.75[a]	54.99+1.98[b]	57.38+1.62[b]	58.40+1.74[b]	64.71+1.73[b]	63.00+1.56	64.08+2.31	60.49+1.17[b]
L-Ascorbic Acid, AA									
AA 0	61.71+1.88	63.16+1.75	54.57+1.98[b]	56.92+1.65[b]	60.86+1.74	70.44+1.73[a]	66.00+1.56[a]	68.13+2.31	63.47+1.17
AA 150	59.18+1.88	59.64+1.75	61.42+1.98[a]	64.69+1.60[a]	60.80+1.74	64.23+1.73[b]	61.37+1.58[b]	65.34+2.31	62.14+1.17
Interactions									
SD3* AA 0	62.95+2.65[b]	60.34+2.47	55.83+2.80	62.71+2.34[c]	63.76+2.46	71.18+2.44	70.87+2.21[a]	71.90+3.26	64.70+1.65
SD3* AA 150	60.48+2.65[c]	65.99+2.47	53.32+2.80	51.14+2.34[d]	57.95+2.46	69.71+2.44	61.13+2.21[c]	64.36+3.26	62.25+1.65
SD5* AA 0	67.39+2.65[a]	55.57+2.47	66.18+2.80	65.75+2.26[a]	62.76+2.46	68.74+2.44	57.88+2.25[d]	66.88+3.26	65.55+1.65
SD5* AA 150	50.96+2.65[d]	63.71+2.47	56.66+2.80	63.62+2.26[b]	58.84+2.46	59.72+2.44	64.86+2.21[b]	63.80+3.26	58.74+1.65
P-SD	0.001**	0.006**	0.034*	0.003**	0.050*	0.034*	0.536	0.112	0.008**
P- AA	0.341	0.156	0.016*	0.001 **	0.983	0.012*	0.039*	0.398	0.427
P-SD*AA	0.010*	0.615	0.214	0.042*	0.701	0.125	0.000**	0.499	0.196

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

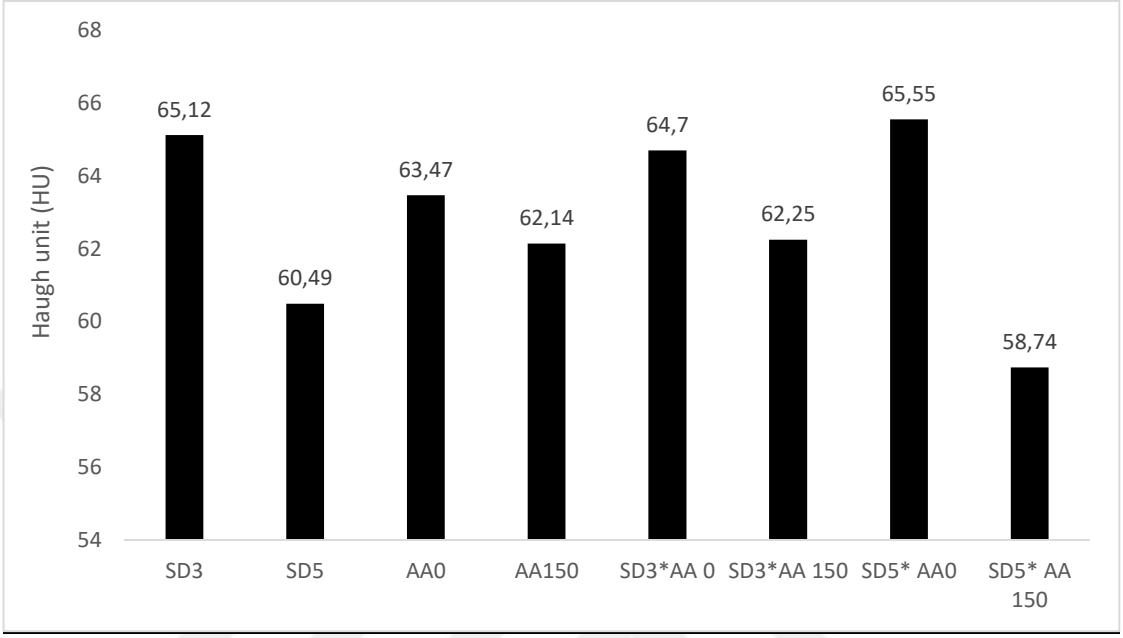


Figure *3.8.* Effects of experimental treatments on Haugh unit in the overall periods

3.9. Effect of experimental treatments on eggshell weight (ESW)

Effects of SD, AA supplementation into laying hen rations and their interactions on eggshell weight are given in Table 3.9 and Figure 3.9. High SD decreased eggshell weight in the 7th period (P<0.05). Supplementation of 150 mg/kg AA decreased ESW in the 3rd period, but increased in the 5th period (P<0.05). SD*AA interactions changed ESW in the 3rd period (P<0.05). SD, AA and their interaction did not affect ESW of hens in overall period.

Table 3.9. Effects of stocking density, L-Ascorbic Acid and their interactions on eggshell weight (ESW)

Treatments	ESW1	ESW2	ESW3	ESW4	ESW5	ESW6	ESW7	ESW8	Overall
Stocking density, SD									
SD 3	5.84+0.08	6.03+0.07	6.27+0.09	6.08+0.07	6.15+0.07	6.19+0.07	6.21+0.07 [a]	6.24+0.15	6.15+0.04
SD 5	5.86+0.08	5.94+0.07	6.21+0.09	5.94+0.07	6.21+0.07	6.37+0.07	5.99+0.07[b]	6.27+0.15	6.12+0.04
L-Ascorbic Acid, AA									
AA 0	5.90+0.08	6.04+0.07	6.38+0.09[a]	6.03+0.07	6.07+0.07[b]	6.19+0.07	6.18+0.07	6.41+0.15	6.15+0.04
AA 150	5.81+0.08	5.94+0.07	6.11+0.09[b]	5.99+0.07	6.29+0.07 [a]	6.36+0.07	6.02+0.07	6.10+0.15	6.12+0.04
Interactions									
SD3*AA0	5.93+0.11	6.05+0.10	6.28+0.13[b]	6.17+0.10	59.11+1.22	6.15+0.10	6.18+0.10[a]	6.42+0.22	6.14+0.05
SD3*AA 150	5.85+0.11	6.03+0.10	6.48+0.13 [a]	5.90+0.10	57.57+1.22	6.22+0.10	6.17+0.10[a]	6.40+0.22	6.17+0.05
SD5*AA 0	5.74+0.11	6.01+0.10	6.27+0.13[b]	6.00+0.10	57.34+1.22	6.22+0.10	6.24+0.10[a]	6.05+0.22	6.16+0.05
SD5* AA 150	5.87+0.11	5.86+0.10	5.95+0.13[c]	5.98+0.10	57.58+1.22	6.51+0.10	5.81+0.10[b]	6.14+0.22	6.08+0.05
P-SD	0.861	0.412	0.645	0.161	0.517	0.078	0.033**	0.872	0.641
P- AA	0.404	0.331	0.043*	0.678	0.009**	0.083	0.140	0.158	0.461
PSD*AA	0.338	0.532	0.046*	0.226	0.658	0.286	0.045*	0.801	0.288

[a,b,c]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.05.

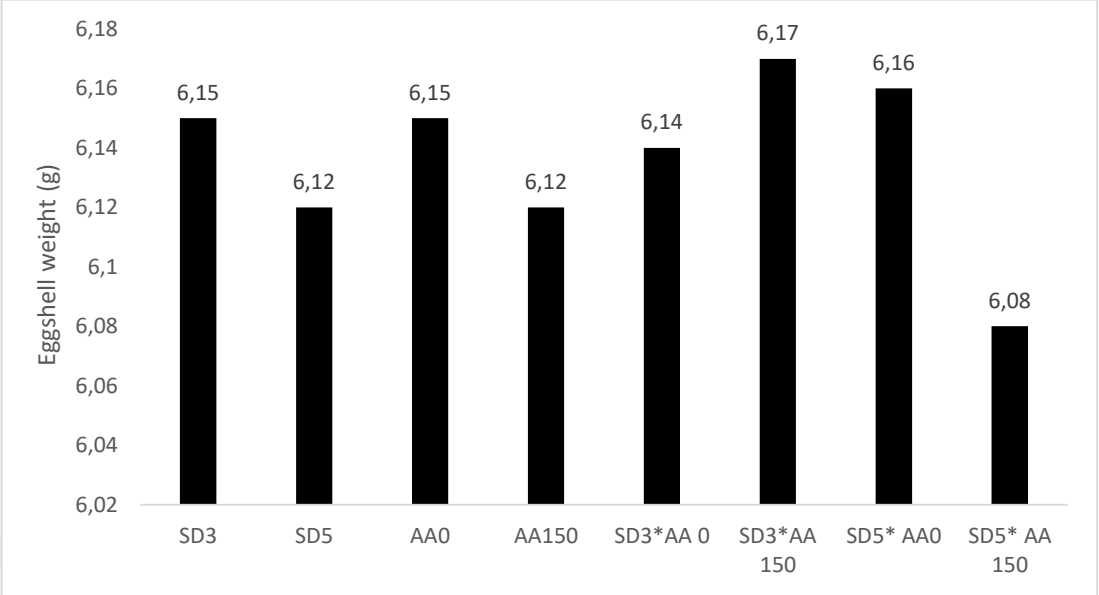


Figure *3.9.* Effects of experimental treatments on eggshell weight in the overall periods

3.10. Effect of experimental treatments on eggshell ratio (ESR)

Effects of SD, AA supplementation to laying hen rations on eggshell ratio are given in table 3.10 and Figure 3.10. High SD increased ESR values in the 4th period ($P< 0.05$) and AA in the 3rd period, however AA decreased in the 5th period ($P< 0.05$). The interactions did not affect ESR values in all periods ($P> 0.05$). The overall values didn't significantly affect by the SD, AA and interactions.

