

## Journal of the Hellenic Veterinary Medical Society

Vol 75, No 1 (2024)



### Effects of outdoor plant varieties on performance, egg quality, behavior, and economic analysis of Turkey local chicken from 20 to 36 weeks of age

A Aygun, D Narinc, A Özköse, H Arisoy, R Acar, S Uzal, C Bulut

doi: [10.12681/jhvms.33984](https://doi.org/10.12681/jhvms.33984)

Copyright © 2024, A Aygun, D Narinc, A Özköse, H Arisoy, R Acar, S Uzal, C Bulut



This work is licensed under a [Creative Commons Attribution-NonCommercial 4.0](https://creativecommons.org/licenses/by-nc/4.0/).

### To cite this article:

Aygun, A., Narinc, D., Özköse, A., Arisoy, H., Acar, R., Uzal, S., & Bulut, C. (2024). Effects of outdoor plant varieties on performance, egg quality, behavior, and economic analysis of Turkey local chicken from 20 to 36 weeks of age. *Journal of the Hellenic Veterinary Medical Society*, 75(1), 7007–7018. <https://doi.org/10.12681/jhvms.33984>

## Effects of outdoor plant varieties on performance, egg quality, behavior, and economic analysis of Turkey local chicken from 20 to 36 weeks of age

A. Aygun<sup>1\*</sup>, D. Narinc<sup>2</sup>, A. Özköse<sup>3</sup>, H. Arisoy<sup>4</sup>, R. Acar<sup>3</sup>, S. Uzal<sup>5</sup>, C. Bulut<sup>1</sup>

<sup>1</sup> Department of Animal Science, Agriculture Faculty, Selçuk University, Konya, Turkey

<sup>2</sup> Department of Animal Science, Faculty of Agriculture, Akdeniz University, Antalya, Turkey

<sup>3</sup> Department of Field Crops, Agriculture Faculty, Selçuk University, Konya, Turkey

<sup>4</sup> Department of Agricultural Economics, Agriculture Faculty, Selçuk University, Konya, Turkey

<sup>5</sup> Department of Farm Structure and Irrigation, Agriculture Faculty, Selçuk University, Konya, Turkey

**ABSTRACT:** This study was carried out to examine the effects of three plant combinations (white clover-perennial ryegrass, bird's-foot trefoil-perennial ryegrass, and alfalfa-perennial ryegrass) planted in the outdoor area of the free-range system on the performance, egg quality, and behavioral characteristics of Turkey local chickens. Performance characteristics of chickens (egg production, feed consumption, feed conversion ratio, broken egg rate, dirty egg rate, and floor egg rate) were evaluated at 4-week intervals from 20 weeks to 36 weeks of age. In the study, the local layer genotype ATAK-S was used. Each group in the study consisted of three different plant varieties, formed from four replications. The mean values of hen-day egg production and hen-day cumulative egg yield of layers in the treatment groups were found to be between 91.2% and 94.8% and between 87.9 and 91.7%, respectively, and there was no statistical difference between the treatment groups. Similarly, there were no statistical differences between the treatment groups in terms of feed consumption, feed conversion ratio, egg weight, egg mass, broken-cracked egg ratio, or internal and external egg quality characteristics. There were no differences between the treatment groups in terms of time budgets allocated to various behavioral characteristics. In this study, outdoor area use was found to be quite high (41-41.6%) in all treatment groups. As a result, it was determined that the use of white clover-perennial ryegrass, bird's-foot trefoil-perennial ryegrass, and alfalfa-perennial ryegrass vegetation combinations in the free-range system encourages layers to go outdoors. In addition, it has been found that these practices have positive effects on birds in terms of both their performance and their well-being.

**Keywords:** Free-range; Egg production; Egg quality; Economic analysis; Poultry behavior; Alfalfa; White clover; Bird's-foot trefoil; Perennial ryegrass

*Corresponding Author:*

Ali Aygün, Department of Animal Science, Agriculture Faculty, Selçuk University, Konya, Turkey  
E-mail address: aaygun@selcuk.edu.tr

*Date of initial submission: 15-03-2023*  
*Date of acceptance: 15-09-2023*

## INTRODUCTION

For decades, certain segments of society have pushed for the abolition of traditional cage systems, claiming that birds raised in conventional cages are unable to exhibit natural behaviors. They have advocated for the use of alternative production systems that allow birds to exhibit their natural behavior while also protecting animal welfare. As an alternative to traditional cage production for laying hens, enriched cage, aviary, extensive indoor, and free-range systems are used (Appleby et al., 2002; Appleby 2003; Rudkin and Stewart 2003). Consumers believe that eggs produced in an alternative system are healthier, more natural, and tastier than conventional caged eggs (Tauson 2002).

It is estimated that around 70% of egg production in the world is done in traditional cages. In the USA, this rate approaches 90%. In particular, the European Union countries have started to produce eggs in alternative systems instead of the traditional cage system (Molnár and Szöllösi 2020). But most countries are in transition, and conventional production has not yet been completely abandoned. Egg production in traditional cages will be terminated in Turkey in 2023. However, the number of eggs produced in conventional systems is still 85%. As a result, comprehensive research is required to determine the best methods for commercial egg production (TEPA, 2021).

The high stocking density and mass production model in intensive poultry houses have greatly benefited the industry, but this model has also revealed many problems. Laying hens raised in conventional cages exhibit health anomalies such as osteoporosis, sudden death syndrome, cage fatigue, and leg problems. In addition, it is known that there are behavioral disorders and deterioration in welfare characteristics in birds (Lay Jr et al., 2011). It has been demonstrated in many studies that battery cages restrict the natural behaviors of birds and cause fear and stress (Dawkins, 1985; Dawkins and Hardie, 1989). Natural behaviors like foraging, exercise, sand bathing, perching, and feather cleaning are crucial for the physical and mental health of laying hens. Other behavioral disadvantages of conventional cages are the absence of nests and perches, and the inability of chickens to escape from other aggressive chickens (CIWF, 1999).

Scientific studies on alternative production models in terms of performance, welfare, and behavioral characteristics in poultry species have increased recently. However, most of these studies have been

done on meat production models, and the number of studies on laying hens is very small. Preisinger and Schmutz (2002) reported that the mortality rate (6.2-8.8%) of chickens raised in enriched cages was lower than that of those in conventional cages (8.7-10.6%), but egg production was lower as well. Appleby (2003) reported that the cost of eggs produced in enriched cages and free-range systems was 15% and 50% higher, respectively, than those produced in conventional cages. Shini (2003) found that the heterophile-lymphocyte ratios of chickens raised in free-range systems were lower than those raised in conventional systems, and these layers were exposed to less stress. Egg production, egg weight, and feed consumption of chickens raised in the free-range system were found to be lower, but with better use of feed conversion ratio, compared to the conventional systems, while their broken-cracked egg ratio was found to be higher (Mostert et al., 1995; Đukić-Stojčić et al., 2009; Petek et al., 2009). It has been found that chicken eggs reared in the free-range system have good qualities, especially in terms of albumen height, Haugh unit characteristics related to shelf life, and yolk color that directly affect consumer preference (Đukić-Stojčić et al., 2009; Petek et al., 2009). Şekeroğlu and Sarıca (2005) reared white and brown hybrid genotypes in conventional cage, enriched cage, and free-range systems, examining the differences in performance and welfare characteristics between rearing systems and genotypes. According to research, chickens reared in a free-range system had the best results in terms of feather score and tibia fracture resistance, but the worst averages in terms of dirty egg ratio, feed consumption, and foot inflammation.

