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Examination of Nesting Behavior of Laying Hens of Different Genotypes Housed in Indoor Alternative Pens Using a Video System

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Abstract: The aim of the research was to examine how the nest selection preference of laying hens with different genotypes differed, the proportion of eggs laid in the litter, eggs laid in the upper and bottom nests, and the number and duration of nest visits. The experiment was conducted with laying hen genotypes provided by Bábolna TETRA Ltd. (Babolna, Hungary) (Commercial hybrid (C); pure-line maternal (Maternal); pure-line paternal offspring group (Paternal)). N = 318; n = 106 hen/genotype; and 53 hens/pen. We placed 53 19-week-old, non-beak-trimmed hens in each of the six 5.52 m^2 alternative pens. We provided 14 nests for the hens at two levels per pen (3.8 hens/nest). We recorded the number of eggs laid in the nests on the bottom and upper levels, as well as within the litter. Infrared cameras were installed above the pens, and we conducted recordings on a test day at the beginning of the third production month. In our results, we found a significant difference in the proportion of litter eggs overall during the 12 months of production (C. hybrid: 30.7%; Paternal: 41.1%; Maternal: 10.2%). A significant difference was found between all genotypes in the proportions of eggs laid at the bottom (B) and upper (U) level during the 12 months of production (C. hybrid: B: 72.2%, U: 27.8%; Paternal: B: 88.0%, U: 12.0%; Maternal: B: 71.4%, U: 28.6%). The evaluation of the video recordings revealed that the C. hybrid and Paternal genotypes visited the bottom nests in 97.2% and 96.0% of the cases, respectively, and the Maternal genotype individuals in a significantly lower proportion, 72.5% of the cases; the Paternal genotype spent significantly more time (13.4 min) on average in the bottom nests compared with the C. hybrid (7.9 min) and the Maternal genotypes (8.6 min). Our conclusion is that it is not enough to ensure the desired ratio of egg nests in egg production, as laying hens may not use nests in certain positions at all. This generates a relative shortage of egg nests and can increase the ratio of eggs laid in the litter, which in turn involves human health risks.

Keywords: laying hen; non-caged; non-trimmed; litter eggs; nesting behavior

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1. Introduction

Nowadays, there is an increasing need to study the behavior of laying hens, including their nesting behavior. This is partly justified by the fact that in the poultry industry, animal welfare aspects come to the fore with regard to the conditions of the laying hens [1], a central element of which the technology is the 'nest use'. The examination of the nesting tendency is also extremely important from an economic point of view, as the egg is the product that is sold commercially and from which the producers achieve sales revenue. Because the discussion concerns a basic food, the most important aspect is first the health and safe care of consumers; these health aspects are mostly influenced by where and under what exact conditions the eggs that reach our tables come from, i.e., 'which nest it comes from'.

In the European Union, a significant change related to the welfare of laying hens was formulated more than two decades ago resulted in the use of traditional cages being banned on 1 January 2012 [2]. In these prohibited, old, traditional cages, there were no nests, which could cause frustration and stress for the hens which, according to some researchers, caused the hens to develop harmful and bad habits [3]. Moreover, it also manifested itself in the retention of egg-laying [4]. Among the many provisions of the European Union directive, there was one provision that wished to support the natural behavior of laying hens by making the use of nests mandatory in various housing systems, even in the case of furnished/modified EU-compliant cages. After these developments, there is no question that the nesting behavior should be given special importance among research of the hen's natural behavioral repertoire.

The changes that affect the housing systems of laying hens may not yet be finished, as in the beginning of the 2020s, the voice of the consumer group in Europe (see the 'End the Cage Age' movement) that has demanded a ban on cage systems that are still licensed has become increasingly louder. In these alternative housing systems, the houses are group-mounted but individual nests are created, which primarily satisfy the seclusion and protection needs of the hens [5]. If these systems are ended, non-caged, i.e., other alternative housing technologies that provide more space and freedom of movement for hens will soon prevail, which will present a new challenge to egg production professionals and farmers. As someone must pay for the 'freedom' of the hen [6], increased production costs will cause price increases at every stage of the egg's journey (from producer to table), and as a result, eggs will become more expensive.

In Europe, due to the decrease in the proportion of cage housing, the 'litter egg' has appeared in alternative-non-cage-keeping technologies that also include littered scratching areas, which is completely unknown to cage keeping. Unfortunately, it must be accepted that in alternative husbandry technologies, to a greater or lesser extent, there are always litter eggs, which, depending on the contamination of the shell, are not easy to sell, not to mention the potential human health risks. All of this significantly worsens the efficiency of egg production and income generation, as well as the production of eggs that are safe for consumers. In alternative systems, in addition to a larger group size, the laying hens have more living space, have a richer behavioral repertoire, and have a much more direct contact between individuals in the group, which is why it is of particular importance to understand the behavior of laying hens with greater depth under the changed housing conditions.

To promote the development of husbandry technology systems and improvement of production efficiency, it would be useful to know why laying hens choose a specific point of the littered scratching area or the nest for oviposition (laying eggs), as well as what preferences prevail when using multi-level egg nests.

Thanks to the ethology of farm animals, we already know much about the behavior of hens. For example, the literature notes that there is a difference in the nesting behavior of laying hens of different genotypes [7–9]. However, due to intensive selection, the egg-producing ability of egg-laying hybrids varies by a fair amount, and thus the housing systems are not considered to be the same either, which can affect the nesting behavior of the birds.