Table 3.10. Effects of stocking density, L-Ascorbic Acid and their interactions on eggshell ratio (ESR)

Treatments	ESR1	ESR2	ESR3	ESR4	ESR5	ESR6	ESR7	ESR8	Overall
Stocking density, SD									
SD 3	11.47+0.17	10.99+0.22	10.57+0.20	10.77+0.20[b]	10.54+0.19	10.97+0.18	10.67+0.22	10.78+0.50	10.82+0.10
SD 5	11.47+0.17	11.28+0.22	10.96+0.20	11.45+0.20[a]	10.62+0.19	10.63+0.18	11.08+0.22	10.23+0.50	10.94+0.10
L-Ascorbic Acid, AA									
AA 0	11.30+0.17	11.08+0.22	10.27+0.20[b]	11.12+0.21	10.94+0.19[a]	10.99+0.18	10.75+0.22	10.68+0.50	10.77+0.10
AA 150	11.74+0.17	11.18+0.22	11.26+0.20[a]	11.10+0.20	10.22+0.19[b]	10.61+0.18	10.99+0.22	10.33+0.50	11.00+0.10
Interactions									
SD3* AA 0	11.13+0.24	11.11+0.31	10.24+0.28	10.74+0.29	10.96+0.27	11.06+0.26	10.73+0.30	10.77+0.71	10.77+0.14
SD3* AA 150	11.48+0.24	11.06+0.31	10.29+0.28	11.50+0.29	10.92+0.27	10.92+0.26	10.78+0.30	10.60+0.71	10.77+0.14
SD5* AA 0	11.81+0.24	10.88+0.31	10.90+0.28	10.80+0.28	10.12+0.27	10.89+0.26	10.62+0.31	10.79+0.71	10.87+0.14
SD5* AA 150	11.67+0.24	11.49+0.31	11.63+0.28	11.41+0.28	10.33+0.27	10.34+0.26	11.37+0.30	9.86+0.71	11.12+0.14
P-SD	0.658	0.351	0.162	0.020*	0.757	0.182	0.191	0.447	0.383
P- AA	0.073	0.744	0.001**	0.945	0.009	0.144	0.436	0.620	0.111
P-SD*AA	0.315	0.284	0.223	0.792	0.658	0.432	0.259	0.592	0.357

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

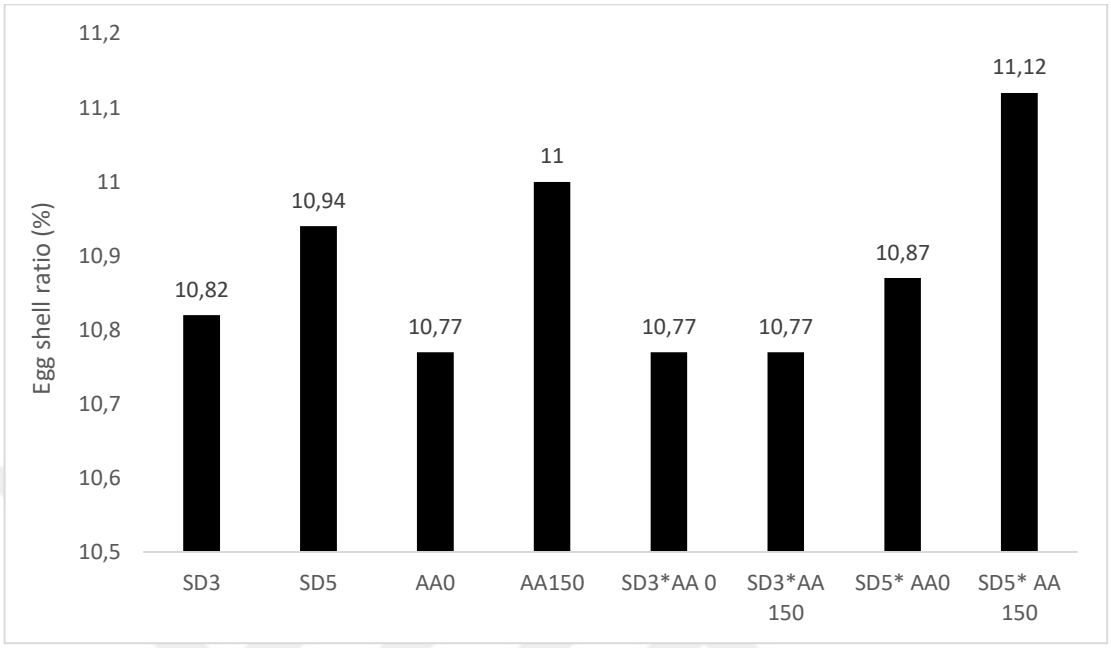


Figure 3.*10.* Effects of experimental treatments on eggshell ratio in the overall periods

3.11. Effect of experimental treatments on eggshell thickness (EST)

Effects of SD, AA supplementation to laying hen rations on eggshell thickness are given in table 3.11 and Figure 3.11. High SD decreased eggshell thickness in the period3 , but increased period4 ($P<0.01$ and $P<0.05$). Supplementation of 150 mg/kg AA increased eggshell thickness in the 2^{nd}, $3^{rd,}$ and 5^{th} periods ($P< 0.01$). There was no significant change in all periods in terms of SD*AA interactions ($P>0.05$).

Table 3.11. Effects of stocking density, L-Ascorbic Acid and their interactions on eggshell thickness (EST)

Treatments	EST1	EST2	EST3	EST4	EST5	EST6	EST7	EST8	Overall
	Stocking density, SD								
SD 3	0.36+0.00	0.34+0.00	0.35+0.00 [a]	0.36+0.00 [b]	0.33+0.00	0.34+0.01	0.33+0.00	0.32+0.00	0.34+0.00
SD 5	0.37+0.00	0.35+0.00	0.33+0.00 [b]	0.37+0.00 [a]	0.33+0.00	0.34+0.01	0.33+0.00	0.33+0.00	0.34+0.00
	L-Ascorbic Acid, AA								
AA 0	0.36+0.00	0.34+0.00[b]	0.33+0.00 [b]	0.37+0.00	0.330+0.00 [b]	0.35+0.01	0.33+0.00	0.32+0.00	0.34+0.00
AA 150	0.37+0.00	0.35+0.00[a]	0.34+0.00[a]	0.36+0.00	0.334+0.00 [a]	0.33+0.01	0.33+0.00	0.33+0.00	0.34+0.00
	Interactions								
SD3* AA 0	0.36+0.00	0.34+0.00	0.34+0.00	0.36+0.00	0.33+0.00	0.36+0.02	0.33+0.00	0.32+0.00	0.34+0.00
SD3* AA 150	0.37+0.00	0.34+0.00	0.32+0.00	0.37+0.00	0.33+0.00	0.34+0.02	0.33+0.00	0.33+0.00	0.34+0.00
SD5* AA 0	0.36+0.00	0.35+0.00	0.35+0.00	0.36+0.00	0.33+0.00	0.33+0.02	0.33+0.00	0.33+0.00	0.34+0.00
SD5* AA 150	0.37+0.00	0.35+0.00	0.33+0.00	0.37+0.00	0.33+0.00	0.33+0.02	0.33+0.00	0.33+0.00	0.34+0.00
P-SD	0.073	0.152	0.000**	0.039*	0.686	0.579	0.671	0.368	0.696
P- AA	0.452	0.000**	0.001**	0.465	0.009**	0.181	0.455	0.232	0.057
P-SD* AA	0.452	0.473	0.222	0.671	0.686	0.730	0.062	0.547	0.696