In the free-range system, there is no obligation to have vegetation in the outdoor area. However, birds usually spend more time indoors or in front of the poultry house when there is no vegetation in the outdoor area, and they do not prefer to go out to the outdoor area. This situation can cause negative effects such as aggression and feather pecking among birds. Therefore, it is essential to designate areas with green plants in order to encourage these animals to use outdoor areas (Mahboub et al., 2004; Horsted et al., 2006; Aydın and Sözcü 2015). Mahboub et al., (2004) found that the frequency of feather problems caused by feather pecking was lower in chickens that spent more time in outdoor areas. Feed consumption costs can be reduced if some of the feed consumed by animals is obtained from the plant vegetation in outdoor areas (Horsted and Hermansen, 2007). Rob-

inson (1951) stated that because the outdoor area of the free-range system contains green plants, approximately 10% of the feed can come from vegetation. Birds not only consume plants, but also organic materials such as seeds, insects, and worms (de Almeida et al., 2012).

There are many studies on the use of plants in the outdoor areas of broilers, turkeys, and laying hens (Mohammed et al., 2013; Kheiralipour et al., 2017; Asaduzzaman 2019; Davoodi and Ehsani 2020; Ianni et al., 2021). In the current studies, only one plant or natural vegetation was used in the outdoor area, and no study was found on the determination of the effects of different plant varieties on the performance and behavioral characteristics of laying hens. The purpose of this study was to determine the effects of three plant combinations (clover-perennial ryegrass, bird's-foot-trefoil-perennial ryegrass, and alfalfa-perennial ryegrass) planted in the outdoor area of the free-range system on the performance, egg quality, and behavioral characteristics of chickens. Thus, the objective is to determine the suitability of different plant materials for egg production in small farms on rural areas.

## MATERIALS AND METHODS

### Animal material

This research was carried out in accordance with

the laws and regulations of the Ministry of Agriculture of the Republic of Turkey on animal management and welfare. In addition, the study fully complied with the SCAHAW (2003) recommendations for the free-range alternative layer rearing system. The study used 240 hens at the age of sixteen weeks of the ATA-K-S layer hybrid genotype developed in Turkey.

### Experimental details and animal husbandry

A total of 12 poultry houses with three different vegetation types were used in the study. These houses were planned as 3 forage plant mixtures and 4 replications in accordance with the factorial treatment design. The combinations of clover-perennial grass (Group A), bird's-foot trefoil-perennial grass (Group G), and alfalfa-perennial grass (Group Y) were used as plant material in the outdoor areas of free-range poultry houses. A total of 20 chickens were placed in each poultry house, with 7 chickens per square meter in the indoor area and 4 square meters of outdoor area for each chicken in the outdoor area. Feed and water were given ad libitum (Table 1). When the chickens were 16 weeks old, they were given 13 hours of light per day, which was gradually increased to 16 hours per day by adding 30 minutes each week. The multi-storey perches of 15 cm per hen were provided separately in the indoor and outdoor areas. Wood shavings were used as litter material in the indoor area for the chickens. A nest box measuring 140x40x40

**Table 1.** Nutritional compositions of the rations for the growth period (16-18 weeks), pre-layer period (18-21 weeks) and layer period (21-36 weeks) of layers.

Ingredients (%)	Growth	Pre-layer	Layer
Corn	58.64	56.92	56.42
Soybean meal (46%)	5.37	13.01	16.35
Sunflower meal (36%)	11.58	4.35	9.04
Wheat Bran	18.09	12.98	-
Gluten	3.35	6.00	5.65
Vegetable oil	-	0.77	1.75
Limestone	1.74	4.04	9.04
DCP	0.81	1.50	1.25
DL-Methionine	-	0.03	0.08
L-Lysine	0.08	-	-
Vit-Min. premix	0.05	0.05	0.05
Salt	0.29	0.35	0.37
Calculated Values			
Crude protein (%)	16.0	17.0	18.0
Metabolic Energy (Kcal/kg)	2700	2750	2800
Ca (%)	1.0	2.00	3.80
Available P (%)	0.36	0.45	0.40
Methionine (%)	0.35	0.38	0.46
Methionine+cystine (%)	0.64	0.67	0.77
Lysine (%)	0.72	0.77	0.84

cm was used in each poultry house. In order for the birds to go out easily during the daytime, the entrance doors were left open from 07:00 in the morning until 30 minutes before dark in the evening. Thus, the birds were provided with at least eight hours of daylight per day.

### Characteristics

From the beginning of the experiment, the eggs produced in the treatment groups were recorded daily, as were the hens' times to reach 5% and 50% yield. To determine the egg weight, all eggs in each poultry house were weighed for two consecutive days every two weeks. The number of broken eggs and floor eggs was determined daily. The Feed conversion ratio was calculated by determining feed consumption during four-week periods. The egg quality characteristics, such as specific gravity, eggshell strength, shell thickness, albumen height, Haugh unit, and yolk color, were measured daily in eggs produced on two consecutive days in the last week of each four-week period. The specific gravity of the egg was determined according to the Archimedes principle (Wells, 1968). Eggshell strength was determined with the Egg Force Reader device (Orka Food Tech. Ltd., China). The eggshell thickness was measured with a micrometer (0.001 precision; Mitutoya, Japan) using samples taken from the pointed, equator, and blunt ends of the shell and averaged. Egg albumen height was measured with the Egg Analyzer device (Orka Food Tech. Ltd., China). The Haugh unit was calculated using albumen height and egg weight values (Haugh, 1937). Egg yolk color was measured with a portable colorimeter device (Konica Minolta, CR-400, Japan).

In the study, focal sampling and scan sampling methods were used to determine the behavioral characteristics and outdoor area usage preferences of the chickens in the treatment groups (Lehner, 1992). In order to provide a source of behavioral sampling methods, the living areas of the chickens were recorded continuously for 24 hours during the experiment by using 24 cameras placed in the indoor and outdoor areas. The behavioral characteristics of 10 randomly selected chickens from each treatment group were monitored for 10 minutes, two days a week. During this period, recordings were made of feeding, drinking, lying, standing, walking, cleaning, pecking, scratching, mating, shaking, wing stretching, and jumping behaviors. In the focal sampling, the percentage of the relevant behavior in the total time was defined as the time budget, and the number of times the behavior

was recorded as the frequency. Instant images were obtained during scan sampling. In these images, the rates of chickens' use of indoor and outdoor areas, use of nests, use of perches, eating feed, drinking water, standing, and lying were determined.

