

Effects of Rooster Presence in Free-Range Systems on Egg Performance, Egg Quality and Fear Response

Ali AYGÜN^{1,*} , Doğan NARİNCİ² 

¹ Selcuk University, Faculty of Agriculture, Department of Animal Science, 42250, Konya, Turkey

² Akdeniz University, Agriculture Faculty, Department of Animal Science, Antalya, Turkey

Article History

Received: Oct 08, 2024
Accepted: Oct 22, 2024
First Online: Nov 30, 2024

*Corresponding Author

Tel: +90 505 547 08 21
E-mail: aaygun@selcuk.edu.tr

Keywords

Free-range system
Rooster
Performance
Egg quality
Tonic immobility

Abstract

The aim of this study was to investigate the effects of having roosters on egg production in free-range system on egg production, egg quality and fear level. In the study, 2 groups (without and with rooster) and each group consisted of 4 replications. The first group will be kept with roosters together with hens and the other group will be kept only with hens. In the research, 15 hens will be kept in each subgroup and 1 rooster will be kept in each replicate in the group with rooster. Egg production, broken-cracked egg ratio, egg weight, feed intake, feed efficiency and livability were determined as egg performance characteristics. Shell strength, Haugh unit and shell thickness were determined as egg quality. Tonic immobility was determined as fear level. The presence of roosters in the free-range flock did not significantly affect egg production, the ratio of broken-cracked eggs, egg weight, feed intake, feed efficiency, livability, eggshell strength, Haugh unit, eggshell thickness, or tonic immobility ($P>0.05$). Consequently, the presence or absence of roosters in a free-range system did not have a significant effect on performance, egg quality, or tonic immobility.

Introduction

The free-range system is an alternative egg production system where chickens can exhibit their natural behaviors (perching, scratching, dust bathing, etc). However, in natural life, chickens form a family with roosters. Red jungle fowl, the ancestors of modern egg hybrids, are highly social animals, forming a family in which roosters fertilize females and protect them from predators (McBride *et al.*, 1969; Odén *et al.*, 2005). Chickens, especially in free-range systems, face many dangers, especially from predators such as foxes, martens, eagles and hawks. Chickens have various behaviors to protect themselves from these predators.

Depending on the type of predator, they may remain motionless, run to hide in a closed area, or escape by jumping to higher levels. While the chickens are doing their behavioral characteristics (foraging, eating food, dust bathing, etc.) in the outdoor area, the roosters protect them against predators that may come from the environment and warn them of danger (Johnson 1963; Sullivan 1991).

Keeping roosters in a free-range system is not preferred due to increased feed consumption. However, in recent years, as a result of the increase in demand for natural food, it is stated that keeping roosters in flocks

has a more improving role in the behavioral characteristics of chickens (Pereira *et al.*, 2017). According to our literature research, there are limited studies on keeping roosters in chicken flocks (Odén *et al.*, 2005; Pereira *et al.*, 2017).

In these studies, mostly behavioral characteristics were examined, there is a lack of literature on the effect of flocks with roosters on egg production, egg quality and tonic immobility. In this study, the effect of the presence of roosters in the flock in a free-range system on egg production, egg quality, and tonic immobility was investigated.

Materials and Methods

This study was carried out at Selcuk University, Faculty of Agriculture, Department of Animal Science (Turkey). In the study, 120 Lohmann sandy layer genotypes and 4 Lohmann Sandy roosters, 13 weeks old, were used. The study was conducted from 13 weeks of age to 43 weeks of age. In the study, there were 2 groups (without and with roosters), and each group consisted of 4 replications. The first group was kept with roosters together with hens, and the other group was kept only with hens. Each subgroup in the research contained 15 hens, and each replicate included 1 rooster.

The hens were reared in a free-range system. The stocking density in the in-door area is 6 hens /m², while the out-door area provides 4 m² per hen. Water and feed are given as ad-libitum. There are clover plants in the outdoor area. The hens were given developer (2700 Kcal kg/ME, 16% CP, 1% Ca and 0.36% available phosphorus) between 13-18 weeks, pre-layer (2750 Kcal kg/ME, 17.50% CP, 2% Ca and 0.45% available phosphorus) between 18-21 weeks and layer (2720 Kcal kg/ME, 17.60% CP, 3.90% Ca and 0.39% available phosphorus) until the end of the experiment. On the day the animals were placed in the housing, 24 hours of lighting was applied on the first day to get them used to the environment; 10 hours of lighting was applied on the following days and the daily lighting period was increased by 30 minutes per week until it reached 16 hours. The photoperiod application of 16 hours of lighting and 8 hours of darkness was continued until the end of the experiment. At least eight hours a day are provided for animals for use in the outdoor area.

The weights of all animals were measured using a scale with a precision of 1 g at the start and end of the trial, on a subgroup basis. Egg production was recorded daily throughout the experiment and egg production (hen-day, %) was calculated for 4-week periods. The number of broken-cracked eggs was recorded daily and the rate of broken-cracked eggs was calculated over 28-day periods. At the end of every four weeks, all eggs produced in subgroups were weighed on a digital scale with 1 g sensitivity on 2 consecutive days and the averages were calculated for 4-week periods. Feed consumption was determined by weighing the feed

consumed in 4-week (28-day) periods using a digital scale with 1 g sensitivity. Feed efficiency was calculated in 4-week periods according to 4-week feed consumption and average egg weight. Mortality was recorded during the trial and livability was calculated from these data.

Tonic immobility was measured to determine the level of fear. Tonic immobility, which is an indicator of welfare, is used to measure and evaluate the level of stress caused by fear in birds (Gallup 1979; Jones 1986). At the end of the experiment, tonic immobility was detected in 8 animals from each group, 2 randomly from each subgroup. In the tonic immobility test, the animal is laid on its back or on its right side in a cradle-like device with its head down, held lightly by the chest for 10 seconds and then released (Elrom 2001). The observer records the time by standing approximately 1 m away from the chicken. It is assumed that tonic immobility is achieved in chickens that do not get up from the cradle within 10 seconds of the animal being released, and the time is recorded until the animal gets up from the cradle. If the animal gets up within 10 seconds and this number is repeated 3 times, the tonic immobility score is recorded as "0". The test period is limited to a maximum of 10 minutes, and in animals that do not stand up at the end of this period, the tonic immobility period is accepted as 600 seconds. The evaluation of the test is based on the time the animal remains immobile. Animals with a longer tonic immobility period are considered more fearful and passive than other animals (Jones and Faure 1980; Zulkifli *et al.*, 2000 a; Mahboub *et al.*, 2004).

For egg quality analyses, egg quality analyses were performed on 5 eggs (10 eggs/subgroup) randomly taken from eggs produced on 2 consecutive days at 50% egg production efficiency, during the peak production period (85-90% chicken-day) and at the end of the trial. Eggs collected daily were stored at room temperature for one day and then analyzed the next day. Eggshell strength (kg) was measured with an ERTEST device (Ankara, Turkey). The height of the albumen was measured using a height gauge. The Haugh unit was calculated using the following formula:

$$\text{Haugh unit} = 100 \times \log(H + 7.57 - 1.7W^{0.37})$$

where H is the albumen height (mm) and W is the egg weight (g) (Haugh 1937). For eggshell thickness, three parts of the egg (pointed, medium and blunt) were measured with a digital micrometer with a sensitivity of 0.001 mm and the average was taken.

Statistical analysis

One-way analysis of variance (ANOVA) was used in the analysis of data. The multiple comparison test Tukey test was used in comparisons between groups. All hypothesis tests will be performed at a significance level of 0.05 and the Minitab 16 package program will be used for statistical analysis.

Results and Discussion

Egg Production

Egg production (hen-day, %) determined throughout the trial for the application with and without roosters are given in Table 1.

During the trial, the effect of the application with and without roosters on hen-day egg production was insignificant ($P>0.05$). Between the twenty and forty-third weeks, the hen-day production was determined as 77.3% in the roosterless group and 78.8% in the rooster group, and the differences between the groups were insignificant ($P>0.05$). This result is inconsistent with the study of Pereira *et al.*, (2017) indicating that egg production obtained in the group with roosters was higher than the group without roosters. This may be due to factors such as Pereira *et al.*, (2017) using a different genotype, the study period being shorter than our study and the different rearing system. Indeed, one of the most important characteristics affecting egg production is genotype (Şekeroğlu and Sarıca 2005; Yetişir and Sarıca 2018; Ketta *et al.*, 2020). Similarly, rearing systems also significantly affect egg production (Baykalır and Şimşek 2014; Dikmen *et al.*, 2016; Dedousi *et al.*, 2020). Baldinger and Bussemas (2021) determined the 16-72 week egg production of the Lohmann Sandy genotype in the organic system as 95%. According to the catalog values of the Lohmann Sandy genotype, the egg production of a 20-43 week old hen is given as approximately 89.2 (Anonymous 2021). Factors affecting egg production include age at sexual maturity, body weight at sexual maturity, genotype, breeding system, feeding, lighting and diseases (Hocking *et al.*, 2003; Englmaierová *et al.*, 2014; Yetişir and Sarıca 2018). According to the results of our research, the presence of a rooster did not have a positive or negative effect on egg production.

Broken-cracked egg ratio (%)

Table 2 presents the broken-cracked egg ratio (%) for rearing with and without roosters throughout the trial. During the trial, the effect of rearing with and without roosters on the hen-day broken-cracked egg rate was insignificant ($P>0.05$). Between twenty and forty-third weeks, the hen-day broken-cracked egg ratio was 3.45% in the group without rooster and 3.74% in the group with rooster and the differences between the groups were insignificant ($P>0.05$).

There is no literature on the effect of rearing with or without rooster on cracked-cracked egg ratio. Feeding, temperature and stress are the main factors affecting the broken-cracked egg ratio (Koelkebeck *et al.*, 1992; Akşit and Özdemir 2002; Konca and Yazgan 2002; Dagher 2008). Küçükyılmaz *et al.*, (2012) determined the rate of broken-cracked eggs as 0.44% in white layer genotypes in organic system. In studies

conducted using different genotypes in free-range systems, the rate of broken-cracked eggs was determined between 0.31% and 4.91% (De Reu *et al.*, 2009; Mugnai *et al.*, 2009; Küçükyılmaz *et al.*, 2012; Tutkun *et al.*, 2018; Aygun *et al.*, 2024). The fracture-fracture rate obtained from our study (3.45%-3.74%) was found to be between the values obtained from these studies. According to Ketta and Tůmová (2016), 8-10% of eggs produced in egg-producing enterprises are cracked, causing financial losses.

Egg weight (g)

Table 3 presents the egg weight (g) for rearing with and without roosters throughout the trial. The effect of treatment with and without rooster on egg weight was significant only at 32-35th hf and 40-43rd weeks. In both periods, the weight of the eggs obtained from the group without rooster was lower than the weight of the eggs obtained from the group with rooster ($P<0.05$). When all periods were examined (24-43 weeks), egg weight was determined as (62.7 g) in the group without roosters and (61.8 g) in the group with roosters, and the differences between the groups were statistically insignificant ($P>0.05$).

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 under 53 g are classified as small, and eggs between 53-63 g are classified as medium (Anonymous 2014). Accordingly, it is seen that the eggs obtained in both groups of our study are medium egg weight. The most important factors affecting egg weight are genotype, flock age, body weight and the amount of methionine in the diet (Koelkebeck *et al.*, 1992; Hocking *et al.*, 2003; Baumgartner *et al.*, 2007; Wolc *et al.*, 2012).

