

Baggio, RA, Lunardi, SJ, Testa, M, Boito, JP, Boiago, MM, Zampar, A, Paiano, D, Petrolli, TG, Silva AS & Zotti, MLAN (2020). Performance and well-being of laying hens subjected to various beak trimming methods and rearing systems. *Research, Society and Development*, 9(7): 1-24, e570974509.

Desempenho e bem-estar de galinhas poedeiras submetidas a diferentes métodos de corte de bico e sistemas de criação

Performance and well-being of laying hens subjected to various beak trimming methods and rearing systems

Rendimiento y bienestar de las gallinas ponedoras sometidas a varios métodos de corte de pico y sistemas de cría

Recebido: 14/05/2020 | Revisado: 14/05/2020 | Aceito: 17/05/2020 | Publicado: 30/05/2020

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Resumo

O objetivo é avaliar se diferentes métodos de corte de bico afetam o desempenho e o bem-estar de galinhas poedeiras criadas em sistemas de gaiola e piso. Durante a fase inicial, foi utilizado um delineamento inteiramente casualizado, com três tratamentos (corte de bico com lâmina quente, corte de bico com radiação infravermelha e sem corte de bico (controle)), com quatro repetições. Na fase de recria e produção, foi utilizado um arranjo fatorial que envolveu dois sistemas de produção (gaiola e piso) e os três métodos de corte de bico citados. As aves foram criadas ao longo de 30 semanas. Avaliamos a produtividade, qualidade dos ovos, parâmetros comportamentais e variáveis bioquímicas. Na fase de cria, os tratamentos não alteraram o desempenho das aves, porém, as aves do grupo controle apresentaram níveis mais altos de glicose no sangue ($p=0,043$). Na fase de recria, as aves submetidas à debicagem por lâmina quente e as aves do grupo controle apresentaram menor consumo de ração e melhor conversão alimentar, e os níveis de triglicerídeos foram maiores no sistema de criação em

gaiola ($p<0,05$). Na fase de produção, o sistema de criação de gaiolas apresentou maior produtividade, peso médio dos ovos, níveis de colesterol e níveis de espécies reativas ao oxigênio ($p<0,05$). Na fase de produção, o sistema de piso possibilitou uma maior frequência de comportamentos indicadores de conforto ($p<0,01$). Concluímos que aves criadas em gaiolas apresentam melhor desempenho e maior eficiência na produção de ovos. A escolha do método de debicagem depende do sistema de criação.

Palavras-chave: Comportamento animal; Livre de gaiolas; Lâmina quente; Radiação infravermelha; Produção de ovos.

Abstract

The aim to evaluate whether different beak trimming methods affected the performance and well-being of laying hens raised in cage and floor systems. During the starter phase, we used a completely randomized design, with three treatments (hot blade beak trimming, infrared beak trimming, and no beak trimming (control)), with four repetitions and in the grower and production phase, we used a factorial arrangement scheme involving two production systems (cage or floor) and three beak management methods (as above). We reared the birds in starter, grower and production phases over a course of 30 weeks. We evaluated productivity, egg quality, behavioral parameters and biochemical variables. In the starter phase, control birds showed higher blood glucose levels ($p=0.043$). In the grower phase, birds subjected to hot blade beak trimming and control birds showed lower feed intake and better feed conversion. Triglyceride levels were higher in the cage rearing system ($p<0.05$). In the production phase, the cage rearing system showed higher productivity ($p<0.05$), mean egg weight ($p<0.01$), cholesterol levels ($p<0.05$) and oxygen reactive species levels ($p<0.05$). In the production phase, the floor system gave rise to a higher frequency of comfort movement behaviors ($p<0.01$). Hens in cage had improved their performance and had greater egg production efficiency. The choice of the beak method depends on the breeding system.

Keywords: Animal behavior; Cage-free; Hot blade; Infrared radiation; Egg production.

Resumen

El objetivo es evaluar si los diferentes métodos para cortar el pico afectan el rendimiento y el bienestar de las gallinas ponedoras criadas en sistemas de jaulas y pisos. Durante la fase inicial, se utilizó un diseño completamente al azar, con tres tratamientos (boquilla cortada con cuchilla caliente, boquilla cortada con radiación infrarroja y boquilla sin corte (control)), con cuatro repeticiones. En la fase de cría y producción, se utilizó una disposición factorial, que

involucró dos sistemas de producción (jaula y piso) y los tres métodos de corte de la boquilla mencionados. Las aves fueron criadas durante 30 semanas. Evaluamos la productividad, la calidad del huevo, los parámetros de comportamiento y las variables bioquímicas. En la fase de crianza, los tratamientos no alteraron el rendimiento de las aves, sin embargo, las aves en el grupo de control tenían niveles de glucosa en sangre más altos ($p=0.043$). En la fase de cría, las aves sometidas a pico de cuchilla caliente y las aves en el grupo de control tuvieron un menor consumo de alimento y una mejor conversión de alimento, y los niveles de triglicéridos fueron más altos en el sistema de jaula ($p<0.05$). En la fase de producción, el sistema de cría en jaulas mostró una mayor productividad, peso promedio del huevo, niveles de colesterol y niveles de especies reactivas de oxígeno ($p<0.05$). En la fase de producción, el sistema de piso permitió una mayor frecuencia de comportamientos de indicadores de confort ($p<0.01$). Concluimos que las aves criadas en jaulas tienen un mejor rendimiento y una mayor eficiencia en la producción de huevos. La elección del método del pico depende del sistema de reproducción.

