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Presence of roosters in an alternative egg production system aiming at animal welfare

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ABSTRACT - The objective of this study was to evaluate the effect of the presence of roosters on welfare and egg production of laying hens reared in an alternative system. Two breeding systems were established: barn 1 - laying hens reared without roosters (4500 birds); and barn 2 - laying hens reared with roosters (4500 layers and 250 roosters). In the poultry facilities, microclimate, egg production, mortality rate, and bird behavior were evaluated. Microclimate analysis showed that the birds were subjected to periods of constant heat stress, except for the morning hours. However, even under these conditions, egg production results and mortality rate were consistent with the indices recommended in the Isa Brown management guide in the barn with roosters; the indices obtained were even better and were characterized by higher egg production and lower mortality rates. In addition to productivity benefits, the presence of roosters broadened the behavioral repertoire of the birds due to the introduction of reproductive behaviors. Moreover, there was a significant decrease in the tolerance-reflex behavior, which is associated with the impossibility of displaying reproductive behaviors. This alternative egg production system proved to promote animal welfare since it provides and stimulates the display of behaviors considered important for birds.

Key Words: behavior, enthalpy, husbandry environment, laying hens

Introduction

Along with issues concerning environmental preservation and food safety, animal welfare is an issue of great impact in the media and productive environments. This scenario has been further impacted by the population awareness of the productive process, as well as by the demand for food with differentiated quality attributes (Pereira et al., 2015b).

With regard to poultry farming, there has been extensive criticism on the practices involved in commercial egg production. Images of beak-trimmed birds and birds reared in the extremely confined spaces of battery cages are truly moving. A major challenge in the layer industry is housing due to the shift from conventional cage housing to alternatives like enriched colonies or cage-free (Thaxton et al., 2016).

In an attempt to make animal welfare a less subjective topic by promoting good welfare and allowing inspection in a productive system, the Farm Animal Welfare Council (FAWC, 1993) has developed the following concept of five freedoms, outlining five aspects of animal welfare under human control. The concept includes: nutritional - freedom from hunger and thirst; environmental - freedom from discomfort; physical - freedom from pain, injury, and disease; behavioral - freedom to express normal behavior; and psychological - freedom from fear and distress. The forth one determines that animals must be reared under conditions in which they can exhibit their natural behaviors, such as scratching, wing-flapping, and perching, among others. Accordingly, the presence of roosters in the production system is an important factor, since reproductive behaviors are natural to this species, although, except in rare cases, egg production systems do not utilize roosters.

Furthermore, the behavior of the birds is strongly influenced by the environment to which they are subjected, because the homeostasis mechanism is only effective when

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the temperature is within the range required by the animals (Pereira et al. 2015a).

In view of the lack of research on this topic, the present study aimed to evaluate the effect of the presence of roosters on the welfare and productivity of laying hens reared in an alternative egg production system.

Material and Methods

This study was carried out according to the ethical principles and was approved by the local Research Ethics Committee. Two adjacent commercial barns located in Ipeúna, SP, Brazil (124° SE and 310° NW) were used in the experiment. Dimensions of barns 1 and 2 were 9.8 × 69.0 × 3.0 and 9.8 × 70.9 × 3.0 m, respectively, both equipped with automatic feeders and drinkers, wooden perches, nests for egg laying, fans, and foggers. There was one nest box for every six laying birds, totaling 750 nest boxes of 0.30 × 0.32 × 0.45 m in size in each barn. The floor area was covered with wood shavings of 0.10 m height in both barns.

The number of feeders and drinkers and the perch space were defined according to the number of birds, in compliance with the Animal Care standards established by the Humane Farm Animal Care (HFAC, 2014).

Following the guidelines developed by the company, artificial light sources were used after the birds reached 50% lay. Accordingly, an intermittent lighting program (15 h of continuous light followed by 9 h of darkness) was used in both barns with fluorescent lights at an intensity of 40 lux.

Isa Brown laying hens and roosters aged 15-31 weeks were investigated to assess the period between the onset and peak of egg production. All birds were initially reared together in the same space. The alternative egg production system was characterized by rearing the birds on the floor, in compliance with the standards established by the HFAC (2014). During the experimental period, the birds in the two barns were provided *ad libitum* food without the addition of antibiotics, chemotherapeutic agents, and products of animal origin. The diets were formulated according to the Isa Brown management guide (Hendrix Genetics, 2012-2013).

