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The effect of different housing systems on the welfare and the parasitological conditions of laying hens

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ABSTRACT: The overwhelming majority of laying hens used for commercial egg production in the Serbia is confined in battery cages. By 2020, conventional cages will no longer be permitted in the Serbia and hens will be housed in furnished cages. The aim of this research was to assess the welfare of laying hens under the influence of different housing systems and analysis of the occurrence, maintenance, and spread of endoparasitic infections. The study was conducted during the winter season 2019/2020 on four rearing systems of Lohman Brown hens. For welfare assessment were used indicators from the Welfare quality assessment protocol for poultry which is grouped into 12 welfare criteria based on principles of good feeding, good housing, good health, and appropriate behavior. All fecal samples for parasites were qualitatively and quantitatively examined. The results showed that each housing system had positive and negative aspects but overall, hens in cage systems had the highest prevalence of poor plumage condition (47 % and 39%). Hens in conventional cages had more skin lesions (27%) than birds in other systems. Keel bone deformation was the most present in the aviary system (56%) while comb pecking wound in a conventional cage (33%) and free-range system (50%). Hens in the non cage had the highest prevalence of foot pad disorders (32% and 40%). In furnished cages, problems occurred in hens are the lowest. Parasitological examination diagnosed four groups of endoparasites: *Coccidia*, *Trichostrongylidae* and *Heterakis* spp, and *Capillaria* spp, with a total prevalence of 64% (64/100) only in free-range system. Laying hens in cage systems have a higher expression of negative emotions in relation to the aviary and free-range system. Evidence of negative hens' emotional condition in cage systems and negative physical condition across all housing systems, suggests that the welfare of modern hens in Serbia is impaired.

Key words: animal welfare; poultry; housing system; coccidia; helminths; behaviour

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INTRODUCTION

In developing countries, such as Serbia, most laying hens are currently produced under the intensive system (mainly in conventional and furnished cages). By 2020, conventional cages will no longer be permitted in Serbia and hens will be housed in furnished cages. These systems should provide nest boxes, perches, a pecking and scratching area, and 750 cm² spaces per hen, or alternative housing systems, such as barn and free-range (RS Regulation 2010; 2014). In recent years in Serbia, there has been an increase in the use of cage-free (aviaries and free-range systems) due to public concern for animal welfare, but still on a low level.

Layer hen welfare is multifactorial and can be affected by the management practices, disease, nutrition, pests and parasites, the external environment, behavior, stress, emotional states, and genetics (Lay et al. 2011; Hartcher and Jones 2017).

Each layer housing system and the technological solution is associated with certain problems such as social stress, the influence of adverse thermal and humidity conditions, inability to express natural behaviors, the threat of zoonoses, risks of exposure to pathogens, and parasites and layers' pathological behavioral reactions, which determine productivity and welfare and resulting in no single housing system being "ideal from a hen welfare perspective" (Sosnowka-Czajka et al., 2010; Lay et al. 2011). Large-scale commercial poultry farms with conventional and furnished cages are characterized by high stocking density, cage housing, lack of outdoor areas, restricted movement, considerable mechanization of handling and permanently deny the opportunity of hens to express most of their basic behavior within their natural repertoire (Sosnowka-Czajka et al., 2010; Lay et al., 2011). On the other hand, free-range systems can comparatively bring an increased risk of disease (Fossum et al., 2009), heat stress (Singh et al., 2017), predation (Bestman and Wagenaar, 2014), parasites (Permin et al., 1999), vent-pecking (Bestman and Wagenaar, 2014), and mortality (Bestman and Wagenaar, 2014; Singh et al., 2017).

The prevention and control of diseases and parasites are widely regarded as fundamental to animal welfare (Fraser et al., 2013). Biosecurity plays a critical role in lowering the risk for infectious diseases to develop and spread (Lay et al., 2011).

The aim of the present work was to analyze the

welfare conditions using Welfare Quality® Assessment protocol for poultry and parasite infection of Lohmann brown hens reared under the different housing systems.

MATERIALS AND METHODS

Ethical approval

Research protocols using animals followed guidelines of the Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade, Serbia, as well as EU Directive 2010/63/EU for animal experiments. The study was reviewed and approved by Animal Ethical Committee of the Faculty of Veterinary Medicine, University of Belgrade, Serbia (01 - 557/2).

