

## The Effect of Feather Condition on Blood Parameters and Egg Quality Traits in Brown Commercial Laying Hens Housed in Enriched Cages

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### Abstract

The study's objective was to investigate the effect of feather condition on blood and egg quality traits of laying hens. A total of 45 Lohmann brown laying hens were randomly selected at 60 weeks of age and grouped based on total feather scores obtained from scoring 6 different body regions of the birds: the head, neck, breast, back, wings, and tail. According to the total feather score, the experimental groups consisted of low feather score (6 - 12, LFS), moderate feather score (13 - 17, MFS), and high feather score (18 - 24, HFS). Blood samples were collected from 15 hens per feather score group at 61 weeks of hen age. Consequently, the number of lymphocytes, monocytes, heterophils, eosinophils, basophil cells, and heterophil/lymphocyte (H/L) ratio was determined. Quality traits of 13-14 eggs from each feather score group were analyzed at 68 weeks of hen age. The results indicated that feather condition significantly influenced monocytes, and heterophils ( $P<0.01$ ), and the impact on H/L ratio nearly reached significant levels ( $P=0.060$ ). However, the impact on lymphocytes and eosinophils was not significant ( $P>0.05$ ). There were differences in the egg weight, redness of eggshell, shape index, and shell thickness among the feather score groups ( $P<0.01$ ;  $P<0.05$ ). Shell-breaking strength, albumen index, Haugh unit, yolk index, and yolk color score were similar among the feather score groups ( $P>0.05$ ). In conclusion, this study revealed slight feather condition-induced changes in stress responses in birds. Furthermore, while the feather condition can modulate some external egg traits, it does not lead to variations in internal egg quality traits. However, further studies over a longer duration are required to refine these findings.

**Keywords:** Laying hen, Feather score, Blood parameters, Stress response, Egg quality traits

### Introduction

The enriched or furnished cages were developed to enhance the well-being of laying hens, following the criticism of the quality of life of hens in conventional cages (Lay et al., 2011; Tainika and Şekeroğlu, 2020). However, Lay et al. (2011) emphasized that every system has its limitations in ensuring a perfect welfare-friendly environment for the birds. To this day, several protocols and approaches have been developed and validated to assess the welfare of hens (EFSA, 2023). Among them, blood parameters, for instance, the heterophil/lymphocyte (H/L) ratio is a significant physiological indicator for stress response in birds and is increased with several stressors (Gross and Siegel, 1983; Scanes, 2016). The H/L ratio has a genetic basis and varies among genotypes reared in cages (Singh et al., 2009). Additionally, Cotter (2015) indicated differences in the H/L ratio in caged hens, which variations might be linked to the environmental changes in the different cage types.

The other approach is the utilization of plumage or feather condition scores (FCS) which are usually determined by a 4-point scoring scale (Tauson et al., 1984). The 4-point feather scoring is based on feather density and a total of scores obtained from scoring the 6 body regions of the bird: neck, breast, cloaca, back, wing, and tail. FCS is a significant physical indicator of hen health and the behaviors expressed within a flock, especially associated with feather pecking (Campe et al., 2018; Janczak & Riber, 2015). The internal stimuli such as aging and genotype of hens are some of the important factors affecting FCS (Albentosa et al., 2003; Tok et al., 2022; Sokołowicz et al., 2023; Tainika et al., 2024). Additionally, FCS can reflect the effect of environmental factors on the birds. For example, Erensoy et al. (2021) and Tok et al. (2022) reported greater feather loss with increasing cage stocking density.

Furthermore, consumer preferences for eggs focus on the traits related to the shell (external egg quality variables) and those linked to the albumen and yolk (internal egg quality variables). Multiple factors influence the quality variables of eggs (Rakonjac et al., 2015; da Silva Pires et al., 2021). Also, cage system properties such as cage tier and direction can influence some egg quality traits in hens (Akkuş and Yıldırım, 2018; Şekeroğlu et al., 2024). While the influence of internal and external stimuli on the H/L ratio and egg quality of hens in cages is fully established, the relationship between feather score, and stress response and egg quality in hens has not been fully investigated.