The effect of the presence of roosters was thus evaluated: 4500 laying hens were housed in barn 1, with housing density of 6.6 birds/m^2; 4500 laying hens and 250 roosters were housed in barn 2, with density of 6.8 birds/m^2. The ratio of eighteen laying hens for each rooster was defined and kept. The number of roosters was determined according to the area available in the barn. The laying hens were not removed from the barn for the introduction of the roosters to ensure greater applicability of this research.

Temperature and relative humidity data were collected inside the barns using data loggers installed at 1.50 m above the floor. Readings were obtained at one-hour intervals. The black globe temperature was measured using a globe-thermometer, which was installed in each barn. Data were recorded once weekly at 09.00, 13.00, and 17.00 h. Black globe humidity index (BGHI) and enthalpy were calculated according to Buffington et al. (1981) and Villa Nova (1999) *apud* Furlan (2001), respectively.

Table 1 - Behavioral patterns evaluated

Behavioral group	Behavior	Behavior description
Physiological	Drinking	Ingestion of water from the drinker.
	Feeding	Ingestion of feed from the feeder.
	Visit to the nest	Hen makes quick trips to the nest.
	Time spent in the nest	Amount of time the bird spends in the nest to lay eggs.
Reproductive	Courtship ritual	Roosters do a half-circle dance around the hen.
	Mating	Mounting.
	Tolerance reflex	Hen lowers itself towards the ground in the presence of humans, similar to the behavior exhibited prior to mating.
Undesirable and agonistic	Agitation	Birds move quickly in an agitated way.
	Fighting	Two or more birds confront each other by bristling up the feathers of the neck and by pecking and scratching each other.
	Cannibalism	Birds peck one another at wounds.
Indicative of welfare	Dust bathing	Birds throw dust over their bodies.
	Flapping wings	Stretching and moving wings up and down.
	Stretching legs and wings	Stretching legs out and wings down.
	Social interaction	Non-agonistic interactions among the birds, expressed by coordinated movements.
	Shaking and fluttering feathers	Birds shake their body and feathers.
	Preening feathers	Birds clean and straighten their own feathers using their beak.

Egg production and mortality rates were evaluated daily. With regard to the study of poultry behavior, the obtained data were grouped as follows: physiological behaviors, reproductive behaviors, undesirable and agonistic behaviors, and behaviors indicative of welfare (Table 1). These evaluations were performed once weekly using the scanning technique in three periods of the day: morning (08.00-09.00 h), midday (12.00-13.00 h), and afternoon (16.00-17.00 h). In addition, a joint analysis of these periods was carried out (08.00-09.00; 12.00-13.00; and 16.00-17.00 h), referred in this manuscript as the "full period". Images of the bird behaviors were captured simultaneously using cameras installed in the center of the barns opposite to each other. Observations lasted 1 min and were made at regular 10-min intervals, totaling twelve observations per hour, considering that each barn had two cameras.

Tolerance-reflex behavior (Figure 1) was evaluated by counting the number of birds exhibiting such behavior within thirty linear meters of the entrance door of the barn. This evaluation was performed after the end of the image-recording period to allow analysis of the other behaviors.

A split-plot design in a randomized block was used. Data on egg production, mortality rate, meteorological variables, and behavioral variables were subjected to analysis of variance using the SAS statistical software package (Statistical Analysis System, version 9.2). Means were compared by Tukey's test at the 0.05 level of significance.

Behavioral variables were subjected to the transformation.

Results and Discussion

Statistical analysis of temperature, humidity, BGHI, and enthalpy data using Tukey's test did not indicate evidence to reject the hypothesis of similarity between the thermal environments inside the barns (Table 2); therefore, the differences found can be attributed to the studied parameters.

The lowest temperature and the highest relative humidity index were recorded in the morning and compared with the other periods of the day (Table 2). The mean and maximum daily temperatures remained above the thermal neutral zone, most part of the day, which, according to the Isa Brown management guide (2012-2013), is in the narrow range of 21-24 ºC, characterizing a heat stress situation. The highest temperatures recorded throughout the experimental period were 35.6 ºC and 35.7 ºC in barns 1 and 2, respectively. As expected, the relative air humidity values were significantly higher in the morning (Table 2).

