

ANIMAL WELL-BEING AND BEHAVIOR

The effect of an enriched laying environment on welfare, performance, and egg quality parameters of laying hens kept in a cage system

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ABSTRACT The aim of this study was to evaluate the welfare and performance of laying hens kept in a furnished cage system equipped with additional feeders. A total of 72 Lohmann Brown hens were randomly assigned to 4 experimental groups. Each group consisted of 6 cages housing 3 birds per cage (18 birds per group). Group I was a control group without an extra feeder in the cages. Experimental groups GII, GIII, and GIV contained one, 2, and 3 additional feeders in the cages, respectively. The assessment of bird welfare was based on production, physiological and behavioral parameters, as well as on the basis of external appearance. The experiment lasted 12 wk. The obtained results suggest that enriching laying hens' cages with additional feeders improved the welfare of the hens. Enrichment of cages significantly reduced the

number of feather pecking and aggressive behaviors in the GII and GIV groups ($P < 0.01$) and the GIII group ($P < 0.05$). Breast plumage was significantly ($P < 0.05$) better in the GII group compared with that in the control group. The control group also had the worst general plumage ($P < 0.01$). The GII and GIV groups were also characterized by significantly ($P < 0.05$) lower blood corticosterone concentrations compared with the control group. No negative changes in egg production and quality parameters were observed in the experimental groups. Only eggs from the GIV group had significantly ($P < 0.05$) lower breaking strength than those from the control and GII group. The results suggest that the best solution is to place 1 additional feeder in furnished laying hens' cages.

Key words: hen, welfare, behavior, corticosterone

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INTRODUCTION

Today's egg production, apart from maintaining high production parameters, should also focus on providing the hens with an appropriate level of welfare. This is mainly because of the growing awareness of consumers about the welfare of livestock. At the center of the welfare controversy is housing laying hens in conventional cage systems (Yilmaz Dikmen et al., 2016).

Conventional cage systems were developed in the 1930s with the main goal to maximize the profit and productivity of egg laying birds by keeping a large number of hens in a small area (Sosnowska-Czajka et al., 2010; Jones et al., 2014; Yilmaz Dikmen et al., 2016).

However, in the 1960s, animal welfare became a growing concern, and conventional cage systems were questioned because they were believed to restrict movement of the birds and have a negative impact on certain important patterns of behavior (Mench et al., 2011). This situation led to the development of furnished cages in the 1980s, which differ from conventional ones in that they provide more space for birds (750 cm² vs. 550 cm² per hen) and are equipped with a perch, nest, and scratcher (Lay et al., 2011). In 2012, public concern led the European Union to ban the use of conventional cage systems and allowed only furnished cages, aviaries, and free-range and organic egg laying systems (EU Directive 1999/74/EC). However, furnished cages also restrict the hens' opportunity to express their full behavioral repertoire, and therefore, animal welfare organizations are fighting to ban these systems as well (Mench et al., 2011). Despite public concern, caged laying hen systems present many advantages such as keeping hens in smaller groups and maintaining higher levels of hygiene (Appleby et al., 2002).

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The aim of this work was to enrich the environment of laying hens kept in furnished cage systems with additional feeders and subsequently assess their welfare on the basis of production, behavioral and physiological parameters, as well as on the basis of external appearance.

MATERIALS AND METHODS

Laying Hen Population and Experimental Design

The research was carried out in an experimental hen-house at the Agricultural Experimental Station "Swojec" in Wrocław, Poland. A total of 72 Lohmann Brown laying hens (23 wk of age) were used for the study. The hens were kept in a furnished cage system under controlled microclimatic conditions (average temperature = 18°C; RH = 65%) with a light regimen of 14 h light and 10 h dark. Hens were allocated to 24 cages (3-tier battery system) and divided into 4 experimental groups consisting of 6 cages with 3 hens per cage (a total of 18 hens per group). The battery system is located in the middle of room. The cages are located on the right and left side of the battery system. Each replication consisted of 3 cages from the first, second, and third floor on the right and left side of the battery system. The surface of each cage was 3,750 cm². Each cage was equipped with a perch, nest, scratcher, feeding trough (20 cm long per hen), and 2 nipples. The hens were randomly assigned into each of the 4 experimental groups. Group I (**GI**) was a control group without an additional feeder in the cages. Experimental groups GII, GIII, and GIV contained 1, 2, and 3 additional feeders in the cages, respectively. All feeders had a capacity of 200 g and were placed above the main trough. All hens were fed once a day with the same amount of feed (120 g/hen). However, in the experimental groups, feed was distributed between the main trough and the additional feeders in the system. In the GI group, 360 g of feed was distributed only in the main trough. In the GII group, 180 g of feed was provided in the main trough and 180 g was provided in the additional feeder. The GIII group had 120 g of feed provided in the main trough and 120 g in each of the additional feeders. Finally, the GIV group received 90 g of feed in the main trough and 90 g in each additional feeder. During the 12-week experimental period, all birds were fed with the same diet based on a complete feed mixture from Tasomix (16.52% CP, 2,700 kcal/kg ME, 0.55% P, 1.8% Ca).