Management and birds

The study was conducted in period December 2019 to February 2020, on four rearing systems of Lohman Brown hens in the Belgrade and Vojvodina region of Serbia (Figure 1): conventional cage system (I), furnished cage (II) system, aviary system (III), and free-range system (IV). Serbia is a continental country in Southeastern Europe, in the central part of the Balkan Peninsula, between 41°53' and 46°11' N and 18°49' and 23°00' E. Due to the Pannonian Plain in the north, it is a part of Central Europe. Table 1 provides characteristics of the 4 housing systems for hens.



Figure 1. Geographic position and regions of farms included in the survey

Table 1. Characteristics of the examined farms

	Conventional cage	Furnished cage	Aviary	Free range
Total number of hens	26850	47341	19648	1164
Age at inspection (in weeks)	43	35	41	36
House Dimensions	100x55x42	360x60x60	360x60x60	
Hens/cage or unit	10-12	84	150	1164
Space/hen (cm ²)	491.62	547.52	1296	
Enclosure furnishings	N/A	Perch, nest boxes	Perch, nest area, scratch pad	Perch, nest area, scratch pad, veranda
Ventilation type	Lateral, tunnel	Lateral, tunnel	Lateral, tunnel	
Manure handling	Belt	Belt	Belt and litter	Litter
Manure removal	Every 2-3 days	Every 2-3 days	Every 2-3 days	End of flock
Feeder length (m ²)	1200	4392	6840	
Feeder space (cm/hen)	4	9	3.5	2.5
Number of water space (nipple/cage)	4	10	2448	/
Water space (hens/nipple)	0.35	0.25	0.12	/
Lighting (light:dark)	14:10	14:10	16 :8	/

Welfare Quality Assessments

One hundred hens from each housing system was assessed using a scoring system based on the WQA protocol for poultry (Welfare Quality 2009). The aim of this research was to assess the welfare of laying hens under the influence of factors from different housing systems and analysis of the impact of biosecurity measures on the occurrence, maintenance, and spread of endoparasitic infections. In this article both physical condition and behavioral measurements are reported. The assessment was conducted between 9h and 16h by 2 assessors overall sampling periods in the same houses at the same time and conferred with one another to reach a consensus any time there was ambiguity about the presence, absence, or severity of a measure. Welfare indicators awarded with a score of 0 when welfare was good, a score of 1 was awarded when there was some compromise of welfare, and a score 2 was awarded when welfare was poor and unacceptable.

Parasitological examinations

During December 2019 and February 2020, parasitological examinations were performed at the Department of Parasitology, University of Belgrade Faculty of Veterinary Medicine, on fecal samples of laying hens from four different housing systems. Coprological testing included both macroscopic and a microscopic examination was done. Group samples were collected from 3 -6 examined birds from the same housing unit, regarding housing systems. In the macroscopic examination, the formation, consistency, col-

or, and odor of fecal samples were investigated. Any changes in these parameters from the typical physiological characteristics of hens' feces were noted. The presence of impurities such as blood, pus, mucus, or undigested food was noted as possible markers of some gastrointestinal pathological disorders. Thereafter, feces was carefully examined using tweezers, and any adult helminths and their parts were transferred to a petri dish, rinsed in saline, and prepared for further analysis. Microscopic examination was performed by qualitative methods of coprological diagnostics with the concentration of parasitic elements. Fecal samples (approximately 5-10 g) were examined by a conventional gravitational flotation method (Mehlhorn et al., 1993) that used saturated aqueous solutions of NaCl (> 97%; Roth, Karlsruhe, Germany) and ZnSO₄ (> 97%; Roth, Karlsruhe, Germany). Flotation solutions were prepared by mixing 210 g of NaCl and 331 g of ZnSO₄. Slides were examined at the magnification of 100x and 400x, and parasite identification at the order, family or genus level was performed according to morphological characteristics of characterized eggs and oocysts (Kassai 1999).

Statistical Analysis

Data were analyzed using Graph Pad Prism software. Results were described by descriptive statistics (mean value, standard deviation, and confidence interval) and as prevalence (the overall number of hens showing the measure regardless of severity). The differences between welfare indicators were analyzed using the nonparametric Kruskal-Wallis test on

the equality of the medians, adjusted for ties. When significant differences were found, Dunn-Bonferroni post hoc test was performed. In all statistical tests, values of $p < 0.05$ and $p < 0.001$ were considered the limit for statistical significance.