Due to the popularity of alternative husbandry in Europe and the prohibition of beak trimming that has already been introduced in many countries [10], a new aspect directs attention to this phenomenon; the abandonment of beak trimming in cage systems causes much less of a problem than in alternative housing methods [11,12]. The aggressive behavior of the hen in non-cage solutions can lead to a not infrequently drastic increase in deaths [13]. That is why breeders have recently been attempting to (also) select their lines based on special criteria and eliminate individuals that, for example, have a crow's beak (see: Lohmann Breeders, Cuxhaven, Germany) or an aggressive tendency (see: Bábolna TETRA Ltd.). The goal is clearly to ensure that the commercial egg hybrid has a calm temperament and produces revenue even in alternative systems. However, if the breeder 'makes' a phlegmatic type out of the previously lively temperament, sanguine hen to reduce the loss resulting from aggressive behavior, it is feared that this bird will lay eggs where it comes to mind in alternative systems and, instead of visiting the nest, the number of litter eggs with contaminated shells will increase.

As a summary of the introduction, the more we understand the behavior related to the use of nests in the new type of alternative husbandry, the more we will learn about hens. Furthermore, it allows us to put the future egg production of Europe on a more secure basis.

2. Materials and Methods

The research was carried out at the Poultry Testing Station of the Kaposvár Campus of the Hungarian University of Agriculture and Life Sciences, with a flock of laying hens of three different genotypes provided by Bábolna TETRA Ltd. (Figure 1). They were:

- Commercial brown layer hybrid (C);
- Purebred paternal offspring group (Paternal);
- Purebred maternal offspring group (Maternal).

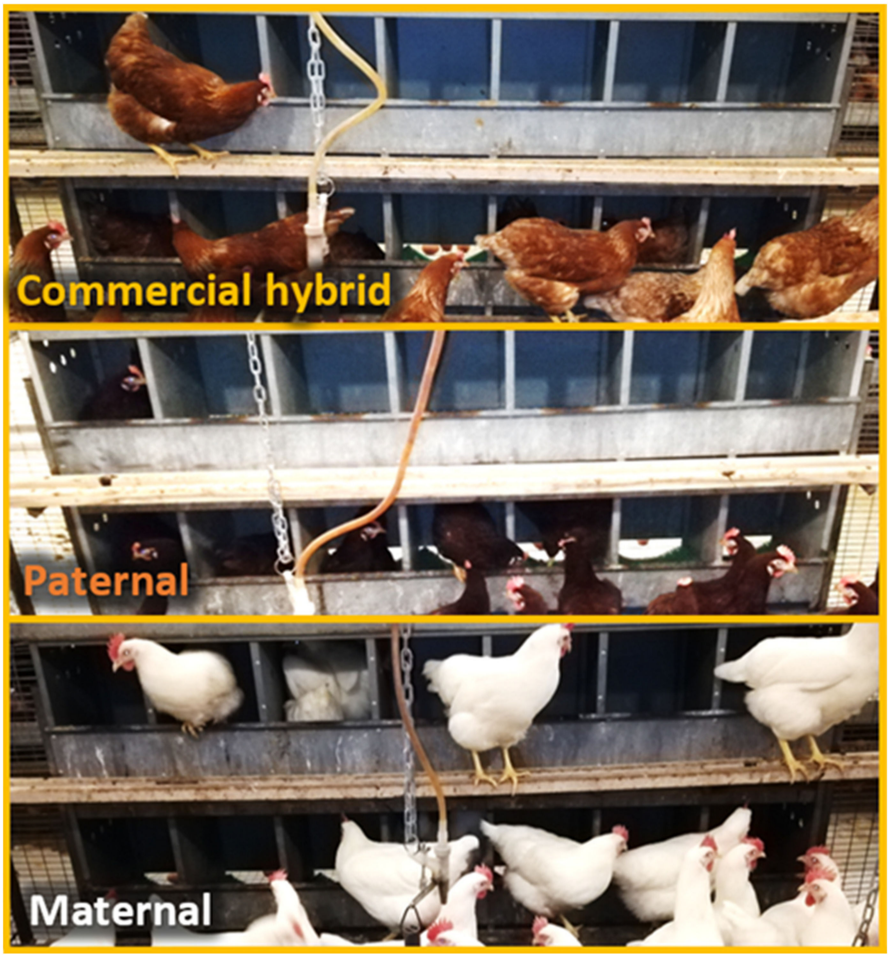

Figure 1. The experimental flocks with the three different genetic backgrounds (commercial hybrid (**above**) and the purebred offspring of the paternal (in the **middle**) and maternal lines (**below**)).

The set numbers were: N = 318; n = 106 hens/genotype; 53 hens/pen, i.e., these were the conditions per group. It is important to emphasize that the laying hens were not beak-trimmed. The temperature in the barn was usually 14–18 °C, where we used 16 h (5:00–21:00) of lighting per day with a light intensity of 30 lux. The laying hens could consume commercially available egg feed and drinking water ad libitum from the suspended feeders and drank water from a suspended open water drinker.

We placed 53 19-week-old pullets in each of the six indoor alternative pens, each with a floor area of 5.52 m^2 (1041 cm^2/hen). One-third of the floor space of the pens were littered with soft wood shavings; the remaining two-thirds of the area were a raised level, plastic grid platform. In the littered part of the scratching area, the thickness of the soft wood shavings was 10 cm, while the raised plastic grid platform was set at a height of 23.5 cm apart from the level of the littered scratching area (Figure 2).