According to the catalog values of the Lohmann Sandy genotype, the egg weight between 20-43 weeks of age was determined as approximately 58.72 g (Anonymous 2021). Alkan (2023) determined the egg weight as 58.84 g in his study with the Lohmann Sandy genotype in the free-range system. Baldinger and Bussemas (2021) determined the egg weight as 65 g in the 16-72 week period of the Lohmann Sandy genotype in the organic system. Kop-Bozbay (2024) determined the egg weight as 60 g in their study with the Lohmann Sandy genotype in the free-range system. Akyol and Denli (2023) determined the average egg weight as 59.9 g in their study with the Lohmann Sandy genotype in the free-range system.

Feed consumption

Table 4 presents the feed consumption (g/hen/day) for rearing with and without roosters throughout the trial. During the trial, the effect of rearing with and without roosters on the feed consumption was insignificant ($P>0.05$).

Table 1. Average daily egg production of hens with and without roosters (%) and statistical analysis results

Treatment	20-23 wk	24-27 wk	28-31 wk	32-35 wk	36-39 wk	40-43 wk	20-43 wk
Without Rooster	27.2	81.3	88.4	89.6	86.2 ^b	91.2	77.3
With Rooster	23.1	83.2	87.9	92.5	93.6 ^a	92.8	78.8
SEM	4.70	3.41	2.80	1.80	1.85	1.95	1.85
P-value	0.554	0.708	0.924	0.308	0.009	0.593	0.582

SEM: Standard of error mean.

Table 2. Average broken-cracked egg ratio of hens with and without roosters (%) and statistical analysis results

Treatment	20-23 wk	24-27 wk	28-31 wk	32-35 wk	36-39 wk	40-43 wk	20-43 wk
Without Rooster	7.22	2.45	2.25	2.51	2.99	3.29	3.45
With Rooster	8.61	3.57	3.44	4.69	1.32	0.82	3.74
SEM	2.25	0.95	1.51	1.81	0.75	1.16	0.95
P-value	0.679	0.448	0.601	0.429	0.172	0.184	0.834

SEM: Standard of error mean

Table 3. Average egg weight of hens with and without roosters (%) and statistical analysis results

Treatment	20-23 wk	24-27 wk	28-31 wk	32-35 wk	36-39 wk	40-43 wk	24-43 wk
Without Rooster	-	56.2	60.5	66.2 ^a	64.1	66.5 ^a	62.7
With Rooster	-	57.5	60.4	64.0 ^b	63.0	64.4 ^b	61.8
SEM	-	0.91	0.38	0.55	0.49	0.47	0.43
P-value	-	0.332	0.898	0.030	0.184	0.018	0.228

SEM: Standard error mean,

^{a,b}The difference between groups with different letters in the same column is significant (P<0.05)**Table 4.** Average egg weight of hens with and without roosters (%) and statistical analysis results

Treatment	20-23 wk	24-27 wk	28-31 wk	32-35 wk	36-39 wk	40-43 wk	20-43 wk
Without Rooster	77.9	105.0	112.3	107.9	107.4	112.8	103.8
With Rooster	78.8	106.9	113.2	107.4	107.6	109.7	104.1
SEM	2.70	2.85	1.35	2.02	2.80	3.30	1.14
P-value	0.815	0.590	0.654	0.854	0.963	0.534	0.889

SEM: Standard error mean

When all periods were examined (20-43 weeks), feed consumption was determined as (103.8 g/chicken/day) in the group without roosters and (104.1 g/chicken/day) in the group with roosters, and the differences between the groups were statistically insignificant ($P>0.05$).

Feed consumption in poultry can be affected by the energy level of the feed, feeding time, feed form, age, genotype, rearing system and environmental conditions (temperature, stress, lighting, stocking density) (McDonald 1978; Küçükyılmaz *et al.*, 2012; Classen 2017; Kahraman *et al.*, 2020). Akyol and Denli (2023) determined the average feed consumption as 123.3 g/day in their study with the Lohmann Sandy genotype in the free-range system. Baldinger and Bussemas (2021) determined the average feed consumption of the Lohmann Sandy genotype in the organic system for 16-72 weeks as 143 g. The feed consumption amounts obtained in our study (103.8 g and 104.1 g) were lower than the values obtained from the studies conducted with Lohmann Sandy.

Feed efficiency

Table 5 presents the feed efficiency (g yum/g feed) for rearing with and without roosters throughout the trial. During the trial, the effect of rearing with and without roosters on the feed efficiency was insignificant ($P>0.05$). When all periods were examined (20-43 weeks), the feed efficiency was determined as (1.74) in the group without roosters and (1.76) in the group with roosters, and the differences between the groups were statistically insignificant ($P>0.05$). Feed efficiency is one of the most important performance characteristics affecting egg cost. It is expressed as the amount of feed consumed per unit product. Akyol and Denli (2023) determined the average feed efficiency as 2.14 in their study with the Lohmann Sandy genotype in the free-range system.

Baldinger and Bussemas (2021) determined the 16-72 week feed efficiency of the Lohmann Sandy genotype in the organic system as 2.35. The feed efficiency value obtained from our study (1.74 and 1.76) was found to be better than the values obtained from the studies conducted with Lohmann Sandy.

Livability

The livability was determined as (96%) in the group without roosters and (89%) in the group with roosters, and the differences between the groups were statistically insignificant ($P>0.05$; data not shown). Pereira *et al.*, (2017) in their study conducted with the Isa Brown genotype and the application with and without roosters, they determined the survival rate as 99.75% in the rooster group and 99.49% in the without rooster group, and stated that the differences between the groups were statistically significant.

Body weight gain

Table 6 presents the body weight gain (g) for rearing with and without roosters throughout the trial. At the beginning of the experiment (13 weeks) body weight was determined as 878 g in the group without roosters and 871 g in the group with roosters and the difference between the groups was statistically insignificant ($P>0.05$). At the end of the experiment (43 weeks) body weight was determined as 1712 g in the group without roosters and 1713 g in the group with roosters and the difference between the groups was statistically insignificant ($P>0.05$). Body weight gain (g) was determined as 834 g in the group without roosters and 832 g in the group with roosters and the difference between the groups was statistically insignificant ($P>0.05$).

Table 5. Average feed efficiency of hens with and without roosters (%) and statistical analysis results

Treatment	20-23 hf	24-27 hf	28-31 hf	32-35 hf	36-39 hf	40-43 hf	20-43 hf
Without Rooster	-	1.87	1.86	1.62	1.67	1.69	1.74
With Rooster	-	1.86	1.87	1.68	1.70	1.70	1.76
SEM	-	0.05	0.02	0.03	0.04	0.06	0.02
P-value	-	0.898	0.599	0.175	0.654	0.905	0.519

SEM: Standard error mean

Table 6. Initial body weight, end of trial body weight and body weight gain of hens with and without roosters (%) and statistical analysis results

Treatment	Initial body weight (g)	End of trial body weight (g)	Body weight gain (g)
Without Rooster	878	1712	834
With Rooster	871	1713	842
SEM	4.74	32.2	32.7
P-value	0.353	0.987	0.877

SEM: Standard error mean

The catalogue body weight value of the Lohmann Sandy genotype grown in alternative systems was given as 1088 g at week 13 (Anonymous 2021). Alimbaev and Ermatov (2022) determined the body weight of the Lohmann Sandy genotype as 951 g at 12 weeks of age. The body weight values obtained from our study (878 g and 871 g) are lower than the catalogue values of the Lohmann Sandy genotype and the value obtained from the study conducted by Alimbaev and Ermatov (2022). This difference may be due to differences in factors such as environmental factors applied during the rearing period, chick weight, and feeding. Chick weight, genotype, feeding, stocking density, lighting, rearing system affect the body weight at the end of the rearing period (Deaton *et al.*, 1979; Hussein *et al.*, 1996; Widowski *et al.*, 2013; Jensen 2019; von Eugen *et al.*, 2019; Işık 2023; Abraham *et al.*, 2024).

Tonic immobilite

Figure 1 presents the tonic immobility (sn) for rearing with and without roosters at the end of the trial. Tonic immobility value was 193 s in the group without rooster and 382 s in the group with rooster and the differences between the groups were statistically insignificant ($P>0.05$).

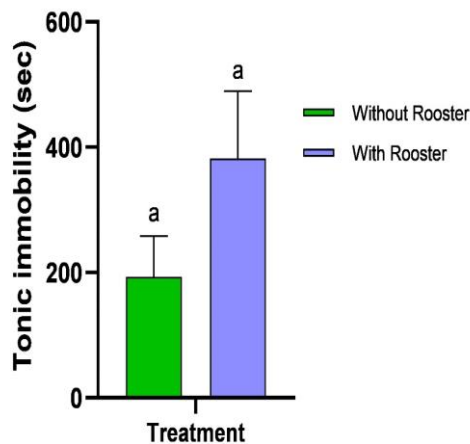


Figure 1. Effect of rearing with and without roosters on tonic immobility (sec)

Odén *et al.*, (2005) conducted a study with the Lohmann LSL Lite genotype in a free-range system, and determined the tonic immobility value as 332 seconds in the group without roosters and 200 seconds in the group with roosters, and the difference between the groups was statistically significant ($P<0.05$). Animals with a long tonic immobility period are considered more fearful and passive than other animals (Jones and Faure 1981). Tonic immobility is affected by factors such as breeding system, genotype, lighting, age, addition of some substances to the feed, and stocking density (Brake *et al.*, 1994; Zulkifli *et al.*, 2000b; Onbaşlılar *et al.*, 2007; Amer *et al.*, 2018; Anderson *et al.*, 2021; Sayin *et al.*, 2022; Işık 2023).

Eggshell strength (kg)

Eggshell strength was 5.355 kg in the group without roosters and 5.400 kg in the group with roosters, and the difference between the groups was statistically insignificant ($P> 0.05$; Figure 2).

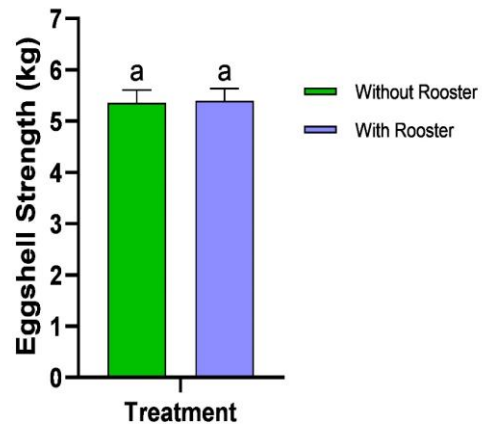


Figure 2. Effect of rearing with and without roosters on eggshell strength

Eggshell strength has an important effect on the collection, transportation and storage processes of eggs. According to the catalog data of the Lohmann Sandy genotype, the eggshell strength was stated to be higher than 4.079 kg, but an average value was not given (Anonymous, 2021). The eggshell strength values obtained from our study (5.355 and 5.400 kg) are better than the catalog data.

Haugh unit

The egg Haugh unit was 97.80 in the group without roosters and 94.73 in the group with roosters, and the difference between the groups was statistically insignificant ($P> 0.05$; Figure 3).

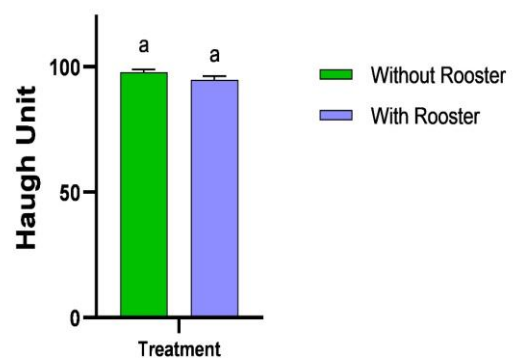


Figure 3. Effect of rearing with and without roosters on Haugh unit

The Haugh unit is an important internal quality trait developed by a scientist named Haugh in 1937 and calculated by egg weight and egg albumen height. The higher the egg Haugh unit, the better the egg quality and the longer the egg can maintain its shelf life during storage. Akyol and Denli (2023) determined the egg Haugh unit as 84.7 in their study with the Lohmann Sandy genotype in the free-range system. Kop-Bozbay (2024) determined the egg Haugh unit as 92.43 in their study with the Lohmann Sandy genotype in the free-range system. Alkan (2023) determined the egg Haugh unit as 92.90 in his study with the Lohmann Sandy genotype in the free-range system.