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

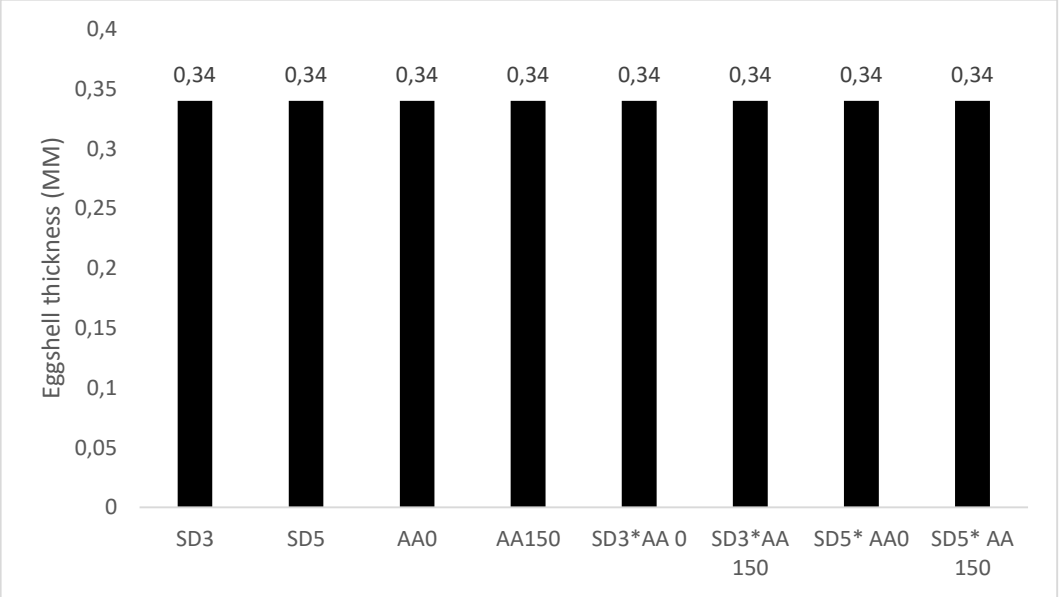


Figure *3.11.* Effects of experimental treatments on eggshell thickness in the overall periods

3.12. Effect of experimental treatments on egg yolk height (HMM)

Effects of SD, AA supplementation into laying hen rations and their interactions on yolk height (HMM) are shown in Table 3.12 and Figure 3.12. High SD decreased egg yolk height in the 1st, 3rd, 4th and 6th periods (P<0.01 and P<0.05), however increased in the 2nd period (P<0.05). Supplementation of AA increased HMM values in the 3 and 4th periods, but it decreased in the 6th period (P<0.01). There was a significant difference only in the 1st period (P<0.01) with SD*AA interaction. There were no significant effects of treatments in overall period.

Table 3.12. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk height (HMM)

Treatments	HMM1	HMM2	HMM3	HMM4	HMM5	HMM6	HM7	HM8	Overall
Stocking density, SD									
SD 3	5.09+0.15[a]	4.46+0.15[b]	4.69+0.15[a]	4.92+0.14[a]	5.72+0.65	5.65+0.15[a]	5.10+0.65	5.24+0.30	5.07+0.14
SD 5	4.29+0.15[b]	5.02+0.15[a]	4.20+0.15[b]	4.44+0.14[b]	4.40+0.65	5.10+0.15[b]	6.14+0.65	4.97+0.30	4.83+0.14
L-Ascorbic Acid, AA									
AA 0	4.70+0.15	4.88+0.15	4.11+0.15[b]	4.32+0.14[b]	5.54+0.65	5.69+0.15[a]	5.53+0.65	5.38+0.30	4.95+0.14
AA 150	4.68+0.15	4.59+0.15	4.77+0.15[a]	5.04+0.14[a]	4.58+0.65	5.05+0.15[b]	5.71+0.65	4.83+0.30	4.95+0.14
Interactions									
SD3* AA 0	4.83+0.21[b]	4.63+0.21	4.18+0.22	4.70+0.20	6.67+0.93	5.82+0.21	5.84+0.92	5.78+0.42	5.05+0.20
SD3* AA 150	4.56+0.21[c]	5.13+0.21	4.05+0.22	3.94+0.20	4.41+0.93	5.56+0.21	5.22+0.92	4.98+0.42	4.86+0.20
SD5* AA 0	5.34+0.21[a]	4.29+0.21	5.19+0.22	5.14+0.20	4.76+0.93	5.47+0.21	4.36+0.93	4.70+0.42	5.10+0.20
SD5* AA 150	4.01+0.21[d]	4.90+0.21	4.35+0.22	4.93+0.20	4.39+0.93	4.63+0.21	7.06+0.92	4.96+0.42	4.80+0.20
P-SD	0.000**	0.009**	0.027*	0.017*	0.159	0.010*	0.261	0.523	0.218
P- AA	0.917	0.171	0.003**	0.001**	0.298	0.003**	0.851	0.198	0.998
P-SD* AA	0.012*	0.800	0.107	0.180	0.307	0.174	0.074	0.214	0.782

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

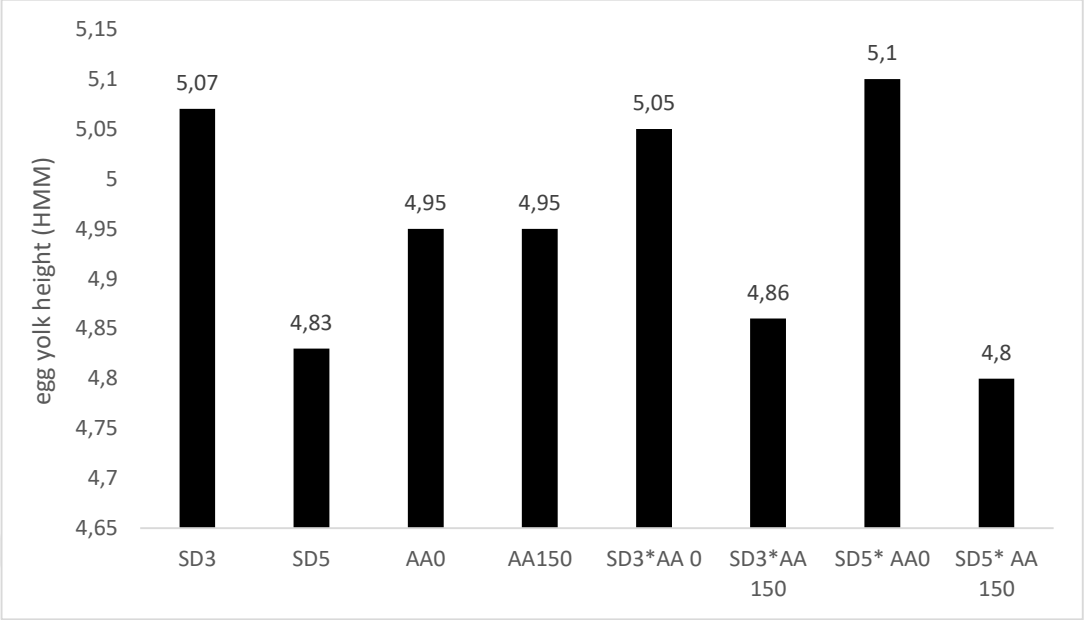


Figure 3.12. Effects of experimental treatments on egg yolk height in the overall periods

3.13. Effect of experimental treatments on Roche color scale values (RRS)

Effects of stocking density, AA supplementation into laying hen rations and their interactions on Roche color scale values (RRS) are shown in Table 3.13 and Figure 3.13. SD decreased RRS value in the 1st period, but increased in the 7th period ($P<0.05$). There was no significant difference of SD in other periods and overall period. Supplementation of AA increased RRS values in the 1st, 3rd and overall periods ($P<0.01$). However, there was no significant difference between the treatments in SD*AA interactions ($P>0.05$).

Table 3.13. Effects of stocking density, L-Ascorbic Acid and their interactions on Roche color scale values (RRS)

Treatments	RRS1	RRS2	RRS3	RRS4	RRS5	RRS6	RRS7	RRS8	Overall
Stocking density, SD									
SD 3	5.92+0.16[a]	5.20+0.13	5.48+0.17	5.04+0.11	5.35+0.13	5.40+0.14	5.29+0.15[b]	8.15+0.33	5.72+0.05
SD 5	5.42+0.16[b]	5.15+0.13	5.55+0.17	5.07+0.11	5.27+0.13	5.50+0.14	5.90+0.15[a]	8.10+0.33	5.79+0.05
L-Ascorbic Acid, AA									
AA 0	5.15+0.16[b]	5.08+0.13	4.62+0.17[b]	5.16+0.11	5.37+0.13	5.42+0.14	5.65+0.15	8.20+0.33	5.56+0.05[b]
AA 150	6.18+0.16[a]	5.27+0.13	6.42+0.17[a]	4.95+0.11	5.25+0.13	5.48+0.14	5.54+0.15	8.05+0.33	5.95+0.05[a]
Interactions									
SD3* AA 0	5.28+0.23	5.07+0.19	4.67+0.24	5.11+0.16	5.33+0.19	5.37+0.19	5.27+0.21	8.40+0.46	5.51+0.07
SD3* AA 150	5.03+0.23	5.10+0.19	4.57+0.24	5.21+0.16	5.40+0.19	5.47+0.19	6.03+0.21	8.00+0.46	5.60+0.07
SD5* AA 0	6.57+0.23	5.33+0.19	6.30+0.24	4.97+0.15	5.37+0.19	5.43+0.19	5.31+0.22	7.90+0.46	5.92+0.07
SD5* AA 150	5.80+0.23	5.20+0.19	6.53+0.24	4.93+0.15	5.13+0.19	5.53+0.19	5.77+0.21	8.20+0.46	5.98+0.07
P-SD	0.029*	0.788	0.781	0.810	0.663	0.602	0.005**	0.914	0.266
P- AA	0.000**	0.326	0.000**	0.172	0.541	0.728	0.603	0.747	0.000**
P-SD* AA	0.242	0.654	0.488	0.648	0.433	0.412	0.470	0.454	0.783

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, *: P<0.01, **: P<0.05.