Palabras clave: Comportamiento animal; Sin jaula; Cuchilla caliente; Radiación infrarroja; Producción de huevos.

1. Introduction

In the internet age, information regarding animal production and management systems are universally available, coincident with increasing concern animal welfare. Several controversies on the subject have appeared in the lay press news, to say nothing of discussions in the technical-scientific literature. In poultry farming, for example, there are controversies surrounding intensive production systems (in cages) as well as handling practices such as beak trimming. It is important to note that beak trimming for laying hens has been criticized because it maims the animal and inflicts pain. According to Gentle (2011), the pain resulting from the procedure can be acute or chronic, with variable durations, according to the beak trimming method as well as the age of the bird undergoing the procedure. However, authors such as Gustafson et al. (2006) and Kuenzel (2007) have stated that no alternative prevents cannibalism and feather pecking between birds with the same efficiency as beak trimming. Furthermore, beak trimming resulted in lower feed wastage with a consequent improvement in feed conversion (Araujo et al., 2005). This may explain why the practice remains common in modern production systems. The conventional cage rearing system reduces the space per animal and does not provide environmental enrichment for the bird. It limits and even

precludes the repertoire of activities considered important for the animal, exacerbating the pain and suffering brought on by beak trimming (Alves et al., 2007).

Previous studies comparing infrared and hot blade beak trimming were developed in a cage rearing system, with limited generalizability to floor rearing systems (Marchant-Forde et al., 2008; Dennis et al., 2009; Vieira Filho et al., 2016; Pelicia et al., 2019); however, the results differ between studies and the benefits of beak trimming in hens and their methods requires more investigation. Our hypotheses were that (a) the infrared radiation beak trimming method would improve animal welfare to a greater extent than the hot blade method; that (b) control birds would show a higher frequency of injurious pecking behavior resulting from having intact beaks; (c) trimmed birds would generate greater zootechnical production than would control birds; and (d) that the floor-rearing system would provide improved well-being.

At the global level, laying hens are nowadays caged in their vast majority; but in order to keep up with trends in animal welfare improvement it is important to research and find alternatives to maintain production efficiency as well as ally animal welfare. Thus, the objective this study was to evaluate whether different methods of beak trimming affected the production and well-being of laying hens in two rearing systems.

2. Materials and Methods

This research had an exploratory and quantitative nature (Pereira et al., 2018). To achieve the objectives of this study, we used a completely randomized design, as detailed below.

2.1. Experiment location

Research with animals was conducted between April and November 2016, in an experimental chicken facility ($27^{\circ}07'S$, $52^{\circ}37'W$, elevation 680 m), total area of 32 m², constructed of wood, with concrete walls and curtain-sided barns (Southern Brazil).

Research on animals was conducted according to the institutional committee on animal use (protocol number 1961120216).

2.2. Animals and experimental units

We used 228 laying hens of the Hy-Line Brown lineage. The experiment was conducted from the first day until 30 weeks of life. This period comprises following phases: starter (1 to 5 weeks), grower (6 to 16 weeks) and production (17 to 30 weeks). In starter phase, the experiment was conducted only in the floor rearing system. In the other phases, grower and production, we used both, a floor rearing system and a cage rearing system.

To simulate a floor rearing system, were constructed 12 boxes (1 x 1 x 1 m) with PVC pipes and screen. Each box was equipped with a tubular-type feeder, a pendulum- drinker, a 125 W infrared heating bulb, and a 10-cm deep pine wood bed. The cage rearing system consisted of a set of 12 cages (0.5 x 0.6 x 0.4 m) equipped with trough-type feeders and cup-type drinkers, positioned in front and at the bottom of the cages, respectively.

The number of birds was changed throughout the experimental period in order to achieve the animal density recommended by the lineage manual. In the starter phase (1 to 5 weeks), 19 birds were housed per box (total of 12 boxes). For the subsequent stages of production, we redistributed the birds. In the grower phase (6 to 16 weeks), ten remained in the same box and seven were transferred to cages; and in the transfer the birds from the same box were allocated in the same cage ($n = 7$); that is, there was no bird mixing to minimize aggressive behavior. During the production phase (17 to 30 weeks), five hens were housed in each box and five in each cage. Therefore, birds were randomly removed from the experiment in the cycle phase changes, that is, growth ($n = 24$) and egg production ($n = 24$ cages, $n = 60$ boxes). All birds removed in the experiment were housed in another experimental room, and were part of another experiment.

2.3 Experimental design

For the starter phase, we used a completely randomized design, with three treatments (hot blade beak trimming, infrared beak trimming, and no beak trimming (negative control)), with four repetitions for each treatment, i.e. each box was considered an experimental unit.