Eggshell thickness (mm)

Eggshell thickness was 0.396 mm in the group without roosters and 0.406 mm in the group with roosters, and the difference between the groups was statistically insignificant ($P > 0.05$; Figure 4).

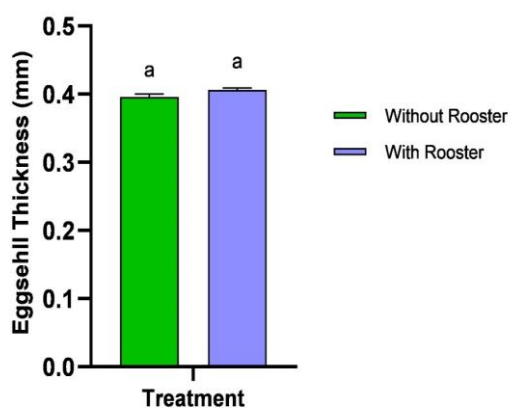


Figure 4. Effect of rearing with and without roosters on eggshell thickness

Alkan (2023) determined the egg shell thickness as 0.400 mm in his study with the Lohmann Sandy genotype in the free-range system. Akyol and Denli (2023) determined the egg shell thickness as 0.360 mm in his study with the Lohmann Sandy genotype in the free-range system. Kop-Bozbay (2024) determined the egg shell thickness as 0.427 mm in his study with the Lohmann Sandy genotype in the free-range system.

Conclusion

Rearing hens with roosters in a free-range system had no positive or negative effects on egg production, egg quality and tonic immobility.

Therefore, raising chickens with roosters as in natural life will reinforce people's idea of natural eggs. Further studies may be recommended to support our results. It may also be recommended to determine the ideal rooster ratio according to the flock size.

Acknowledgement

This study was supported by Selcuk University BAP with project number 22401015.

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Determination of the General Structure of the Enterprises Producing Eggs in Organic System in Ordu Province

Kardelen GÜÇCÜK¹ , Sezai ALKAN^{2, *} 

¹Directorate of Agriculture and Forestry, Ordu, Turkey

²Ordu University, Faculty of Agriculture, Department of Animal Science, Ordu, Turkey

Article History

Received: Apr 24, 2024

Accepted: Jun 10, 2024

First Online: Nov 30, 2024

*Corresponding Author

Tel: +90 505 7372480

E-mail: sezaialkan61@gmail.com

Keywords

Survey

Egg production

Organic System

Ordu province

Abstract

In this study, it was aimed to determine general structure of the enterprises engaged in organic system egg production in Ordu province. For this purpose, the data obtained from face-to-face surveys with 47 breeders who produce eggs in organic system in Ordu province were used in this study. In the study, 68.1% of organic system poultry breeders are older than 41 years and 46.8% of all breeders are farmers by occupation. It was determined that 83% of the egg production enterprises were established in the under-hazelnut land and 55.3% of them used sandwich panels in poultry house construction. 85.1% of the enterprises provided ventilation in the poultry house with a fan, 68.1% used individual nest boxes, 93.6% used nipple type drinkers and 83% preferred paddy husk as litter material. The number of hens was 750 or more in 68.1% of the enterprises and Lohman Brown breed hens were used in 97.9% of the enterprises. 51.1% of the enterprises had 6 or more hens per square meter (m²) in the roaming area and 48.9% had 4 hens per square meter (m²) in the indoor area. At the same time egg yield was higher than 81% in 70.2% of the enterprises.

Introduction

Most consumers prefer eggs produced through alternative production systems that apply food safety regulations since animal rights started to be considered important in egg production systems (Anderson, 2009). There are various alternative production systems available such as free range, organic, enriched cage, aviary, and deep-litter systems (Şekeroğlu et al. 2010; Türker and Alkan, 2018). Among these, organic system egg production has gained more importance especially in recent years. In developed countries, there has been a demand in organic system poultry breeding by the consumers, because of welfare concerns associated with farming of poultry under intensive conditions. For the “best positive welfare outcome”, hens should be free from hunger, thirst, discomfort, pain, injury,

disease, fear, and distress and able to express normal behaviors (Brambell, 1965).

Ordu province is located between 40°-41° North Parallels and 37°-38° East Meridians. The geographical structure of Ordu province is hilly and almost all the agricultural lands are hazelnut gardens. While producers provide their income from hazelnut in certain periods of the year, they remain inactive in the remaining months. In addition to hazelnut, the development and implementation of other alternative production activities in Ordu province is very important in preventing migration from the village to the city. In this regard, producers can combine hazelnut cultivation and organic system egg poultry farming in their lands and benefit from the synergistic effect of these two forms of

production and provide additional income. With this production combination, chickens graze in hazelnut gardens and save approximately 5-10% in terms of feed consumption, while the weeds in the gardens are cleaned by chickens without additional labor costs (Anonymous, 2014; Derebaşı and Alkan, 2018).

Organic agriculture is a form of production based on soil fertility and food safety without the use of artificial inputs such as chemical fertilizers and pesticides, which is sustainable, does not harm the environment and human health and at the same time supports, registered, controlled, and most importantly certified from production to consumption. Organic agriculture is a system developed and developing with principles such as protecting future generations, ensuring the continuity of water existence, minimizing chemical pollution, preventing soil loss, making more profit from the products produced, contributing to economic development, increasing biodiversity, and bringing rich-flavored natural products to consumers. Unlike conventional agriculture, organic agriculture combats pests and diseases without the use of synthetic pesticides, herbicides, chemical fertilizers, growth hormones, antibiotics, and modified genes.

Breeders who prefer organic farming support the reduction of pollution while protecting the existing ecological system (Öztürk and Türkoğlu, 2012; Bardakçı, 2021). Organic laying hen breeding differs from the poultry breeding referred to as village hens (natural hens) in that hens can roam freely in the area with organic soil surrounded by certain boundaries and fed with organic feed (Balık, 2016). Although there are some similarities between free-range poultry breeding and organic system poultry breeding, the compulsory certification process in organic system poultry breeding causes it to be less preferred (Balık, 2016). For the organic system egg poultry producers to market their products organically, they must first make a mutual contract with any certification body authorized by the Ministry of Agriculture and Forestry. In the process following the contract, the area, and animals to be farmed organically are taken into the transition process and organic activity is officially started. In organic farms, land and grasses are taken into transition period for 2 years and poultry animals to be used in egg production are taken into transition period for 6 weeks (Öztürk and Türkoğlu, 2012).

Hen breeds to be selected for use in the organic system should be selected from breeds that can adapt to the conditions of the environment where the poultry house is located and are relatively more resistant to diseases. The breeders to be selected should be selected from breeds whose genetic structure has not been modified, completely fed with organic feed, and especially adapted to the region where they will be raised (İpek and Sözcü, 2015). The hens needed for organic egg production should be obtained from enterprises with proven organic production or from breeding enterprises that do not pose salmonella risk for

organic production. The enterprise to be established for organic system egg production can be realized from conventional enterprises, provided that the hens are not older than a certain week, as well as from organic enterprises (Öztürk and Türkoğlu, 2012).

In organic system laying hens breeding, the hen houses should be planned in such a way that the hens can go out of the hen house whenever they want, easily access food, and water, and perform some physical and physiological behaviors comfortably (such as self-cleaning, scratching, stretching, flapping wings) and thus minimize stress. The roaming area should be sheltered with vegetation, enough feeders should be provided, and 16 hours of lighting time should be applied to the hens (İpek and Sözcü, 2015; Öztürk and Türkoğlu, 2012; Durmuş and Alkan, 2015; Sözcü and Yılmaz, 2014).

The indoor part of the hen house should be of sufficient size to protect the hens from diseases, clean, provide adequate air flow and protect them from dust. The floor of the poultry houses should be covered with materials such as straw, sawdust, sand, or short grass and more than 1/2 of it should be designed to be suitable for collecting feces. Entry-exit holes at least 4 m in length and 30-35 cm in height should be planned for every 100 m² of the poultry house floor area so that chickens can easily enter and exit the poultry house.

The poultry houses should be positioned in a way that is favorable for natural ventilation and light entry. A perch area of 18-20 cm per hen and a nesting box for 6-7 hens or 120 cm² nesting box area per hen should be provided (Öztürk and Türkoğlu, 2012; Durmuş and Alkan, 2015; İpek and Sözcü, 2015; Sözcü and Yılmaz, 2014). In this study, it was aimed to determine the general structure of the enterprises engaged in organic system egg production in Ordu province.

Material and Method

Material

In this study, face-to-face interviews were used as data collection method and questionnaire forms prepared to measure research variables were used as data collection tool. After the questionnaire was explained to the owners of the enterprises to which the questionnaires were to be applied, it was ensured that the participants answered the questions in a healthy way. In this study, 9 of the questions in the questionnaires are about demographic characteristics, 29 of them are about poultry enterprises, 11 of them are about health and nutrition of hens, 11 of them are about sales and marketing of eggs, 10 of them are egg producers' association, 3 of them are about Covid-19 epidemic, and a total of 75 questions were used in the survey.

Method

Sample of the Study

The sample of the study consisted of 47 enterprises that produce eggs in organic system in Ordu province.

Statistical Analysis

First, frequency values (n and %) were calculated by frequency analysis of the answers given by the participants to all the questions in the survey. SPSS (2008) statistical package program was used in the calculations.

Findings and Discussion

Demographic Characteristics of Producers

The basic findings (n and %) on the individual characteristics of the breeders practicing laying hens in the organic system and the socio-demographic and economic characteristics of the families are given in Table 1. In study, 68.1% of the enterprise owners are older than 41 years and 46.8% of all breeders are farmers by occupation. At the same time, 55.3% of the breeders have less than 4 people in their households. It was determined that 2.1% of the breeders included in the study were illiterate, 42.6% were primary and secondary school graduates, 38.3% were high school graduates and 17% were university graduates.

While 74.5% of the enterprise owners stated that they did not participate in any training or course on poultry, 85.7% of those who did not participate stated that they would like to participate in a training or course on hen breeding. It was also determined that 61.7% of the breeders' social security is the Social Security Institution. At the same time, 85.1% of the breeders stated that they own the ownership of their enterprises, while 61.7% stated that they do not use agricultural loans. Çimrin *et al.* (2019), in their study on egg poultry enterprises in Hatay province, stated that approximately 40% of the producers were between 36-50 years old and 35% were over 51 years old, and Cönk (2006) stated that 42.6% of the producers were between 36-50 years old and 50% were 51 years old and over. In the same study, 55.6% of the breeders stated that they were engaged in breeding as an occupation and 63% stated that the number of households was less than 4 people. It was determined that 1.9% of the breeders included in the study were illiterate, 35.2% were primary and secondary school graduates, 46.3% were high school graduates and 16.7% were university graduates. In the study conducted by Sarıca *et al.* (2020) average age of the producers was found to be 53. It was also determined that 50.7% of the enterprise owners were primary school graduates, 33.6% were middle school graduates, 13.6% were high school graduates, and 5.8% were university graduates. Aydın and Çelen (2011) in Gaziantep, Diyarbakır, Şanlıurfa, Batman, Adıyaman, Kilis and Mardin provinces, it was found that all poultry enterprise owners in Batman province were primary school graduates, whereas all poultry enterprise owners in Gaziantep province were university graduates. Köse and Durmuş (2014) reported that 31.5% of the producers were primary school graduates, 58% were secondary and high school graduates, and 10.5% were

university graduates in their study conducted in poultry enterprises in Ordu province. While 74.1% of the breeders stated that they did not participate in training or courses related to poultry, 70% of those who reported that they did not participate in training stated that they wanted to participate. In the study, it was determined that 38.9% of the breeders were affiliated to the Social Security Institution in terms of social security. While 81.5% of the breeders stated that the ownership of the enterprise belonged to them, 63% of them stated that they had never used agricultural credit.