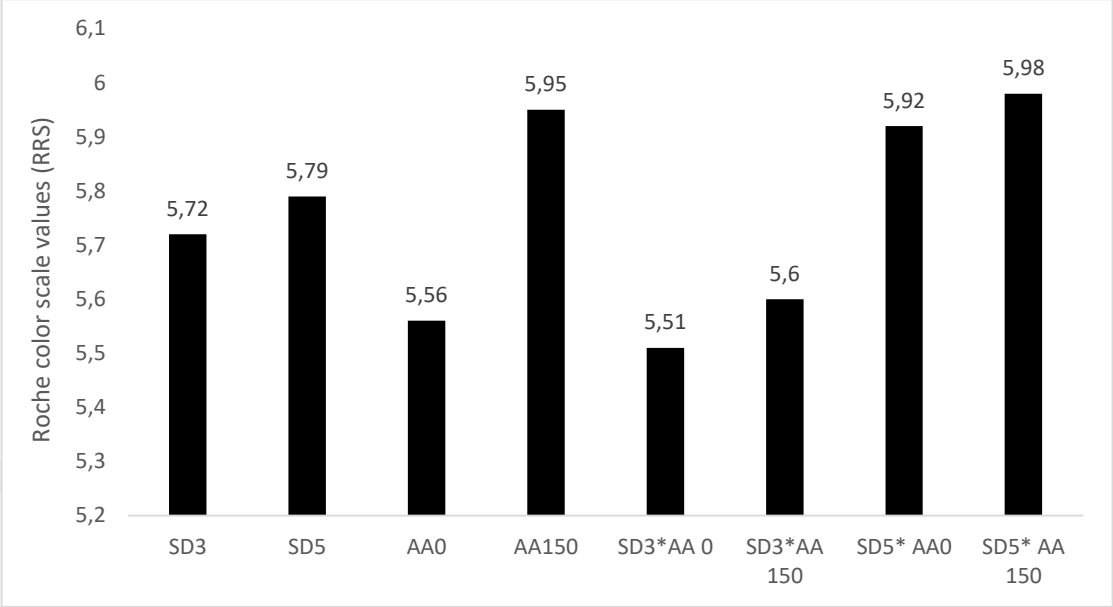


Figure *3.13.* Effects of experimental treatments on Roche color scale values in the overall periods

3.14. Effect of experimental treatments on egg yolk brightness (L*)

Effects of SD, AA supplementation into laying hen rations and their interactions on eggshell brightness (L*) values are shown in Table 3.14 and Figure 3.14. High SD increased L* value in the 8th period (P<0.01), however, did not change in the other periods and overall period. Supplementation of AA significantly decreased L* values in the 1st, 7th period and overall periods (P<0.05), but increased in the 4th period (P<0.01). The interactions significantly changed L* values in the 2nd and 7th periods.

Table 3.14. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk brightness (L*)

Treatments	L*1	L*2	L*3	L*4	L*5	L*6	L*7	L*8	Overall
Stocking density, SD									
SD 3	60.00+0.35	58.68+0.55	60.59+0.50	60.12+0.49	90.43+0.75	89.63+0.63	89.92+0.65	93.15+0.99^{b}	75.50+0.23
SD5	59.19+0.35	59.22+0.55	61.42+0.50	60.50+0.49	89.99+0.75	91.31+0.63	91.22+0.64	96.59+0.99^{a}	75.87+0.23
L-Ascorbic Acid, AA									
AA 0	60.60+0.36^{a}	58.80+0.55	61.63+0.50	59.14+0.50^{b}	90.35+0.75	89.74+0.63	90.89+0.64^{a}	94.45+0.99	76.08+0.23^{a}
AA 150	58.57+0.35^{b}	59.10+0.55	60.38+0.50	61.48+0.48^{a}	90.07+0.75	91.20+0.63	90.25+0.65^{b}	95.29+0.99	75.28+0.23^{b}
Interactions									
SD3* AA 0	60.97+0.50	57.46+0.77^{d}	61.28+0.71	58.71+0.70^{b}	90.62+1.07	89.05+0.90	90.01+0.91^{b}	92.02+1.41	75.62+0.32
SD3* AA 150	60.28+0.50	60.15+0.77^{a}	61.97+0.71	59.58+0.70^{a}	90.09+1.07	90.43+0.90	91.78+0.91^{a}	96.88+1.41	76.54+0.32
SD5* AA 0	59.04+0.50	59.90+0.77^{b}	59.90+0.71	61.54+0.68	90.25+1.07	90.22+0.90	89.84+0.92^{c}	94.29+1.41	75.37+0.32
SD5* AA 150	58.10+0.50	58.29+0.77^{c}	60.87+0.71	61.42+0.68	89.90+1.07	92.19+0.90	90.66+0.91^{b}	96.29+1.41	75.20+0.32
P-SD	0.108	0.491	0.245	0.586	0.683	0.064	0.832	0.020*	0.259
P- AA	0.000**	0.706	0.083	0.001**	0.794	0.106	0.029*	0.553	0.019*
P-SD* AA	0.804	0.006**	0.836	0.477	0.932	0.744	0.000**	0.317	0.196

$^{a, b}$: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, **: P<0.01, *: P<0.05.

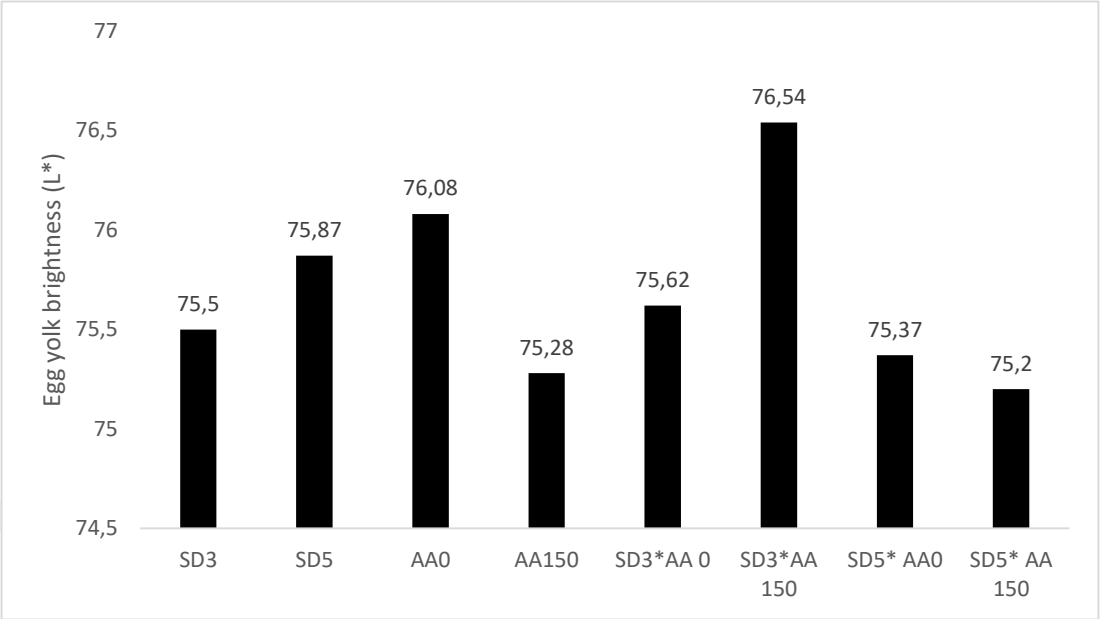


Figure 3.*14.* Effects of experimental treatments on egg yolk brightness in the overall periods

3.15. Effect of experimental treatments on egg yolk redness (a*)

Effects of SD, AA supplementation into laying hen rations and their interactions on eggshell redness (a*) are shown in Table 3.15 and Figure 3.15. High SD decreased egg yolk redness in the 6th and 8th periods (P<0.05), but not overall and the other periods. The AA supplementation increased a* values in the 2nd, 3rd, 7th and overall periods (P<0.05 and P<0.01), but decreased in the 4th period (P<0.01). There were significant variations in the 3rd, 6th, and 7th periods in interactions of SD*AA.