During the rearing, grower and production phases, the birds were transferred from place to place, because a new cage system was included in our study; that is, a new treatment where the cage was the experimental unit. In these phases, because we had two systems (cage and box) and three beak trimming methods (hot blade, infrared and control) we use a factorial

arrangement (2 X 3), with two rearing systems (floor and cages) and three beak trimming methods (as above), for a total of six treatments, with four experimental unit (repetitions).

2.4. Beak trimming methods

Infrared beak trimming was performed on the first day of life using the Poultry Service Processor (HY-LINE: Nova Tech Engenharia device, 2016). Hot blade beak trimming (no. 950-08, Uniquímica, Brazil) was performed by a trained individual, using a commercial trimmer on the 12th day of life, with second trimming in the 12th week of life in order to repair uneven beak growth. We chose to perform revision in this manner to approximate real-life field operations.

2.5. Performance

Identical diets were used for all groups, with adjustments throughout the experimental period according to nutritional requirements (Rostagno et al. 2011). Birds were fed ad libitum throughout the experiment. Bird density, photoperiod, air temperature, height and number of animals per feeder/drinking and other factor were according to recommendations of the lineage manual (HY-LINE: Manual de manejo de poedeiras comerciais, 2014).

In the starter phase, we evaluated performance (feed intake, weight gain and feed conversion) by weighing the animals and feed using an electronic scale (Model 2095/23, Toledo do Brasil, Brazil) with a ± 5 g error. We weighed the birds on days of life 1, 12, 13, and 40. These periods (1–12 days, 13–40 days) were defined as such because we performed hot blade beak trimming on day 12, and the because the interval after infrared irradiation required for the tip of the beak to fall off was seven to 10 days (Marchant-Forde et al. 2008). In the grower phase, we evaluated the same variables. In addition, we evaluated batch uniformity in the 12th and 16th weeks of life. We evaluated performance in the production phase at the end of three productive cycles of 28 days, at which time we considered the following: daily feed intake; feed conversion (kg of feed/kg of egg produced, and kg of feed/dozen eggs produced); average egg weight; egg mass; percentage of production; and average final weight of the birds.

To calculate mean egg weight, we used a semi-analytical balance (Model ARC120, OHAUS Corporation, Brazil), with an error of ± 0.1 g. We calculated egg production (%) by counting and collecting eggs daily from each experimental unit. To calculate the mass of eggs

in the final two days of each productive cycle, all eggs in each experimental unit were individually identified and weighed. The egg mass was then calculated as follows: egg mass = mean weight (g) x day production (%). Sexual maturity was estimated by age on days when 50% of the birds in each unit laid one egg per day. We analyzed and reported data from three cycles of 28 days each.

2.6. Egg quality

At the end of each productive cycle (28 days), two eggs per experimental unit were collected to evaluate specific gravity (Freitas et al. 2008) and shell resistance (kgf) using a texturometer (Model TA.XT Plus, Extragel, Brazil), height of albumen, height and width of the yolk, with the use of a digital caliper and weight of the yolk and albumen, Haugh units were calculated as follows: $HU = 100 \log (\text{mean albumen height} / \text{egg weight}) + 7.57 - 1.7 \times \text{egg weight} / \text{grams} \times 0.37$ (Haugh, 1937); yolk index was calculated as follows: yolk index = yolk height ÷ yolk width.

Yolk color was determined using the DSM colorimetric spectrum, ranging from 1 to 15, from the lightest to the reddest. Because of the subjectivity of this method, yolk color was also evaluated using colorimeter (Model CR400, Konica Minolta Sensing Americas, Inc, USA) that evaluated luminosity (L^*), intensity of red (a^*) and intensity of yellow (b^*).

The egg yolks were separated from the albumen, weighed separately, and the shells were washed for complete removal of any albumen adhered to the inner membrane. Shells were set to dry at room temperature for later weighing. Using these weights, we calculated the percentage of yolk, albumen and shell. Dry and wet shells were measured in three distinct segments (basal, equatorial and apical) with the aid of a digital caliper. From measurements of each segment, average shell thickness was calculated for each egg. The pH of the yolk and albumen was obtained using a digital pH meter (Model testo 205, Testo, Brazil).

2.7. Behavioral measures

Behavioral measures was performed weekly throughout the experimental period. Behavior was recorded between 0900 h and 1100 h by two trained observers. All animals in the experimental units were evaluated continuously for a period of five minutes, according to Marchant's adapted methodology (Marchant-Forde et al., 2008) to calculate the frequency of feather pecking behaviors (forcing down the head or neck, compulsively pecking another

bird), environmental pecking (floor pecking (Dennis et al., 2009), comfort movements (batting and spreading the wings, cleaning and shaking the feathers) and “sand baths” (scratching and throwing sand over its body, or performing simulations of these movements, while in the cage) (Barbosa-filho et al., 2007).

The “sand bath” was evaluated even in the cage rearing system, where there was no “sand” with which to “bathe” because the longer the bird is deprived of the opportunity to perform normal movements in its behavioral repertoire, the greater the likelihood of the animal attempting to simulate these behaviors (Zuanon 2007).