Performance and Egg Quality Parameters

Throughout the experiment, all groups were monitored for egg production. Eggs were collected and weighed daily. Egg production was determined by dividing the number of eggs laid over the course of the experiment by the number of hens in the same period (expressed as percentage of egg production). Feed intake was recorded once per week. Feed conversion ratio

(**FCR**) was calculated by dividing the feed intake by the mass of eggs. To determine thickness and breaking strength of eggshells, 72 eggs were collected randomly from each group at the end of the experiment. The eggshell thickness was determined using a micrometer. It was measured at 3 egg-measuring points (small end, large end, and equator), and the arithmetic mean of 3 measurements was taken as the final result. The eggshell breaking strength was measured by using an Egg Force Reader from ORKA Food Technology (West Bountiful, Utah, USA).

Welfare Assessment

The behavior of the hens was observed during the whole study period. One person carried out observations. A different replication (cage) was observed every day for 1 h. Therefore, each cage was subjected to observations 3 times during the experiment, and the arithmetic mean of these 3 observations was taken as the final result. The observed behaviors included comfort behaviors (balancing wing flaps, tail wagging, and bill wiping), feather pecking, and aggressive behaviors (pecking and scratching). The behaviors were summarized as a number of individual behaviors/hen/h. At the end of the experiment, 24 laying hens (35 wk of age) were randomly selected (1 hen from each cage) and evaluated based on the state of their plumage and body wounds according to method described by [Tauson et al. \(2005\)](#). As per this system, hens with higher scores have better plumage and fewer body wounds. In addition, 24 randomly selected birds (35 wk of age, 1 from each cage) were observed for their response to a tonic immobility test. The test was carried out based on the methods described by [Campo et al. \(2005\)](#) and [Ghareeb et al. \(2014\)](#). For the tonic immobility test, the hens were caught and transferred to a separate room. Individual birds were placed on the table in a supine position. The handlers held the sternum with 1 hand and the head and neck with the other. The bird was held for 10 s. After removing the hands, the stopwatch was started, and the observer retreated. If the bird remained motionless for at least 10 seconds, it was considered that tonic immobility was induced, and total duration of tonic immobility was recorded for a maximum of 600 s. If the bird moved within 10 s, it was assumed that the tonic immobility was not induced, and the procedure was repeated. The procedure was repeated a maximum of 4 times for each studied bird. At the end of the experiment (35 wk of age), the blood samples from 24 randomly selected hens (1 from each cage) were collected postmortem to determine the concentration of basal corticosterone. In accordance with the Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes, the hens were killed by percussive blow to the head and then exsanguinated ([European Parliament and Council of the European Union, 2010](#)). Then, 2 mL blood was placed

into disposable nonpyrogenic and nonendotoxic tubes. Samples were left at room temperature for 2 h and then centrifuged for 20 min at about $1,000 \times g$. The supernatant was collected and protected by placing in a freezer at -20°C . After thawing the samples and bringing them to room temperature, corticosterone determinations were made. Two measurements were made for each sample. The level of corticosterone was measured at the Biochemical Laboratory of the Department of Environment Hygiene and Animal Welfare (Wroclaw, Poland) using the absorbance, fluorescence, and luminescence reader of the BioTek Instruments, Inc. (Winooski, VT, USA) and the Chicken CORT (Corticosterone) ELISA kit from ImmunoGen (ImmunoGen, Warsaw, Poland).

Statistical Analysis

The mean, SD, and SEM were calculated for production parameters (egg production, egg mass, and FCR), egg quality parameters (strength and thickness), behavioral parameters, plumage conditions and skin injuries, tonic immobility, and the concentration of corticosterone in the blood. The normality of the data distribution was checked using the Shapiro–Wilk test. If the distribution was normal, a one-way ANOVA was performed, where the differences between the groups were assessed using the Duncan test. If the distribution was not normal, the Kruskal–Wallis test was carried out. Differences were statistically significant when $P < 0.05$ (significant difference) or $P < 0.01$ (a very significant difference). All data were analyzed using Statistica ver.13.1.