Main Findings Regarding the Enterprises Producing Eggs in Organic System

The main findings (n and %) related to the general characteristics of laying hen enterprises in organic system are given in Table 2. In this study, 83% of the breeders stated that their enterprises were established under hazelnut land and 17% in open land. At the same time, it was determined that 55.3% of the breeders preferred to use sandwich panels in poultry house construction, while 44.7% of them used concrete material. While 85.1% of the organic system poultry breeders use fans to provide ventilation in the poultry house, 12.8% use chimney and window, and 2.1% use only window. Keeping the temperature and relative humidity at optimum levels in poultry houses, removal of harmful gases released by animals and animal welfare are directly related to the ventilation capacity and quality of the poultry house (Akkaya and İlgüzar, 2006).

While 68.1% of the breeders in the study stated that they used individual nesting boxes and the remaining 31.9% used group nesting boxes in their poultry houses, 93.6% of the breeders stated that they gave water to the hens with nipple type drinkers, 2.1% with hanging round type drinkers, and the remaining 4.2% with trough type and other type drinkers. As litter material, 83% of the breeders stated that they used paddy husk, 12,8% thick sawdust, 2,1% fine sawdust, and the remaining 2,1% other materials. Also, 78,7% of the breeders stated that they applied 16 hours of lighting to the hens, 12,8% 12 hours, and 8,5% daylight. In this study, 91,5% of the breeders stated that hens can find green grass in the grazing area throughout the year. Again, 68.1% of the breeders stated that the number of hens in their holdings was 750 and above, 25.6% stated that it was between 250-750, and 6.4% stated that it was 250 and below. Lohman Brown and Atak'S genotypes were used in 97.9% and 2.1% of the enterprises, respectively. In this study, it was determined that Lohman Brown breed hens were mostly (97.9%) used in organic system poultry enterprises in Ordu province.

Similarly, in the study conducted by Köse and Durmuş (2014) in Ordu province, it was reported that Lohman Brown and Hy-Line Brown breeds of hens were used in most of the poultry enterprises, and in the study conducted by Çimrin *et al.* (2019) in Hatay province, it was

reported that Atak-S and Lohman Brown and Nick Brown breeds of hens were raised in 52.18% and 47.82% of the enterprises, respectively. In this study, 83% of the breeders stated that they purchased their hens at the age of 16-18 weeks, 10.6% at the age older than 18 weeks, and 6.4% at the age younger than 14 weeks. Again, 87.2% of the breeders stated that they used their hens in production for 71 weeks and over, 10.6% between 61-70 weeks of age and 2.1% for less than 50 weeks. At the same time, it was determined that 83% of the breeders purchased the hens as pullets. Of the breeders included in the study, 57.4% of the breeders reported that they purchased the hens by their own means and the remaining 42.6% of the breeders reported that they purchased the hens through the Egg Producers' Association. Also, 78.8% of the breeders who participated in the survey stated that they did not want to raise the chicks they used themselves. It was determined that 51.1% of the enterprises had 6 or more hens per m² in the grazing area, 23.4% had 4, 14.9% had 3 and 10.6% had 5 hens. Again, 48.9% of the breeders stated that there were 4 hens per m² in the indoor area, while 40.4% stated that there were 6 or more hens and 10.6% stated that there were 5 hens. At the same time, 72.3% of the breeders stated that the mortality rate in their enterprises was less than 5%, 25.5% stated that it was between 6-10% and 2.1% stated that it was between 11-15%.

In the study conducted by Tuğluk and Yalçın (2004) in Nevşehir/ Kozaklı, it was reported that the mortality rate in laying hen enterprises was 5.9% on average. While all the breeders stated that they regularly calculate egg yield in their enterprises, 70.2% of them stated that egg yield was more than 81% and the remaining 29.8% stated that it was between 61-80%. While 57.4% of the breeders stated that they did not use any worker in their enterprises, 36.2% stated that they employed 1 (6.4%) or 2 (29.8%) people. Köse and Durmuş (2014) reported in their study conducted in poultry enterprises in Ordu province that 82.4% of the enterprises employed 1 person and 17.6% employed 2 people. According to this result, it is seen that the poultry enterprises in the organic egg production system in Ordu province continue egg production by using very few workers. Tuğluk and Yalçın (2004) reported that chickens were used in production for an average of 71 weeks, between 52 and 86 weeks, except for the pullets' period, in their study on egg poultry enterprises in Kozaklı/Nevşehir. Among the breeders participating in the study, 89.2% stated that they had a feed store in their enterprises. While 63.8% of the breeders stated that they were engaged in egg poultry breeding for additional livelihood, 14.9% of the breeders reported that they were engaged in egg poultry breeding as their main livelihood. While 51.1% of the breeders have been poultry breeding for 4 years or more, 19.1% have been poultry breeding for 1 year, 19.1% for 2 years and 10.6% for 3 years. While 85.1% of the breeders stated that they would continue to keep organic laying hens, 70.2% of them stated that they were planning to expand their enterprises. Cönk (2006) reported in his study on egg poultry production in Afyonkarahisar that approximately 67% of the breeders engaged in egg production would not continue egg

production. While 66% of the breeders reported that they do not breed any other animal species other than poultry, it was determined that 75% of the breeders who breed an additional animal other than poultry breed cattle and 25% of them breed small ruminants. At the same time, while 83% of the breeders reported that their enterprises were adequately inspected, it was determined that 78.8% of those who expected feed support from public institutions and organisations, 14.9% of those who expected pullets support and 6.4% of those who demanded cash aid.

Main Findings on the Health and Nutritional Status of the Hens Used in Egg Production

The main findings (n and %) on the health and nutritional status of the hens used in the organic system are given in Table 3. While 70.2% of the breeders stated that they received help from a freelance veterinarian in the fight against diseases, 97.9% of them stated that they routinely disinfected their poultry houses to prevent diseases. In addition, 63.8% of the breeders stated that the quality control of the water given to the hens is carried out at certain intervals. At the same time, 93.7% of the breeders stated that they obtained the feed they used in their enterprises from any feed factory (51.1%) and from the feed factory of the Egg Producers' Association (42.6%), while 6.4% of the breeders reported that they made the feed themselves. Consistent with the data obtained in this study, Çimrin *et al.* (2019) reported that 86.96% of the poultry enterprises in Hatay and Tuğluk and Yalçın (2004) reported that 67% of the poultry enterprises in Nevşehir/Kozaklı preferred to buy feed. On the other hand, Cönk (2006) stated in his study that approximately 76% of the hen's breeders procure the feed they use by their own means. Breeders who try to gain an economic advantage by producing feed themselves face high raw material costs. While 66.7% of the breeders who met their feed needs with their own means stated that they preferred this way to have healthier feed, 52.3% of the breeders who met their feed needs by purchasing feed stated that they preferred to buy feed because they did not have sufficient technical knowledge. At the same time, 87.2% of the breeders stated that they had information about the content of the feed they used. In animal husbandry, especially in egg poultry enterprises, the most important part of the costs in the enterprises is feed expenses (65-70%). Therefore, in order to solve the feed problem of poultry enterprises in Turkey, projects should be developed to increase the production of feed raw materials and breeders should be supported in this regard. Again, 93.6% of the breeders stated that they always keep feed in front of their hens and 95.7% of the breeders stated that they do not feed their hens other than the recommended feed. At the same time, 100% of the breeders stated that they calculated the amount of feed consumed by the hens and in 95.8% of the enterprises, the average feed consumption per hen was 100-130 grams.

Table 1. Demographic characteristics of breeders

No	Demographic characteristic	Options	Frequency	
			n	%
1	Age of breeders	< 30 ages	6	12,8
		31- 40 ages	9	19,1
		41-50 ages	17	36,2
		> 51 ages	15	31,9
		Total	47	100,0
2	Occupation of breeders	Farmer	22	46,8085106382978750
		Self-employment	9	19,1489361702127670
		Veterinary technician/Physician	0	0
		Agricultural technician/Engineer	0	0
		Civil Servant/Worker	7	14,8936170212765950
		Retired	9	19,1489361702127670
Total	47	100,0		
3	Breeder's education status	Illiterate	1	2,1
		Primary-Secondary School	20	42,6
		High School	18	38,3
		University	8	17,0
		Total	47	100,0
4	Number of breeder's households	< 4 persons	26	55,3
		4 – 6 persons	17	36,2
		7 < persons	4	8,5
		Total	47	100,0
5	Ownership of the enterprise	My own	40	85,1
		Rent	2	4,3
		Mine - Rent	4	8,5
		State land	1	2,1
		Total	47	100,0
6	Breeder's social security	None	11	23,4
		Social Security Organization for Artisans and the Self-Employed	6	12,8
		Social Security Institution	29	61,7
		Green Card	1	2,1
		Total	47	100,0
7	Participation of breeders in training/course on poultry breeding	I joined	12	25,5
		I did not participate	35	74,5
		Total	47	100,0
8	If you have not attended the training or course, would you like to attend?	Yes	30	85,7
		No	4	11,4
		I have no idea	1	2,9
		Total	35	100,0
9	Agricultural credit utilization status of breeders	Bank	18	38,3
		Agriculture and Credit Co-Operative	0	0
		Chamber of Agriculture	0	0
		I not used	29	61,7
		Total	47	100,0

Table 2. Main Findings Regarding the Enterprises Producing Eggs in Organic System

No	Questions	Options	Frequency	
			n	%
1	What is your reason for keeping egg poultry?	Main source of livelihood	7	14,9
		Additional livelihood	30	63,8
		Because it's snowy	6	12,8
		I don't have anything else to do	4	8,5
		Total	47	100,0
2	How many years have you been keeping egg poultry?	1 year	9	19,149
		2 years	9	19,149
		3 years	5	10,638
		4 years and more	24	51,064
		Total	47	100,0
3	Do you breed animals other than hens?	Yes	16	34,0
		No	31	66,0
		Total	47	100,0
4	If yes, what is it?	Cattle	12	75,0
		Sheep or Goat	4	25,0
		Bee	0	0
		Others	0	0
		Total	16	100,0
5	Do you plan to expand your enterprises?	Yes	33	70,2
		No	13	27,7
		I'm thinking about quitting	0	0
		I have no idea	1	2,1
		Total	47	100,0
6	What is the structure of the land where your enterprise is located?	Under hazelnut	39	83,0
		Open land	8	17,0
		Other	0	0
		Total	47	100,0
7	What materials did you use to build the hen house?	Concrete	21	44,7
		Sandwich Panel	26	55,3
		Other	0	0
		Total	47	100,0
8	What type of nest box do you use in your hen house?	Individual	32	68,1
		Group	15	31,9
		I do not use	0	0
		Total	47	100,0
9	How do you provide ventilation in your hen house?	Fan	40	85,1
		Window	1	2,1
		Chimney	0	0
		Chimney + Window	6	12,8
		Total	47	100,0
10	How do you give water to the chickens in your hen house?	With gutter type drinker	1	2,1276595744680850
		With nipple type drinker	44	93,6170212765957500
		With hanging round drinker type	1	2,1276595744680850
		Other	1	2,1276595744680850
		Total	47	100,0
11	How much lighting time do you apply to the hens during the laying period?	As daylight	4	8,5
		12 hours	6	12,8
		16 hours	37	78,7
		24 hours	0	0
		Total	47	100,0
12	Do hens have year-round access to green grass in the roaming/grazing area?	Yes	43	91,5
		No	4	8,5
		Total	47	100,0
13	How many people work in your enterprises?	None	27	57,4
		1 worker	3	6,4
		2 workers	14	29,8
		> 3 and above	3	6,4
		Total	47	100,0
14	How many hens do you have in your enterprises?	< 250 hens	3	6,3829787234042550
		250 to 500 hens	6	12,7659574468085100
		500 to 750 hens	6	12,7659574468085100
		>750 hens	32	68,0851063829787200
		Total	47	100,0