Table 3.15. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk redness (a*)

Treatments	a*1	a*2	a*3	a*4	a*5	a*6	a*7	a*8	Overall
Stocking density, SD									
SD 3	-2.46+0.82	-3.77+0.08	-3.51+0.09	-3.81+0.08	-5.05+0.17	-5.32+0.15^{a}	-5.24+0.12	0.40+0.49^{a}	-3.84+0.05
SD 5	-3.36+0.82	-3.89+0.08	-3.49+0.09	-3.76+0.08	-5.01+0.17	-5.85+0.15^{b}	-5.27+0.12	-1.49+0.49^{b}	-3.97+0.05
L-Ascorbic Acid, AA									
AA 0	-3.02+0.82	-3.95+0.08^{b}	-4.49+0.09^{b}	-3.38+0.09^{a}	-5.03+0.17	-5.77+0.15	-5.44+0.12^{b}	0.12+0.49	-4.22+0.05^{b}
AA 150	-2.81+0.82	-3.71+0.08^{a}	-2.51+0.09^{a}	-4.19+0.08^{b}	-5.02+0.17	-5.40+0.15	-5.07+0.12^{a}	-1.22+0.49	-3.59+0.05^{a}
Interactions									
SD3* AA 0	-1.98+1.16	-3.82+0.12	-4.33+0.13^{b}	-3.47+0.12	-5.01+0.24	-5.72+0.21^{c}	-5.89+0.17^{a}	0.53+0.69	-4.14+0.07
SD3* AA 150	-4.05+1.16	-4.08+0.12	-4.65+0.13^{a}	-3.30+0.12	-5.06+0.24	-5.83+0.21^{b}	-4.99+0.17^{c}	-0.29+0.69	-4.29+0.07
SD5* AA 0	-2.95+1.16	-3.72+0.12	-2.69+0.13^{c}	-4.15+0.12	-5.09+0.24	-4.92+0.21^{d}	-4.58+0.17^{d}	0.26+0.69	-3.54+0.07
SD5* AA 150	-2.66+1.16	-3.70+0.12	-2.33+0.13^{d}	-4.23+0.12	-4.96+0.24	-5.87+0.21^{a}	-5.55+0.17^{b}	-2.69+0.69	-3.64+0.07
P-SD	0.444	0.304	0.872	0.700	0.870	0.014	0.832	0.010*	0.094
P- AA	0.857	0.042*	0.000**	0.000**	0.974	0.080	0.029*	0.062	0.000**
P-SD* AA	0.312	0.236	0.012*	0.319	0.697	0.050*	0.000**	0.133	0.733

^{a,b}: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, **: P<0.01, *: P<0.05.

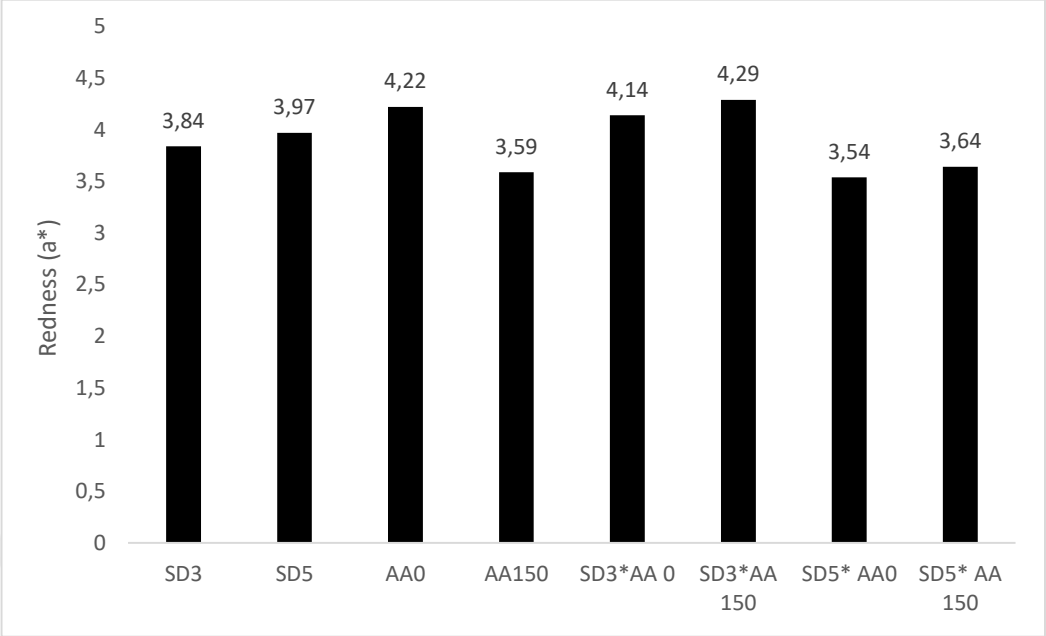


Figure *3.15.* Effects of experimental treatments on egg yolk redness in the overall periods

3.16. Effect of experimental treatments on egg yolk yellowness (b*)

Effects of SD, AA supplementation into laying hen rations and their interactions on egg yolk yellowness (B*) are shown in Table 3.16 and Figure 3.16. The high SD decreased b* values in the 1st period (P<0.05), however, it increased b* values in the 7th period (P<0.01). Supplementation of AA decreased b* values in the 1st period (P<0,05) but increased in the 7th period (P<0.01). SD*AA interactions affected b*values in the 7th and 8th periods (P<0.05). There was no significant effect on b* values at overall period.

Table 3.16. Effects of stocking density, L-Ascorbic Acid and their interactions on egg yolk yellowness (b*)

Treatments	b*1	b*2	b*3	b*4	b*5	b*6	b*7	b*8	Overall
Stocking density, SD									
SD 3	39.97+0.48[a]	38.54+0.62	39.84+0.39	38.94+0.67	58.23+0.86	57.13+2.37	58.07+0.97[b]	62.51+1.52	49.26+0.43
SD 5	38.59+0.48[b]	39.11+0.62	40.14+0.39	39.36+0.67	57.57+0.86	62.23+2.37	62.08+0.96[a]	64.92+1.52	50.15+0.43
L-Ascorbic Acid, AA									
AA 0	40.00+0.48[a]	38.96+0.62	40.47+0.39	38.58+0.68	58.34+0.86	60.72+2.37	58.20+0.96[b]	64.16+1.52	49.89+0.43
AA 150	38.55+0.48[b]	38.69+0.62	39.51+0.39	39.72+0.66	57.46+0.86	58.64+2.37	61.95+0.97[a]	63.28+1.52	49.52+0.43
Interactions									
SD3* AA 0	40.25+0.68	38.04+0.87	40.07+0.55	38.96+0.96	-5.01+0.24	56.55+3.36	58.12+1.36[b]	60.23+2.15[d]	49.21+0.61
SD3* AA 150	39.76+0.68	39.89+0.87	40.87+0.55	38.20+0.96	-5.06+0.24	64.90+3.36	58.28+1.36[b]	68.08+2.15[a]	50.57+0.61
SD5* AA 0	39.69+0.68	39.05+0.87	39.61+0.55	38.93+0.93	-5.09+0.24	57.71+3.36	58.01+1.38[b]	64.80+2.15[b]	49.31+0.61
SD5* AA 150	37.41+0.68	38.33+0.87	39.42+0.55	40.51+0.93	-4.96+0.24	59.56+3.36	65.89+1.36[a]	61.77+2.15[c]	49.73+0.61
P-SD	0.044*	0.518	0.581	0.665	0.596	0.131	0.004**	0.269	0.151
P- AA	0.035*	0.754	0.084	0.232	0.474	0.536	0.007**	0.687	0.549
P-SD* AA	0.190	0.141	0.370	0.218	0.471	0.335	0.005**	0.016*	0.443

[a, b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, **: P<0.01, *: P<0.05.

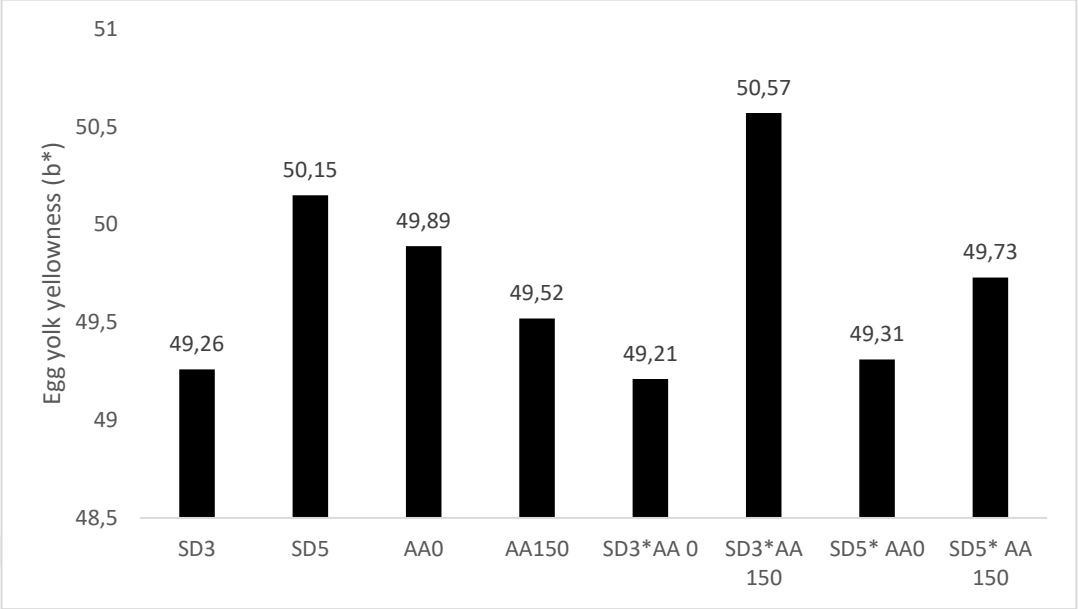


Figure 3.*16.* Effects of experimental treatments on egg yolk yellowness in the overall periods

3.17. Effect of experimental treatments on blood parameters (BP)

Effects of SD, AA supplementation into laying hen rations and their interactions on blood parameters are shown in Table 3.17. High SD caused an increase in serum triglyceride, AST and cortisol concentrations, however, there was no significant differences in terms of serum glucose, cholesterol and ALT values. AA supplementation and interactions didn't significantly affect of serum components.