2.8. Feather score

We evaluated the animals by visual feather score on the final day of the experimental period, considering lesions in seven body regions: head, neck, chest, abdomen, wing, back and tail. We employed a scale of 0 to 5, with the best score being 0 (complete plumage), and the worst score being 5 (area completely plucked with skin lesions), according to the methodology described by Dennis et al. (2009).

This methodology was chosen by the authors due to international acceptance, as well as having an ethogram with appropriate behavioral definitions for our study.

2.9 Blood variables

We collected five blood samples on days of life 4, 16, 88, and 182. The first and second collections were carried out to verify possible negative physiological effects of the beak trimming procedures. For the first and second collections, we sacrificed one bird per experimental unit using inhaled isoflurane, followed by cervical dislocation. We performed the third blood collection on day 88 to determine the possible effects of the trimming revision (the second hot blade treatment).

We performed the fourth collection to evaluate the effects of possible chronic stress derived from the treatments. In the third and fourth collections, one bird was sampled per experimental unit, using the cutaneous ulnar vein.

We measured serum levels of glucose, triglycerides, cholesterol and reactive oxygen species (ROS), as well as the activity of antioxidant catalase (CAT) and superoxide dismutase (SOD). Serum cholesterol, triglyceride and glucose levels were evaluated using a semi-automated analyzer (Bioplus 2000®, São Paulo, Brazil) with specific kits. Serum levels of

ROS were evaluated according to the methodology described by Ali et al. (1992) using 2',7'-dichlorofluorescein diacetate (DCFH-DA). Briefly, we measured formation of a fluorescent oxidized derivative of DCFH, called 2',7'-dichlorofluorescein (DCF), with an excitation wavelength of 488 nm and an emission wavelength of 525 nm on an LS-50 spectrophotometer.

Result was expressed as FU/mg protein. We measured CAT activity according to Nelson and Kiesow (1972) methodology. The decrease in hydrogen peroxide (H_2O_2) was measured by spectrophotometry at 240 nm, and CAT activity was expressed as nmol/mg protein. We measured superoxide dismutase using the methodology described by McCord and Fridovich (1969). The speed of adrenochrome formation observed at 480 nm was used to determine SOD activity, expressed as U/mg protein.

2.10. Statistical analysis

The data were first evaluated for normality of the errors using the Shapiro-Wilk and Kolmogorov-Smirnov tests. The Fisher-Snedecor test ($p < 0.05$) and Tukey's test (when necessary) were used for the analysis of variance. Non-parametric tests were used for the behavioral variables.

The Friedman non-parametric method was used for the rearing data in the initial observations. Rearing and production data were subjected to aligned rank transforms for each factor and interaction, as suggested by Wobbrock et al. (2011). The aligned rank transforms were then analyzed using ANOVA. For the comparison of the interactions, the Friedman test was used. The Wilcoxon benchmark method was used to compare the means of the ranks for two samples, and the Dunn-Bonferroni test (Dunn 1964) was used for paired comparisons.

The aligned rank transforms were performed using ARTTool software. Analyses of variance were performed in SAS software, version 9.1 SAS/2012 for data with normal distribution. SPSS software, version 22, 2013 was used for Friedman's nonparametric tests and Wilcoxon's randomized blocks. Significant statistical differences were assumed when $P < 0.05$.

3. Results

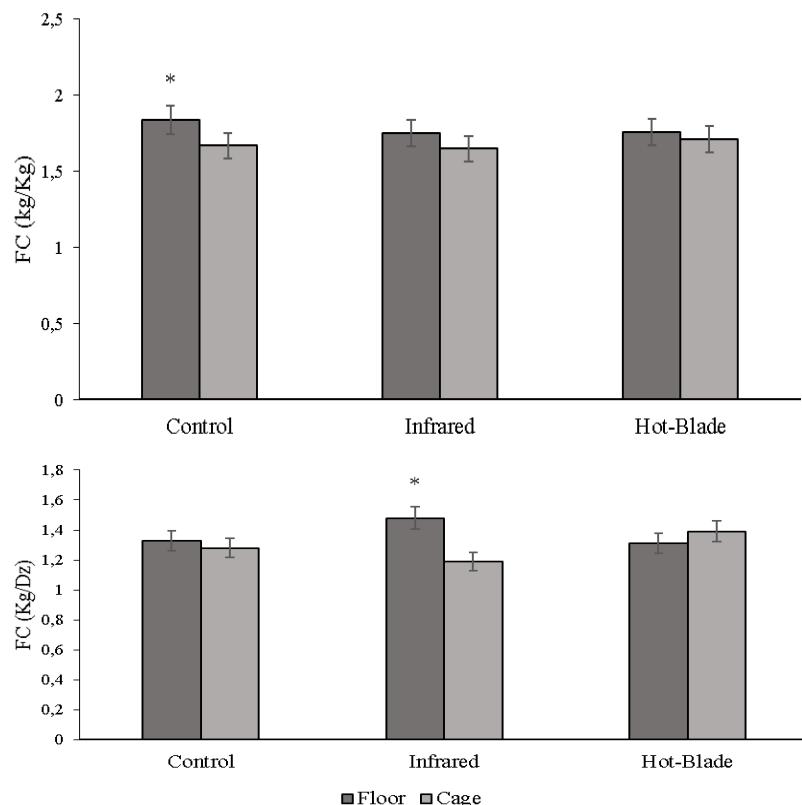
3.1. Performance

The treatments did not alter bird performance during the starter phase. In the grower phase, the birds in the floor rearing system showed higher daily weight gain, higher daily feed intake, and higher average weight at 12 weeks (Table 1).