15	What do you use as litter material in your enterprises?	Thick sawdust	6	12,8
		Fine sawdust	1	2,1
		Paddy husk	39	83,0
		Other	1	2,1
		Total	47	100,0
16	Which hen breed do you use in your enterprises?	Tinted	0	0
		Lohman Brown	46	97,9
		Nick-Brown	0	0
		Atak-S	1	2,1
		Hy-Line Brown	0	0
Total	47	100,0		
17	At what age do you buy the hens you use in production?	< 12 weeks	2	4,3
		12-14 weeks	1	2,1
		16-18 weeks	39	83,0
		> 18 weeks	5	10,6
		Total	47	100,0
18	Would you like to breed chicks yourself?	Yes	10	21,3
		No	37	78,7
		Total	47	100,0
19	Do you calculate egg yield at certain intervals in your enterprise?	Yes	47	100,0
		No	0	0
		Total	47	100,0
20	What is the average egg yield in your enterprise?	< % 60 %	0	0
		61-70 %	3	6,4
		71-80 %	11	23,4
		> %81 %	33	70,2
		Total	47	100,0
21	What is the mortality rate from various causes in your hen house?	Less than 5%	34	72,3404255319149000
		6-10 %	12	25,5319148936170200
		11-15 %	1	2,1276595744680850
		More than 15 %	0	0
		Total	47	100,0
22	How many weeks of age do you use the hens in production (excluding the pullet period)?	< 50 weeks	1	2,1276595744680850
		51-60 weeks	0	0
		61-70 weeks	5	10,6382978723404250
		>71 weeks	41	87,2340425531915000
		Total	47	100,0
23	Where do you buy your hens?	By my own means	27	57,4
		From the Egg Producers' Association	20	42,6
		From the Chamber of Agriculture	0	0
		Total	47	100,0
24	Do you plan to continue egg poultry breeding?	Yes	40	85,1
		No	7	14,9
		Total	47	100,0
25	How many hens per m ² in the grazing area of your hen house?	3 hens	7	14,9
		4 hens	11	23,4
		5 hens	5	10,6
		6 and more hens	24	51,1
		Total	47	100,0
26	How many hens per m ² in the indoor area of your hen house?	3 hens	0	0
		4 hens	23	48,9361702127659600
		5 hens	5	10,6382978723404250
		6 and more hens	19	40,4255319148936100
		Total	47	100,0
27	Is there an egg and feed storage unit in your enterprise?	Yes	42	89,4
		No	5	10,6
		Total	47	100,0
28	Is your enterprise adequately audited?	No	39	83,0
		Yes	8	17,0
		Total	47	100,0
29	What are your expectations from public institutions and organisations?	Feed support	37	78,7
		Training/course support	0	0
		Pullets support	7	14,9
		Cash aid	3	6,4
		Total	47	100,0

Table 3. Main findings related to the health and nutritional status of the hens used in egg production

No	Questions	Options	Frequency	
			n	%
1	Is regular disinfection applied in your poultry house?	Yes	46	97,9
		No	1	2,1
		Total	47	100,0
2	How do you fight against diseases in your poultry house?	With the help of the Provincial/District Directorate of Agriculture and Forestry	6	12,8
		With the help of a freelance veterinarian	33	70,2
		With my own means	8	17,0
		Total	47	100,0
3	Do you always keep feed in front of the hens?	Yes	44	93,6
		No	3	6,4
		Total	47	100,0
4	Where do you get the feed?	I make it myself	3	6,3829787234042550
		I buy from any feed factory	24	51,0638297872340400
		I buy from the feed factory of the Egg Producers' Association	20	42,5531914893617000
		Toplam	47	100,0
5	Do you feed your hens other than the recommended commercial feed?	Yes	2	4,3
		No	45	95,7
		Total	47	100,0
6	Do you calculate the feed consumption of hens?	Yes	47	100,0
		No	0	0
		Total	47	100,0
7	Do you have any information about the content of the feed you use?	Yes	41	87,2
		No	6	12,8
		Total	47	100,0
8	What is your average daily feed consumption per hen?	Less than 100 g	2	4,2553191489361700
		100-120 g	20	42,5531914893617000
		121-130 g	25	53,191489361702250
		131-141 g	0	0
		More than 140 g	0	0
Total	47	100,0		
9	If you meet the need for feed by producing it with your own means, what is the most important reason for this?	To be able to produce cheaper	0	0
		To provide additional income by selling feed to other producers	0	0
		To be able to produce better quality feed from feed factory	1	33,3
		To produce healthier feed	2	66,7
		Total	3	100,0
10	What is the most important reason for purchasing feed?	Cheaper	2	4,5
		I do not have enough space to store raw materials	13	29,5
		I do not have sufficient technical knowledge	23	52,3
		I think it is healthier	6	13,6
Total	44	100,0		
11	Is the quality of the water you give to the hens checked at regular intervals?	Yes	30	63,8
		No	17	36,2
		Total	47	100,0

Main Findings on Sales and Marketing of Eggs Produced

The main findings on the sales and marketing of eggs produced in the organic system are given in Table 4. In this study, 97.9% of the breeders who are engaged in organic system poultry breeding stated that the eggs obtained from hens raised in the organic system are better. When the breeders were asked why consumers should prefer eggs obtained from hens raised in the organic system, 85.1% of the breeders stated that these eggs were healthier and 14.9% stated that they thought that these eggs were more nutritious. It was determined that 91.5% of the breeders who participated in the questionnaire preferred open viol and the remaining 8.5% preferred closed cardboard viol when marketing the eggs. It was stated that 55.3% of the breeders marketed the eggs they produced themselves and 40.4% of them sold them to the Egg Producers' Association.

In the study conducted by Köse and Durmuş (2014), it was determined that only 23% of the poultry breeders in Ordu province marketed the eggs with their own means, while the remaining 77% marketed the eggs through cooperatives. On the other hand, in a study conducted by Tuğluk and Yalçın (2004) in laying hen enterprises, it was determined that only 20% of the eggs produced were marketed by the producers' own means. When the study conducted in Ordu province is examined, it is determined that there is a decrease in the preference of the enterprises in Ordu province to market the eggs they produce with institutions such as unions or cooperatives compared to the study conducted in previous years. It is thought that this situation encountered in Ordu province is caused by the problems experienced by producers with institutions such as unions or cooperatives. Both the Covid-19 pandemic, and the global economic crisis have a direct impact on egg prices and current prices are constantly changing. When the breeders were asked "What measures do you take in periods when egg prices experience large decreases in the short term and you have to sell at a loss?", 87.2% of the breeders stated that they desperately wait for egg prices to rise and continue egg sales without reducing feed, 6.4% of them keep the eggs in cold storage, and the remaining 6.4% stated that they dispose of the hens at a loss and stop egg production. Therefore, alternative systems should be established to make use of the processability of the egg for the producer to skip the egg production process with the least damage during the periods when the egg market is moving, and the producer should be prevented from stopping the egg production by disposing the hens. When the breeders were asked "What do you suggest increasing egg sales?", 31.9% of the breeders stated that they thought that television-radio advertisements should be made to encourage egg consumption and 31.9% of the breeders stated that

Information activities should be carried out to explain the health benefits of eggs. At the end of the production period, 34% of the breeders who participated in the survey stated that they sent their hens to a slaughterhouse, 36.2% of them sold them to a wholesale company and the remaining 29.8% of them sold them through the Egg Producers' Association. Also, 70.2% of all breeders stated that they use the manure obtained from hens on their own land. To the question "How do you see the future of organic system poultry breeding?", 72.3% of the breeders answered that it will be better.

Main Findings Regarding the Egg Producers' Association

The main findings related to the opinions of the breeders about the Egg Producers' Association are given in Table 5. In this study, 55.3% of the organic system poultry breeders stated that they were not members of the Egg Producers' Association. When the breeders were asked "Does the Egg Producers' Association meet your expectations?", 66% of the breeders answered no and 57.7% of the breeders who were not members of the Egg Producers' Association stated that they became members because they thought that the Egg Producers' Association was not well managed. To the question "What are your suggestions?" asked in order for the Egg Producers' Association to meet the expectations of the breeders, the breeders verbally stated that the contracted production model should be switched to give a purchase guarantee to the breeders, expert veterinarian support should be provided in poultry, the feed provided to the breeders by the association should be of high quality and continuity, and the importance of organic eggs should be conveyed to the citizens more clearly both in social media and in billboards and advertisements with prepared conversions.

All the breeders who are members of the Egg Producers' Association reported that they received their payments in the form of money-feed-violet and at the appropriate time without any problems. To the question "Do you think that there are enough meetings in the Egg Producers' Association?", 81% of the breeders answered yes, while 75% of those who said no stated that there should be at least one meeting per month. Again, 90.5% of the breeders answered yes to the question "Are you aware of the dates of the meetings held or to be held in the Egg Producers' Union and the decisions taken?"

Main Findings Regarding Covid-19 Pandemic

The main findings regarding the level of the organic system poultry breeders being affected by the Covid-19 pandemic are given in Table 6. To the question "Has the Covid-19 pandemic affected your egg sales prices?", 76.6% of the breeders answered yes, and 72.2% of the breeders affected by the pandemic stated that egg prices were positively affected. Also, 77.8% of the same breeders stated that they estimated that the effect of the Covid-19 pandemic would last longer than 12 months.