RESULTS

Based on the results, the highest prevalence of keel bone deformation was obtained in the aviary system (56%) with no evidence in the furnished cage system (Table 2). The average score of keel bone deformation in the aviary system (1.12 ± 1.00) was significantly higher ($p < 0.001$) compared with other housing systems (Table 3). Prevalence of skin lesions with a score of 1 was the highest in conventional cage system (27%) followed by free-range (15%), aviary (3%), and furnished cage (1%). Regarding skin lesions, for all systems, no birds received a score of 2 (Table 2). The average score of skin lesions in conventional cage systems (0.27 ± 0.45) and free-range (0.15 ± 0.35) were significantly higher ($p < 0.05$; $p < 0.001$) compared with other housing systems (Table 3). Minor foot pad disorders (score 1) in free-range and aviary system were the highest (40%, 32%), as well as severe lesions (score 2) (7%, 5%) (Table 2). The average score of foot pad disorders was significantly higher ($p < 0.001$; $p < 0.05$) in free-range (0.55 ± 0.63) and aviary system (0.42 ± 0.59) compared with cage systems (Table 3).

The highest score 1 and 2 of plumage damage observed in conventional cage system (47%, 53%) with pronounced feather loss on beak/rump (26%, 33%) (Table 4). The average score for plumage damage was significantly higher ($p < 0.001$) in conventional cage systems (1.53 ± 0.50 , 0.69 ± 0.72) compared with other systems. Hens in the conventional cage had a significantly higher ($p < 0.05$) average score for head/neck compared with aviary and free-range system and significantly higher ($p < 0.001$) average score for beak/rump plumage damage compared with other systems (Table 5). The prevalence of less than 3 comb peck (score 1) was observed in hens from free-range system (50%), while more than 3 comb pecks in a conventional cage system (16%). The average score for comb pecking was significantly higher ($p < 0.05$; $p < 0.001$) in free-range (0.76 ± 0.66), and conventional cage system (0.65 ± 0.74) compared with other systems (Table 5). The highest prevalence of aggressions was seen in the conventional cage system (42%) while the lowest was in the aviary (25%).

Figure 2 presents the average Qualitative Behavioural Assessment (QBA) scores per housing system. Positive scores of positive emotional status were the most expressed in free-range (9.81 ± 1.28) and aviary system (9.18 ± 1.62), while negative in cage systems (-10.07 ± 1.38 ; -7.15 ± 2.5). Depressed, bored, and distressed were the most prevalent and manifested in hens in the conventional cage system,

Table 2. Prevalence of welfare parameters (good health, absence of injuries) in 400 individual laying hens examined on four farms in Serbia

Welfare parameters	Score	Housing systems			
		I (N=100)	II (N=100)	III (N=100)	IV (N=100)
Keel bone deformation	2	3	0	56	10
	1	27	1	3	15
Skin lesions	2	0	0	0	0
	1	19	20	32	40
Foot pad disorders	2	0	0	5	7

N - total number of samples

Table 3. Mean (\pm SD) scores of welfare parameters (good health, absence of injuries) in laying hens examined on four farms in Serbia

Housing systems	Welfare parameters					
	Keel bone deformation		Skin lesions		Foot pad disorders	
	Mean \pm SD	95% CI	Mean \pm SD	95% CI	Mean \pm SD	95% CI
I	0.06 ± 0.35^A	-0.00 - 0.06	0.27 ± 0.45^{Aa}	0.18 - 0.36	0.19 ± 0.39^{AB}	0.11 - 0.27
II	0	0	0.01 ± 0.10^{AB}	-0.01 - 0.03	0.20 ± 0.40^{AB}	0.12 - 0.28
III	1.12 ± 1.00^A	0.92 - 1.32	0.03 ± 0.17^{Ab}	-0.00 - 0.06	0.42 ± 0.59^B	0.30 - 0.54
IV	0.24 ± 0.66^A	0.05 - 0.43	0.15 ± 0.35^{Ba}	0.05 - 0.27	0.55 ± 0.63^A	0.42 - 0.67

a, b- $p < 0.05$; A, B- $p < 0.001$

while fearful, tense, and unsure in a furnished cage (Figure 3). Hens in the aviary and free-range system were the most expressed happy, energetic, positively occupied, and comfortable (Figure 4).