No effects of rearing systems on feed conversion was observed. In the same phase, infrared beak trimming resulted in higher feed intake than did no beak trimming. Likewise, no beak trimming reduced feed conversion more than did infrared beak trimming.

Feed conversion variables (expressed as kg of feed/kg of eggs produced and kg of feed/dozen eggs produced) showed an association between rearing system and beak trimming method (Table 1 and Supplementary Figure 1).

Supplementary Figure 1: Variation of variable interaction: Feed conversion kg/kg (upper) and feed conversion kg/dozen eggs (lower) of laying hens in production phase subjected to infrared radiation beak trimming, hot blade beak trimming, and non-trimmed birds (control).



* Columns differ according to Fisher-Snedecor test ($P < 0.05$). Source: Authors.

This interaction showed that lighter birds had better feed conversion values when reared in cages (Supplementary Figure 1). Thus, in terms of production, when birds were not trimmed, the cage system was better than the floor rearing system. This result is related to higher average weight of the eggs, egg mass and productivity.

3.2. Egg quality

For qualitative variables, only the intensity of red and the percentage of yolk were altered for treatments (date not-showed). The intensity of red showed a significant difference in terms of rearing system: birds in the floor rearing system produced eggs with more reddish coloration than did those in the cage rearing system. The percentage of yolk was significantly different for the evaluated treatments: eggs produced by birds treated with hot blade beak trimming returned higher values compared with those produced by birds treated with infrared radiation beak trimming. However, neither type of beak trimming returned results different from those of non-beaked birds.

Table 1. Performance of laying hens subjected to infrared, hot blade beak trimming and untrimmed (control).

Variables	Rearing systems (RT)		Beak trimming methods (BT)			P-value		
	Floor	Cage	Infrared ¹	Hot-blade ²	Control	RT	BT	RT*BT
Grower phase								
Feed intake, g/day	57.34A	53.16B	56.55 b	55.31 ab	53.89 a	<0.01	<0.05	0.77
Weight gain, g/day	15.87 B	14.74A	14.96	15.29	15.67	0.01	0.40	0.51
Feed conversion, kg/kg	3.63	3.61	3.78 b	3.62 ab	3.46 a	0.76	0.01	0.30
Initial body weight, g	486.3	486	473.55	500.42	484.5	0.98	0.14	0.81
Body weight at 12 weeks, g	1227B	1203.26A	1207.6	1225.4	1212.6	0.03	0.35	0.06
Uniformity at 12 weeks, %	91.55	90.28	94.96	88.89	88.89	0.80	0.51	0.36
Body weight at 16 weeks, g	1580.26	1559.39	1571.05	1571.84	1566.58	0.21	0.96	0.18
Uniformity at 16 weeks, %	93.75	87.5	96.35	89.06	86.46	0.30	0.38	0.60
Production phase								
Feed intake, g/day	105.32	103.14	102.85	104.99	104.86	0.22	0.54	0.11
Egg mass, g	59.82 B	61.09 A	60.62	60.72	60.02	0.02	0.50	0.26
Average egg weight, g	55.22 B	58.87 A	56.72	57.2	57.21	0.01	0.92	0.62
Feed conversion, kg/kg	1.76	1.7	1.7	1.73	1.75	0.02	0.33	0.02
Feed conversion, kg/dz	1.37	1.28	1.33	1.35	1.31	0.13	0.82	0.04
Productivity, %	92.29 B	96.37 A	93.52	94.15	95.33	0.02	0.71	0.61
Final Body weight, g	2003.67 A	1899.13 B	1946.97	1969.13	1938.09	<0.01	0.68	0.30
Sexual maturity, days	133.83	132.83	132.37	133.75	133.87	0.33	0.41	0.38

Note: ¹Infrared beak trimming; ²Hot-blade beak trimming; Means followed by different uppercase letters in the line differ from each other by the Fisher-Snedecor test ($P < 0.05$); Means followed by different lowercase letters in the line differ from each other by the Tukey test ($P < 0.05$). Source: Authors.

3.3. Blood variables

Among the variables analyzed in the starter phase, only glucose showed a significant difference, specifically at the second collection (four days after hot blade beak trimming, 16 days old, Table 2). In the production phase, glucose levels showed significant differences with respect to the evaluated treatments, with lower concentrations observed in the birds subjected to hot blade beak trimming than with birds that underwent infrared radiation beak trimming. However, levels did not differ from those of control birds. In the grower phase, triglycerides levels differed significantly depending on the rearing system. Triglyceride levels were higher in the cage rearing group (Table 2). Variables such as glucose and triglycerides fluctuate rapidly in the blood after feeding; therefore, we cannot rule out the possibility that beak trimming may have adversely affected food intake because of pain.