Effects of SD, L-Ascorbic acid supplementation into laying hen rations and their interactions on blood heterophil, eosinophil, basophil, monocyte, lymphocyte and H/L values are shown in Table 3.18. SD, AA supplementation and their interactions didn't affect blood heterophil, eosinophil, basophil, monocyte, lymphocyte and H/L values.

Table 3.17. Effects of stocking density, L-Ascorbic Acid and their interactions on blood parameters (BP)

Treatments	Glucose, mg/dL	Triglyceride, mg/dL	Cholesterol, mg/dL	AST, U/L	ALT, U/L	Cortisol, mcg/dL
Stocking density, SD						
SD 3	162.20+13.63	775.25+137.55^{b}	103.55+13.74	158.10+14.77^{b}	3.30+0.72	0.04+0.00^{b}
SD 5	190.85+13.63	1203.25+137.55^{a}	103.35+13.74	208.80+14.77^{a}	2.50+0.72	0.05+0.00^{a}
L-Ascorbic Acid, AA						
AA 0	184.25+13.63	1004.00+137.55	100.00+13.74	191.00+14.77	2.00+0.72	0.00+0.00
AA 150	168.00+13.63	973.00+137.55	106.00+13.74	175.00+14.77	3.00+0.72	0.00+0.00
Interactions						
SD3* AA 0	180.00+19.27	894.60+194.53	107.80+19.43	175.40+20.89	3.40+1.02	0.05+0.00
SD3* AA 150	188.50+19.27	1115.20+194.53	93.60+19.43	207.10+20.89	1.80+1.02	0.05+0.00
SD5* AA 0	144.40+19.27	655.90+194.53	99.30+19.43	140.80+20.89	3.20+1.02	0.04+0.00
SD5* AA 150	193.20+19.27	1291.30+194.53	113.10+19.43	210.50+20.89	3.20+1.02	0.05+0.00
P-SD	0.146	0.034*	0.992	0.020*	0.437	0.035**
P- AA	0.428	0.873	0.779	0.460	0.559	0.281
P-SD* AA	0.303	0.293	0.476	0.369	0.437	0.281

^{a,b}: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, **: P<0.01, *: P<0.05.

Table 3.18. Effects of stocking density, AA and their interactions on blood parameters (BP)

Treatments	Heterophil	Lymphocyte	Monocytes	Eosinophil	Basophils	H/L
Stocking density, SD						
SD 3	17.64+2.18	23.10+2.17	19.55+1.90	17.72+2.26	21.97+1.82	0.78+0.11
SD 5	20.53+2.32	25.40+2.32	19.33+2.02	16.59+2.41	18.10+1.94	0.88+0.12
L-Ascorbic Acid, AA						
AA 0	17.69+2.32	23.79+2.32	20.63+2.02	17.08+2.41	20.76+1.94	0.74+0.12
AA 150	20.47+2.18	24.71+2.17	18.25+1.90	17.23+2.26	19.31+1.82	0.92+0.11
Interaction						
SD3* AA 0	14.43+2.93	22.95+2.93	21.65+2.56	16.97+3.05	23.95+2.46	0.65+0.15
SD3* AA 150	20.95+3.59	24.63+3.59	19.60+3.13	17.20+3.73	17.58+3.01	0.83+0.18
SD5* AA 0	20.84+3.21	23.24+3.21	17.44+2.80	18.48+3.34	19.98+2.69	0.91+0.16
SD5* AA 150	20.10+2.93	26.18+2.93	19.07+2.56	15.98+3.05	18.63+2.46	0.93+0.15
P-SD	0.377	0.477	0.940	0.736	0.166	0.549
P- AA	0.395	0.775	0.404	0.965	0.592	0.270
P-SD* AA	0.270	0.844	0.516	0.684	0.359	0.616

SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability.

3.18. Effect of experimental treatments on respiratory count (RC), tonic immobility (TI) and feather scoring (FS)

Effects of SD, AA supplementation into laying hen rations and their interactions on respiratory count, tonic immobility (TI) and feather scoring are given in Table 3.19 and Figure 3.19, 3.20 and 3.21. SD affected respiratory rate ($p<0.05$) and feather loose score ($P<0.01$). AA addition into feed and SD*AA interactions didn't affect these parameters.

Table 3.19. Effects of stocking density, L-Ascorbic Acid and their interactions on respiratory rate, Tonic immobility (TI) and Feather score (FS)

Treatments	Respiratory rate	Tonic immobility	Feather score
Stocking density, SD			
SD 3	65.58+1.42[b]	168.35+34.38	0.46+0.69[b]
SD 5	71.63+1.51[a]	241.25+34.38	0.89+0.69[a]
L-Ascorbic Acid, AA			
AA 0	67.96+1.51	230.75+34.38	0.71+0.69
AA 150	69.25+1.42	178.85+34.38	0.65+0.69
Interaction			
SD3* AA 0	63.17+1.91	184.00+48.61	0.53+0.98
SD3* AA 150	72.75+2.34	152.70+48.61	0.40+0.98
SD5* AA 0	68.00+2.10	277.50+48.61	0.88+0.98
SD5* AA 150	70.50+1.91	205.00+48.61	0.90+0.98
P-SD	0.010*	0.142	0.001**
P- AA	0.542	0.293	0.563
P-SD* AA	0.106	0.674	0.435

[a,b]: Values with different superscript in a column differ significantly, SD: stocking density, AA: L-Ascorbic Acid, FI: feed intake, P: probability, **: P<0.01, *: P<0.05.

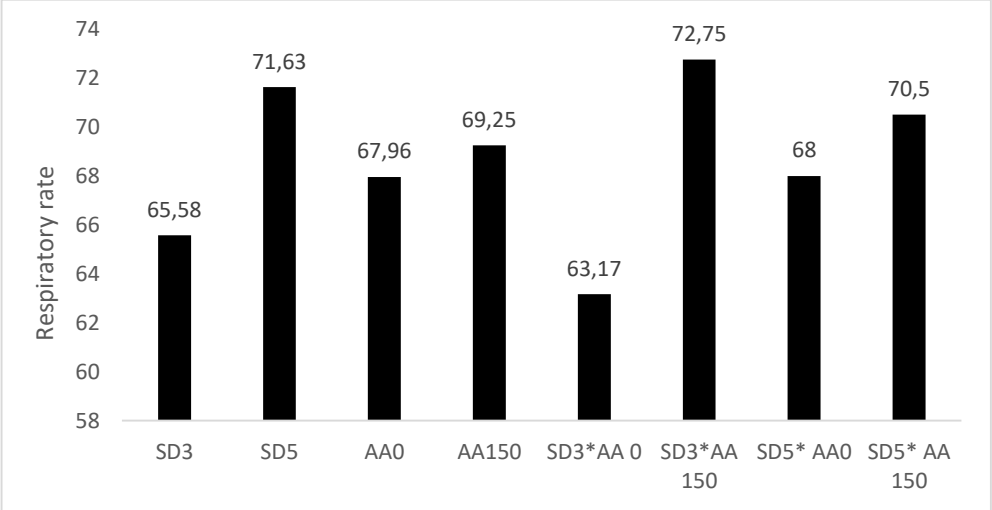


Figure 3.*17.* Effects of experimental treatments on respiratory rate in the overall periods

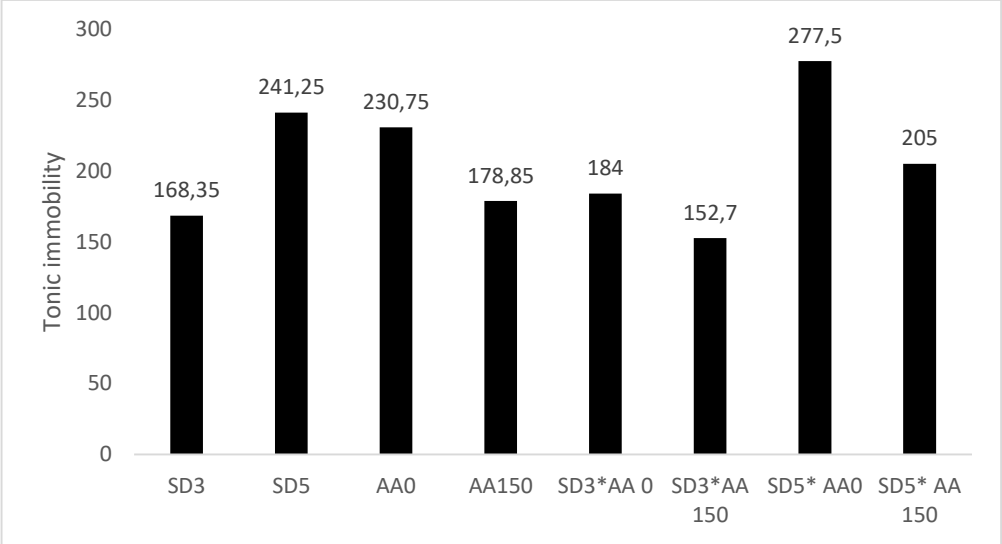


Figure 3.*18.* Effects of experimental treatments on tonic immobility in the overall periods