According to Ferreira (2005), adult birds produce more when reared in environments with relative humidity in the range of 40-70%. In the present study, except for the morning hours, a misting system was necessary so that relative humidity would not reach critical levels; i.e., values below 40%.

The BGHI was significantly lower in the morning. Considering the values established by Tinôco (1998), in which a BGHI value of up to 75 is considered as thermal comfort value for laying hens, it was verified that the values obtained in the periods of midday and afternoon, between 77 and 78, were above the recommended values. Baêta and

Source: private archive.

Figure 1 - Manifestation of the behavior named tolerance reflex.

Table 2 - Mean values of temperature, air relative humidity, black globe humidity index, and enthalpy in three periods of the day (8.00-9.00, 12.00-13.00, and 16.00-17.00 h) and in the full period

Barn	Temperature (°C)			Air relative humidity (%)			Black globe humidity index			Enthalpy (Kj/kg dry air)		
	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h
With roosters	24.0±0.4Ba	29.9±0.6Aa	30.1±0.9Aa	78.8±2.5Aa	52.0±3.8Ba	52.2±4.3Ba	73.7±0.5Ba	78.2±0.7Aa	78.4±0.8Aa	67.4±0.8Ba	72.9±0.7Aa	73.5±0.9Aa
Without roosters	23.9±0.4Ba	30.0±0.7Aa	30.3±0.9Aa	78.8±2.7Aa	51.5±4.0Ba	50.7±4.4Ba	73.3±0.4Ba	78.3±0.6Aa	77.3±0.9Aa	67.3±0.7Ba	72.8±0.8Aa	73.4±0.9Aa
CVp (%)		1.33			5.38			3.08			0.85	
CVs (%)		1.38			3.28			2.22			2.02	
Full period												
With roosters		28.0±0.6a			61.0±3.2a			76.8±0.6a			71.3±0.7a	
Without roosters		28.1±0.6a			60.3±3.2a			76.3±0.6a			72.7±0.7a	
CV (%)		7.53			19.95			2.74			3.83	

CVp - coefficient of variation of the plot (rooster); CVs - coefficient of variation of the subplot (time); CV - coefficient of variation.
Means followed by the same uppercase letter within rows and lowercase letter within columns are not significantly different by Tukey's test at 5% probability level.

Souza (1997) reported an alert situation with BGHI value above 74. Armstrong (1994) considered BGHI of 78 as critical limit.

The analysis of the thermal conditions from the perspective of enthalpy confirms heat stress condition, characterized by values above 70. According to Barbosa Filho et al. (2007) and Silva et al. (2006), the upper and lower enthalpy limits of the comfort zone are 70 and 64, respectively.

The egg production of the hens reared with roosters was significantly higher ($P<0.05$) than that of the hens reared without rooster and higher than the value recommended in the Isa Brown management guide (2012-2013) (Table 3). The hens housed with roosters also had lower mortality rate.

The laying hens of both barns started laying eggs at 15 weeks of age. At the 18th week of age, according to the Isa Brown management guide, the birds were supposed to reach an egg production rate of 2% (Hendrix Genetics, 2012-2013). At this age, layers housed without roosters reached egg production rates of 10.94% and those housed with roosters reached production rates of 18.87%. It can be inferred that the presence of roosters was the factor determining these results, corroborating Leonard et al. (1993), who reported an improvement in the productivity of birds due to exposure to the opposite sex.

According to Newberry (1995), the presence of rooster plays an important role in environmental enrichment, providing improvements in the biological functioning of birds in captivity and consequent improvements in their health. This result can explain the lower mortality rate recorded in the barn with roosters. In absolute terms, at the end of the experimental period in the treatment with roosters, the mortality rate recorded was 0.25%, whereas in the treatment without roosters the rate was 0.51% (Table 3).

It should be stressed that, although mortality was higher in the barn without roosters, the rate was not higher than the 1.11%, as described in the Isa Brown management guide for the end of the 31st week of age of the birds.