Table 2. Serum levels of triglycerides (mg/dL), cholesterol (mg/dL), glucose (mg/dL), Superoxide dismutase (SOD) (IU/mg of protein) and catalase (CAT) (protein nmol/g) and reactive oxygen species (ROS) (FU/mg protein) of laying hens subjected to infrared radiation beak trimming, hot blade beak trimming, and non-trimmed (control).

Variables	Rearing systems (RS)			Beak trimming methods (BT)		P-value		
	Floor	Cage	Infrared ¹	Hot-blade ²	Control	RS	BT	RS* BT
16 days old (2 nd blood sample- growing)								
Triglycerides	-	-	167.25	134.75	176	-	0.66	-
Cholesterol	-	-	120.0	123.0	115.5	-	0.81	-
Glucose	-	-	256.7 ^a	277.7ab	316.7b	-	0.04	-
SOD	-	-	14.74	18.16	17.45	-	0.68	-
CAT	-	-	15.94	16.42	16.82	-	0.43	-
ROS	-	-	18.32	16.90	12.01	-	0.05	-
88 days old (3 rd blood sample - production)								
Triglycerides	114.54 A	145.92 B	122.57	140.5	128.5	0.03	0.47	0.57
Cholesterol	96.54	97.5	102.28	89.75	99.75	0.77	0.36	0.10
Glucose	246.81	237.42	248.43	254.62	223.5	0.46	0.23	0.34
SOD	14.86	17.71	18.76	15.92	14.81	0.24	0.30	0.38
CAT	16.08	16.6	16.59	15.79	16.64	0.44	0.51	0.35
ROS	16.78	16.43	16.66	16.92	16.23	0.74	0.87	0.33
182 days old (4 th blood sample- final)								
Triglycerides	716.09	981.18	794.37	853.57	905.71	0.13	0.90	0.87
Cholesterol	104.0A	201.3B	133.25	144.57	183	0.01	0.59	0.69
Glucose	245.18	249.9	273.25 b	213.29 a	252.43 ab	0.86	0.01	0.42
SOD	14.13	18.03	15.13	19.39	14.36	0.17	0.46	0.22
CAT	23.73	23.84	23.84	23.47	24.01	0.81	0.84	0.40
ROS	13.93 A	17.48 B	14.68	16.85	15.5	0.03	0.45	0.25

Note: ¹Infrared beak trimming; ²Hot-blade beak trimming; Means followed by different uppercase letters in the line differ from each other by the Fisher-Snedecor test ($P<0.05$); Means followed by different lowercase letters in the line differ from each other by the Tukey test ($P<0.05$). Source: Authors.

3.4. Behavioral measures

Among the behavioral measures in the starter phase, the only significant difference was observed for environmental pecking. The control birds showed higher levels of this behavior than did birds subjected to beak trimming. In the grower phase, neither feather pecking nor environmental pecking showed significant differences with respect to the beak management. In the production phase, there was a significant difference in terms of rearing system for comfort movements, as well as for the environmental interaction and for sand bathing. In the floor rearing system, there was a greater occurrence of comfort movements. We observed that, in the floor rearing system, the birds that were treated with infrared radiation and those that were not trimmed showed a higher frequency of this behavior than

did birds subjected to hot blade beak trimming (Table 3). On the other hand, in cage rearing systems, environmental pecking was a more frequent behavior in birds treated by hot blade than in other groups. This suggests that the rearing system determined the way in which the trimming method affects behavioral pattern. Birds in the floor rearing system had a higher frequencies of sand bathing behavior (Table 3).

Table 3. Non-parametric analysis¹ (splitting) of the variables environmental pecking and sand bathing in laying hens subjected to infrared radiation beak trimming, hot blade beak trimming, and in non-trimmed birds (control) in the production phase.

Behavior	Rearing systems	Infrared ²	Hot-blade ³	Control
Environmental pecking	Floor	2.71 Aa	1.62 Ab	3.42 Aa
	Cage	1.13 Ba	0.69 Ba	0.29 Ba
“Sand baths”	Floor	2.47 Aa	2.28 Aa	2.47 Ab
	Cage	0.00 Ba	0.11 Ba	0.08 Ba

¹Friedman test for randomized blocks (development); ²Infrared beak trimming; ³Hot-blade beak trimming; Means followed by different letters in the columns uppercase (Floor vs Cage) and lowercase lines (Infrared vs Hot-blade vs Control) differ by the Bonferroni method ($P < 0.05$). Source: Authors.

3.5. Feather score

There was an effect of the rearing system on the feather score in the head and abdomen regions, with worse results for the cage system (Table 4).