It is argued that the differences in feather condition would result in variations in the bird's response to the environment. This would consequently impact the bird's physiological mechanisms, leading to differences in the H/L ratio and egg quality traits. Therefore, this study examined the effect of feather condition on blood parameters and egg quality in the Lohmann brown laying hens.

## Materials and methods

### Animal materials and housing

This research was carried out at the laying unit of Niğde Ömer Halisdemir University Ayhan Şahenk Agricultural Research Application and Research Center. This study was approved by the Animal Experiments Local Ethics Committee of Niğde Ömer Halisdemir University, Ayhan Şahenk Agricultural Research Application and Research Center (Decision no: 2023/08).

In this study, animal materials comprised Lohmann brown laying hen hybrids. The initial feather condition was evaluated at 61 weeks of age, using a 4-point scoring scale as described by Tauson et al. (1984). While score 1 is for the lack of feathers, score 4 shows perfect feathering. The score of the 6 different body regions was determined and thereafter, the total feather score of each hen was calculated, with a total score of 24 as the maximum. A total of 45 birds were sampled, 15 birds for each group. Group 1 involved poor feathering birds, with a score of less than 12 (low feather score, LFS). Group 2 consisted of moderate feathering, with a score between 13- 18 (moderate feather score, MFS). Group 3 comprised high feathering, with a score of >18 (high feather score, HFS). The hens in each group had been previously raised in the commercial poultry unit from 16 weeks of age in pens comprising 20 birds/ cage unit.

Based on the feather score groups, the birds were then housed in the first three cage units of the second cage tier. There was an egg conveyor belt and a feeder in front of the cage tier. The cages were made from galvanized wires. Each cage unit size measured 240 cm in length, 63.5 cm in width, and 60 cm in height, and was furnished with a stainless-steel nipple drinking system, and two parallel perches, each measuring 180 cm. The perches were equipped with a claw-shortening device. Additionally, each cage unit was enriched with a dark blue curtained nesting area, measuring 40 cm in length, 33.5 cm in width, and 30 cm in height, a scratch pad, and a wire floor.

The photoperiod of 16 hours light:8 hours darkness was offered to the birds, between 06:00 - 22:00. 24-W led bulbs (20 lux -3.2w/m<sup>2</sup>) were the light sources. Standard concentrate feed (2. period layer feed) purchased from a commercial firm was offered to the birds during this period. The nutrient content of the feed used in the study is shown in Table 1. Feed and water were provided *ad libitum*.

Before the blood samples were collected at 61 weeks of hen age, the plumage condition of birds in their respective were again scored using a 4-point scale (Tauson et al., 1984), and the data is indicated in Table 2. During the second scoring, both the feather score groups were recorded, and the accuracy of the group distribution was checked. On both occasions, scoring was done by the same observer, thus, avoiding significant differences in the scores.

**Table 1.** The nutrient composition of feed provided to the hens

Ingredients	%
Crude Protein	15.6
Crude Cellulose	4.4
Ash	12.4
Crude oil	3.8
Calcium	3.83
Phosphorus	0.35
Sodium	0.16
Lysine	0.76
Methionine	0.37
Metabolic Energy	2720 Kcal/Kg

**Table 2.** Feather score of different body regions laying hens 7 days after grouping based on feather density.

Group	Neck	Back	Tail	Cloaca	Breast	Wing	Total
LFS (6-12)	1.33 <sup>a</sup>	2.13 <sup>a</sup>	1.40 <sup>a</sup>	1.47 <sup>a</sup>	1.67 <sup>a</sup>	2.00 <sup>a</sup>	10.0 <sup>a</sup>
MFS (13-17)	2.07 <sup>b</sup>	3.07 <sup>b</sup>	2.67 <sup>b</sup>	2.53 <sup>b</sup>	2.33 <sup>b</sup>	2.73 <sup>b</sup>	15.40 <sup>b</sup>
HFS (18-24)	2.40 <sup>b</sup>	3.60 <sup>c</sup>	3.33 <sup>c</sup>	3.80 <sup>c</sup>	2.73 <sup>c</sup>	3.00 <sup>b</sup>	18.87 <sup>c</sup>
SEM	0.079	0.081	0.074	0.072	0.078	0.069	0.186
p-value	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***	0.000***