Table 3 - Mean values of egg production and mortality of Isa Brown laying hens reared with and without roosters and values of the Isa Brown management guide

Barn	Egg production (%)	**Mortality (%)
With roosters	84.40±6.56A	0.25±0.04B
Without roosters	76.21±8.12B	0.51±0.10A
Isa Brown management guide	75.33±9.62B	0.52±0.10A
Coefficient of variation (%)	9.56	33.30

** Highly significant.
Means followed by the same letter within columns are not significantly different by Tukey's test ($P<0.05$).

Table 4 - Physiological behaviors (%) recorded in the barns with and without roosters in three periods of the day (8.00-9.00, 12.00-13.00, and 16.00-17.00 h) and in the full period

Barn	Behavior											
	Drinking			Feeding			Visit to the nest			Time spent in the nest		
	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h
With roosters	31.3±5.2Ab	36.8±3.2Aab	45.1±5.6Aa	80.5±4.0Aa	77.1±5.4Aa	81.9±3.5Aa	91.7±3.3Aa	61.1±5.8Ab	64.6±4.1Ab	91.0±2.8Aa	16.6±3.2Ab	11.8±4.2Ab
Without roosters	29.2±4.5Aa	35.8±3.8Aa	37.8±4.3Aa	84.7±2.3Aa	77.1±4.6Aa	84.7±4.1Aa	82.6±2.6Aa	50.0±4.0Ab	41.0±5.5Bb	83.3±4.7Aa	15.9±5.3Ab	11.8±1.2Ab
	Full period											
With roosters		37.7±2.9A			79.9±2.5A			72.5±3.4A			39.8±6.4A	
Without roosters		34.2±2.7A			82.2±2.2A			57.9±3.8B			34.3±6.4A	

Means followed by the same uppercase letter within columns and lowercase letter within rows are not significantly different by Tukey's test at 5% probability level.

It is important to highlight that, contrary to what is observed in the fertile egg production system, using a 1:10 (Grunow et al., 2009) and 1:12 ratio of males to females (Embrapa, 2007), there was no plumage damage in the laying hens at the end of the experimental period.

The drinking behavior changed considerably only according to the observed period in the treatment with the presence of rooster. There was no significant statistical difference due to the introduction of roosters; drinking frequency remained the same in the full period (Table 4).

The increased water intake at the hottest time of the day is directly related to the increased demand for water to be used in the process of heat loss through evaporative processes. Many studies have suggested that endogenous heat production is associated with the lean tissue mass accrual of the individual (Brown-Brandl et al., 2004; Ball et al., 2008). This result can explain the increase in drinking behavior only in the barn with roosters, since they were heavier than the laying hens, which probably caused greater sensitivity to heat stress and dependency on the methods of latent heat dissipation.

The feeding behavior was not influenced by the presence of roosters or by the period of time observed (Table 4). This was found to be a high-priority behavior and, therefore, was displayed with a high frequency rate of 77.1-84.7%.

In the evaluated production system, it was apparent that the greatest stimulus to food intake came from the sound produced with the activation of the automatic feeder. After hearing the sound, the birds approached the feeder and, for a few minutes, this was the prevailing behavior in the barns. Using the scanning technique, Pereira et al. (2015a) did not find a significant difference in the frequency of feeding behavior either. However, other authors, such as Barbosa Filho et al. (2007) and Silva et al. (2006), using the individual bird banding technique, reported a decrease in the frequency of feeding behavior with temperature increase.

The presence of roosters changed the frequency of the visit to the nest behavior only at 17.00 h, when this activity was more frequent (64.6%) in the barn with roosters. This behavior was directly associated with egg production and, therefore, it was displayed more frequently ($P<0.05$) in the morning. The frequency values in the barns with and without roosters were 91.7% and 82.6%, respectively. The analysis in the full period demonstrated that this behavior changed considerably due to the presence of roosters (Table 4). In the barn with roosters, the frequency of 72.5% was recorded, whereas in the barn without roosters, the frequency was only 57.9%.

Duncan (1998) also reported the relationship between the nest visit frequency and egg laying. The author identified that nest visit is more frequent in the pre-laying period (1-2 h before laying) and reported that, when the birds are prevented from visiting the nest, they become frustrated and demonstrate it by crouching.