Parasite infections observed only in free-range systems (Table 6). Parasitological examination of laying hen feces samples diagnosed four groups of endoparasites: one at the order level (Coccidia - A), one at the

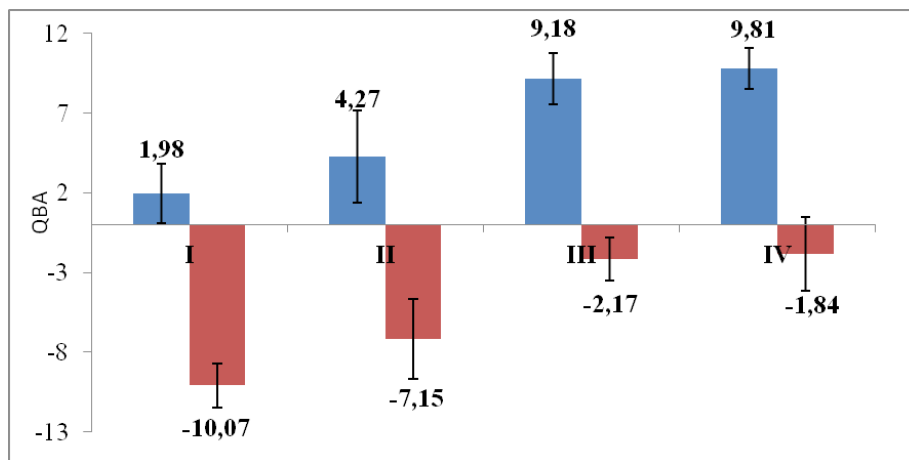


Figure 2. Average results of Qualitative Behavioural Assessment (QBA) per housing system. Positive scores indicate positive emotional status. Negative scores indicate negative emotional status.