Table 4. Main findings on the sales and marketing of eggs produced in organic system

No	Questions	Options	Frequency	
			n	%
1	Do you think that eggs produced in the organic system are better?	Yes	46	97,9
		No	0	0
		I have no idea	1	2,1
		Total	47	100,0
2	Why should consumers prefer eggs produced in the organic system?	Because it's more nutritious	7	14,9
		Because it's healthier	40	85,1
		Because animal welfare is considered	0	0
		No opinion	0	0
		Total	47	100,0
3	How do you see the future of egg production in the organic system?	It'll get better	34	72,3404255319149000
		It's gonna get worse	3	6,3829787234042550
		No change	5	10,6382978723404250
		No opinion	5	10,6382978723404250
		Total	47	100,0
4	How much do you sell the eggs you produce in the organic system?	< 50 cents	1	2,1
		50-60 cents	1	2,1
		60-70 cents	5	10,6
		>70 cents	40	85,1
		Total	47	100,0
5	What do you think the unit price of the eggs you sell should be?	70 cents	1	2,1
		80 cents	3	6,4
		90 cents	4	8,5
		More than 90 cents	39	83,0
		Total	47	100,0
6	Which type of packaging do you use when selling the eggs?	Open viol	43	91,5
		Gelatine-coated viol	0	0
		Closed cardboard viol	4	8,5
		Foamed viol	0	0
		Plastic viol	0	0
		Total	47	100,0
7	How do you market your eggs?	I market myself	26	55,3
		I give to the Egg Producers Association	19	40,4
		I market it myself + I give it to the Egg Producers' Association	2	4,3
		Total	47	100,0
8	How do you utilize the chickens at the end of the production period?	I sell to any slaughterhouse	16	34,0
		I sell to any company	17	36,2
		I'm selling to the Egg Association	14	29,8
		Total	47	100,0
9	How do you utilize the manure from your chickens?	I do not evaluate	2	4,3
		Selling	12	25,5
		I use it on my own land	33	70,2
		Total	47	100,0
10	What measures do you take when you must sell your eggs at a loss?	I'm reducing the feed I give to the hens	0	0
		Desperate, I wait for egg prices to rise and continue sales without cutting feed	41	87,2
		Hoping that egg sales will increase in the short term; I keep them in cold storage	3	6,4
		I'm disposing of the hens at a loss and stopping production	3	6,4
		Total	47	100,0
11	What do you suggest increasing the sales of organic eggs?	Making television and radio advertisements to encourage egg consumption	15	31,9148936170212780
		Carrying out information activities on the benefits of eggs for health	15	31,9148936170212780
		Promotional studies should be carried out on the processability of eggs with different products	5	10,6382978723404250
		The benefits of eggs should be better explained in schools and egg consumption of students should be encouraged	4	8,5106382978723400
		Negative and false news about eggs should be prevented	8	17,0212765957446800
		Total	47	100,0

Table 5. Main Findings Regarding the Egg Producers' Association

No	Questions	Options	Frequency	
			n	%
1	Are you a member of the Egg Producers Association?	Yes	21	44,7
		No	26	55,3
		Total	47	100,0
2	If you are not a member of the Egg Producers' Association, what are the reasons for this?	Egg purchase prices set by the Egg Producers' Association are low	4	15,4
		The payment term set by the Egg Producers' Association is too long	0	0
		The Egg Producers' Association is monopolized by certain producers, and I am not satisfied with this situation	2	7,7
		I think the Egg Producers' Association is not well managed	15	57,7
		Not all egg producers produce eggs of the same quality	1	3,8
		I can market better with my own means	4	15,4
		Total	26	100,0
3	Does the Egg Producers' Association meet your expectations?	Yes	16	34,0
		No	31	66,0
		Total	47	100,0
4	If no, what is your suggestion?	-	-	-
5	How do you receive your payment from the Egg Producers' Association?	In money	0	0
		As feed and viol	0	0
		As money, feed and viol	21	100,0
		Total	21	100,0
6	Do you receive your payments at the appropriate time after egg sales?	Yes	21	100,0
		No	0	0
		Total	21	100,0
7	If you have not received payment at the appropriate time, how many months are you experiencing disruption?	1 ay month	0	0
		3 ay months	0	0
		5 ay months	0	0
		12 months and over	0	0
		Total	0	100,0
8	Do you think that enough meetings are held in the Egg Producers' Association?	Yes	17	81,0
		No	4	19,0
		Total	21	100,0
9	If no, how many meetings should be held per month?	1 time	3	75,0
		2 times	1	25,0
		3 times	0	0
		Total	4	100,0
10	Are you aware of the dates of the meetings held or to be held and the decisions taken?	Yes	19	90,5
		No	2	9,5
		Total	21	100,0

Table 6. Main findings on the impact of the Covid-19 pandemic

No	Questions	Options	Frequency	
			n	%
1	Has the Covid-19 pandemic affected your egg sales prices?	Yes	36	76,6
		No	11	23,4
		Total	47	100,0
2	If so, in what direction?	Positive impact	26	72,2
		Negative impact	10	27,8
		No opinion	0	0
		Total	36	100,0
3	How long do you think the impact of the Covid-19 pandemic on your enterprise will last?	3 months	4	11,1
		6 months	3	8,3
		9 months	1	2,8
		>12 months	28	77,8
		Total	36	100,0

Main Findings of Digital Marketing (DITAP)

The main information about the level of knowledge of the organic system poultry breeders about digital marketing is given in Table 7. To the question "Do you have information about digital marketing?", 59,6% of the breeders answered yes and 74,5% of all breeders stated that they wanted to sell without intermediaries through digital marketing.

Conclusion and Recommendations

Organic egg production system has its own advantages and disadvantages. Factors such as the storage conditions of eggs produced in the organic system and the time until sale are very effective. For this reason, it is very important to determine the general structure of the enterprises producing eggs in the organic system and to reveal the deficiencies correctly and the results of such studies should be well examined. According to the results obtained from this study; 46,8% of the breeders who are engaged in organic system egg poultry breeding are farmers. According to the results obtained from this study, it can be said that the main problem of the enterprises engaged in organic system egg poultry breeding in Ordu province is economic problems. The development of any sector is in parallel with the export power. For this reason, the planning of how these enterprises will market the eggs they produce should be made and signed in advance. If the existing marketing opportunities are not sufficient, new marketing opportunities should be searched. Thus, both the economic burden of the enterprises producing eggs in the organic system can be reduced and the motivation of the producers to make production can be increased.

It has been determined that the success of the enterprises in terms of animal welfare and egg production in organic system poultry breeding is related to good care-feeding and management techniques. The sustainability of the enterprises is closely related to the analysis of factors such as the system used, the preferred genotype, the age of the genotype, care-feeding and environmental conditions. For this reason, organic egg producing enterprises in Ordu province

should be closely monitored and necessary studies should be carried out to determine their status precisely. People need to consume enough animal origin nutrients to sustain their lives in a healthy way. One of the nutrients of animal origin that they should consume is eggs. For this reason, first, egg production enterprises should be properly controlled, the deficiencies seen should be eliminated and sufficient egg production should be realized. At the same time, consumers should be sufficiently informed about the organic system egg production, which is an alternative egg production system. In order to be able to market the eggs produced by the enterprises producing eggs in the organic system in Ordu province, firstly, continuity and uniformity in the domestic market should be ensured. This is only possible if the producers can work in harmony with the union or cooperative in Ordu province. Thanks to a fair and active union-cooperative structure, both breeders and the poultry sector in Turkey will win. For this reason, it should be aimed that all unions and co-operatives throughout the country should carry out joint works with a common mission and vision and should be brought together routinely to create an internal power for the foreign market by being supervised by the relevant institutions. Sectoral trainings should be organized by the relevant institutions to focus on the deficiencies in the province of Ordu, taking into consideration the high rate of requests of the breeders to attend training/courses related to poultry breeding. In order to minimize the damage to the breeders from the possible supply-demand imbalance, sufficient number and quality of egg storages should be established in Ordu province to store the eggs produced for a certain period of time. Based on the fact that eggs are a processable foodstuff, advanced processing technologies should be used and made widespread to make the eggs produced more suitable for export. In this way, the transportation of raw eggs, which are transformed into forms such as liquid yellow and liquid white, frozen eggs, and dry egg powder, will be facilitated and the yield and quality of the unit product will increase. In this context, necessary innovative and technological steps should be taken in Ordu province, studies on this subject should be supported, and investors should be encouraged.

Table 7. Main findings of Digital Marketing (DITAP)

No	Questions	Options	Frequency	
			n	%
1	Do you have information about digital marketing?	Yes	28	59,6
		No	19	40,4
		Total	47	100,0
2	Would you like to sell without intermediaries through digital marketing?	Yes	35	74,5
		No	12	25,5
		Total	47	100,0

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The Effect of Applying Different Coloured Lighting to Partridge (*Alectoris Chukar*) Eggs in Incubators on Hatching Results and Responses of Open Field Test

Samet Can DONMEZ¹, Ahmet CEYHAN¹, Nilgün YAPICI¹ *, Dogan NARINC¹

¹Akdeniz University, Agriculture Faculty, Department of Animal Science, Antalya, Turkey

Article History

Received: Oct 08, 2024
Accepted: Nov 01, 2024
First Online: Dec 03, 2024

*Corresponding Author

Tel: +90 05357235398
E-mail: nilgunya@gmail.com

Keywords

Monochromatic light
Photoperiod
Incubation
Poultry
Behaviours

Abstract

The aim of this study is to ascertain the impact of dark and two distinct monochromatic lighting applications during incubation on the hatching characteristics of Chukar partridge (*Alectoris chukar*) eggs, as well as certain open field test characteristics following hatching. A total of 300 hatching eggs collected three consecutive days from partridges that mated by chance and were not subjected to selection were used in the study. All eggs were placed equally in same incubators and applied dark, blue, and green illumination. The highest hatchability of fertile eggs and hatchability of total eggs were found to be 89.7% and 87.9% in the blue group ($P<0.05$). It was determined that the reason for this situation was that embryonic deaths in the late period were higher in the group without illumination and in the groups with green monochromatic illumination. In addition, the effects of the lighting applications on the open field test performed after hatching on the characteristics such as movement, jumping and calling were found to be insignificant. As a result, it was determined that the incubation results obtained from the incubator illuminated with blue colour had a better effect than the other groups.

Introduction

Wildlife is damaged due to reasons such as global temperature change, wild animal trade, poaching and pesticides. According to the data of International Union for Conservation of Nature (IUCN), there are more than 157000 species on the IUCN red list and more than 44000 of them are in danger of extinction (IUCN, 2024). One of the ways to prevent this extinction in wildlife is to ensure that species produced and grown under intensive conditions are released into their wild environments and continue to survive. Protecting wildlife is important for protecting our genetic resources. Partridge, one of the birds of wildlife, especially unconscious hunting activities are one of the

most important factors that reduce the number of this species. Every year, thousands of partridges raised in enterprises within the Ministry and Universities are released into nature after reaching a certain size. Considering that the production period for partridges is only 3-4 months and that only 20-30 eggs are produced per partridge, eliminating egg losses during incubation will be important for both the farmer and the country's economy (Şengül *et. al.*, 2016). When Türkiye's incubator enterprises were examined, it was determined that there was no lighting in the incubators and the eggs were incubated in a dark environment.

Studies have shown that different lighting colours positively affect hatching results. Zhang *et al.* (2014), a total of 880 chicken eggs with an average weight of 68 g were exposed to green (560 nm) monochromatic light in the incubator. The embryo that developed in the dark environment and the embryo exposed to green light were also examined at the molecular level. According to these results, it was observed that green light increased post-hatching muscle development by accelerating cellular division. In a different study, the effects of white light, green light and blue-green combinations on the embryo in the incubator were investigated. Green or green-blue light combinations have been shown to have an effect on growth performance and carcass development. LED light sources and their intensity are important in terms of energy consumption, cultivation costs and raising healthy individuals. In addition, the use of such lights provides calmer individuals (Gonngtruttananun, 2014). In a different study conducted on brown layers (261 eggs) in the Aegean region, it was aimed to investigate the effect of white and green LED light application during incubation (21 days) on the production performance, behaviour and physiological stress and feather pecking behaviour of laying hens. It has been determined that green LED light application does not negatively affect egg production and may help reduce pecking behaviour, stress and anxiety (Dayioğlu, 2018).

As a result of comparative statistical analysis of the data of 55 different experimental research articles, considering the effects of monochromatic green light stimulation on embryonic development, chick quality and hatching characteristics in broiler chickens; It was determined that green light stimulation did not affect chick weight and hatching performance, but significantly reduced hatching time (Tainika, 2019).

This study aimed to examine the effects of different coloured lighting (dark, blue and green) applied to partridge eggs in the incubator on hatching results and chick behaviour. In addition to high hatching efficiency, the hatching time of the chicks being close to each other (mass hatching) is also an important criterion for a business. In addition, an open field test was applied to chicks that had completed hatching and dried out.