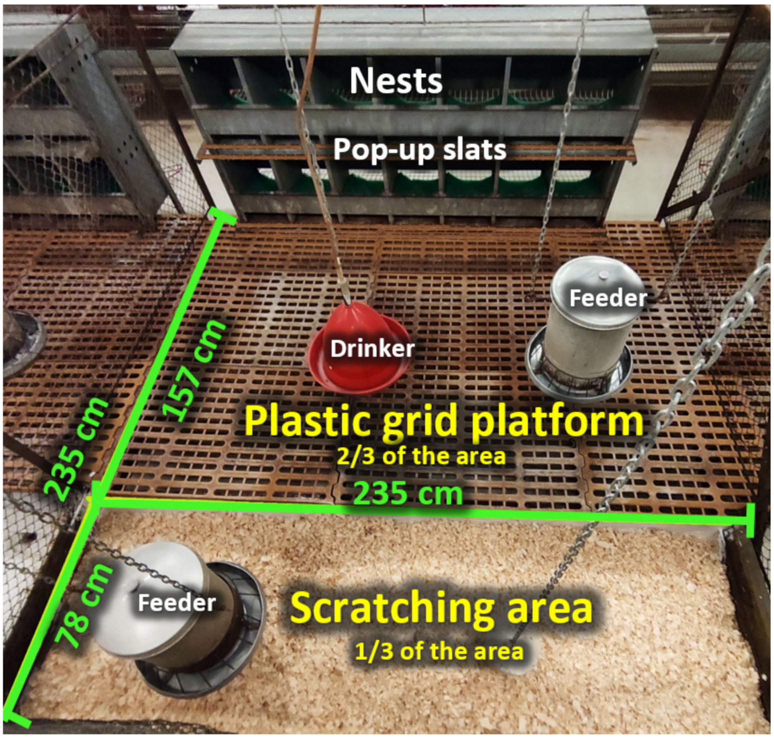

Figure 2. The floor plan of the alternative pen and the layout of the technological devices.

Figure 3 clearly depicts that 14 nests with artificial grass were provided for the hens on two levels per pen (3.8 hens/egg nest), whose design and dimensions (W: 24.5 cm; H: 18.5 cm; D: 33 cm) can be seen in the Figure 4. At the entrance of each nest, there was a 10 cm high plate cover (=threshold). The entrance to the bottom and upper egg nests were 24 cm and 65 cm above the level of the plastic grid platform, respectively.

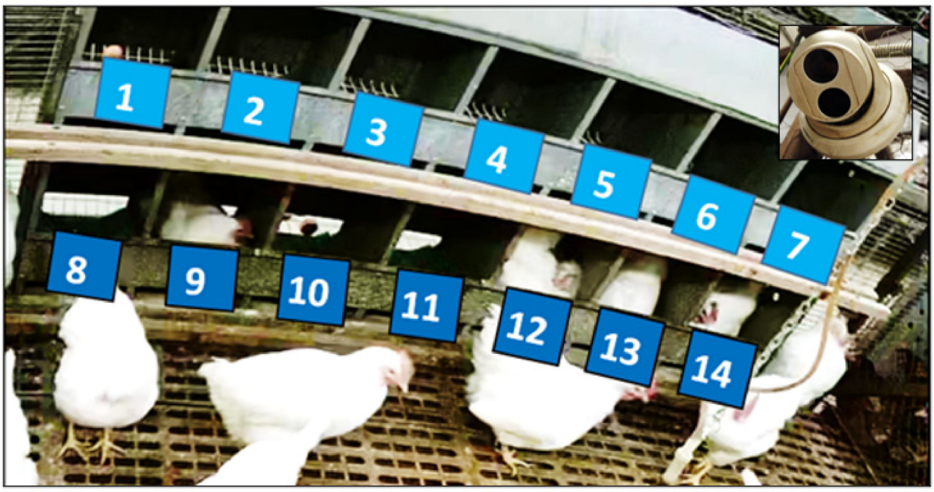

Figure 3. The image taken by an HD camera shows the position of the nests located on two levels in one of the pens installed for the Maternal Line; an infrared camera was installed above the nests.

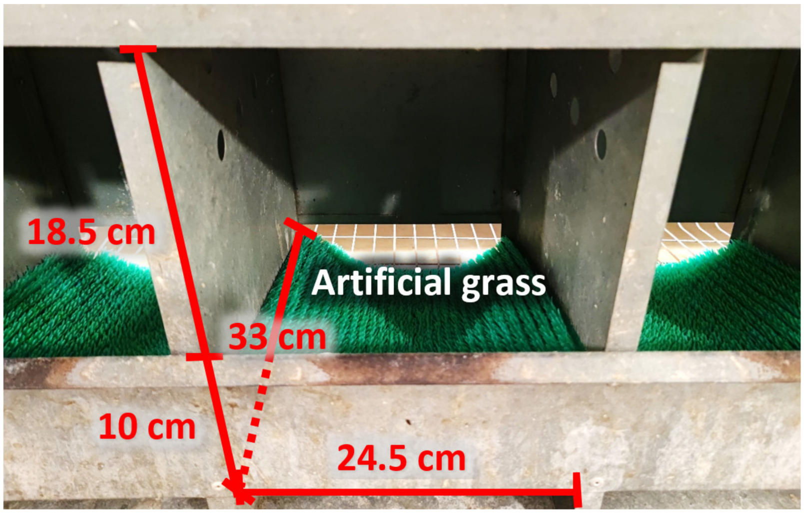

Figure 4. The design and dimensions of the nest with artificial grass.

In front of the row of nests, 2-2 pop-up slats per level (Figure 3) assisted the approach to the nests. The laying hens were able to enter the nests from the plastic grid platform. The height placement of the nests and pop-up slats made it easier for the laying hens and did not hinder them from entering the nests. During the whole test period (12 production months), the eggs were collected at exactly 10 o'clock every day. We separately recorded the number of eggs laid in the nests on the lower and upper levels, as well as the number of eggs laid in the scratching area in the litter.

Infrared cameras (GeoVision Target H.265 4.0 Megapixel outdoor IP Eyeball dome camera) were installed above the row of nests in the alternative pens (Figure 3), and we captured 24 h recordings using a special software (GeoVision GV-NVR System). The video recordings were a continuous recording. When studying the nesting behavior of laying hens, the number of times the birds visit the nests in different positions is telling information (see: Figure 2). That is why we considered it important to be able to track the number of times the laying hens visited the 7 egg nests placed on each of the two levels, i.e., a total of 14 nests, even over the course of a test day. Because the 12-month egg production period is quite long, we took special care to choose the appropriate time for the examination. We decided to carry out the evaluation of the recordings at the beginning of the third production month, as based on the literature recommendations of Bábolna TETRA Ltd., the tested genotypes reach their peak production intensity as a breeder around that time. Because the greatest number of eggs are expected to be laid during this period, it is logical that this is also the time when most nests will be used, thus ensuring a good opportunity to observe the nest-using behavior of the different genotypes. A minor aspect is that we were also still at the beginning of the production period, when the mortality in the barn is smaller, meaning that we could still carry out the evaluation with a relatively large number of elements due to few losses in the vicinity of the production peak. In our experiment, we have evaluated one day in two repeats per genotype.