The time spent in the nest was statistically similar, regardless of the presence of roosters, but at 09.00 h, it was statistically higher in both barns, which is consistent with literature data reporting a higher egg-laying rate in the morning hours. At 09.00 h, frequencies of 91.0% and 83.3% were observed in the barns with and without roosters, respectively. In the other periods of time evaluated, frequency was lower than 17.0%, characterizing the great influence of the period of the day.

According to Riber (2010), the use of nest boxes is an important part of the behavioral repertoire of laying hens kept under commercial conditions and, thus, they must be granted this right to minimize their stress and improve their welfare. Accordingly, it is worth mentioning that the egg laying rate on litter was low, which is in agreement with the findings of Barbosa Filho (2004), who found that after a period of acclimation, birds "learn" to use the nests.

With regard to the reproductive behaviors (Table 5), statistical analyses to compare courtship rituals and mating behavior data between the barns were not carried out because these behaviors were not displayed in the barn without roosters.

Both the courtship rituals and the mating behaviors changed significantly according to the period of time observed. Courtship rituals were less frequent in the morning and were performed at a frequency lower than 5%.

The mating frequency was significantly higher ($P<0.05$) in the late afternoon than in the morning. During these periods of time, the frequency increased from 8.3% to 19.4% (Table 5). At 13.00 h, the recorded frequency was similar to that of 9.00 and 17.00 h (Table 5).

Studying the behavior of birds, Campos (2000) suggested that the highest frequency of mating in the afternoon is associated with the lower egg production in this period of the day, which can be a strategy to facilitate the ascent of spermatozoa through the female reproductive tract, allowing fertilization. Thus, the results obtained in the present study corroborate those found by this author.

The tolerance-reflex behavior was more frequent in birds reared without the presence of roosters in all periods of time evaluated (Table 5). This behavior changed significantly ($P<0.05$) according to the observation time only in the shed without roosters; the highest frequency was observed in the afternoon (55.1%) and the lowest in the morning (22.5%). The analysis of the full period showed that this behavior changed considerably due to the presence of roosters.

The mating behavior reinforces the idea that the tolerance-reflex mechanism is indeed related to the lack of mating opportunities, since tolerance-reflex behaviors are more frequently displayed without the presence of roosters, and in the afternoon, when mating was also higher (Table 5). This result confirms that roosters are an important tool for behavioral freedom.

The frequency of agitation among the birds was lower than 5.0% in both barns and it did not change significantly due to the presence of roosters and the observation time ($P>0.05$) (Table 6). It is possible that the roosters contributed to the low frequency observed, because even the birds housed without roosters were exposed to visual and auditory stimuli due to the proximity to the experimental barns, which might have given these birds a greater feeling of safety. Odén et al. (2015) found that laying hens reared with roosters demonstrated shorter periods of tonic immobility and low frequency of vigilance behavior, which are typical behavioral responses to fear. The authors concluded that the presence of roosters reduced fear in the laying hens.

Table 5 - Reproductive behaviors (%) recorded in the barns with and without roosters in three periods of the day (8.00-9.00, 12.00-13.00, and 16.00-17.00 h) and in the full period

Barn	Behavior								
	Courtship rituals			Mating			Tolerance reflex		
	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h
With roosters	4.9±2.8b	12.5±1.6a	13.2±3.5a	8.3±2.3b	13.2±3.3ab	19.4±3.6a	0.4±0.2Ba	0.4±0.2Ba	0.4±0.2Ba
Without roosters	-	-	-	-	-	-	22.5±3.7Ac	36.6±2.4Ab	55.1±3.7Aa
	Full period								
With roosters		10.2±1.7			13.6±1.9			0.4±0.1B	
Without roosters		-			-			38.1±2.6A	

Means followed by the same uppercase letter within columns and lowercase letter within rows are not significantly different by Tukey's test at 5% probability level.
(-): absent behavior.

The frequency of fights changed significantly due to the presence of roosters and also according to the observed period. The barn with roosters showed higher frequency of fights in the late afternoon (6.2%), differing statistically from the frequency recorded in the barn without roosters (0.7%) (Table 6). Interestingly, during that period of time, most of the fights involved roosters only. The higher frequency of mating recorded in the late afternoon (Table 5) is likely to have intensified the disputes over females, causing increased conflicts among roosters. However, it should be noted that this was a sporadic behavior, with frequency lower than 10%, corroborating the reports of D'Eath and Keeling (2003), who also found lower frequency of aggressive behavior in birds reared in large groups.