Materials and Methods

The study was carried out in the summer of 2022 at the Livestock Facilities of Akdeniz University Faculty of Agriculture, Department of Animal Science, and partridge (*Alectoris chukar*) was used as animal material. Animal material was taken from Coşkunlar pheasant and partridge production farm, recommended by the 5th Regional Directorate of Nature Conservation and National Parks. A total of 300 hatching eggs obtained from a breeding partridge herd in 3 days were used in the experiment. Hatching eggs were brought to Akdeniz University Faculty of Agriculture Department of Animal Husbandry Facilities after a 300 km journey under

suitable conditions. The eggs were placed in the incubator without any storage process, by measuring their width, length and weight. Three eggs were broken during the transfer. For this reason, trial groups were created with 99 eggs each. The averages of hatching egg characteristics are presented in Table 1. The average egg weight was found to be similar as 40.96 g in the dark group and 40.75 g and 40.61 g in the green and blue lighting groups. The similar averages indicate that the eggs were successfully randomized. The width of hatching eggs is 21.37-20.99-21.30 mm in the dark, green and blue groups, respectively. The length of the eggs falling in the dark and blue treatment groups was 31.28 and 31.17 mm, respectively, and they were higher than the length of the eggs falling in the green light group (30.85 mm). The shape index averages of the eggs belonging to the groups are 67.17 and 68.29, and the averages are similar to each other.

In the study, a VGS 490 brand (54×67×150) cm incubator with a capacity of 840 partridge eggs, a combined development and hatching compartment, full environmental control and automatic cooling and heating motor was used. This machine has 10 shelves. Each shelf has a separate motor and individual hatching basket. After the incubation room and incubation machines are cleaned and disinfected, blue and green monochromatic (light intensity average of 200 lux) LED lighting mechanisms were prepared inside the incubators. The eggs in the groups were randomly placed in 3 different machines that were identical to each other.

1. Dark group (Control): There was no lighting in the incubator.

2. Green group: The incubator was illuminated with green light (~560 nm wavelength).

3. Blue group: The incubator was illuminated with blue light (~480 nm wavelength).

The temperature and humidity in the incubators were adjusted to 37.5 ± 0.1 °C - 55% during the development of the embryo and 37.2 ± 0.1 °C - 70% in the hatch (last 3 days). The shelves were rotated automatically every hour. As a result of the 24-day incubation period, hatched chicks, dead embryos under the shell and infertile eggs were calculated. In order to reveal differences in embryonic deaths in the experimental groups, macroscopic examination was performed on the eggs. In this process, embryos that were determined to die between days 0-14 of incubation were recorded as early stage, and embryos that were determined to die between days 15-24 were recorded as late stage.

- Hatchability of total eggs (%): (number of chicks hatched/number of total eggs) × 100
- Hatchability of fertile eggs (%): (number of hatched chicks/numbers of fertilized eggs) × 100
- Fertility: (number of fertilized eggs/number of eggs placed in the machine) × 100
- Hatching window: The periods between the first chick hatched and the last chick hatched were calculated (Kızılaslan and Şimşek 2019).

Table 1. Egg external quality characteristics*

Egg characteristics	Dark	Green	Blue	P
Weight, g	40.96 ± 0.28	40.75 ± 0.28	40.61 ± 0.28	0.689
Width, mm	21.37 ± 0.14	20.99 ± 0.14	21.30 ± 0.14	0.144
Length, mm	31.28 ± 0.08	30.85 ± 0.08	31.17 ± 0.08	0.082
Shape index %	68.27 ± 0.34	67.17 ± 0.34	68.29 ± 0.34	0.763

*The table gives averages values with standard error

Open field test (chick's first call and first movement time, first defecation time, total number of squares navigated, total number of jumping movements) was applied by randomly taking 30 samples from chicks that had hatched and dried in each group. In this test, an all-white wooden box containing 25 (5×5) squares of size (25×25) cm was used. For the open field test, 10 min of song and behaviour recording was made after the chick was released into the square in the middle of the box (Rodenburg *et al.*, 2003). These video recordings were watched three times to determine the chick's first call and first movement time, first defecation time, total number of frames visited, total number of calls and total number of jumping movements.

In the study, hypothesis tests were analyzed with one-way ANOVA, and 95% probability value was accepted as the acceptance limit of H0 hypothesis. Statistical analyses of the study were performed using SPSS 26 software.

Results and Discussion

Incubations results of the groups

The hatching results, early and late embryonic mortality detected in the experimental groups are summarized in Table 2. As can be seen from the relevant table, the fertility rate in the control, green and blue groups is 90.9%-88.9% and 99.0%, respectively; differences between means were insignificant. Early embryonic death was found to be similar at 4.4% in the control (dark) group, 5.3% in the green group and 4.1% in the blue group.

Differences between groups were found to be significant ($P < 0.05$) in terms of late embryonic period deaths. The rate of late embryonic deaths detected in the blue group (6.2%) was lower than the rates detected in control (dark) and green light applications (17.8%-18.1%) ($P < 0.05$). Total embryonic mortality rate was also significantly ($P < 0.05$) affected by blue light application. The lowest embryonic mortality rate (10.3%) was average for the blue group. Hatchability of fertile eggs and hatchability characteristics were also significantly affected by lighting ($P < 0.05$ for both). The highest hatchability of fertile eggs was found to be 89.7% in the blue group. The highest hatchability of total eggs was measured as 87.9% in the blue group.

When the hatching window of the incubation results is examined, at the end of the 24-day incubation period, hatching occurs on the 22nd - 24th day in the dark group, and on the 24th - 28th day in the green group and hatching in the blue group were collected between the

predicted day and lasted a total of 48 hours. In the green light group, chicks started hatching on the predicted day and the total hatching window lasted 98 hours. In the blue group, hatching started 2 days ago and the last chick hatching was recorded at 75 hours.

Behavioural test

The results of the open field test used to reveal the anxiety level of the animals are given in Table 3. Among the behaviours taken into account in the open field test, only the time of first defecation (seconds) was significantly ($P < 0.05$) affected by the lighting application. The average time to first defecation was found to be similar in the dark and green groups (157.00 sec and 106.66 sec). The average determined in the blue lighting group (81.09 sec) is similar to the average in the green group, but lower than the average in the dark group ($P < 0.05$).

Discussions

It is known that lighting practices positively affect the embryo before incubation and chick development afterwards (Karen *et al.*, 2017; El-Sabroun and Khalil, 2017). Many studies have been conducted to reveal the best lighting colour, light intensity and duration in incubators (Yildirim *et al.*, 2008; Yu *et al.*, 2018; Wang *et al.*, 2020; Li *et al.*, 2021).

In this study, which investigated the effects of monochromatic blue and green light application in incubators on hatching results and behaviour, the highest hatchability of fertile eggs and hatchability of total partridge eggs was found in the blue group (89.7% and 87.9%) ($P < 0.05$) (Table 2). Zhang *et al.* (2014), in a study they conducted on chicken eggs, determined that green light accelerated cell division and increased muscle development after hatching (Shavey and Al-mohsen 2002). Tainika (2019) conducted a study on the comparative statistical analysis of the data of 55 different experimental research articles, considering the effects of monochromatic green light stimulation on embryonic development, chick quality and hatching characteristics in broiler chickens, and found that green light stimulation did not affect chick weight and hatching performance, but reduced the incubation period. The hatching window of partridge eggs in the dark group spanned a shorter period (48 hours) than the hatching window of chicks in incubators illuminated with blue and green light and started 2 days earlier than expected. The hatching window for the eggs in the blue group also started 2 days earlier than expected but was spread over

Table 2. Fertility, hatchability of set eggs, hatchability of fertile eggs, and embryonic mortality according to light coloured*

Hatchability (%)	Dark	Green	Blue	P
Fertility**	90.9 ± 2.6	88.9 ± 2.6	99.0 ± 2.6	0.054
Early embryonic mortality	4.4 ± 2.2	5.3 ± 2.2	4.1 ± 2.1	0.922
Late embryonic mortality	17.8 ± 3.6 ^a	18.1 ± 3.5 ^a	6.2 ± 3.5 ^b	0.025
Total embryonic mortality	22.2 ± 44.1 ^a	23.4 ± 4.0 ^a	10.3 ± 3.9 ^b	0.036
Hatchability of fertile eggs***	77.8 ± 4.1 ^b	76.6 ± 4.0 ^b	89.7 ± 3.9 ^a	0.036
Hatchability of total eggs****	70.7 ± 4.2 ^b	72.7 ± 4.2 ^b	87.9 ± 4.2 ^a	0.007

^{a,b} Means with different superscript letters in rows differ significantly (P < 0.05)

*The table gives averages values with standard error

** Fertility (%) = (number of fertilized eggs/number of eggs set) ×100

***Hatchability of fertile eggs (%) = (number of hatched chicks/total number of fertile eggs) ×100

**** Hatchability of set eggs (%) = (number of hatched chicks/total number of set eggs) ×100

Table 3. Effect of different light applications on responses of open field test*

	Dark	Green	Blue	P
First movement time, sn	35.16 ± 7.37	33.76 ± 7.88	19.78 ± 7.59	0.288
First call time, sn	20.4 ± 4.32	23.66 ± 4.9	19.69 ± 4.68	0.824
First defecation time, sn	157.00 ± 21.6 ^a	106.66 ± 25.92 ^{ab}	81.09 ± 19.14 ^b	0.044
First jump time, sn	104.16 ± 17.5	67.37 ± 21.43	123.61 ± 23.78	0.200
Total number of frames visited pieces	67.4 ± 12.9	62.88 ± 13.41	75.25 ± 12.67	0.793
Total number jumps, pieces	8.25 ± 1.68	8.00 ± 2.06	6.23 ± 2.29	0.766
Total call number, pieces	23.07 ± 1.83	17.42 ± 2.07	18.26 ± 1.98	0.086

^{a,b} Means with different superscript letters in in rows differ significantly (P < 0.05)

*The table gives averages values with standard error

a 75-hour period. Blue light application reduced embryo mortality and increased hatchability compared to dark and green light applications. Studies have shown that such illumination studies conducted with eggs from different species are also effective in the results (Shavey *et al.*, 2004; Cooper *et al.*, 2011).

It has been determined that different lighting applied to incubators not only positively affects the development of the embryo, but also affects stress factors (Archer and Mench, 2013). In this study, an open field test was applied to chicks that completed their development and hatched in environments with different colours. When Table 3 was examined, it was determined that the time to first defecation was shorter in the blue group. It is thought that this creates negative stress for the animal. In a different study, the relationship between the number of defecations and timidity in rats was examined. And it was concluded that taking animals from their accustomed environments and placing them in a new environment (separating from the group or herd and leaving them in an open area) causes anxiety (Çalışkan *et al.*, 2017).

As a result, it is thought that a more detailed investigation of the effects of different lighting applications on stress and productivity in partridges will be economically important for businesses.

Ethical statement

This research was approved by Akdeniz University, Experimental Animals Application and Research Center local ethics committee for animal experiments.