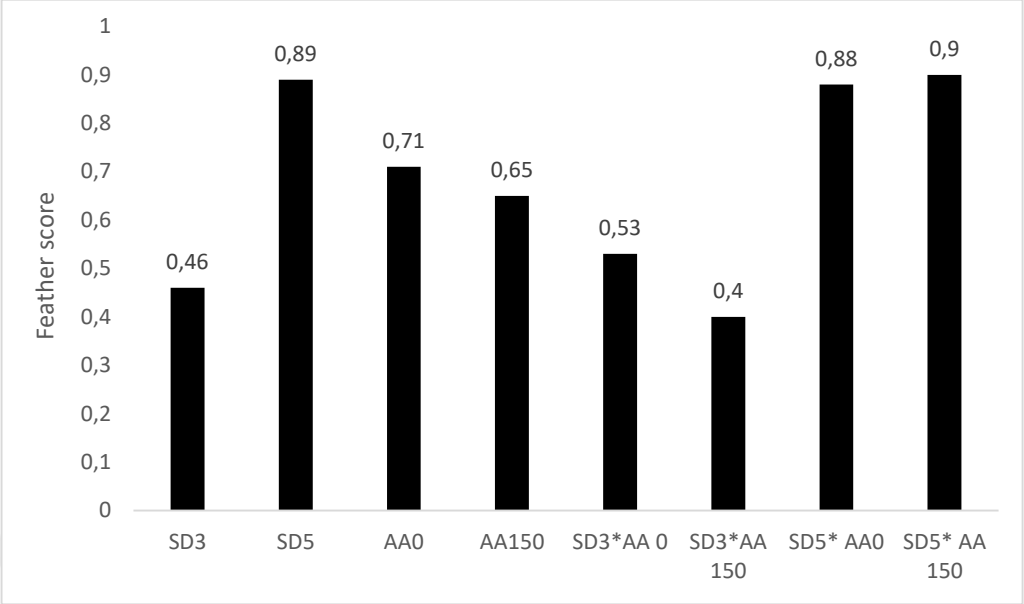


Figure 3.19. Effects of experimental treatments on feather score in the overall periods

CHAPTER 4

DISCUSSION - CONCLUSION AND RECOMMENDATIONS

4.1. Effect of stocking density on performance traits

Stocking density of laying hens may affect the production performance, egg quality, blood and stress parameters. According to general experience, especially in hot summers, high SD and heat stress can result in a low feed intake, egg production, egg quality and high mortality. The objective of this study was to determine the effects of stocking density and AA supplementations into laying hen rations on laying performance, egg quality, tonic immobility, respiratory rate, feather score and blood hematological parameters. When laying hens are fed in conventional cages, it is very important for animal welfare to provide sufficient feeder area per hen and sufficient place for perching/laydown together at the same time to rest. Personal observation showed that when the hens reared in the cages at high SD, the animals cannot find enough feeding space and they consume their feed sequentially one by one. Feeding priority is usually given to the stronger and dominant animals in the cage and big feed particles are selected by those animals. In this case, hard grain feeds and coarse particles such as maize are selected by the dominant animals, the rest of feeds, fine feed particles, such as soybean meal and wheat in the feeder are selected by the others. In this way, coarse particles cause energy rich feeds and result in greater weight gain and small particles cause protein rich feeds and result in unbalanced nutrition.

In this experiment, SD (high 560cm^{2,} and low 336cm^{2}) caused a lower final body weight. At the same time, high SD animals (5 hens/cage) had more feed consumption than the low SD animals (3 hens/cage). Weimer et al. (2019) noted that high stocking densities (465 vs 955 cm^{2}) decreased final body weight. Saki et al. (2012) reported that the high SD (500 vs. 2000 cm^{2}) decreased final body weights and Wang *et al.* (2020) also reported

reduced body weights at high stocking density (506 cm^2/hen space). In contrast to present findings, Kang *et al.* (2016) reported that laying hens with high SD (10 hens/m^2) caused a lower feed intake. Similarly, Benyi et al. (2006) reported that low SD of laying hens (773 vs 1100 cm^2) increased final body weight and feed intake. However, these researchers claimed that high SD did not affect FCR values. In contrast to their findings, in the current experiment, it was seen that FCR values were negatively affected by high SD. Hanafy, (2022) experimented different stocking densities (6, 9 or 12 m^2) and indicated that the final body weight was enhanced by 6 m^2, the FCR improved by 12 m^2 and egg production, egg weight and egg mass were improved by 6-9 m^2. There is also another contrast related to feed intake and body weigh change that reported by Hanh *et al.* (2021). They explained that the SD of conventional cage (45×40x40cm), low density (540 cm^2), middle density (450 cm^2) and high density (386 cm^2) of laying hens didn't affect final body weight, feed intake increased in all density groups and FCR increased in high density group. In another study, Kaya et al. (2021) experimented different cage densities (5 and 7 hens/cage) and reported increased feed intake and egg production at 5 hens per cage. Similarly, Kahraman et al. (2020) noted that the SD of 412.5 and 495 cm^2/hen increased feed intake, FCR and egg production of laying hens. In a similar study, Erensoy et al. (2021) reported that FCR increased at medium (736.3 cm^2) and low (1104.5 cm^2) cage density and decreased at high density (552.3 cm^2). Simitzis (2012) reported that SD did not any significant effects on FCR of laying hens.

4.2. Effect of stocking density on egg yield and egg quality traits

In the present study, high SD decreased egg production, however, egg weight and mass were not influenced by the SD. In high SD conditions, animals may not consume enough feed and calcium, but it depends on animal density per area. If the animals do not consume enough feed to maintain the egg yield, it may cause reductions in egg production, weight, mass and eggshell characteristics. In this study, there was a slight increase in feed consumption of high SD hens, however, egg production was low. In a similar experiment, Weimer et al. (2019) noted that the high stocking densities (465 vs 955 cm^2) decreased egg production. Erensoy *et al.*, (2021) reported that egg production decreased at high and medium density but increased at low density. Similar observations were noted by Sarica et al. (2008) and they noted that the stocking densities of 2000, 1000, 667 and 500 cm^2 per hen decreased egg production and egg quality traits. Benyi et al. (2006) confirmed

that low SD of laying hens (773 vs 1100 cm^2) increased egg production. El-Tarabany (2016) experimented different stocking densities (200, 167 and 143 cm^2) and reported that low SD increased egg production, egg mass, egg weight and egg quality traits of quail. At the same time, different SD conditions may change egg external and internal quality.

Geng et al. (2020) noted that indoor SD values (5, 6, 7 and 8 hens/m^2) did not affect egg quality traits of laying hens. In another study, Son et al. (2020) discovered that SD in conventional cage system (750 and 500cm2/ birds) did not have any significant effects on egg quality traits. Haque et al. (2006) found that stocking densities (0.186, 0.279 and 0.372m^2/bird) of laying hens resulted in different seasonal HU, egg weight, shape index, shell color and yolk color value and shell weight was better in winter than in summer, but albumen index and shell thickness was not affected by season. Rakonjac (2019) reported that SD in laying hen influenced egg yolk height. The egg albumen height (HMM) of SD was higher in some periods, however, didn't affect in the other periods and overall period.

In present study, the Roche color scale (RRS) values did not influenced by the experimental treatments. Wang et al. (2020) experimented high stocking density (338 cm^2) and low density (506 cm^2) and indicated that Roche color scale value decreased at high SD as compared to low SD. It was also reported that high SD reduced egg weight, feed intake and increased eggshell weight, decreased shell redness and yellowness value, strength, thickness and relative weight as compared to low SD. Wang et al. (2021) explained that the stocking densities of 450 cm^2/bird, 675 cm^2/bird, 900 cm^2/bird had no effect on eggshell thickness, shell breaking strength and egg shape index, while the greatest SD reduced body weight, yolk color and egg weight. In another study, Altan et al. (2002) noted that the SD of 640 and 480 cm^2 did not have any significant effects on egg production and egg weight, however, 384cm^2 SD reduced HU and egg production, but, no impact on shell quality and egg weight.

Egg yolk lightness (L*), redness (a*) and yellowness (b*) did not change with SD. In a similar study, Sekeroglu (2010) reported that SD of 40x42x45 cm^2 did not affect yolk color (L*, a*, b*) of laying hens. In another study, El-Shafei et al. (2012) reported that SD of 140, 170, 200, and 233 cm^2 did not affect egg weight and quality, except that shell weight and thickness increased in 140 cm^2 SD and the respiratory rate of laying hens improved by 170 cm^2 and 140 cm^2 SD.

Eggshell traits (weight, percentage, thickness and ratio) were not influenced by the SD. Mirfendereski and Jahanian (2015) stated that the SD of 40x45cm^2 had no effect on eggshell weight, eggshell thickness and eggshell ratio of laying hens. Similarly, Erensoy (2021) claimed that layer hens with low and medium SD had greater egg yield than those with high SD (high 552.3, medium 736.3, and low 1104.5 cm^2 cage floor space per hen). Saki et al. (2012) found that the cage densities of 2,000, 1,000, 667 and 500 cm^2 per hen with four hens per cage had significantly lower eggshell weight than the hens in the control group with one hen per cage and egg quality and egg production increased at 1,000 cm^2. It was reported in a similar study that cage density increased eggshell thickness (Şekeroğlu and Altuntaş, 2009).