### Economic analysis

For each treatment group, the gross production value, gross profit, and egg cost were calculated separately. In addition, all calculations were made for each forage plant combination. Relative profitability levels in each group were determined with the aid of gross profit. In the short term, gross profit is considered a measure of success in business activity branches (Erkuş et al., 1995).

### Statistical analysis

In the study, the variance analysis technique was used to compare the groups for continuous data (egg yield, egg internal-external quality characteristics, feed consumption, and feed conversion ratio) obtained from chickens in four replication poultry houses with three different forage plant mixtures. First of all, it was determined whether the collected continuous data met the parametric test assumptions, and for this, the normal distribution of the data and the homogeneity of variance were checked. The rank data transformation was applied to some data that did not show a normal distribution, and then hypothesis tests were carried out. When the group means were compared with the parametric method, a significant difference at the level of 0.05 was detected between the groups, and Duncan's multiple comparison test revealed from which group or groups the difference originated. The statistical analysis of discrete data with a binomial or poisson distribution, such as mortality, focused behavioral characteristics, or egg production, was done using the GLIMMIX procedure of the SAS program (Narinç et al., 2016). In this statistical test, the Tukey-Kramer multiple comparison test was used in order to determine from which group or groups the difference between the groups resulted in the rejection of the null hypothesis at a significance level of 0.05 (Zhu, 2014).

## RESULTS

### Body Weight and Viability

The mean body weights of hens in groups A, G, and Y at 16 weeks of age were 1015 g, 1003g, and 1014 g, respectively (at the beginning of the experiment). The average body weights at the end of the



experiment (36 weeks of age) were found to be 1812 g, 1833 g, and 1853 g, respectively. The relative body weight gains in the treatment groups were 78.4%, 82.9% and 82.8%, respectively. There were no statistical differences between the treatment groups in terms of both body weights and relative weight gains (all  $P>0.05$ , not presented in any table). The effect of different plant varieties on the viability of hens was found to be insignificant ( $P=0.296$ ). The mean values of viability were determined as 96.3% in the A group, 97.5% in the G group, and 100% in the Y group (not presented in any table).

### Egg Production Traits

It was determined that flocks reached 5% egg production at ages of 137, 141, and 138 days in groups A, G, and Y, respectively. The ages at which the flocks produced 50% the total number of eggs were determined to be 144 days in group A, 147 days in group G, and 148 days in group Y (all  $P>0.05$ , not shown in any table).

In terms of hen-day egg production, the differences between the treatment groups in all periods were statistically insignificant (all  $P>0.05$ , Table 2). Hen-day egg production in the last period was 94.8% in the A group, 93.5% in the G group, and 92.6% in the Y group. Hen-housed egg production in the last period was 91.3% in the A group, 91.2% in the G group, and 92.6 in the Y group, and the differences were not significant among treatment groups (Table 3).

The average values of the broken-cracked egg ratio in 4-week periods in the treatment groups and the statistical analysis results are given in Fig. 1. There was no statistically significant difference between the treatment groups in terms of broken-cracked egg averages in any period. The mean of broken-cracked eggs during the whole experiment varied between 1.78% and 4.91%. The percentages of floor eggs in 4-week periods in the treatment groups and the statistical analysis results are given in Fig. 2. There was no statistically significant difference between the treatment groups in terms of the average number of floor eggs in any period. The mean number of floor eggs during the whole experiment varied between 0.00% and 0.98%.

### Feed Intake and Feed Conversion Ratio

The hen-day feed consumption and feed conversion ratio averages of the chickens in the treatment groups and statistical analysis results are given in Tables 4 and 5, respectively. There was no difference between the treatment groups in any period in terms of both feed consumption and feed conversion ratio ( $P>0.05$ ).

### Egg weight

The mean values of egg weight in the treatment groups and the statistical analysis results of the chickens are given in Table 6. The differences between groups in egg weight averages were statistically insignificant in all periods ( $P>0.05$  for all). In the last

**Table 2.** The hen-day egg production and statistical analysis results of hens with different plant mixtures in the outdoor area (%)

Group	21-24 wk	25-28 wk	29-32 wk	33-36 wk
A	48.4	89.2	93.8	94.8
G	47.1	89.9	94.4	93.5
Y	41.7	90.1	90.5	92.6
SEM	7.02	1.28	1.05	0.95
<i>P</i> -value	0.775	0.875	0.064	0.287

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

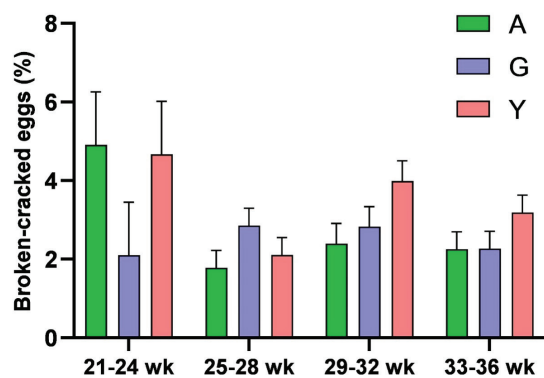
SEM: Standard error of mean

**Table 3.** The hen-housed egg production and statistical analysis results of hens with different plant mixtures in the outdoor area (%)

Group	21-24 wk	25-28 wk	29-32 wk	33-36 wk
A	48.4	88.1	91.1	91.3
G	47.1	89.1	92.6	91.2
Y	41.7	90.1	90.5	92.6
SEM	7.02	1.36	1.15	1.35
<i>P</i> -value	0.775	0.598	0.404	0.705

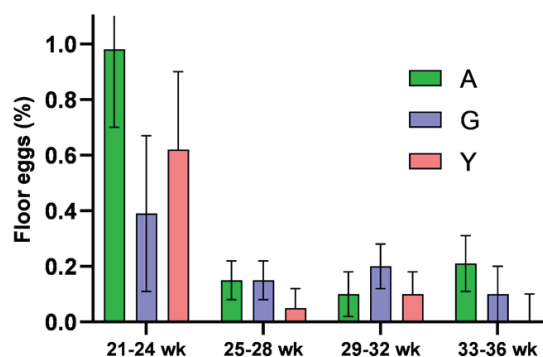
A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

SEM: Standard error of mean



**Fig. 1** The mean values of broken-cracked eggs in treatment groups (%)

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.