Abbreviations; LFS: low feather score, MFS: moderate feather score, HFS: high feather score, SEM; standard error of mean; Means within the same column with different letter superscripts significantly differ (\*\*\*) p < 0.001).



### **Blood sample collection and Blood cell count**

Blood samples were collected from a total of 45 hens, 15 per feather condition score group from the wing vein and 2 cc sterile syringes were used for this purpose. Approximately 1 drop of blood was taken and dropped on the base or smear slide to ensure a thin blood smear was drawn. This was obtained by running the drop of blood on the smear slide thinly using another slide, which is called a spreader slide. The process started by ensuring that the drop of blood on the smear slide was run to form a thin line between the smear slide at an angle of 30 - 45 ° C with the spreader slide as presented.

Afterward, the blood samples, labeled with a pencil were taken to Niğde Ömer Halisdemir University, Faculty of Medicine for staining and blood cell counting. The staining process was carried out using May-Grunwald and Giemsa (Lukas 1961). Briefly, during the staining process:

- Blood smear slides were kept in methyl alcohol for 2 minutes and 45 seconds.
- The smear slides were removed from methyl alcohol and allowed to dry.
- Dried smear slides were then kept in May Grunwald for 3 minutes.
- The smear slides were then dipped in distilled water or tap water 3-4 times and removed.
- The smear slides were then kept in Giemsa solution (1ml of Giemsa 9ml of water) for 16 minutes.
- Lastly, the smear slides taken out of Giemsa were again immersed 3-4 times in water, removed, and left to dry.

In the mounting process, 1-3 drops of balm or entellan mounting media were added to the smear slide using an entellan injector depending on the slide size to be mounted on the blood smear slide. The mounting was done slowly so that an air gap was not left, or the adhesive did not form a layer on the slide and dry.

After the staining process, blood cell counting was done in the same place. In this procedure, lymphocytes, monocytes, heterophiles, eosinophils, and basophils were counted as blood parameters. First, the blood smear slides were examined under the microscope at a 40x setting, and the areas where blood cells were found were determined. Then, the microscope was adjusted to 100x, and blood cells were counted starting from the far right or left most of the blood smear, and again from the top or bottom, depending on preference. The counted cells were recorded in order. The counting of blood cells was completed when a total of 100 cells was reached. The H/L ratio was determined according to Gross and Siegel (1983).

### **Egg quality analyses**

A total of 40 eggs, approximately 13-14 eggs per feather condition score group were analysed at 68 weeks of hen age. All the egg samples were collected on the same day and analysed after 24 hours of storage at room temperature as described by Altan (2015) and Şekeroğlu et al. (2024).

**Egg weight (EW, g):** a scale with an accuracy of 0.1 grams was used to measure the weight of eggs.

**Shape index (SI, %):** Egg width (EW, mm) and egg length (EL, mm) were measured using a digital caliper. The shape index was calculated with the formula;  $SI = (EW/EL) \times 100$ .

**Shell color:** was based on the average of three measurements obtained from blunt, center, and pointed egg regions. Each region was determined for lightness (L\*), redness (a\*), and yellowness (b\*) for each region was taken with a Minolta CR400 chromameter (Konica Minolta Sensing Inc., Osaka, Japan). Color differences ( $\Delta E^*$ ) were determined by the L\*, a\*, b\*, and indicated as the color of an egg (Ingram et al., 2008).