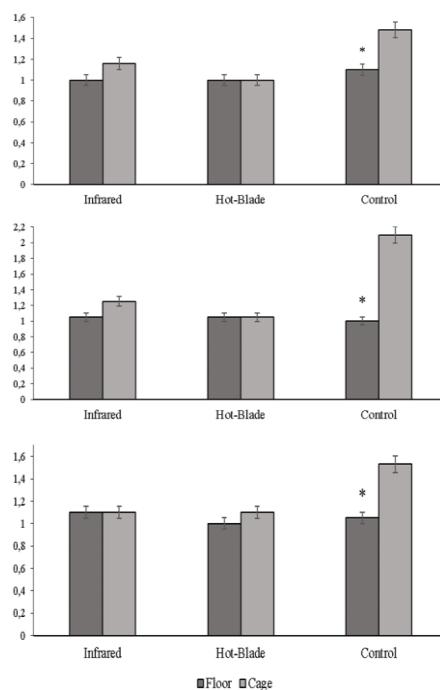
Table 4. Feather score in laying hens subjected to infrared beak trimming, hot blade beak trimming, and in non-beaked birds (control).

Body regions	Rearing Systems (RS)			Beak trimming method (BT)		P-value		
	Floor	Cage	Infrared ¹	Hot-blade ²	Control	RS	BT	RS*BT
Head	1.00 A	1.16 B	1.06	1.07	1.12	<0.01	0.69	0.69
Neck	1.03	1.47	1.15	1.05	1.55	<0.01	0.14	0.03
Chest	1.03	1.15	1.08	1.00	1.29	<0.01	<0.01	0.03
Back	1.2	1.25	1.12	1.09	1.43	0.28	0.60	0.86
Abdomen	1.02 A	1.25 B	1.06	1.14	1.20	0.01	0.31	0.54
Wings	1.05	1.00	1.00	1.02	1.16	0.65	0.04	0.87
Tail	1.05	1.17	1.10	1.05	1.29	<0.01	<0.01	<0.01

¹Infrared beak trimming; ²Hot-blade beak trimming; Means followed by different letters in the line differ from each other by the Fisher-Snedecor test ($P < 0.05$). Source: Authors.

There was interaction between rearing systems and beak management for feather scores in the chest, neck and tail (Supplementary Figure 2).

Supplementary Figure 2: Splitting of the interactions of the feather score variables by region: chest (upper); neck (middle); tail (lower), in laying hens subjected to infrared radiation beak trimming, hot blade beak trimming and non-trimmed birds (control). * Columns differ according to Fisher-Snedecor test ($P < 0.05$).



Source: Authors.

4. Discussion

Bird performance during the starter phase not changes between treatments, although differences between treatments were expected at this stage, because considerable stress is generated by beak trimming, especially by the hot blade treatment. Dennis and Cheng (2010) attributed differences in performance to chronic pain from wounds caused by the treatment, with consequent decreased feeding and poor body development.

However, neither group (without beak trimming, infrared beak trimming) differed from hot blade beak trimming, neither in terms of feed intake and feed conversion. Our results conflict with those of a previous report, in which the infrared beak trimming method led to higher body weight and higher feed efficiency than did hot blade beak trimming (Dennis and Cheng, 2010). On the other hand, our results were similar to those of Marchant-Forde et al.

(2008) and Petrolli et al. (2017), who suggested that the two methods of beak trimming affected production in similar ways.

Hens in the floor rearing system produced eggs with more reddish coloration than did those in the cage rearing system. This factor may be related to the selectivity of the animals, because altering the morphology of the beak or the peculiarities of the feeders of each rearing system might affect the consumption of ingredients rich in carotenoids (such as corn), altering the yolk coloration. This selectivity is one of the justifications for beak trimming, bringing benefits such as lower selectivity and feed wastage (Prescott et al., 2004). According to Awang et al. (1992) and Hencken (1992), the main factor affecting egg yolk color is the carotenoid content of chicken diets. The higher the consumption of feeds rich in these compounds, the higher the deposition of these compounds in yolk, and the greater the intensity of its coloration. Another result that differed between treatments was the yolk percentage; eggs produced by birds treated with hot blade beak trimming returned higher values than those produced by birds treated with infrared radiation beak trimming. This factor is primarily related to the age of the bird, because the younger the bird, the greater the percentage of albumen and the lower the percentage of yolk (Carvalho et al., 2007). It is also related to egg storage time, because, with the increase of the days of storage, there is an increase in the percentage of yolk, and a decrease in the percentage of albumen and shell (Carvalho et al., 2007) because of loss of albumen liquid to the yolk. Nevertheless, it should be noted that the age of the birds in all groups was identical, and the eggs used in the analysis were all fresh.

The birds without beak trimming had higher blood glucose levels than did those who underwent infrared radiation break trimming; however, neither group differed from the hot blade beak trimming group. The lower glucose value in the infrared radiation beak trimming group may be related to the fall of the beak tip that occurred most often at the end of the second week of life, soon before the second blood collection. The determination of blood glucose level is closely associated with animal feeding, because this variable is used to assess carbohydrate metabolism (Jardim-filho et al., 2010). In the production phase, glucose levels showed significant differences with respect to the evaluated treatments, with lower concentrations observed in birds subjected to hot blade beak trimming, than in birds that underwent infrared radiation beak trimming. However, levels did not differ from those of control birds. Stress factors act quickly to raise glucose levels (Puvadolpirod and Thaxton, 2000) because of the increased secretion of corticosterone (Nicol et al., 2009). Despite the fact that the birds treated with infrared beak trimming had higher concentrations of glucose than

did those treated with hot blade beak trimming, neither differed from glucose levels of control birds, suggesting that the procedure should not be considered a stressful procedure in the production phase.