**Fig. 2** The mean values of floor eggs in treatment groups (%)

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

**Table 4.** The mean values of feed consumption of hens in different treatment groups, and statistical analysis results (g/hen/day)

Group	21-24 wk	25-28 wk	29-32 wk	33-36 wk
A	97	114	120	127
G	102	113	121	125
Y	99	110	119	123
SEM	1.35	2.48	1.85	1.68
<i>P</i> -value	0.207	0.598	0.761	0.341

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

SEM: Standard error of mean

**Table 5.** The mean values of feed conversion ratios of hens in different treatment groups, and statistical analysis results

Group	21-24 wk	25-28 wk	29-32 wk	33-36 wk
A	2.07	2.22	2.18	2.23
G	2.15	2.11	2.16	2.16
Y	2.04	2.04	2.08	2.10
SEM	0.041	0.063	0.053	0.042
<i>P</i> -value	0.256	0.297	0.323	0.134

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

SEM: Standard error of mean

**Table 6.** The mean values (g) of eggs weight in treatment groups, and statistical analysis results

Group	21-24 wk	25-28 wk	29-32 wk	33-36 wk
A	49.3	51.6	54.5	57.7
G	49.9	53.6	54.9	58.3
Y	51.5	52.8	56.2	58.3
SEM	0.633	0.771	0.591	0.482
<i>P</i> -value	0.111	0.237	0.207	0.603

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

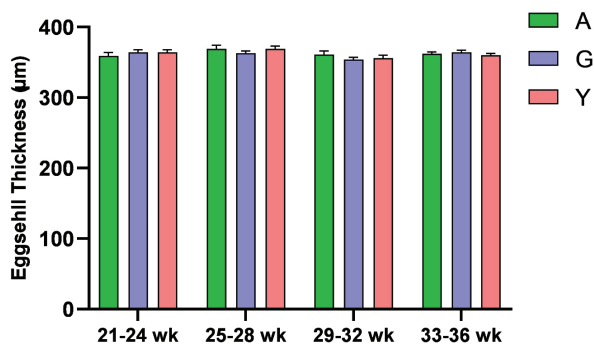
SEM: Standard error of mean

period, egg weights were determined to be 57.7 g, 58.3 g, and 58.3 g in groups A,G, and Y, respectively.

### Egg Quality Traits

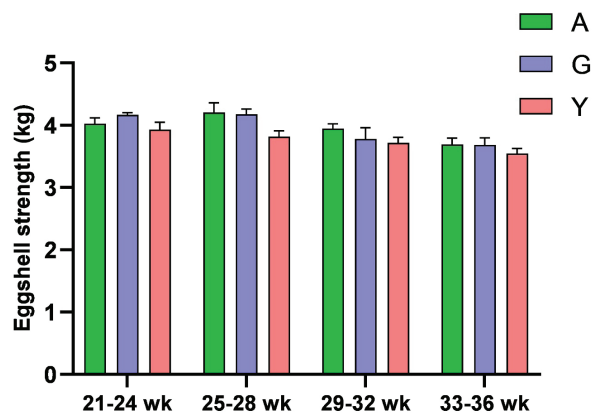
The mean values of eggshell thickness, eggshell

strength, and Haugh Unit in the treatment groups according to the periods and the statistical analysis results of the hens are given in Figs. 3, 4, and 5, respectively. In terms of eggshell thickness and eggshell strength, there were no differences between the



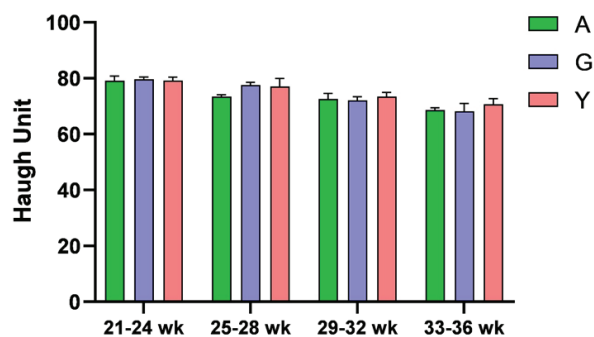
**Fig. 3** The mean values of eggshell thickness in treatment groups (µm)

A: Clover-Perennial grass, G: Bird’s-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.



**Fig. 4** The mean values of eggshell strength in treatment groups (kg)

A: Clover-Perennial grass, G: Bird’s-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.



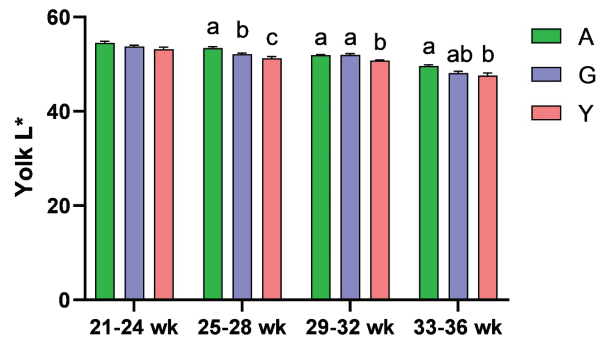
**Fig. 5** The mean values of Haugh unit in treatment groups

A: Clover-Perennial grass, G: Bird’s-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

averages found in the treatment groups in all periods ( $P>0.05$  for all). In the study, the average values of shell thickness of the eggs obtained from all groups varied between 354 µm and 369 µm, while the mean values of eggshell strength were found between 3.55

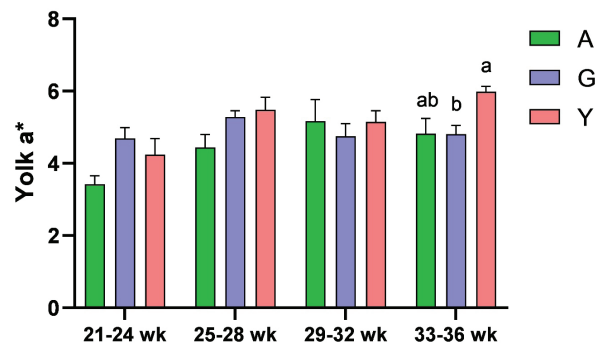
kg and 4.21 kg. In the last period of the study, Haugh Unit averages were determined as 68.6, 68.2, and 70.7 in the A, G, and Y groups, respectively.

The mean values of lightness ( $L^*$ ), redness ( $a^*$ ), and yellowness ( $b^*$ ) in egg yolk in the treatment groups according to the periods, and statistical analysis results are given in Figs. 6, 7, and 8, respective-



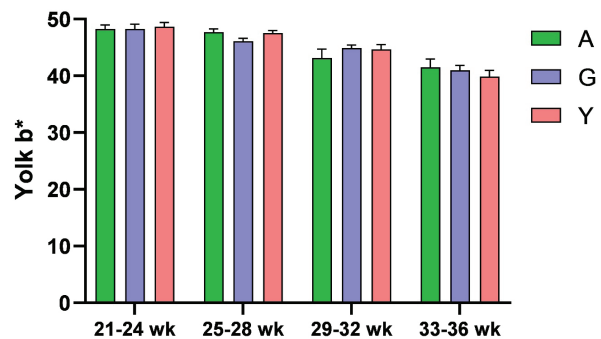
**Fig. 6** The mean values of yolk  $L^*$  in treatment groups

A: Clover-Perennial grass, G: Bird’s-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.