**Shell-breaking strength (Kg. force):** was taken by an Egg force reader (orka food tech. FGV-10XY (5.000 kg) EFO493/2013).

**Shell thickness (mm):** was determined as the average of shell thickness values obtained from three different regions of an egg, using a metrica manual micrometer (0.01mm). The shell thickness values at the blunt, center, and pointed portions of eggs were measured on shells without the shell membrane.

Thereafter, each egg sample was broken on a special glass table to measure internal egg quality variables. Here, after waiting for 10 minutes, the albumen height (AH) and yolk height (YH) were measured with a tripod 0.01 mm sensitivity manual micrometer. The albumen length (AL), albumen width (AW), and yolk diameter (YD) values were taken by a digital caliper (0 – 150 mm). The following formulas were used to calculate the albumen and yolk index, AI and YI, respectively, and the Haugh unit.

**AI, %** = ((AH (mm)) / ((AL (mm) + AW (mm))/2)) × 100.

**YI, %** = (YH (mm) / YD (mm)) × 100.

**HU** = 100 × log (AH (mm)+7.57 – 1.7 EW 0.37) AH: observed albumen height (mm) (Haugh, 1973).

**Yolk color:** was scored by DSM yolk color fan.

### **Statistical analysis**

The normality assumption of the data was found to provide the normality assumption of the data, except for the feather score, which was examined with the Kolmogorov Smirnov test ( $p>0.05$ ). According to these conditions, it was determined that the data other than the feather score were suitable for analysis of variance, and statistical analyses were performed according to one-way analysis of variance (One-way ANOVA). Differences between groups were determined by Duncan's multiple comparison test. The Mann-Whitney U test was used to analyze the data of the feather score. However, since there was no difference, no multiple comparison test was used. SPSS package program was used in the analysis of the data. The difference was accepted as statistically significant at  $P<0.05$ .



## Results and discussion

Recently, the increased public concern for the welfare of hens has influenced the laying hen housing systems in many developed countries. Welfare issues such as stress and fear responses and feather damage are still critical to the poultry industry. The H/L ratio is widely used as a physiological indicator of stress responses in birds (Dinçer et al, 2011; Scanes, 2016; Scanes et al., 2019). Table 3 indicated that feather condition had a significant influence on monocytes and heterophils ( $P < 0.009$ ), and the effect on H/L ratio approached a significant level ( $P = 0.060$ ).

**Table 3.** Blood parameters (%) of laying hens based on feather score

Group	Lymphocytes	Monocytes	Heterophils	Eosinophils	Basophils	H/L ratio
LFS (6-12)	50.73	18.87 <sup>a</sup>	25.20 <sup>b</sup>	2.67	0.33	0.51
MFS (13-17)	55.33	21.07 <sup>a</sup>	22.73 <sup>b</sup>	2.27	0.80	0.43
HFS (18-24)	53.27	26.93 <sup>b</sup>	16.53 <sup>a</sup>	3.00	0.27	0.33
SEM	1.047	0.727	1.121	0.404	0.114	0.029
P-value	0.211	0.000***	0.009***	0.761	0.128	0.060

Abbreviation: SEM; standard error of mean, LFS: low feather score, MFS: moderate feather score, HFS: high feather score. Means within the same column with different letter superscripts significantly differ (\*\*\*) ( $P < 0.001$ ).

The monocytes and heterophils increased and decreased, respectively with the increase in feathering of birds. The H/L ratio increased with the decrease in feather score. However, the impact of feather condition on lymphocytes, and eosinophils was not significant ( $P > 0.05$ ). This study is in partial agreement with Campo et al. (2001), who reported a higher H/L ratio for poor feathering compared to perfect feathering hens. It can be argued that the slight variations in results might be connected to the differences in age when blood samples were taken, genotypes, handling of birds, study places, housing system, etc.