RESULTS

Performance and Egg Quality Parameters

The experimental results of the production parameters of laying hens are presented in Table 1.

Placement of additional feeders in furnished cages for laying hens did not have a significant impact on egg production. Egg mass was significantly lowest ($P < 0.01$) in the GIII group, where 2 additional feeders per cage were provided. Egg mass obtained from the GIV group was significantly lower ($P < 0.05$) than that from the GII group. The highest eggs mass was observed in the GII group, where one additional feeder per cage was provided. There is no evidence that

providing enrichment in the form of additional feeders in furnished cage systems had a significant impact on the FCR, $P = 0.188$.

The effect of additional feeders in furnished cage systems on egg quality parameters is presented in Table 2.

The results of this study have shown that equipping the furnished cage systems with additional feeders had a significant impact on the eggshell breaking strength. Eggs from the GIV group had significantly ($P < 0.05$) lower strength than those from the control and GII group. Enrichment of the cages also did not have a significant impact on eggshell thickness.

Welfare Assessment

The results of the behavioral observations are presented in Table 3.

Enrichment of studied cages with additional feeders had no impact on the hens' comfort behaviors. Providing additional feeders significantly reduced the number of feather pecking cases and aggressive behaviors in the GII and GIV groups ($P < 0.01$) and also in the GIII group ($P < 0.05$) compared with those in the control group.

The results relating to the condition of the plumage and skin injuries are presented in Table 4.

The results of the study did not show a significant effect of additional feeders on the plumage condition of the hens' necks. The results do show that the breasts of the hens from the GII group obtained a higher score than those in the control group ($P < 0.05$). There were no differences in plumage condition score on the cloaca, back, wings and tail ($P > 0.05$). The birds from the GI group scored lower on overall plumage condition than hens from the other experimental groups ($P < 0.01$). Additionally, hens from the GII group had higher plumage condition scores than hens from the GIII group ($P < 0.05$). There were no statistical differences in the number of wounds on the hens' bodies.

The results relating to the duration of tonic immobility and the level of corticosterone marked in the blood samples are presented in Table 5.

There was no significant influence of the applied treatments on the duration of tonic immobility in the studied birds ($P > 0.05$). The hens from the GII and GIV groups showed lower concentrations of corticosterone in the blood than the birds from the control group ($P < 0.05$).

Table 1. Impact of the enrichment laying environment on the production parameters of laying hens.

Parameter	GI (control)		GII (1 feeder)		GIII (2 feeders)		GIV (3 feeders)		SEM	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Egg production (%)	96.61	1.72	97.14	1.14	96.77	1.28	96.78	0.87	0.26	0.926
Egg mass (g)	62.42 ^A	4.05	62.51 ^{A,a}	4.61	59.75 ^{B,C}	3.69	61.38 ^{D,b}	3.43	0.14	0.00001
FCR (g feed/g egg)	1.95	0.17	1.90	0.04	1.97	0.07	1.89	0.07	0.02	0.188

^{a,b}Statistically significant differences between the groups for individual parameters, at $P < 0.05$.

^{A-D}Statistically significant differences between the groups for individual parameters, at $P < 0.01$.

Abbreviation: FCR, feed conversion ratio.

Table 2. The effect of additional feeders in furnished cage systems on egg quality parameters.

Parameter	GI (control)		GII (1 feeder)		GIII (2 feeders)		GIV (3 feeders)		SEM	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Strength (N)	55.25 ^a	6.50	53.92 ^{a,b}	8.85	56.67 ^a	9.01	50.85 ^b	7.68	0.75	0.0411
Thickness (µm)	0.43	0.01	0.42	0.02	0.42	0.03	0.41	0.02	0.002	0.182

^{a,b}Statistically significant differences between the groups for individual parameters, at $P < 0.05$.

DISCUSSION

The assessment of the farm animal welfare can be based on production, economic, behavioral, and physiological parameters (Kołacz and Dobrzański, 2019). The assessment of the welfare of laying hens kept in cage systems is of particular importance because this system is very controversial. The welfare of laying hens in cage systems is viewed as negative because of the large number of birds being kept in a relatively small area and the restriction on hen movement and behavioral expression. On the other hand, this system provides better monitoring of flock health (Rodenburg et al., 2008). The welfare of laying hens in cage systems can be improved by enriching the cages with nests, perches, and scratchers (Lay et al., 2011).

It is assumed that a high level of welfare has a positive effect on the production parameters of livestock animals. The results obtained from this study did not show an impact of cage enrichment, with the provision of additional feeders, on egg production. Similar results were obtained by Thogerson et al. (2009), who reported that feeder space did not affect egg production. The results of this study are consistent with the results of other studies that reported similar egg production in both conventional and furnished cage systems (Neijat et al., 2011; Yilmaz Dikmen et al., 2016).