Funding information

This study was supported by The Scientific and Technological Research Council of Turkey (Tübitak) within the scope of University Students Research Projects Support Program. Project number: 2209/A

Note: The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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Alternative Application for Fumigation: Ozone Treatment During Incubation*

Bilgehan YILMAZ DİKMEN^{1,*} , Arda SÖZCÜ¹ , Aydın İPEK¹ 

¹Bursa Uludağ University, Faculty of Agriculture, Animal Science Department, 16059 Bursa, Türkiye

*Presented In: International Poultry Congress 2024, Bursa, Turkey

Article History

Received: Oct 18, 2024
Accepted: Dec 02, 2024
First Online: Dec 05, 2024

*Corresponding Author

Tel: +902242941569
E-mail: bilgehan@uludag.edu.tr

Keywords

Ozone
Incubation
Fumigation
Eggshell microbial load
Broiler

Abstract

In this study it was aimed to investigate effects of ozone treatment during incubation of broiler eggs as an alternative application for fumigation. A total of 240 eggs from 45-week-old Ross 308 broiler breeder flocks were utilized. The eggs were weighed and randomly assigned to two groups: one without ozone and one with ozone. A commercial ozone generator was placed in the incubator, with ozone gas generated for 1 minute every hour to provide ozone gas at a concentration of 0.050 ppm. Throughout the 18-day incubation phase, ozone gas was applied in three-day cycles. On the 18th day of incubation, six randomly selected eggs from each experimental group were placed in sterile bags to assess the microbial load of the eggshell. Furthermore, six eggs were randomly selected from each experimental group to assess embryo growth. The total aerobic bacteria and Coliform sp. count were found higher in control than ozone group ($P < 0.01$). The yeast mold count, egg weight, embryo weight and embryo yolk sac weight were found to be similar between the groups ($P > 0.05$). It can be determined that using ozone instead of fumigation during incubation in broiler chicken eggs reduces eggshell total aerobic bacteria and coliform counts, but not affected to embryo traits such as weight, yolk sac weight and length.

Introduction

In poultry, from the moment the egg is laid until it is incubated, it is exposed to many environmental conditions and interventions such as collection, transportation and storage, and the eggshell can be contaminated by microorganisms. As a matter of fact, it has been emphasized in various studies that many different microorganisms such as *Salmonella*, *Streptococcus*, *Escherichia coli*, *Staphylococcus* and *Yersinia* can be found on eggshells (Jones *et al.*, 2004; Musgrove *et al.*, 2008; Koç, 2015). Microorganisms that multiply under incubation conditions quickly enter the egg through the pores on the eggshell, damaging the developing embryo and reducing success in incubation

(Berrang *et al.*, 1999). Therefore, sanitation is an extremely critical and necessary issue in hatching eggs. Many different methods are used for sanitation of hatching eggs. Among these include fumigation with formaldehyde gas (Ledoux, 2002), immersion in disinfectant solutions, and spraying with disinfectant solutions (Moats, 1981). Among these methods, especially the use of disinfectant solutions causes wetting of the eggshell and therefore an increase in bacterial permeability, and the fumigation method with formaldehyde gas has decreased in use due to its toxic and carcinogenic effects. Therefore, in recent years, emphasis has been placed on alternative methods

for sanitation of hatching eggs. Among these; use of UV light (Al-Shammari *et al.*, 2015), application of colloidal silver to eggshells (Batkowska *et al.*, 2017) and use of various natural/herbal extracts (Uluçay and Yıldırım, 2010; Çopur *et al.*, 2011; Batkowska *et al.*, 2018). In recent studies, the use of ozone gas for sanitation has been mentioned (Wlazlo *et al.*, 2020; Gogaev *et al.*, 2021). One well-known very reactive antibacterial agent is ozone (O₃). Ozone is in gaseous form at room temperature and is colorless and has a distinctive odor. FDA accepted ozone application as an antimicrobial agent for foods in 2001. Ozone has other benefits in addition to its bactericidal action, such as low toxicity and ease of handling (Braun *et al.*, 2011). The embryo development is an important factor. Therefore, we applied the ozone treatment as continuously during the 18 day of incubation period in 3 days-cycle to bring to light effects on embryo development, as stimulating effect or inhibiting effect. This study was conducted to investigate effects of ozone treatment during incubation of broiler eggs as an alternative application for fumigation.

Materials and Methods

This study was carried out in Bursa Uludağ University Faculty of Agriculture Research and Application Unit. Practices regarding the care and use of animals for research purposes were in accordance with the laws and regulations of Turkey and approved by the Animal Use and Ethical Committee of Uludağ University (Approval Number 2023-12/02). A total of 240 eggs obtained from 45-week-old Ross 308 broiler breeder flocks were used in the study.

The non-fumigated eggs were weighed and randomly divided into two groups: the group without ozone (control group) and the group with ozone (n: 3 trays, 40 eggs/tray). The eggs were placed into two incubators with the same features, which were calibrated before the experiment. A commercial ozone generator placed in the incubator and ozone gas activated for 1 minute per hour and provide ozone gas at the level of 0.050 ppm. Ozone gas was applied in 3-day cycles during the 18-day incubation period. During this period, a temperature of 37.2-37.5°C and 55% of relative humidity were applied in incubators.

On the 18th day of incubation, randomly selected six eggs from each experimental group were sampled to determine the microbial load of the eggshell. Egg samples were placed in sterile containers containing 50 mL of phosphate buffered saline solution. Serial dilutions of samples in phosphate buffered saline were placed on sterile substrates to obtain total aerobic bacteria, coliforms, yeast-molds (Gentry and Quarles, 1972; Jones *et al.*, 2002). After the incubation period, colonies were counted, and the result was expressed as colony forming unit (CFU)/1 mL of egg liquid. The plate count agar, violet, red bile agar, potato malt agar was

used for total aerobic bacteria, coliforms, yeast-molds count respectively. On the 18th day of incubation, besides, six eggs were randomly sampled from each experimental group for determination of egg weight at transfer, embryo weight, embryo yolk sac weight and embryo length. The embryo length was measured from the tip of the beak to the tip of the longest toe by placing the embryo face down on a flat surface and straightening the left leg. The embryo parameters were determined by the formula given below;

$$\text{Embryo ratio (\%)} = \frac{\text{Embryo weight without yolk sac} \times 100}{\text{Egg weight}}$$

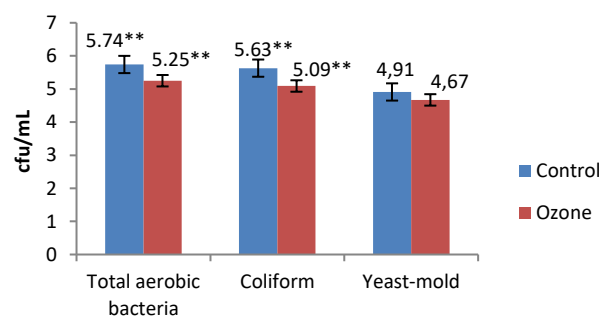
$$\text{Embryo yolk ratio (\%)} = \frac{\text{Embryo yolk sac weight} \times 100}{\text{Egg weight}}$$

Statistical analysis

The study was conducted on a completely randomized design and data was analyzed by analysis of variance using General Linear Models (Minitab, 2013). Analysis of percentage data were conducted after arcsine square root transformation of the data. Differences in investigated traits were analyzed by two sample T-test (Minitab, 2013). Data were presented as mean \pm standard error in all the tables. Differences were considered significant at $P \leq 0.05$ and the statistical difference at $P < 0.10$ was described as a tendency.

Results

The effect of ozone application on eggshell microbial load at 18 days of incubation are given in figure 1. The total aerobic bacteria count was found higher in control group than ozone group (5.74 versus 5.25 cfu/mL respectively; $P < 0.01$). The number of *Coliform sp.* was found higher in control group than ozone group (5.63 versus 5.09 cfu/mL respectively; $P < 0.01$). The yeast mold count was found similar between the groups ($P > 0.05$).



Bacterial count of eggshell at 18 day of incubation

Figure 1. The effect of ozone application on eggshell microbial load

The effect of ozone application on embryo parameters at 18 days of incubation are given in Table 1. The egg weight, egg weight at transfer, embryo weight, embryo yolk sac weight, embryo length, embryo ratio and embryo yolk ratio were found to be similar between the groups ($P > 0.05$). But egg weight at transfer was numerically lower in ozone group than control group ($P=0.069$). The embryo length was numerically higher in ozone group than control group ($P=0.082$).

Table 1. The effect of ozone application on embryo parameters

Embryo parameters	Control	Ozone	P - Value
Egg weight, g	68.84 ± 0.39	68.77 ± 0.29	0.802
Egg weight at transfer, g	63.43 ± 0.48	62.32 ± 0.24	0.069
Embryo weight, g	28.26 ± 1.81	30.28 ± 0.37	0.202
Embryo yolk sac weight, g	17.46 ± 1.37	15.74 ± 1.49	0.237
Embryo length, cm	14.00 ± 0.10	14.98 ± 0.50	0.082
Embryo ratio, %	44.55 ± 2.53	48.57 ± 0.52	0.115
Embryo yolk ratio, %	27.54 ± 2.36	25.26 ± 2.40	0.324

Discussion

Braun *et al.*, (2011) suggested that ozone gas is suitable for treatments in hatcheries. Thus in the study, total aerobic bacteria and *Coliform sp.* count were higher in control group than ozone group. Similar to our findings Koç and Aygün (2021) reported that the 7 ppm ozone application reduced total aerobic mesophilic bacteria on egg shell. But, they also reported that 1, 3, 5 ppm ozone application did not have any effect on total aerobic mesophilic bacteria on egg shell. However, Rodriguez-Romo and Yousef (2005) reported that after ozone application reduction in egg shell microbial load. And, Whistler and Sheldon (1989) reported that the application of ozone gas to eggs reduced the number of *E. coli*, *Pseudomonas*, *Fluorescens*, *S. typhimurium*, *Proteus* species and *Aspergillus fumigatus* species on the egg shell. However, Bailey *et al.* (1996) found that after treatment with 0.2-0.4ppm ozone, 90.9% of egg shells remained contaminated with *Salmonella* until hatching. In the study there was no any differences for yeast mold count og egg shells between the groups.

In the study, embryo length was tend to be higher in ozone group than control group. Thus, Gogaev *et al.* (2021) discovered that the weight and length of quail embryos in incubated eggs treated with ozone for 20 minutes at a dosage of 10 mg/m³ were considerably greater than the control, 10- and 30-minute treated groups. However, in the study there were no any differences for egg weight, embryo weight, embryo yolk sac weight, embryo ratio and embryo yolk ratio between the groups. Thus, according to Fuhrmann *et al.* (2010) lower than 50 mL L⁻¹ ozone dosages resulted in oxidative reactions at the egg surface, which are likely innocuous to the developing embryo. Ozone exhibits dosage

dependence. The survival and development of chick embryos exposed to ozone in ovo are significantly influenced by their impact and reaction time (Hoffman *et al.*, 2005). Oxygen is typically depleted from the embryo during egg incubation. As ozone disintegrates into its constituent atoms, ideal conditions for embryonic development are created (Gogaev *et al.*, 2021). Low ozone levels (10 ppm) totally damaged the egg cuticle proteins, according to Fuhrmann *et al.* (2010). More water escapes the eggshell through its pores if the cuticle layer is compromised (Peebles, 1998). Thus, in the study, egg weight at transfer was tend to be lower in ozone group than control group. It may also be due to the use of very low ozone doses in our study. However, Koç and Aygün (2021) reported that there was no any differences for egg weight and transfer egg weight between the ozone groups and control, but the transfer egg weight was numerically lower in the 7 ppm ozone application group than control and 1, 3, 5 ppm ozone groups.

Conclusion

Ozone has considerably decreased the amount of microorganisms on egg surfaces. It can be concluded that ozone application (0.050 ppm) as an alternative to fumigation during incubation in broiler chicken eggs is effective in reducing of eggshell microbial load without harmful effect on embryo development. However, it should be recommended to perform extended studies to investigate the various application ways of ozone in egg fumigation with considering effecting mechanism related to embryo development and hatchability.

Ethical Statement

This study was approved by the Bursa Uludağ University Animal Experiments Local Ethics Committee (Approval no: 2023-12/02).

Funding Information

The authors received no specific funding for this work.

Presented In: International Poultry Congress 2024, (8-11 May 2024) Bursa, Turkey

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