Furthermore, Gross and Siegel (1983) reported that stress is considered low when the H/L ratio is about 0.2, moderate when it is 0.5, and high when it is 0.8. In the present study, it was determined that the H/L ratio of the high feathering group was 0.33. Thus, the stress response of the high feathering group was low (value close to 0.2) compared to the H/L ratio of the low feathering group with the highest value of 0.51, showing moderate stress. Generally, the findings of this study would highlight that poor feathering birds might indeed be more stressed than high-feathering hens.

Notably, the mechanisms underlying the effect of feather condition on some blood parameters need to be fully investigated. Furthermore, the birds that originated from different cage tiers of the enriched were mixed to form the different feathering groups. Therefore, the results of this study might speculate the potential of hens to cope with the new environment and flock mates after the separation.

Studies have found an inverse relationship between feather condition and performance or egg production traits (Bentsen, 1983; Hagger et al., 1989; Glatz, 1998; Yamak and Sarica, 2012; Taşkın et al., 2015). Feather damage is of economic importance in chicken production especially due to its influence on feed intake which in turn impacts egg quality. Meanwhile, the association between feather condition and egg quality traits is not fully investigated. In the present study (Table 4), egg weight ( $P < 0.05$ ), redness of eggshell ( $P < 0.05$ ), shape index ( $P < 0.01$ ), and shell thickness ( $P < 0.05$ ) were significantly affected by feather condition.

**Table 4.** Effect of feather score on egg quality traits in laying hens based on feather score

Feather score	Egg weight (g)	L*	a*	b*	L*-a*-b*	Shape index (%)	Shell breakin	Shell thickness (mm)	Albumen index (%)	Haugh unit	Yolk index (%)	Yolk color (COXAS)	Albumen pH
n	40	40	40	40	40	40	40	40	40	40	40	40	40
LFS	69.48 <sup>b</sup>	59.18	17.52 <sup>a</sup>	31.64	10.03	77.53 <sup>ab</sup>	4.13	0.2440 <sup>a</sup>	1.96	78.09	41.48	13.13	9.00
MFS	66.95 <sup>ab</sup>	59.27	19.03 <sup>b</sup>	31.85	9.06	79.10 <sup>b</sup>	4.17	0.26 <sup>ab</sup>	2.17	80.33	43.28	12.87	8.97
HFS	63.59 <sup>a</sup>	59.70	18.86 <sup>b</sup>	32.11	8.72	76.17 <sup>a</sup>	4.55	0.27 <sup>b</sup>	1.96	79.12	41.94	13.00	9.11
SEM	0.944	0.561	0.270	0.254	0.988	0.379	0.167	0.004	0.078	1.590	0.498	0.080	0.030
P	0.05*	0.936	0.030*	0.777	0.863	0.007**	0.604	0.020*	0.435	0.837	0.279	0.363	0.166

Abbreviations: n; number of eggs; LFS: low feather score, MFS: moderate feather score, HFS: high feather score, SEM; standard error of mean, L\*; brightness, a\*; redness, b\*; yellowness; The difference between the means indicated with different Letters in the same column is statistically significant (\*;  $P < 0.05$ , \*\*;  $P < 0.01$ )

Egg weight decreased with an increase in feather condition, higher in the poor feathering compared to the moderate and high feathering groups, whose values were statistically similar. The redness of the shell color was higher in the moderate feathering group compared to the poor and high feathering groups. The egg shape index was higher in the moderate feathering group compared to the poor and high feathering groups. Shell thickness increased with the increase in feather condition scores.



The influence of feather condition on some egg quality traits is probably linked to the individual variability in the ability of hens to cope with the challenges of feather scores. These variabilities can be related to the association between feed intake and feather condition. However, the lack of significant differences in the shell-breaking strength and internal egg quality parameters among feathering groups shows that individual differences within a strain in coping with the challenges of feather loss seem insignificant for these traits.

## Conclusions

This study was able to reveal that feather condition does influence stress response in birds. In addition, there might be feather condition-induced variations in external egg quality traits. It is suggested that further studies are warranted to explore the association between feather loss and stress response and egg quality traits.

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