Cannibalism was not observed (Table 6), which can be attributed mainly to the low idleness among the birds, because they were able to display several different behaviors. Rocha et al. (2008) stated that environmental enrichment is an important tool to minimize cannibalism. Janczak and Riber (2015) also pointed out that, as a result of a continuous genetic selection, it is possible that the current genotypes reared for egg production are less susceptible to feather-plucking and cannibalism, emphasizing that new studies are necessary to investigate the need to practice debeaking.

In the present study, we identified that the egg production system, rearing the birds on the floor, allowed the display of a wide range of behaviors indicative of welfare. The frequency of the following behaviors was recorded: dust bathing, flapping wings, stretching legs and wings, social interaction, shaking and fluttering feathers, and preening feathers (Table 7).

The display of the dust bathing behavior changed only according to the period of time observed. This variable was less frequent ($P<0.05$) in the morning in both barns. The analysis of the full period demonstrated that the frequency of this behavior was similar in the two barns.

Table 6 - Undesirable and agonistic behaviors (%) recorded in the barns with and without roosters in three periods of the day (8.00-9.00, 12.00-13.00, and 16.00-17.00 h) and in the full period

Barn	Behavior								
	Agitation			Fighting			Cannibalism		
	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h
With roosters	-	2.8±1.2Aa	2.8±1.9Aa	2.1±1.1Ab	1.4±0.9Ab	6.2±2.1Aa	-	-	-
Without roosters	2.1±1.5a	4.2±1.9Aa	4.7±1.6Aa	1.4±0.9Aa	0.7±0.7Aa	0.7±0.7Ba	-	-	-
					Full period				
With roosters		1.8±0.8A			3.2±0.9A			-	
Without roosters		3.5±1.0A			0.9±0.4B			-	

Means followed by the same uppercase letter within columns and lowercase letter within rows are not significantly different by Tukey's test at 5% probability level.
(-): absent behavior.

Table 7 - Behaviors indicative of welfare (%), recorded in the barns with and without roosters, in three periods of the day (8.00-9.00, 12.00-13.00, and 16.00-17.00 h) and in the full period

Barn	Behavior								
	Dust bathing			Flapping wings			Stretching legs and wings		
	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h	9.00 h	13.00 h	17.00 h
With roosters	2.8±1.2Ab	36.8±10.4Aa	45.1±4.8Aa	70.8±7.4Aa	72.2±3.3Aa	68.8±5.6Aa	4.2±1.9Aa	4.2±1.9Aa	3.5±1.2Aa
Without roosters	2.1±1.6Ac	30.6±7.4Ab	53.5±7.3Aa	62.5±7.6Aa	45.8±6.5Ba	51.4±5.8Ba	6.2±2.1Aa	4.2±1.3Aa	2.8±1.2Aa
					Full period				
With roosters		28.7±4.9A			70.6±3.2A			3.9±1.0A	
Without roosters		28.2±4.9A			53.2±3.9B			4.4±0.9A	
	Social interaction			Shaking and fluttering feathers			Preening feathers		
With roosters	13.9±3.0Aa	24.3±4.0Aa	24.3±3.3Aa	31.9±3.1Aa	34.7±4.5Aa	32.6±4.2Ba	86.1±3.5Aa	65.3±5.4Ab	55.6±6.5Ab
Without roosters	13.1±2.6Aa	22.9±3.7Aa	17.4±2.6Aa	36.8±3.2Aa	36.1±3.9Aa	47.2±4.3Aa	86.1±5.2Aa	70.1±5.7Aa	61.8±6.5Ab
					Full period				
With roosters		19.9±2.2A			33.1±2.2B			72.7±2.2A	
Without roosters		18.0±1.8A			40.0±2.3A			69.0±1.8A	

Means followed by the same uppercase letter within columns and lowercase letter within rows are not significantly different by Tukey's test at 5% probability level.