During the evaluation of the recordings, we separately recorded the time of entry and exit from the upper (numbered 1–7) and lower (numbered 8–14) (Figure 3) nests, from which we determined the length of time spent in the nests. We observed two pens per genotype, and thus evaluated the data of six pens total.

The frequency of nest visits and the frequency of eggs laid in different places were evaluated using the likelihood ratio test, and the difference between the average durations spent in the nest was evaluated using a one-way ANOVA analysis using the SPSS 10.0 program package.

3. Results and Discussion

3.1. Proportion of Eggs Laid in Nests and in Litter

In Table 1, we have shown the total relative percentage of eggs laid in the nests and in the scratching area (in the litter) during the 12 production months, organized by the genotype. There was a significant difference between the examined genotypes in terms of the occurrence rate of eggs laid in the nests and in the litter, which was also statistically proven. It is well-known that the occurrence of litter eggs is undesirable, as eggs laid in litter are usually more polluted, meaning their sale is also more difficult; it is forbidden to wash edible eggs because of the contamination, even though the risk to human health is greater (It is no coincidence that the cleanest eggs can be produced in cage systems). Based on the data, it is striking that the Maternal genotype mostly used the nests, as a result of which the occurrence rate of eggs laid in the litter was the lowest, barely around 10%.

Table 1. The total ratios of the eggs laid in the nests and in the litter during the 12 months of production in percentages (%).

Genotypes	Distribution of Eggs (%)		
	Total in the Nests	Total in the Litter	Prob.
Commercial hybrid	69.3 ^{b}	30.7	<0.001
Paternal	58.9 ^{a}	41.1	<0.001
Maternal	89.8 ^{c}	10.2	<0.001
Prob.	<0.001		

$^{a–c}$ indicate significant differences among the different genotypes ($p < 0.05$).

The Commercial hybrid laid 69.3% of the eggs in the nest, but in the case of the pure-line Paternal genotype, this pattern was observed to be below 60% (58.9%), which was the weakest performance in the ranking. Looking at the data, it seems at first glance that the Commercial genotype produced an intermediate result compared with the pure-line parental genotypes. However, this is only an appearance that was a result of the nature of the experiment that we conducted (see: pure-line parental generation vs. crossed hybrid). If we recall the classic definition of the heterosis effect and perform the simple calculation of how (P1 + P2)/2 compares (<?>) with the phenotypic value of F1, we experience a negative heterosis compared with the average parental phenotypic performance. The parental average was 74.4%, which was 5.1% higher than the 69.3% measured for the Commercial hybrid, thus the negative heterosis was 7.4%. Because the calculated $SzD_{5\%}$ value during the test was of the order of 10% for the eggs laid in the egg nest, the degree of heterosis in the negative range cannot be considered significant.

Nevertheless, it is thought-provoking that in the breeding of egg-laying hybrids, the selection of pedigree lines is based on the performance shown in the cage—and it will likely remain so for a while—but we expect the hybrids from these lines to know exactly where to lay their eggs in non-cage conditions. As a solution, the selection of pure lines could be performed in similar conditions (floor) using with trap nests, as it was possible in the very early times of poultry breeding. However, it will lead to an increase in costs.

Overall, it can be stated that the Paternal especially and, to a lesser extent, the Commercial hybrid, were more inclined to lay eggs in the litter; it would be worthwhile to take practical steps to eliminate this from happening (the optimization of stocking density, selection and proper placement of the appropriate nest type, correct selection of nest material, correct impact of light intensity at the level of the nests, etc.).

The upper portion of Figure 5, shows the percentage changes in the eggs laid in the litter and in the egg nests in the Commercial hybrids during the entire egg production period. The height of the columns shows that in the first month of production, the ratio of eggs laid in the nest and in the litter was almost 50%:50%, which is very unfavorable. However, in the second half of the egg production period, the ratio of eggs in the litter already reached well below the 10% level.