Triglyceride levels also were higher in the cage rearing group. Triglycerides act indirectly in the formation of glucocorticoids (cortisol and corticosterone). When elevated, glucocorticoids suggest greater stress suffered by the animals. According to Bonamigo et al. (2011), high concentrations of triglycerides can be considered indicators of acute stress. In addition, these authors observed higher concentrations of triglycerides in animals produced in higher density, a condition that is a proxy for worse well-being. In the production phase, hens go through daily metabolic stress to produce eggs. We found that cholesterol and ROS levels were significantly different depending on the rearing system, with higher plasma concentrations of these two variables in the cage rearing system than in the floor rearing system. However, there were no changes in the activity of the antioxidant enzymes CAT and SOD (Table 2). Cholesterol and ROS levels are thought to be physiological indicators of stress, because for birds, corticosterone, derived from cholesterol is the primary glucocorticoid synthesized by the adrenal gland in conditions of stress (Siegel, 1995). When birds are subjected to stress, levels of free radicals such ROS increase. At high concentrations, ROS are toxic to cells (McDowell et al., 2007). Despite the increase in ROS, there was no change in the activity of the antioxidant enzymes CAT and SOD in the blood. These data suggest, that regardless of the life stage of the animals raised in cages or on the floor, as well as different types of beak trimming, there was no significant oxidative stress, a common consequence of redox imbalance. Therefore, we believe that the stress suffered by the birds was not substantial enough to cause these biochemical changes that affect the quality of life of the birds in different breeding systems and also exposed to the different handling of debiting.

In the starter phase, control birds showed higher levels of environmental pecking than did birds subjected to beak trimming. Some investigators suggest that increased animal activity (or in this case, elements of the rearing system) may determine animal well-being (Siegel, 1995), because low levels of activity may indicate pain and discomfort (Marchant-Forde et al., 2008). In the production phase in the floor rearing system, there was a greater occurrence of comfort movements. According to Barbosa-Filho et al. (2007), comfort movements are more evident in floor rearing systems, because the dimensions of the cages and the environmental conditions of the rearing systems do not allow expression of the behaviors to the same extent. This higher expression of natural behaviors by birds in floor

system may correlate with less levels of cholesterol and oxygen reactive species in the cage system group, suggesting that these birds were in better welfare.

Birds in the floor rearing system had a higher frequencies of sand bathing behavior. According to Petherick et al. (1993), the use of a substrate for bedding provides an important stimulus for sand bathing and is a key triggering factor. This behavior that control birds presented at lower frequency when raised in floor rearing systems may be related to a greater expression of exploratory behavior in this rearing system. That is, control birds raised on the ground abandoned this behavior in favor of environmental exploration.

The cage system negatively affected the feather score in the head and abdomen regions. This fact can be explained by the limitation of movements when the birds are housed in cages, preventing the bird from performing typical movements (Barbosa-filho et al., 2007; Shimmura et al., 2010), making them more aggressive. Another explanation for the worst feather score was seen in control birds. Birds with intact beaks who were subjected to restriction of their natural behavior by cages performed a higher frequency of aggressive behaviors, resulting in damage to plumage, than did animals that had been trimmed, independent of the method used. Hartcher et al. (2015), demonstrated worse feather scores in control chickens compared with hot-bladed chickens (1.66) when reared in cages. Our results suggest that the type of beak management did not influence feather score when laying hens were raised on the floor, contrary to the situation in the cage system.

5. Final Considerations

The welfare of laying hens is a current and worldwide discussion, as the of animal protection groups have defended the beak trimming and called for a system of breeding and production free of cages. According to experts, these methodologies can have negative effects on the health and welfare of birds. Faced with doubts regarding this matter, we propose an experiment, which had its objectives achieved.

Birds in cage had improved their performance and had greater egg production efficiency. Also, chicken performance was not affected by beak trimming. Conversely, floor system had more frequency of comfort movement behaviors and better feather score as a result of lower aggressive behavior. The beak trimming methods to used must considered the rearing type, with indications that the floor rearing system allows raising chickens without beak trimming. The various methods of beak trimming did not affect the performance of the birds, but they affected bird welfare, depending on the breeding system. We showed that

laying hens had a higher frequency of negative (aggressive) behaviors when their beaks were not trimmed in a cage rearing system, suggesting worse animal welfare. Confirming one of our hypotheses, the floor system improved bird welfare by increasing positive behaviors such as sand bathing, and reducing negative behavior, such as pecking other birds in the cage. Although all birds consumed the same diet, we found that small metabolic alterations occurred in the blood of the birds because of the various breeding systems or beak cutting processes; nevertheless, these did not affect health, growth or productivity. These results represent additional information for transformation of current rearing models of egg production in free cage systems.

Conflict of Interest

The authors declare that they have no conflict of interest.

Financial Support and Acknowledgments

We thank CAPES, CNPq and FAPESC for their technical and financial support.

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