According to Santos et al. (2010), dust bathing is a mechanism of heat exchange with the environment. This may explain the fact that this behavior was more frequent at the hottest times of the day, between 13.00 and 17.00 h, whereas at 9:00 h, its frequency was lower than 3.0%. Pereira et al. (2007) reported that the frequency of dust bathing is positively correlated with temperature. According to the European Commission (2000), this is considered important for birds because it helps maintain plumage in good condition.

It was noted that when given the chance to choose, birds prefer to take dust baths in sunny places. It is believed that the main factors influencing such preference are: the litter is usually drier and softer, facilitating the bedding material to spread over the legs of the birds; the exposure to sunlight aids in the control of ectoparasites; and when loose bedding material is thrown into the air it reflects sun light, which can stimulate the curiosity of the birds. Petherick et al. (1995) reported that litter is an important visual stimulus to birds and is one of the main factors that trigger the dust bathing behavior.

There was a significant difference in the frequency of the flapping wing behavior due to the presence of roosters between 13.00 and 17.00 h. During this period, their presence significantly increased the display of this behavior, which reached 72.2% and 68.8%, respectively, whereas in the barn without roosters, the frequency was, respectively, 45.8% and 51.4%. The evaluation of the full period indicated that the frequency of this behavior was different in the two barns, but no difference (P>0.05) was found between the observation times in the analysis of the same barn.

The observations made in the present study demonstrated that this behavior was more frequently displayed by the roosters, in agreement with the report by Wood-Gush (1956), according to whom this is a typical behavior of roosters during aggressive contacts and when courting females. Leonard and Zanette (1998) studied the behavior of roosters and identified an increase in the frequency of this behavior when laying hens were around. The authors defined it as a mechanism of body exhibition that influences the choice of roosters to mate.

The frequency of stretching legs and wings behavior was similar (P>0.05) in both barns and it was not significantly different over the observation period (Table 7). Pereira et al. (2013) evaluated the behavior of laying hens and, similarly to our findings, they also observed low frequency of this behavior, 2.8%-6.2%. Among all behaviors indicative of welfare, flapping wings was the one with the lowest frequency.

The frequency of social interactions behavior did not change according to the studied variables (presence of roosters and different periods of time); there was no significant difference in the full period either (P>0.05). The occurrence of this behavior varied between 13.1% and 24.3%; therefore, it can be said that it was a very frequent behavior among the birds.

The hens showed strong tendency to interact with the roosters by cleaning their feathers and giving them many delicate quick pecks, usually on the face side. These forms of interaction were less frequent in the barns without roosters. Without the males, the predominant forms of interaction were collective dust baths and the act of scratching the ground together searching for insects and/or objects in the litter.

The presence of roosters caused a significant decrease in the frequency of the shaking and fluttering feathers behavior in the afternoon. During this period of time, this behavior was displayed at a frequency of 32.6%, whereas in the barn without roosters, the frequency was 47.2%. Considering that the allocation of time and resources to different physical or behavioral activities is controlled by motivational mechanisms, it is hypothesized that the frequency reduction observed is associated with the higher frequency of other behaviors performed during this period, e.g., the reproductive behaviors.

The same behavior was displayed with a relatively similar frequency in the different periods of the day; in other words, the frequency of the behavior did not change according to the observation time, probably because it was more closely related to the act of straightening the feathers than to the attempts to dissipate heat. Pereira et al. (2005) did not identify the influence of period of time on the frequency of this behavior either. Preening feathers was also described as a behavior inherent to the species and indicative of welfare. Its frequency changed according to the observation period and the presence of roosters (P>0.05). The barn with roosters showed higher frequency in the morning (86.1%), whereas the barn without roosters showed higher frequencies in the morning and midday, 86.1% and 70.1%, respectively (Table 7).

It was found that, among all behaviors that indicate welfare, this was the most frequently displayed behavior by the birds in the morning. Furthermore, it was possible to identify that this behavior was systematically more frequently exhibited by the birds sitting on the perch, whereas the birds on the floor were more engaged in other activities such as feeding, drinking, and interacting with the litter.

Conclusions

This alternative egg production system proved to promote animal welfare since it provides and stimulates the display of behaviors considered important for birds. In this production system, the presence of roosters stimulates the display of reproductive behaviors, thus broadening the repertoire of natural behaviors of birds. Moreover, their presence significantly reduces tolerance-reflex behavior, which is associated with the lack of mating opportunities.

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