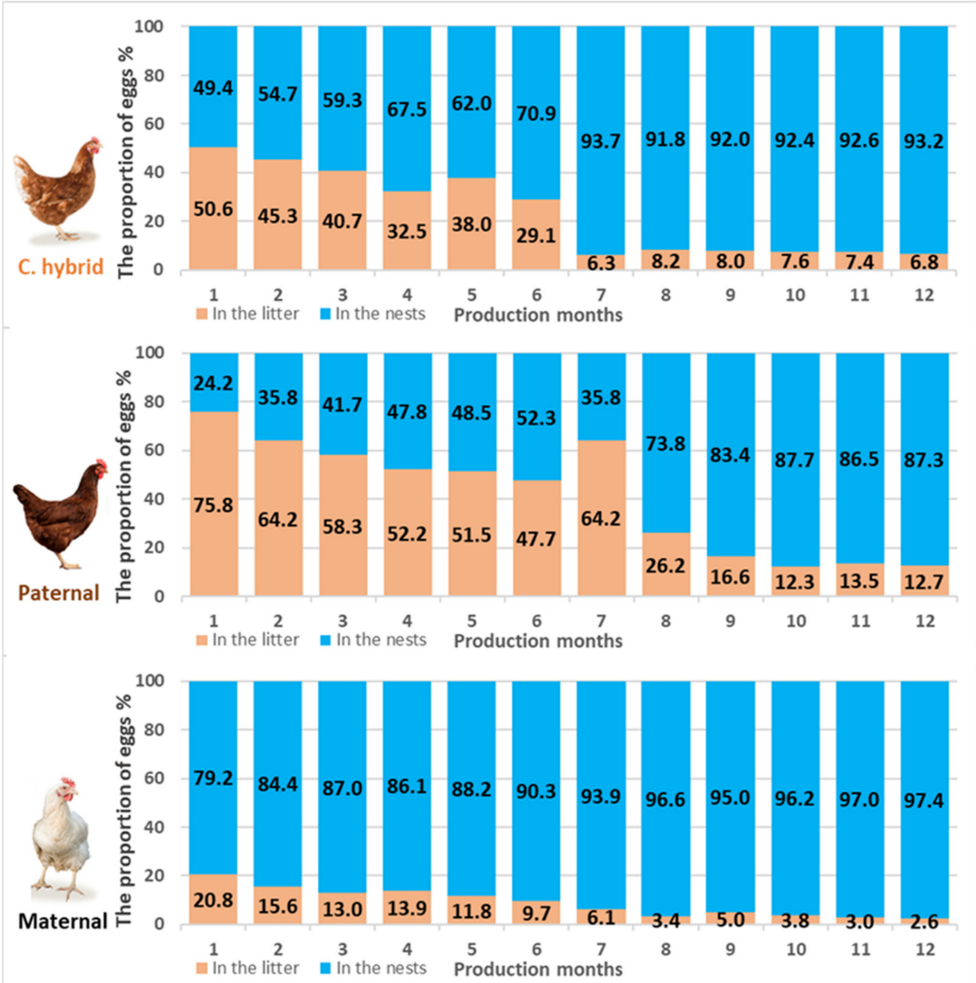

Figure 5. Changes in the proportion of eggs laid in the litter and in the nest during the 12 months of egg production in the case of the different genotypes.

The middle portion of Figure 5 shows the same changes, this time in the Paternal genotype. Even in the first month of production, the percentage of eggs laid in the litter was much higher (75.8%) than in the Commercial hybrid (50.6%). In the first seven months of the egg production period, the purebred Paternal group, with one exception, laid more than half of the eggs in the litter of every month, and this decreased to only around 10–15% in the last three months.

The bottom of Figure 5 shows the values that were characteristic of the Maternal genotype, along with their changes. It can be clearly seen from the size of the columns that, compared with the previous two genotypes, this genotype clearly preferred the nests for laying their eggs. Moreover, after the learning process of the initial months, less than 5% of the eggs were laid in the litter at the end of the egg production period.

3.2. The Preference of Laying Hens between Nests Located on the Lower and Upper Levels

It is important to compare the nesting behavior observed with the video assessment in the produced data of each group of hens to see how many eggs the hens laid in the upper and bottom nests. Therefore, the observations and the daily collected production data can confirm each other and allow conclusions to be drawn on a solid basis.

The higher number of eggs in each nest can be used to determine the nests preferred by the hens [14].

Table 2 shows the total relative percentage of eggs laid in the nests of the bottom and upper levels during the 12 production months. The Commercial hybrid and Maternal genotype laid their eggs in one of the lower nests in a ratio slightly below three-quarters (72.2% vs. 71.4%, respectively), while slightly above a quarter (27.8% vs. 28.6%, respectively) in the upper level. The nest selection preference is clear, as nearly three times as many eggs could be collected from the lower nests as from the upper ones during the 12 months of production.

Table 2. The total ratios of eggs laid in the lower and upper nests during the 12 production months in percentages (%).

Genotypes	Distribution of Eggs (%)		
	Bottom Nests	Upper Nests	Prob.
Commercial hybrid	72.2 [b]	27.8	<0.001
Paternal	88.0 [a]	12.0	<0.001
Maternal	71.4 [c]	28.6	<0.001
Prob.	<0.001		

[a–c] indicate significant differences among the different genotypes ($p < 0.05$).

The performance of the Paternal genotype differed from that of the other two tested genotypes in that 88% of the eggs were laid in the lower nests. Based on the nest selection preference, seven times more eggs were 'placed' on the lower level compared with the upper one (Figure 6). The results clearly show that the Paternal genotype mostly preferred the lower places as it laid its eggs here, which is also reflected in the choice of nest and the number of litter eggs, as this genotype is the one that laid its eggs to the greatest extent in the litter-covered scratching area.

Figure 6. The significant preference for bottom nests in the Paternal genotype.

Figure 7 shows the changes in the proportion of eggs laid in the lower and upper nests during the 12 months of egg production, organized by the tested genotype. A clearly visible and clear trend can be observed, where each genotype produced more than 90% of the eggs collected in the bottom nests during the first month of production.