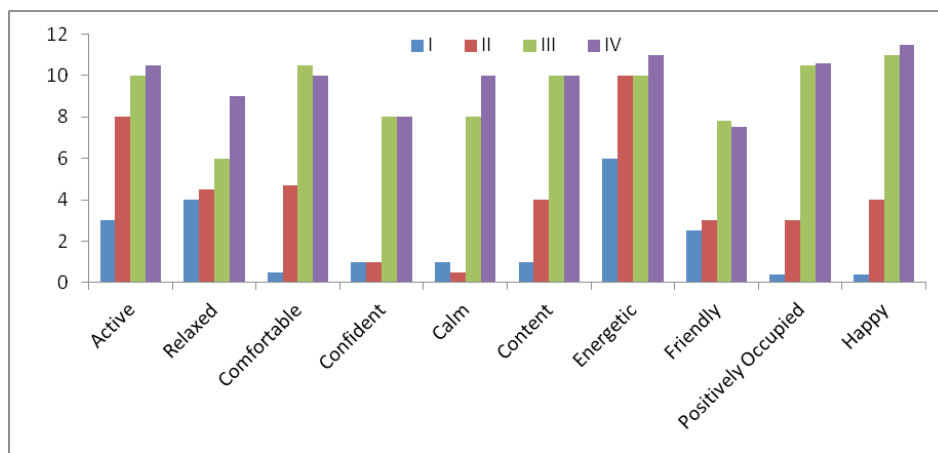


Figure 3. Average results of positive emotional status per housing system

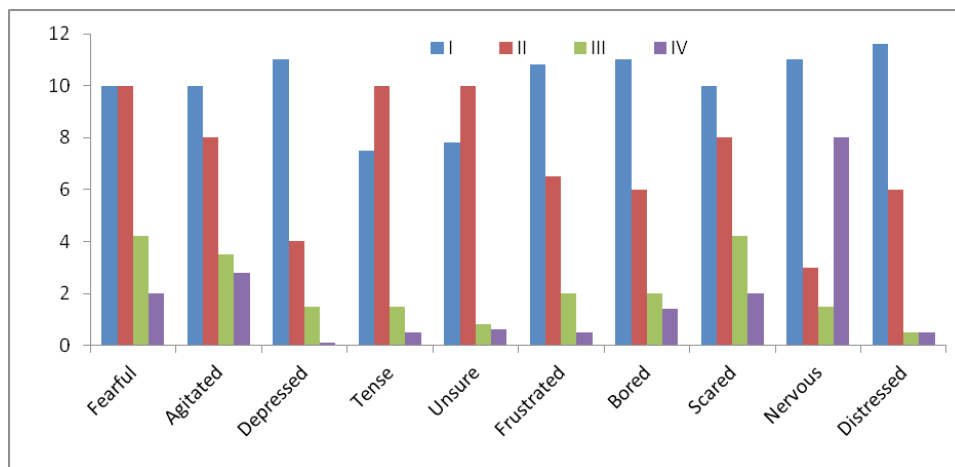


Figure 4. Average results of negative emotional status per housing system

Table 4. Prevalence of welfare parameters (appropriate behaviour, expression of social behavior) in 400 individual laying hens examined on four farms in Serbia

Welfare parameters	Score	Housing systems			
		I	II	III	IV
		(N=100)	(N=100)	(N=100)	(N=100)
		%	%	%	%
Plumage damage	1	47	39	21	20
	2	53	15	10	15
Head/neck plumage damage	1	18	19	10	12
	2	15	9	6	5
Beck/rump plumage damage	1	26	13	8	6
	2	33	5	4	8
Belly plumage damage	1	3	4	3	2
	2	5	1	0	2
Comb pecking wounds	1	33	13	12	50
	2	16	0	0	13
Aggressive behaviour	1	42	35	25	34

N - total number of samples

Table 5. Mean (\pm SD) scores of welfare parameters (appropriate behaviour, expression of social behavior) in laying hens examined on four farms in Serbia

Housing systems	Welfare parameters				
	Plumage damage	Head/neck	Beck/rump	Belly plumage damage	Comb pecking wounds
	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD	Mean \pm SD
I	1.53 \pm 0.50 ^A	0.48 \pm 0.75 ^a	0.92 \pm 0.86 ^A	0.13 \pm 0.46	0.65 \pm 0.74 ^A
II	0.69 \pm 0.72 ^{ABC}	0.37 \pm 0.56	0.23 \pm 0.53 ^A	0.06 \pm 0.28	0.13 \pm 0.34 ^{AB}
III	0.41 \pm 0.67 ^{AB}	0.22 \pm 0.54 ^a	0.16 \pm 0.45 ^A	0.03 \pm 0.17	0.12 \pm 0.33 ^{AC}
IV	0.50 \pm 0.74 ^{AC}	0.22 \pm 0.52 ^a	0.22 \pm 0.58 ^A	0.06 \pm 0.31	0.76 \pm 0.66 ^{BC}

a-p<0.05; A, B, C-p<0.001

Table 6. Prevalence of parasite infections in laying hens in free range system

Monoinfection	N=100	
	n	% CI 95
<i>Capillaria</i> spp	16	16 (8.81-23.18)
Coinfection	n	% CI 95
Coccidia - Trichostrongylidae	27	27 (18.30-35.70)
<i>Heterakis</i> spp - <i>Capillaria</i> spp - Trichostrongylidae	21	21 (13.02-28.98)
Total	64	64

N - total number of samples; n - number of positive samples

family level (Trichostrongylidae - B), and two at the genus level (*Heterakis* spp - C and *Capillaria* spp - D) (Figure 5), with a total prevalence of 64% (64/100). In 16% (16/100) of the tested laying hens only *Capillaria* spp. were identified as monoinfection. Other endoparasites were diagnosed as coinfections with a prevalence of 48% (48/100) (Table 6). By analyzing the quantitative results of coprological diagnostics, a

low (50 - 500 opg / epg) to medium degree (550 - 1000 opg / epg) of diagnosed endoparasitosis was determined (Table 7). The majority of laying hens was dominated by a low degree of coccidiosis - in 85.19% (23/27), heterakiosis - in 85.71% (18/21), capillariosis - in 91.89% (34/37) and trichostrongylidosis - in 97.92% (47/48) (Table 7).