4.3 Effect of SD on blood parameters, feather score and tonic immobility

In present study, the high SD increased serum triglyceride, AST and cortisol levels. However, there was no significant difference in cholesterol, glucose, alanine transaminase (ALT) levels of SD treatments. Hanafy et al. (2022) and Guo et al. (2012) indicated that SD (543 cm^2 and 586 cm^2) did not affect glucose, triglycerides and cholesterol levels of laying hens. Gholami et al. (2020) reported that aspartate aminotransferases (AST) and alanine aminotransferase (ALT) were affected by SD. Osadcha et al. (2021) noted that high SD had no impact on cholesterol level of blood.

In present experiment, treatments did not affect blood heterophil lymphocyte monocytes, eosinophil, basophils and H/L values. Tactacan et al. (2009) noted that the conventional cage of 561.9 cm^2 and enriched cages of 642.6cm^2 didn't affect heterophil, lymphocyte monocytes, eosinophil and basophils levels of laying hens. In another experiment, Kang et al. (2011) noted that the heterophil (H), lymphocytes (L), monocytes and H:L ratios were not influenced by different SD values (12 - 44 kg of body weight/m^2).

In this experiment, respiratory rate and feather score increased at high SD. Widowski et al. (2017) and Onbaşilar and Aksoy (2005) indicated that the SD of 520 cm^2 (low density) or 748 cm^2 (high density) increased feather score. In a previous study, Sanotra (2001) reported that tonic immobility (TI) duration of high SD was significantly higher in broilers. In another similar study (Hrabcakova, 2012), the duration of TI was considerably shorter in pheasant hens maintained in enriched cages than in hens kept in conventional cages. Şekeroğlu (2014) noted that cage density of 600 cm^2 significantly increased TI of laying hens.

4.4. Effect of L-ascorbic acid supplementation into diets on performance traits

In laying hen production, supplementation of AA is the most common practice and AA was proven to be effective in protection against heat stress (Konca et al., 2002; Khan et al., 2012). Also, AA is now under use in chickens exclusively to treat diseases, strengthen the immune system and produce high-quality eggs. Birds activate catecholamine hormones to resist stress conditions and as a result of this rapid response, the body's vitamin C reserves are rapidly depleted (Butcher and Miles, 2002). If there is not enough vitamin C at this stage, animals enter into a stage of exhaustion and the death rate increases as a result (Siegel and Kampen, 1984). In present study, AA supplementations did not have significant effects on the final body weight of laying hens. Similarly, Puthpongsiriporn (2001) added vitamin C into feed (0 to 1000 ppm) and Niu et al. (2009) added vitamin C (0, 100 or 200 mg/kg) and vitamin E together and reported that vitamin C and E supplementations did not have significant effects on the body weight. In contrast to these findings, Çiftçi et al. (2005) reported that 200 mg vitamin C supplementation increased final body weight and egg production. Büyükkılıç Beyzi et al. (2020) reported that body weight, egg weight and feed intake of the hens were not affected by the 250 mg/kg AA addition into laying hen diets under heat stress conditions.

In current experiments, addition of AA (150 mg/kg) increased feed intake in overall period. In a previous study, Seven et al. (2008) reported that the vitamin C supplementation (250 mg/kg of L- ascorbic acid/kg) into the diets increased feed intake. Another similar studies (Seven et al., 2012; Del Barrio et al., 2020) showed that the supplementation of vitamin C into drinking water (200 g/1.000 L) significantly increased feed intake and final body weight, while reduced FCR values. Abudabos et al. (2018) found that the vitamin C supplementation (100, 200 mg/kg feed) significantly improved feed intake and body weight gain. However, in the current experiment, the addition of vitamin C into laying hen diets did not affect FCR values. Cinar et al. (2014) and Niu et al. (2009) found that vitamin C (250 mg/kg diet), E (250 mg/kg diet) and C+E (250 mg/kg + 250 mg/kg diet) supplementations reduced feed conversion of laying hens. Another study (Torki et al., 2014) found that vitamin C supplementation (250 mg/kg) did not affect the feed conversion of laying hens.

4.5. Effect of L-ascorbic acid treatments on egg yield and quality traits

The addition of vitamin C didn't affect egg weight, HMM, eggshell quality traits in this experiment. Vitamin C is normally synthesized in kidney of chickens, however, when animals are exposed heat stress, its synthesis decrease and requirements increase (Butcher and Miles, 2002). Effects of vitamin C on laying performance and quality parameters depend on the level of heat stress degree. On the other hand, vitamin C has a role in calcification (Farquharson et al., 1998; Konca et al., 2008). Büyükkılıç Beyzi et al. (2020) reported that under heat stress conditions, egg quality traits were not affected by 250 mg/kg AA addition.

Seven et al. (2008) and Torki et al. (2014) reported that vitamin C supplementation did not affect egg mass of laying hens. El-Gendi et al. (1999) experimented vitamin C (ascorbic acid) supplementations (200, 300 and 400 g/ton) into poultry diets and reported that 300 and 400 mg/kg AA decreased egg production rate, however, increased egg weight and egg mass of laying hens. Skřivan et al. (2013) reported that the supplementation of Vitamin C (0 or 200 mg/kg) did not affect egg production and feed consumption. Çiftçi et al. (2005) reported that the addition of 200 mg/kg vitamin C into diet did not affect albumen height, HU or eggshell thickness.

Saki et al. (2010) reported that vitamin C supplementation of 250, 500 and 750 mg/kg and resulted in greater albumen index and HU and 500 mg resulted in a higher yolk height and shape index.

Vitamin C supplementation into laying hen diets didn't effect eggshell thickness in overall period. Asli et al. (2007) reported that the supplementation of 200 mg/kg vitamin C into basal diet had no significant effect on eggshell thickness, shell percentage and HU values.

Roche Scale Scores (RRS) were influenced by AA supplementation into laying hen diets. With AA supplementations, egg yolk brightness (L*) decreased in the 1^{st}, 4^{th}, 8^{th} and overall periods. Egg yolk redness (a*) also significantly increased in overall periods. Egg yolk yellowness (b*) was also significantly different among the treatments in the 1^{st} and 8^{th} periods. Based on the present study, differences in the age of the hens and the amount of vitamin C administered to the animals could be a reason for this. Çiftçi et al. (2005) reported that addition of vitamin C (200 mg) caused an increase in egg yolk color.

However, Büyükkılıç Beyzi et al. (2020) reported that AA addition (250 mg/kg) didn't change yolk color parameters of laying hens under heat stress.

4.6. Effect of AA on blood parameters, feather score and tonic immobility

In this experiment, blood parameters, heart rate and feather score weren't influenced by vitamin C supplementation into laying hen diets. The serum triglycerides, AST, cortisol, cholesterol, glucose, ALT, heterophil, lymphocytes, monocytes, eosinophils, basophils and H/L were not affected by the experimental treatments. Sahin et al. (2002) found that the addition of vitamin C and E did not affect blood glucose and cholesterol levels.

As a result, it was determined that the addition of vitamin C to the rations of laying hens reared in two different plans adversely affected the live weight, feed consumption and feed efficiency values. At the same time, it was determined that it increased the respiratory rate and negatively affected the feather score and increased the AST, cortisol and triglyceride values of the blood serum. It was determined that although the stocking density affects the egg production, weight and egg internal and external quality traits in some periods, it does not significantly affect the average values of all traits.

4.7. Conclusion and recommendations

Vitamin C added to the ration in order to eliminate the negativities caused by the SD did not affect the performance, egg quality, blood values, tonic immobility and feather score values. The SD and combinations of vitamin C did not significantly affect the measured parameters in general.

Present findings revealed that placing 3 animals per cage in the housing of laying hens raised in conventional cages will provide improvements in yield and animal welfare parameters and these findings support the European Union animal welfare practices.

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Master of Animal Science at Erciyes University/ Kayseri-Turkey 2020-2022.

Bachelor of Veterinary Science, Departments of Medicine and Husbandry Production at Benadir University/ Mogadishu-Somalia 2012-2016.

Diploma of Medical Laboratory Science at Juba University/Mogadishu-Somalia 2015-2017.

Diploma of Principle of Computer programs at Diba College institute/Baladweyne 2009-2010.

WORK EXPERIENCES AND TRAINING

Currently a member of the Central Regions Livestock Professional Association, Baladweyn\Somalia (CERELPA) since September 2017 as an animal treatment and livestock production development.

CERELPA is a Somali organization in the central regions Association for animal health and production formed by Somali veterinarians from July 2018 to August 2018.

Has attended Master Seminar Training held by Food and Agriculture Organization of the United Nations FAO Community Animal Health Workers in Mogadishu-Somalia July 2018 - August 2018.

Has attended Seminar Training program of crop improvement held by Somali agriculture technical group (SATG), from June 2015 to August 2015 at AFGOOYE\Lower Shabelle\Somalia

Worked as Laboratory technician in Saacid General Hospital at Mogadishu\Somalia from February 2017 to August 2017.

Attended Seminar for Human Development Program at Furkan Human Development Center, in Mogadishu\Somali from September 2015 to November 2015.

LANGUAGES

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