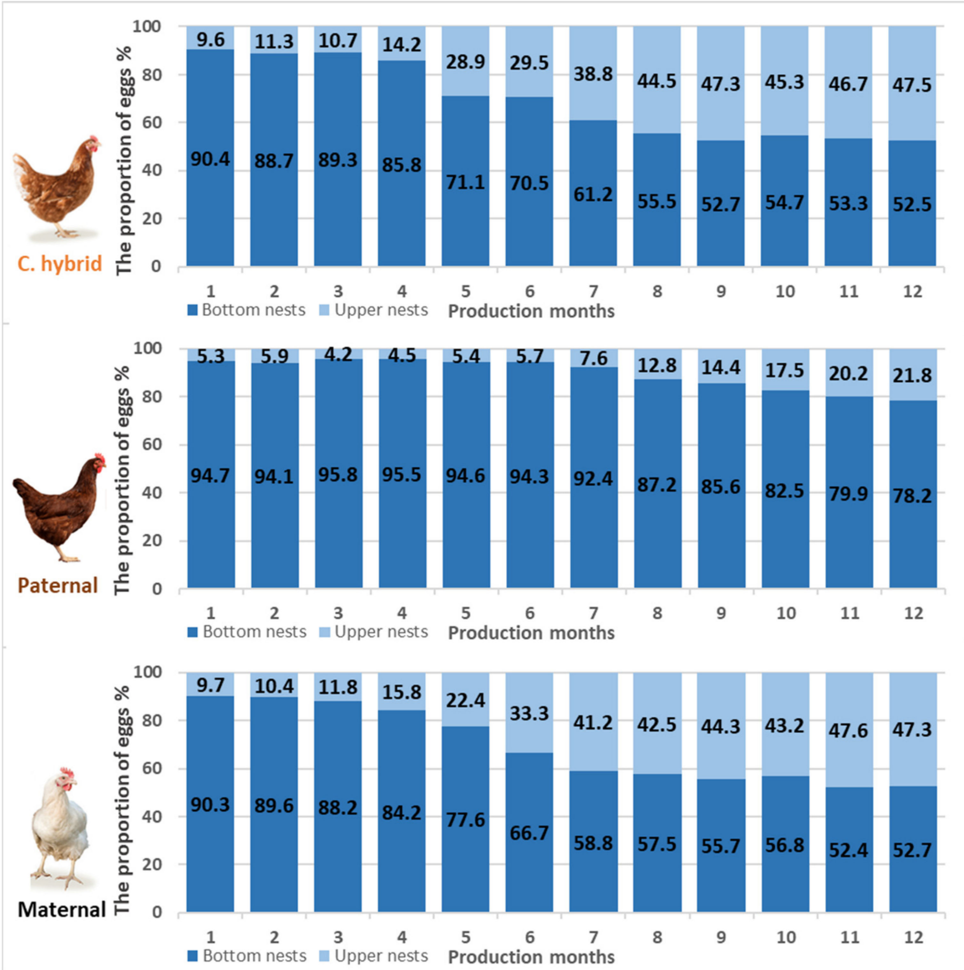

Figure 7. Changes in the proportion of eggs laid in the lower and upper nests during the 12 months of egg production in the case of the different genotypes.

In the case of the Commercial hybrid and the Maternal genotypes, the use of the upper nests and the proportion of eggs that collected there gradually increased. This rose above 20% at the fifth month, then reached or approached 40% by the seventh month of production and maintained or even slightly increased this level towards the end of the study period. Practically, for these two genotypes, the proportion of eggs laid in the upper nests increased at the same rate, and at the eighth month, 45–47% of the eggs were laid in the upper nests, while 53–55% were laid in the lower nests.

Based on our observations, the proportion of eggs laid in the bottom and upper nests of the Paternal genotype differed greatly from this trend, which is an eye-catching feature of the data.

In the first month of egg production, 94% of the eggs from the Paternal genotype were collected from the lower nests, which not only stagnated, but sometimes even increased. This rate only dropped below 90% after the eighth month of production, but the rate of eggs collected from the bottom nests was only less than 80% in the last two months.

This experience significantly coincides with the results of Figure 5 and Tables 2 and 3 (which shall be discussed later) in which we can observe that the Paternal genotype clearly used the upper nests less; in fact, if a hen had the opportunity, she laid her eggs to a greater extent in the scratching area located 'lower' in height than even the lower nests.

Table 3. The distribution of nest visit occasions of laying hens based on the position of the nests, in percentages (%).

Distribution of Laying Hens' Nest Visits per Nest, %									
	Commercial Hybrid							**Prob.**	**Total**
Upper Nests	**1**	**2**	**3**	**4**	**5**	**6**	**7**	<0.001	
	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	1.1 ^{a}	0.6 ^{a}	1.1 ^{a}		2.8 ^{A} (5)
Bottom Nests	**8**	**9**	**10**	**11**	**12**	**13**	**14**		
	29.1 ^{c}	12.8 ^{b}	8.9 ^{b}	13.4 ^{b}	12.8 ^{b}	10.6 ^{b}	9.5 ^{b}		97.2 (174)
Prob.	-	-	-	-	-	-	-		<0.001
	Paternal								
Upper Nests	**1**	**2**	**3**	**4**	**5**	**6**	**7**	<0.001	
	1.0 ^{a}	0.0 ^{a}	0.0 ^{a}	0.0 ^{a}	1.0 ^{a}	0.0 ^{a}	2.0 ^{a}		4.0 ^{A} (4)
Bottom Nests	**8**	**9**	**10**	**11**	**12**	**13**	**14**		
	15.8 ^{b}	12.9 ^{b}	11.9 ^{b}	9.9 ^{b}	16.8 ^{b}	18.8 ^{b}	9.9 ^{b}		96.0 (97)
Prob.	-	-	-	-	-	-	-		<0.001
	Maternal								
Upper Nests	**1**	**2**	**3**	**4**	**5**	**6**	**7**	<0.001	
	9.6 ^{c}	9.6 ^{c}	2.4 ^{b}	2.4 ^{b}	2.4 ^{b}	0.0 ^{a}	2.4 ^{b}		27.5 ^{B} (46)
Bottom Nests	**8**	**9**	**10**	**11**	**12**	**13**	**14**		
	15.0 ^{c}	10.8 ^{c}	9.0 ^{c}	10.2 ^{c}	8.4 ^{c}	9.0 ^{c}	10.2 ^{c}		72.5 (121)
Prob.	-	-	-	-	-	-	-	-	<0.001
Prob.	-	-	-	-	-	-	-	-	<0.001

$^{a–c}$ indicate significant differences between the number of visits to different nests ($p < 0.05$). ^{A,B} indicate significant differences among the different genotypes ($p < 0.05$).

In a study by Krause and Schrader [15], laying hens could choose between nests placed at different heights (0 cm, 39 cm, 78 cm, and 117 cm). Similar to our results, all three of the tested genotypes in their study preferred egg nests placed at the level of the floor. In the other part of their study, when the hens had the opportunity to choose between four levels, each group preferred the one which they were previously accustomed to.