**Fig. 7** The mean values of yolk  $a^*$  in treatment groups

A: Clover-Perennial grass, G: Bird’s-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.



**Fig. 8** The mean values of yolk  $b^*$  in treatment groups

A: Clover-Perennial grass, G: Bird’s-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.



ly. In the study, no difference was observed between the treatment groups in terms of the mean value of yolk L\* only in the 21-24-week period, while the differences between the treatment groups in all other periods were found to be statistically significant. At 25-28 weeks of age, the highest yolk L\* value was found in the A group (53.4) and the lowest in the Y group (51.3). Similarly, the highest averages in terms of yolk L\* at 29-32 weeks and at 33-36 weeks of age were found in the eggs of group A. In terms of egg yolk a\* averages, higher values were found only at 33-36 weeks of age in the Y group ( $P < 0.05$ ). There were no differences between the treatment groups in all periods in terms of the b\* means representing yellowness in egg yolk ( $P > 0.05$  for all).

### Behavioral Traits

The time budget averages determined by the focal sampling method for chickens' feeding, walking, drinking, scratching, standing, shaking, cleaning, pecking, wing stretching, lying, and jumping traits are presented in Tables 7 and 8. There were no statistically significant differences between the treatment groups in terms of the time budgets of all behavioral traits ( $P > 0.05$  for all).

According to the treatment groups of hens in the indoor and outdoor areas, the scan sampling method was used to determine the mean values of area usage,

equipment usage, and certain behavioral characteristics (scan sampling results are not presented in a table). According to the scan sampling method, the outdoor area usage rates of chickens in groups A, G, and Y were found to be 41%, 41.6%, and 41%, respectively. In the same order, perch usage in the outdoor area was found to be 1.35%, 1.67%, and 3.02%, while these rates were determined to be 7.29%, 4.79%, and 5.42% in the indoor area. The rates determined in the A, G, and Y treatment groups for the use of nests located only in the closed area were 7.19%, 6.98%, and 5.42%, respectively. There were no statistical differences between the treatment groups in terms of the averages determined for indoor and outdoor use, nest, and perch use ( $P > 0.05$  for all).

### Economic analysis

The chickens in the study were raised in three different forage planted areas, and a total of 12 poultry houses were used. Gross production value, gross profit, and egg costs were calculated for each poultry house separately, and egg costs were determined for each feed plant mixture. Gross production value was calculated as 224 dollars in group A, 225 dollars in group G, and 228 dollars in group Y, and the differences between groups were found to be statistically insignificant ( $P > 0.05$ , not presented in any table). Gross profit was calculated as 82.5 dollars in group A, 80.2 dollars in group G, and 82.7 dollars in group

**Table 7.** The mean values of time budgets of some behavioral characteristics of chickens in treatment groups determined by focal sampling method

Group	Feeding	Drinking	Lying	Standing	Walking
A	30.9	6.67	15.6	68.6	2.71
G	24.5	5.94	25.7	68.2	2.57
Y	21.9	14.5	27.9	70.7	2.42
SEM	3.41	3.28	8.55	1.84	0.25
<i>P</i> -value	0.180	0.125	0.385	0.656	0.705

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

SEM: Standard error of mean

**Table 8.** The mean values of time budgets of some behavioral characteristics of chickens in treatment groups determined by focal sampling method

Group	Cleaning	Pecking	Stretching	Scratching	Jumping
A	6.48	4.67	0.00	68.6	1.67
G	8.11	2.20	0.67	68.2	0.75
Y	13.3	2.36	0.67	70.7	0.78
SEM	3.29	0.712	0.001	1.84	0.122
<i>P</i> -value	0.424	0.061	0.087	0.656	0.084

A: Clover-Perennial grass, G: Bird's-foot trefoil-Perennial grass, Y: Alfalfa-Perennial grass.

SEM: Standard error of mean

Y, and the differences between groups were found to be statistically insignificant ( $P>0.05$ , not presented in any table). Egg unit cost was calculated as 10.7 cents per egg in group A, 10.9 cents per egg in group G, and 10.9 cents per egg in group Y, and the differences between groups were found to be statistically insignificant ( $P>0.05$ , not presented in any table).

## DISCUSSION

In the study, the differences in egg production between the treatment groups in all periods were found to be insignificant. The egg yields obtained in this study are consistent with the results obtained in previous studies in the free-range system (Campbell et al., 2017; Küçükyılmaz et al., 2012; Tutkun et al., 2018; Türker and Alkan 2018). The 50% yield age determined in the study was found to be lower than the 50% yield age obtained by Türker et al., (2017) and Sekeroglu et al., (2010) in the studies where they used the ATAK-S genotype.

In the study, the differences between the groups in terms of broken-cracked egg ratio were found to be statistically insignificant. The rates of broken-cracked eggs were determined to be between 1.78% and 4.91% in all periods. De Reu et al., (2009) found the rate of broken eggs to be 4.10% in non-cage systems. Küçükyılmaz et al., (2012) found the rate of broken-cracked eggs to be 0.310% in their study using the ATAK-S genotype in the organic system. Mugnai et al., (2009) reported that the rate of broken-cracked eggs in the organic system was 2.0%. Tutkun et al., (2018) found that the rate of broken-cracked eggs of the ATAK-S genotype in the free-range system was 0.890%.

In the study, the differences between the groups in terms of feed consumption were found to be statistically insignificant. The amount of feed consumed per determined animal varied between 123 and 127 g. Sözcü et al., (2021) determined the feed consumption of the ATAK-S genotype in a free-range system to be 117 g/day.

Similarly, Tutkun et al., (2018) found hen-day feed consumption to be 124 g in their study, where they used the ATAK-S genotype in the free-range system. Differences between groups in terms of feed conversion ratio were found to be statistically insignificant. Feed conversion ratios obtained in the treatment groups were between 2.10 and 2.23. The study's average feed conversion ratio was lower than the 2.43

reported by Küçükyılmaz et al., (2012), the 3.4 reported by Mugnai et al., (2009), the 2.42 reported by Tutkun et al., (2018), the 1.71 reported by Sharma et al., (2022), the 2.08 reported by Kop-Bozbay et al., (2021), and the 2.54 reported by Sözcü et al., (2021). The main factors limiting the evaluation of forage plants in the outdoor area are low palatability, high fiber content, low energy, and high moisture content. In addition, the presence of non-nutritive substances (tannins, saponins, mimosin, trypsin inhibitors, hemoglobin, phytate, and hydrogen cyanide) may limit the use of these forages (Ndelekwute et al., 2018).

