PROOF RESEARCH PAPER



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

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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 $/m^2$, 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 4week 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:

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 fortythird 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 (Sekeroğlu 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; Daghir 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 fracturefracture 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 comsumption

Table 4 presents the feed comsumption (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).

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	93.6ª	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

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

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	66.2ª	64.1	66.5ª	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; Onbaşılar *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.

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