Because the individuals of the Paternal purebred offspring group chose the nests closest to the ground level to the greatest extent (the bottom row of nests), it is logical that this genotype was also characterized by a high rate of laying eggs in the litter. It is quite certain that the observed differences are to be found in the different genetic background of the examined offspring groups, where primarily the Paternal (RIR) and Maternal (RIW) lines have different, yet characteristic properties. Nesting at ground level is characteristic of wild hens, and this is much more pronounced in the red-colored Rhode Island Red (RIR) Paternal line than in the white-feathered Maternal genotype.

3.3. Number of Times Laying Hens Visited the Nest Based on Video Evaluation

Table 3 shows the distribution of the nest visiting occasions of the laying hens based on the position of the egg nests (1–14). Regarding all three genotypes, it can be established that there was a spectacular and significant difference in the number of times the laying hens visited the nest, depending on the position of the egg nest.

From the data in the table, certain nests were preferred by the hens over others. We know that domestic hens are influenced by several factors in the place that they choose to lay their eggs, e.g., the safety of the area, the quality of the nest, and if there is a complex life situation caused by the social ranking (pecking order). The nest is mostly made in places such as under a bush or at the foot of slopes, which are secluded from disturbing factors, but most importantly hidden from predators [16].

Overall, the Commercial hybrid and the Paternal genotype visited the lower nests at rates of 97.2% and 96.0%, respectively, while the individuals of the maternal line did so in a significantly lower proportion, where less than three-quarters (72.5%) visited the lower nests. This result significantly coincides with Figure 6, where we reported that individuals of the Commercial hybrid and the Paternal genotype laid their eggs in the lower nests to a very similar extent, and the Maternal genotype was the one in this period that laid its eggs to the greatest extent in the upper nest (11.8% in the third production month).

Examining the basic data further, it can be clearly seen that there was a statistically verifiable difference between the individual egg nests in the proportion of nest visit occasions for all three genotypes. The phenomenon observed during egg production can therefore be perceived here as well, where the laying hens preferred—or even avoided—the different nests based on the position of the nest; a nest preferred by others could cause more traffic, even a tumult, perhaps to a disproportionately greater extent, as would be expected based on the number of hens and available nests. The phenomenon could even be referred to as a 'relative lack of nests', which can easily influence the increase in the number of eggs laid in the litter.

It is a well-known fact that these housing systems still have problems that arise from the number of nests and the space per hen [17]. In order for pre-laying behavior to be properly expressed in cages, a sufficient number of suitable nests is needed [18].

According to other researchers [16], it is also logical, there are more potential nesting sites in a housing system than in the wild, hens cannot move away from their mates, so social factors may be more important in the expression of nesting behavior. Before laying eggs, laying hens are highly motivated to search for a suitable nest [19], if there is enough nesting space, hens spend more time resting and less time dust bathing than hens that do not have access to an egg nest [20]. Laying hens tend to prefer certain nests, so competition for egg nests may develop [8]. A limited number of nests increases the frequency of aggressive interactions [21] and a smaller holding area also accounts for this phenomenon [22].

Due to the group nesting tendency, we believe that in order to ensure the well-being of the laying hens and ensure uninterrupted production, it may be worth increasing even the size of the nests, as already noted by another researcher [23]; alternatively, the nests located in the middle of the rows should be made more attractive by changing their appearance, which can reduce group nesting, a suggestion that has also been previously mentioned by others [24].

In all three genotypes, the most visited egg nest was by far the one on the bottom left (it fell in the corner of the pen), which was nest number 8. The individuals of the Commercial hybrid 'produced' nearly one-third (29.1%) of the nest visits at nest number 8, which was significantly more compared with the visits to all other nests.

It is known from several studies that in alternative housing systems, laying hens choose a nest every day—in which they lay their eggs from many identical nests—and often prefer the corner nest [25,26]. In this regard, Villanueva et al. [8] made an interesting observation that the brown hens preferred the nests on the right side and laid more eggs in that place, while the white hens preferred the compartments on the left side. These results clearly show that different genotypes show different nest use and egg-laying behavior.

In the case of the Maternal genotype, it was observed that a significantly higher proportion of laying hens stayed in the leftmost nests in the upper level than in one of the middle or rightmost nests. In the case of the Paternal and Maternal genotypes, we found no statistically verifiable difference in the relative proportion of visits to the egg nests located on the lower level.

As mentioned earlier, our tests were carried out in the third month of production, so it is likely that the level of group nesting may decrease as time progresses as other experiments note that the occurrence of group nesting is more frequent in the first half of the egg production period than in the second half [27].

To evaluate the results, it must be added that a hen could naturally visit the nests several times a day, and these nest visits did not always end with egg laying. Some laying hens, for example, could visit the nests on a very different number of occasions. As the laying hens were not individually marked (as can be seen in the video recordings), the average nest visit occasions were presented in this study. In the future, it could be interesting to check the correlations between the number of visits or duration and number of eggs laid in the nests.

Domestic hens show unique differences in pre-laying behavior, including in the final choice of their nest site. Most hens make small but extended visits to the nest and lay their eggs there, but some hens often make only short visits and occasionally lay eggs outside of the nest box [28].

3.4. Duration of Nest Visits of the Laying Hens Based on Video Evaluation

It is worth emphasizing that the time spent in the nest is mostly, but not always, the time of egg-laying. It is possible for the hen to simply be resting there, or to have entered the nest to seek shelter.

A significant difference was found in the average duration of the use of the lower nest between the studied genotypes (Table 4). This means that the laying hens that belonged to the Paternal genotype spent more time on average in the lower nests (13.4 min) than the Commercial hybrid (7.9 min) and the Maternal genotypes (8.6 min). The measured difference is also statistically proven.