The differences in egg weight between the groups were found to be statistically insignificant in the experiment. Egg weight is an important criterion for consumers. Egg prices in Turkey are determined according to egg weight classes. According to the Turkish Food Codex, eggs weighing less than 53 g are classified as small, and eggs weighing between 53 and 63 g are classified as middle-class. Accordingly, while the G group reached the medium size class in the second period, the A and Y groups reached the medium egg weight in the third period. The egg weight value found in our study was comparable to the average egg weight (55.4 g) reported by Mugnai et al., (2009) in the organic system but less than the averages (61.6 g and 63.2 g, respectively) found by Sharma et al., (2022) and Kop-Bozbay et al., (2021) in the free-range system.

The differences between the groups in terms of eggshell strength were statistically insignificant. The eggshell's strength is important for minimizing losses during egg laying, collection, storage, and transportation. In this study, the eggshell strength varied between 3.55 kg and 4.21 kg. These findings were found to be compatible with the eggshell strengths (3.62 kg to 4.36 kg) determined by Samiullah et al., (2014) in the free-range system. On the other hand, Campbell et al., (2017) found the eggshell strength to be between 51.4 N and 53.1 N in the free-range system. Additionally, Kop-Bozbay et al., (2021) found a slightly lower value (3,23 kg) than our study.

Eggshell thickness is affected by several factors, including hen age, egg size, stress, strain, prevalence of disease, and nutrition (Al-Batshan et al., 1994; Aygun 2017; Sözcü et al., 2021). Due to a variety of factors, eggshell thickness varies between 300 and 400  $\mu\text{m}$  in chickens. The thickness of the shell acts as a significant barrier, preventing bacteria from passing through (Chen et al., 2019). The shell thickness value

(354-369 m) obtained in our study is comparable to the shell thickness value (371  $\mu\text{m}$ ) reported by Sözcü et al., (2021) for the ATAK-S hybrid grown in a free-range system.

The Haugh unit value obtained in the study ranged from 68 to 79. The Haugh unit is one of the important internal egg quality characteristics calculated based on egg weight and albumen height. A high Haugh unit means good egg quality. Samiullah et al., (2014) stated that the Haugh unit value of eggs produced in the free-range system varies between 63.3 and 96.5. On the other hand, Petek et al., (2009) and Golden et al., (2012) found the Haugh unit values to be 83.4 in the free-range system, which were higher than the values obtained in our study.

$L^*$ ,  $a^*$ , and  $b^*$  values were used to determine egg yolk color. As the egg yolk  $L^*$  value approaches 100, its color becomes brighter, and as it approaches 0, it becomes darker (McGuire, 1992). According to the results of the study, it can be said that the yellow color of the Y group eggs is less bright than the other groups in all periods except at 21-24 weeks of age. A positive egg yolk  $a^*$  value indicates an increase in redness, and a negative value indicates an increase in greenness (McGuire, 1992). Narinç et al., (2015) stated in their study on the Roche yolk color scale and  $a^*$  value that the  $a^*$  value increases as the yolk color gets darker. Therefore, it can be said that egg yolk darkness was higher in the Y group (5.98) and the G group (4.81) at 33-36 weeks of age, but there was no statistical difference with in the A group (4.82). The carotenoids in a chicken's diet primarily determine the color of the yolk. However, differences in the amount of carotenoids in feed and their bioavailability from different sources can significantly affect egg yolk (Zurak et al., 2022). Egg yolk color can also be determined according to the Roche scale. In this method, it is detected visually according to the color spectrum from 1 to 15. Several of the researchers who used the Roche scale value to determine the yolk color in the free-range system, Sözcü et al., (2021) reported a value of 12.4, Mugnai et al., (2009) reported a value of 7.40, Campbell et al., (2017) reported a range of 11.7-12.3, Iqbal et al., (2018) reported a value of 7.0, and Kop-Bozbay et al., (2021) reported a value of 11.8.

A free-range system with a well-designed outdoor area has great potential to improve poultry welfare as it provides additional outdoor and open-air as well as opportunities to meet behavioral needs such as perching, foraging, and dust bathing (Lay Jr et al.,

2011; Weeks and Nicol, 2006). In the relevant literature, there is no study investigating the effects of different vegetation types in the outdoor area on the behavioral characteristics of laying hens raised in the free-range system. In a study conducted by Gebhardt-Henrich et al., (2014) using 12 different genotypes of laying hens on 8 farms, it was determined that the average usage of outdoor areas by chickens was 15.7% using the scan sampling method. Gilani et al., (2014) who carried out a similar study in 33 flocks on 28 farms, reported that this rate was 13%. Based on the results of many studies, Pettersson et al., (2016) reported that the percentage of outdoor area usage by hens reared for egg production in free-range systems rarely exceeds 50% of the flock and sometimes decreases to 10%. In this study, outdoor usage was found to be between 41.1% and 41.6% in all treatment groups. It is thought that the reasons for the high outdoor preferences of the birds in the study were that the free-range system was well implemented and that all three vegetation types applied motivated the birds to go to the outdoor area.

Eggs are an important food source that meets the protein needs of people. The easy availability and cheapness of eggs are of great importance in terms of feeding middle- and low-income families. There is no study in the literature on the calculation of egg production costs in the free-range system. In addition, there are studies on the cost of eggs produced in the conventional system. Gültekin (2019) found the average egg cost of some enterprises engaged in egg production activities in Turkey to be 2.94 cents per unit. A similar study was conducted by Karasioglu et al., (2021) for enterprises engaged in egg and poultry farming. In the study, the cost of eggs in 2018 was calculated at 2.67 cents per egg. According to the annual sector reports prepared by the Egg Producers Central Union, the egg cost in Turkey in 2017 was 6.37 cents per egg, and the egg cost in 2018 was 6.23 cents per egg (TEPA, 2021).

## CONCLUSIONS

In the free-range system, it was observed that different plant varieties in the outdoor area did not differ in terms of egg performance, egg quality, or egg cost. For this reason, it would be appropriate to use the plant variety that can be economically grown in the region. In future studies, it may be suggested to determine the plant utilization rate in the outdoor area of hens.

**ACKNOWLEDGEMENTS**

The Turkish Scientific and Technological Research Council (TÜBİTAK) provided funding for this study (grant number: TOVAG-1170792).

**CONFLICT OF INTEREST**

The authors declare that they have no conflicts of interest.