The results of the present study showed that egg mass from cages with 2 additional feeders in the cage was significantly lower than egg mass from the other experimental groups with 1 and 3 additional feeders. Different results were obtained by Tactacan et al. (2009), who reported that the egg mass in furnished and conventional cages was similar. The same results were presented by Yilmaz Dikmen et al. (2016). Onbaşlar et al. (2015) also did not observe that the mass of eggs differed depending on the type of cage. In contrast to these results, Englmaierova et al. (2014) showed that the weight of eggs obtained from hens in furnished cages were higher

than the weight of eggs obtained from hens in conventional cages.

The results of this study did not show an effect of enrichment on the FCR of laying hens in cages. Other results were obtained by Thogerson et al. (2009), who found that reducing feeder space significantly increased feed intake and worsened FCR. Sirovnik et al. (2018) also obtained other results. They found that increasing the feeder space significantly reduced the FCR. Based on the research of other authors, it can be concluded that the feeder space has a significant impact on the FCR. This is probably because of the fact that the use of larger feeder space reduces the waste of feed. However, the results of our own research did not confirm this. This may be owing to the fact that the feed in the experimental groups was divided into several feeders, therefore, despite the theoretically larger feeder space, the waste of feed could be similar.

The results of this study have shown that cage enrichment with additional feeders had a significant impact on eggshell breaking strength. Eggs from the GIV group had a significantly lower breaking strength than those from the control and GII group. This may be owing to the fact that in the GIV group, the feed was distributed to the largest number of feeders and therefore more of it could be wasted, which affected the quality of eggs. Similar results were presented by Englmaierova et al. (2014), who showed that eggs obtained from hens kept in conventional cages were more durable and thick than eggs obtained from hens housing in furnished cages.

Poor welfare has a negative effect on animal behavior. The number of comfort behaviors is lower in animals with poor welfare; on the other hand, the number of aggressive and stereotypic behaviors increases (Kołacz and Dobrzański, 2019). There was no effect of cage enrichment on the number of comfort behaviors (balancing wing flaps, tail wagging, and bill wiping) in the studied hens. Different results were obtained by Appleby et al. (2002), who showed that cage enrichment

Table 3. The effect of additional feeders in furnished cage systems on behavioral parameters.

Behavioral parameters (number/hen/h)	GI (control)		GII (1 feeder)		GIII (2 feeders)		GIV (3 feeders)		SEM	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Balancing wing flaps	2.0	0.73	2.16	0.71	2.08	0.66	2.08	0.67	0.09	0.948
Tail wagging	1.16	0.71	1.08	0.66	1.08	0.51	1.00	0.60	0.08	0.922
Bill wiping	1.16	0.57	1.33	0.49	1.08	0.51	1.25	0.45	0.07	0.683
Feather pecking	3.08 ^{A,a}	0.66	1.83 ^B	0.57	2.00 ^b	0.60	1.91 ^B	0.51	0.11	0.0001
Aggressive behaviors	3.91 ^{A,a}	0.66	2.58 ^B	0.79	2.66 ^b	0.77	2.58 ^B	0.66	0.13	0.0003

^{a,b,c}Statistically significant differences between the groups for individual parameters, at $P < 0.05$.

^{A,B}Statistically significant differences between the groups for individual parameters, at $P < 0.01$.

Table 4. Evaluation of plumage condition and skin injuries.

Plumage	GI (control)		GII (1 feeder)		GIII (2 feeders)		GIV (3 feeders)		SEM	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Neck	3.00	0.63	3.66	0.51	3.83	0.40	3.66	0.51	0.12	0.0839
Breast	2.83 ^a	0.40	3.83 ^b	0.40	3.50 ^{a,b}	0.83	3.50 ^{a,b}	0.54	0.13	0.0407
Cloaca	3.16	0.40	3.66	0.51	3.50	0.83	3.33	0.81	0.13	0.455
Back	3.33	0.51	3.83	0.41	3.50	0.54	3.66	0.51	0.10	0.349
Wings	3.16	0.40	3.66	0.51	3.33	0.51	3.50	0.54	0.10	0.350
Tail	2.33	0.51	3.33	0.51	3.00	0.89	3.33	0.51	0.14	0.0534
Total	17.83 ^A	1.16	22.00 ^{B,a}	0.89	20.66 ^{B,b}	1.21	21.16 ^B	0.75	0.38	0.000005

Wounds	GI (control)		GII (1 feeder)		GIII (2 feeders)		GIV (3 feeders)		SEM	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Rear part of the body	2.50	0.54	2.83	0.40	2.66	0.51	2.83	0.40	0.09	0.546
Comb	2.50	0.54	2.66	0.51	2.83	0.40	2.66	0.51	0.09	0.696

^{a,b}Statistically significant differences between the groups for individual parameters, at $P < 0.05$.