The basic data reveal that the values for the individual nests show a rather large variation, which unfortunately significantly increased the margin of error of the statistical calculations; therefore, no further significant difference could be verified. At the same time, it is thought-provoking that some nests were quite exceptional in the duration of stays among the different egg nests. For example, the top level nest number five used by the Paternal line and the top level nest number one used by the Maternal line. The time spent in these areas were far more than what is necessary for oviposition.

In the case of the Commercial hybrid, the laying hens did not spend an average of 1 min in the upper nests, while the individuals of the Paternal genotype spent roughly twenty times that, but here the three ‘honored’ nests (number 1, 5, and 7) played a special role for a reason. One thing is for sure: the duration of stay there sometimes showed huge differences in the case of some egg nests, but it is very difficult to clarify the hen’s preference, as nest 1 and 7 are in extreme positions, while nest 5 is almost in the middle.

Giersberg et al. [9] also similarly used a video system in an aviary-type barn and investigated the nest use patterns and nest behavior of two genotypes of laying hens, the dual-purpose Lohmann Dual and egg-type Lohmann Brown Plus, and also examined the effect of the placement of the nests.

The results showed that the patterns of nest use and behavior in the nest differed between the conventional, dual-purpose, and egg-laying hens. Dual-use hens, for example, used the same nests in greater numbers. Both genotypes used nest number one more than nest number six, but spent more time in the latter. Based on their experience, dual-purpose hens were more affected by the location of the nest than egg-laying hens.

Table 4. The change of the average duration of the nest visits of the laying hens per egg nest (in minutes).

Duration of Nest Visits by Laying Hens per Nest (minutes)								
	Commercial Hybrid							**Combined Average**
Upper Nests	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**Upper Nests**
	-	-	-	-	1.3	1.0	0.5	0.9
Bottom Nests	**8.**	**9.**	**10.**	**11.**	**12.**	**13.**	**14.**	**Bottom Nests**
	8.0	7.1	4.6	11.1	8.0	9.1	4.6	7.9 [a]
Prob.	-	-	-	-	-	-	-	0.266
SE	-	-	-	-	-	-	-	1.028
	Paternal							
Upper Nests	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**Upper Nests**
	5.1	-	-	-	38.7	-	13.5	17.7
Bottom Nests	**8.**	**9.**	**10.**	**11.**	**12.**	**13.**	**14.**	**Bottom Nests**
	14.4	5.7	9.3	7.6	19.3	17.8	11.1	13.4 [b]
Prob.	-	-	-	-	-	-	-	0.703
SE	-	-	-	-	-	-	-	2.160
	Maternal							
Upper Nests	**1.**	**2.**	**3.**	**4.**	**5.**	**6.**	**7.**	**Upper Nests**
	25.3	4.2	7.1	4.4	1.3	-	2.0	7.4
Bottom Nests	**8.**	**9.**	**10.**	**11.**	**12.**	**13.**	**14.**	**Bottom Nests**
	7.3	2.8	6.1	10.3	7.6	18.1	7.7	8.6 [a]
Prob.	-	-	-	-	-	-	-	0.894
SE	-	-	-	-	-	-	-	1.052
Prob.	-	-	-	-	-	-	-	**0.018**
SE	-	-	-	-	-	-	-	**0.812**

[a] and [b] indicate significant differences in the bottom nests among the different genotypes ($p < 0.05$).

4. Conclusions

Based on the results for the entire egg production period, we determined that there was a significant difference in the rate of egg-laying outside of the nest, i.e., the occurrence rate of litter eggs, between the tested genotypes. Regarding the proportion of eggs laid in the litter, the most favorable result was observed for the Maternal genotype (10.2%), followed by the Commercial hybrid (30.7%) and the Paternal genotype (41.1%). We believe that the most favorable result of the Maternal genotype was due to the favorable maternal traits developed during the breeding of the Rhode Island White (RIW) line.

In the case of the Commercial hybrid, we did not receive a value close to the parental average, but here we measured a positive heterosis of around 7% in terms of the proportion of eggs laid in the litter, an assessment that is unfortunately particularly unfavorable and undesirable.

As the time spent in production progressed, we found that the proportion of eggs laid in the litter gradually decreased for all three genotypes according to our conclusions. This is because the laying hens learned to use the nests over time and became accustomed to the nests. At the same time, the marked difference between the tested genotypes typically remained with respect to the nesting behavior.

Because different nests were preferred by the laying hens based on the position of the nest (or even avoided), more 'traffic' (tumult) could develop at the nests preferred by others, even to a greater extent than what would be expected based on the number of hens and available nests. This phenomenon could also be referred to as a 'relative lack of egg nests', which can easily influence the increase in the number of eggs laid in the litter.

With the help of recorded video, we determined how much time the laying hens spent in the nest on average, and we found a significant difference in the duration of the use of the lower nests between the tested genotypes. Laying hens that belonged to the Paternal genotype spent more time on average in the lower nests (13.4 min) than the Commercial hybrid (7.9 min) and the Maternal genotypes (8.6 min).

Based on the tests, it can be said as a summary that in the alternative housing method equipped with egg nests, the nest selection preference of the investigated laying hens with different genetic backgrounds was significant showed significant differences in several cases. The results are extremely instructive and draw attention to the fact that in egg production, it is not enough to only ensure the desired ratio between the number of hens and the number of egg nests. It is possible that the laying hens, due to their genetic background, do not use nests that are placed in certain positions (e.g., on top) at all. they are not used, which generates a relative shortage of egg nests and can even cause an enormous increase in the proportion of eggs laid in the litter, which in turn involves human health risks.

The lesson of the study is that a laying hen should be selected based on the method of housing and its characteristics, and it would not hurt to 'ask the hen' about several questions.

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Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to sensitive data about some pedigree lines.

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Conflicts of Interest: A.O. is an employee of Bábolna TETRA Ltd., which is the owner of the examined genotypes. The remaining authors declare no conflict of interest. The funders had no role in: the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

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