**REFERENCES**

- Al-Batshan H, Scheideler S, Black B, Garlich J, Anderson K (1994) Duodenal calcium uptake, femur ash, and eggshell quality decline with age and increase following molt. *Poult Sci* 73: 1590-1596. <https://doi.org/10.3382/ps.0731590>
- Appleby M, Walker A, Nicol C, Lindberg A, Freire R, Hughes B, Elson H (2002) Development of furnished cages for laying hens. *Br Poult Sci* 43: 489-500. <https://doi.org/10.1080/0007166022000004390>
- Appleby MC (2003) The European Union ban on conventional cages for laying hens: History and prospects. *J Appl Anim Welf Sci* 6: 103-121. <https://doi.org/10.1207/S15327604JAWS060203>
- Asaduzzaman M (2019) Effect of napier grass on economic turkey production in Bangladesh. *Indian Journal of Science and Technology* 12 (24): 1-10. <https://doi.org/10.17485/ijst/2019/v12i24/144894>
- Aydın İ, Sözcü A (2015) Management Practices and Welfare Standards of Alternative Poultry Production Systems. *J Agr Faculty Uludag Univ* 29: 133-146.
- Aygun A (2017): The Eggshell Microbial Activity. In: *Egg Innovation and Strategies for Improvement*. Hester P (Editor), Academic Press, Elsevier, Oxford, United Kingdom: pp 135-144.
- Campbell D, Lee C, Hinch G, Roberts J (2017) Egg production and egg quality in free-range laying hens housed at different outdoor stocking densities. *Poult Sci* 96: 3128-3137. <https://doi.org/10.3382/ps/pex107>
- Chen X, Li X, Guo Y, Li W, Song J, Xu G, Yang N, Zheng J (2019) Impact of cuticle quality and eggshell thickness on egg antibacterial efficiency. *Poult Sci* 98: 940-948. <https://doi.org/10.3382/ps/pey369>
- CIWF (Compassion in World Farming) (1999) Europe agrees ban on battery cages: Huge victory for animal welfare. *AgsScene*, 135, 10.
- Davoodi P, Ehsani A (2020) Characteristics of carcass traits and meat quality of broiler chickens reared under conventional and free-range systems. *J World Poult Res* 10 (4): 623-630. <https://dx.doi.org/10.36380/jwpr.2020.71>
- Dawkins MS (1985) Cage height preference and use in battery-kept hens. *Vet Record* 116: 345-347. <https://doi.org/10.1136/vr.116.13.345>
- Dawkins MS, Hardie S (1989) Space needs of laying hens. *Br Poult Sci* 30: 413-416. <https://doi.org/10.1080/00071668908417163>
- De Almeida GF, Horsted K, Thamsborg SM, Kyvsgaard NC, Ferreira JF, Hermansen JE (2012) Use of *Artemisia annua* as a natural coccidiostat in free-range broilers and its effects on infection dynamics and performance. *Vet Parasitol* 186: 178-187. <https://doi.org/10.1016/j.vetpar.2011.11.058>
- De Reu K, Rodenburg T, Grijspeerdt K, Messens W, Heyndrickx M, Tuytens F, Sonck B, Zoons J, Herman L (2009) Bacteriological contamination, dirt, and cracks of eggshells in furnished cages and noncage systems for laying hens: An international on-farm comparison. *Poult Sci* 88: 2442-2448. <https://doi.org/10.3382/ps.2009-00097>
- Dukić-Stojić M, Perić L, Bjedov S, Milošević N (2009) The quality of table eggs produced in different housing systems. *Biotechnol Anim Husb* 25: 1103-1108.
- Erkuş A, Bülbül M, Kırıl T, Açıl AF, Demirci R (1995) *Tarım Ekonomisi*. Ankara Üniversitesi Ziraat Fakültesi Eğitim, Araştırma ve Geliştirme Vakfı Yayınları, 5. Ankara Üniversitesi Ziraat Fakültesi, Ankara, 298 pp
- Gebhardt-Henrich SG, Toscano MJ, Fröhlich EK (2014) Use of outdoor ranges by laying hens in different sized flocks. *Appl Anim Behav Sci* 155: 74-81. <https://doi.org/10.1016/j.applanim.2014.03.010>
- Gilani A-M, Knowles T, Nicol C (2014) Factors affecting ranging behaviour in young and adult laying hens. *Br Poult Sci* 55: 127-135.
- Golden J, Arbona D, Anderson K (2012) A comparative examination of rearing parameters and layer production performance for brown egg-type pullets grown for either free-range or cage production. *J Appl Poultry Res* 21: 95-102. <https://doi.org/10.3382/japr.2011-00370>
- Gültekin N (2019) Yumurta Üretim Faaliyetinde Bulunan İşletmelerin Tarımsal Faaliyetler (TMS 41) Standardına Göre Muhasebeleştirilmesi. *Ekonomi, İşletme ve Maliye Araştırmaları Dergisi* 1: 246-265.
- Haugh RR (1937) The Haugh unit for measuring egg quality. *The US Egg Poultry Magazine* 43: 522-573.
- Horsted K, Hammershøj M, Hermansen JE (2006) Short-term effects on productivity and egg quality in nutrient-restricted versus non-restricted organic layers with access to different forage crops. *Acta Agric Scand Section A* 56: 42-54. <https://doi.org/10.1080/09064700600866072>
- Horsted K, Hermansen J (2007) Whole wheat versus mixed layer diet as supplementary feed to layers foraging a sequence of different forage crops. *Animal* 1: 575-585. <https://doi.org/10.1017/S175173110769418X>
- Ianni A, Bartolini D, Bennato F, Martino G (2021) Egg Quality from Nera Atriana, a Local Poultry Breed of the Abruzzo Region (Italy), and ISA Brown Hens Reared under Free Range Conditions. *Animals* 11: 257. <https://doi.org/10.3390/ani11020257>
- Iqbal Z, Roberts J, Perez-Maldonado R, Goodarzi Borojani F, Swick RA, Ruhnke I (2018) Pasture, multi-enzymes, benzoic acid and essential oils positively influence performance, intestinal organ weight and egg quality in free-range laying hens. *Br Poult Sci* 59: 180-189. <https://doi.org/10.1080/00071668.2017.1403566>
- Karasioglu F, Göktürk İE, Çetintaş ME (2021) Accounting Procedures in the Framework of Tas 41 in Egg Poultry Enterprises. *Electron J Soc Sci* 20: 590-613. <https://doi.org/10.17755/esosder.724709>
- Kheiralipour K, Payandeh Z, Khoshnevisan B (2017) Evaluation of environmental impacts in turkey production system in Iran. *Iran J Appl Anim Sci* 7 (3): 507-512.
- Kop-Bozbay C, Akdag A, Bozkurt-Kiraz A, Gore M, Kurt O, Ocak N (2021) Laying Performance, Egg Quality Characteristics, and Egg Yolk Fatty Acids Profile in Layer Hens Housed with Free Access to Chicory-and/or White Clover-Vegetated or Non-Vegetated Areas. *Animals* 11: 1708. <https://doi.org/10.3390/ani11061708>
- Küçükylmaz K, Bozkurt M, Herken EN, Çınar M, Çatlı AU, Bintaş E, Çöven F (2012) Effects of Rearing Systems on Performance, Egg Characteristics and Immune Response in Two Layer Hen Genotype. *Asian-Australas J Anim Sci* 25: 559-568. <https://doi.org/10.5713/ajas.2011.11382>
- Lay Jr D, Fulton R, Hester P, Karcher D, Kjaer J, Mench JA, Mullens B, Newberry RC, Nicol CJ, O'Sullivan NP (2011) Hen welfare in different housing systems. *Poult Sci* 90: 278-294. <https://doi.org/10.3382/ps.2010-00962>
- Lehner PN (1992) Sampling methods in behavior research. *Poult Sci* 71: 643-649. <https://doi.org/10.3382/ps.0710643>
- Mahboub H, Müller J, Von Borell E (2004) Outdoor use, tonic immobility, heterophil/lymphocyte ratio and feather condition in free-range laying hens of different genotype. *Br Poult Sci* 45: 738-744. <https://doi.org/10.1080/00071660400014267>
- McGuire RG (1992) Reporting of objective color measurements. *Hort Science* 27: 1254-1255.
- Mohammed KA, Sarmiento-Franco L, Santos-Ricalde R, Solorio-Sanchez JF (2013) Egg production, egg quality and crop content of Rhode Island Red hens grazing on natural tropical vegetation. *Trop Anim Health Prod* 45: 367-372. <https://doi.org/10.1007/s11250-012-0225-y>