^{A,B}Statistically significant differences between the groups for individual parameters, at $P < 0.01$.

caused an increase in the frequency of balancing wing flaps, tail wagging, and bill wiping. The present research showed that cage enrichment, in the form of additional feeders, reduced feather pecking and aggressive behaviors. This is likely owing to greater availability of feed and faster hierarchy formation as a result. Different results were presented by [Appleby et al. \(2002\)](#), who showed that aggressive behaviors occurred more often in hens from furnished cages in comparison with those from conventional cages. Similar results were obtained by [Sirovnik et al. \(2018\)](#), who stated that increasing the feeder space significantly reduces the number of aggressive behaviors.

The hens from the experimental groups had better overall plumage conditions than hens from the control group. In addition, hens from the group with 1 additional feeder in the cage had better feather breast plumage than those from the control groups. It probably results from the fact that the number of aggressive behaviors among birds from experimental groups was reduced. These suppositions seem to be confirmed by the other authors, for example similar results were obtained by [Abrahamsson and Tauson \(1997\)](#), who showed that hens kept in furnished cages were characterized by better plumage condition than those kept in conventional cages. Similar results were also presented by [Yilmaz Dikmen et al. \(2016\)](#) and [Blatchford et al. \(2016\)](#). Other results were obtained by [Tactacan et al. \(2009\)](#), who did not observe differences in the feathering of birds kept between conventional and furnished cages. In this study, the addition of feeders did not have an impact on the degree of skin injury observed on the hens. However, the hens from the control group were

observed to have the worst skin injury scores on the comb and rear parts of the body. This is likely owing to the smaller number of aggressive behaviors. Similar results were showed by [Abrahamsson and Tauson \(1997\)](#) that hens kept in furnished cages were observed to have a lower degree of injury to the back of the body.

Tonic immobility is a reliable indicator of the level of fear in poultry. This parameter can, therefore, be successfully used as a welfare indicator of laying hens ([Larsen et al., 2018](#)), especially when it is combined with immunologic, hematologic, or corticosterone concentrations in the blood. The results of this study have not indicated that cage enrichment with additional feeders had an impact on the duration of tonic immobility. The concentration of corticosterone was higher in the blood of hens from the control group, as compared with GII and GIV groups. This may be related to the differences in aggressive behaviors in the groups. Other results were obtained by [Thogerson et al. \(2009\)](#), who stated that changing the feeding space does not affect the heterophils/lymphocytes ratio. Similar results were obtained by [Yilmaz Dikmen et al. \(2016\)](#). These authors also found that the duration of tonic immobility was similar in conventional and furnished cages. However, hens kept in conventional cages are characterized by the highest ratio of heterophils and lymphocytes.

In summary, the results of this study provide evidence that providing additional feeders in furnished cage systems improves the welfare of laying hens. In this study, enrichment was provided in the form of additional feeders in the experimental groups. The additional feeders provided reduced the number of aggressive behaviors and feather pecking. Additional feeders had a positive effect

Table 5. Duration of tonic immobility and the concentration of corticosterone in the blood of laying hens.

Parameter	GI (control)		GII (1 feeder)		GIII (2 feeders)		GIV (3 feeders)		SEM	P-value
	Mean	SD	Mean	SD	Mean	SD	Mean	SD		
Tonic immobility (s)	216.00	57.05	161.16	45.01	176.33	27.51	177.66	26.34	8.87	0.154
Corticosterone (ng/mL)	74.32 ^a	43.41	43.69 ^b	22.25	50.36 ^{a,b}	10.45	44.59 ^b	29.25	4.42	0.0166

^{a,b}Statistically significant differences between the groups for individual parameters, at $P < 0.05$.

on the plumage and skin condition of the studied hens and also reduced stress, which is reflected in the concentration of corticosterone in the blood of the examined hens. The provision of additional feeders in the enriched caged systems also maintained high egg production and quality parameters of the hens. Based on the results of this study, it is recommended to place 1 additional feeder in caged egg laying systems. This is owing to the fact that hens from the GII group were characterized by a smaller number of feather pecking and aggressive behaviors, had better breast and general plumage, and also had lower concentration of corticosterone in the blood compared with those from the control group. Such a large number of positive effects were not found in the other groups.

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