- Molnár S, Szöllösi L (2020) Sustainability and quality aspects of different table egg production systems: A literature review. *Sustainability* 12: 7884. <https://doi.org/10.3390/su12197884>
- Mostert BE, Van der Walt J, Bowes E (1995) Influence of different housing systems on the performance of hens of four laying strains. *S Afr J Anim Sci* 25: 80-86.
- Mugnai C, Dal Bosco A, Castellini C (2009) Effect of rearing system and season on the performance and egg characteristics of Ancona laying hens. *Ital J Anim Sci* 8: 175-188. <https://doi.org/10.4081/ijas.2009.175>
- Narınç D, Aygün A, Küçükönder H, Aksoy T, Gürcan EK (2015) An application of bootstrap technique in animal science: Egg yolk color sample. *Kafkas Univ Vet Fak* 21: 631-637. <https://doi.org/10.9775/kvfd.2014.12693>
- Narınç D, Erdoğan S, Tahtabiçen E, Aksoy T (2016) Effects of thermal manipulations during embryogenesis of broiler chickens on developmental stability, hatchability and chick quality. *Animal* 10: 1328-1335. <https://doi.org/10.1017/S1751731116000276>
- Ndelekute E, Enyenihi G, Akpan I (2018) Potentials and challenges of utilizing forage resources for chicken production. *J Anim Sci Livest Prod* 2: 4. <https://doi.org/10.21767/2577-0594.100014>
- Petek M, Alpay F, Gezen SS, Cibik R (2009) Effects of Housing System and Age on Early Stage Egg Production and Quality in Commercial Laying Hens. *Kafkas Univ Vet Fak* 15: 57-62. <http://doi.org/10.9775/kvfd.2008.65-A>
- Pettersson I, Freire R, Nicol CJ (2016) Factors affecting ranging behaviour in commercial free-range hens. *World PoultSci J* 72: 137-150. <https://doi.org/10.1017/S0043933915002664>
- Preisinger AL, Schmutz UN (2002) Commercial layer breeding with special focus on alternative housing. In: *Proceedings of 11<sup>th</sup> European Poultry Conference*, Bremen, Germany.
- Robinson L (1951) *Modern poultry husbandry*. 2<sup>nd</sup> ed, Crosby Lockwood and Son Ltd., London: pp 550.
- Rudkin C, Stewart GD (2003) *Behaviour of hens in cages: a pilot study using video tapes*. Rural Industries Research and Development Corporation, Queensland, Australia.
- Samiullah S, Roberts J, Chousalkar K (2014) Effect of production system and flock age on egg quality and total bacterial load in commercial laying hens. *J Appl Poultry Res* 23: 59-70. <https://doi.org/10.3382/japr.2013-00805>
- SCAHAW (2003) Report of the Scientific Committee on Animal Health and Animal Welfare Adopted 24-25<sup>th</sup> April 2003.
- Sekeroglu A, Sarica M, Demir E, Ulutas Z, Tilki M, Saatci M, Omed H (2010) Effects of different housing systems on some performance traits and egg qualities of laying hens. *J Anim Vet Adv* 9:1739-1744.
- Sharma MK, McDaniel CD, Kiess AS, Loar II RE, Adhikari P (2022) Effect of housing environment and hen strain on egg production and egg quality as well as cloacal and eggshell microbiology in laying hens. *Poult Sci* 101: 101595. <https://doi.org/10.1016/j.psj.2021.101595>
- Shini S (2003) Physiological responses of laying hens to the alternative housing systems. *Int J Poult Sci* 2: 357-360.
- Sözcü A, İpek A, Oguz Z, Gunnarsson S, Riber AB (2021) Comparison of performance, egg quality, and yolk fatty acid profile in two Turkish genotypes (Atak-S and Atabey) in a free-range system. *Animals* 11: 1458. <https://doi.org/10.3390/ani11051458>
- Şekeroğlu A, Sarıca M (2005) The effects of free range system on egg productions and egg quality of brown and white layer genotypes. *J Poult Res* 6: 10-16.
- Tauson R (2002) Furnished cages and aviaries: production and health. *World Poult Sci J* 58: 49-63. <https://doi.org/10.1079/WPS20020007>
- TEPA (Turkish Egg Producers Association) (2021). <https://www.yum-bir.org/yumurta/FiyatListele.aspx?Aranan=1.1.2018>. [Assessed 20 December 2021].
- Tutkun M, Denli M, Demirel R (2018) Productivity and egg quality of two commercial layer hybrids kept in free-range system. *Turkish JAF Sci* 6: 1444-1447. <https://doi.org/10.24925/turjaf.v6i10.1444-1447.2070>
- Türker İ, Alkan S, Akçay S (2017) Comparison of Domestic and Foreign Commercial Brown Layer Hens in Terms of Yield Characteristics in FreeRange Raising System. *Turkish JAF Sci* 5: 814-821. <https://doi.org/10.24925/turjaf.v5i7.814-821.1216>
- Türker İ, Alkan S (2018) Comparison of Hens Reared in Free-Range and Deep-Litter Systems in Terms of Certain Production Characteristics. *J Agric Sci* 24: 179-184. <https://doi.org/10.15832/ankutbd.446434>
- Weeks C, Nicol C (2006) Behavioural needs, priorities and preferences of laying hens. *Worlds PoultSci J* 62: 296-307. <https://doi.org/10.1079/WPS200598>
- Wells R (1968): A study of the hen's egg. In: *British Egg Marketing Board Symposium*, Edinburgh: pp 207-249
- Zhu M (2014) Analyzing multilevel models with the GLIMMIX procedure. SAS Institute Inc. SAS026-2014.
- Zurak D, Slovenec P, Janječić Z, Bedeković X, D, Pintar J, Kljak K (2022) Overview on recent findings of nutritional and non-nutritional factors affecting egg yolk pigmentation. *World Poult Sci J* 78: 531-560. <https://doi.org/10.1080/00439339.2022.2046447>