Table 7. Quantitative assessment of fecal samples in laying hens in free range system

Endoparasites	N	Degree of infection (Quantitative FEC method)					
		Low			Medium		
		n	%	Mean \pm SD	n	%	Mean \pm SD
<i>Coccidia</i>	27	23	85.19	215.2 \pm 130.1	4	14.81	650 \pm 168.3
<i>Heterakis</i> spp.	21	18	85.71	236.8 \pm 143.2	3	14.29	625 \pm 35.36
<i>Capillaria</i> spp.	37	34	91.89	213.2 \pm 113	3	8.11	583.3 \pm 57.74
Trychostrongylidae	48	47	97.92	218.8 \pm 126.7	1	2.08	550

Low: <50-500 opg/epg; Medium: 550-1000 opg/epg; (opg/epg - number of oocysts/eggs calculated per 1g faeces); N - total number of samples; n - number of positive samples

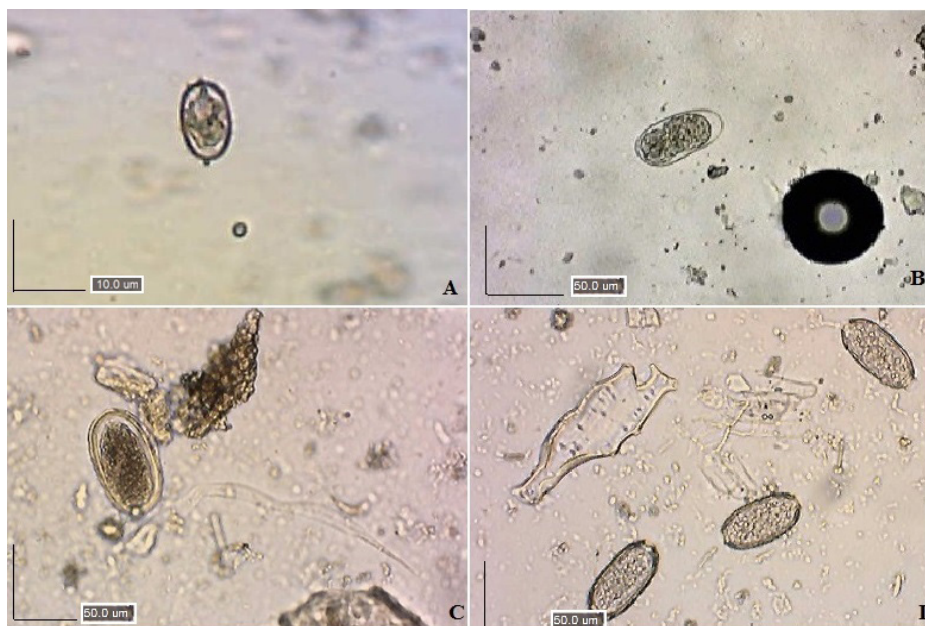


Figure 5. Copromicroscopic finding in laying hens from the free-range system - A) oocysts of coccidia (400x); B) egg of trichostrongylids (100x); C) egg of *Heterakis* spp (100x); D) eggs of *Capillaria* spp. (100x)

DISCUSSION

The present screening of laying hens' welfare and parasitological status in different housing systems provides an overview of the health and welfare state of the hens, as well as evidencing previous or potential welfare concerns.

Commercial laying hens are selectively raised to increase egg production. In addition to higher carrying capacity, today's laying hens have a higher growth rate, the higher body weight of adults, earlier sexual maturity, and larger eggs than their ancestors. Higher carrying capacity also requires increasing calcium deposition due to the formation of the eggshell, which leads to calcium loss in the bones and consequently to a high rate of osteoporosis, skeletal weakness, and increased sensitivity to fractures (Hocking et al., 2003). Although wing and keel bones are stronger in laying hens in avian and floor free-range systems compared to cage systems, keel bone fractures are more com-

mon in alternative systems (Sherwin et al., 2010). The results in the present study revealed significantly more ($p < 0.001$) keel bone deformity in laying hens in the aviary system (56%) compared to other housing systems. The higher incidence of keel bone deformity in alternative systems can be explained by the fact that although laying hens in the avian system can exhibit movement, flapping, and flying, which increases musculoskeletal strength and reduces osteoporosis, fractures, and deviations represent a risk when hens fall and were injured during landing from feeders, drinkers, perches or nests (Lay et al., 2011; Fraser et al., 2013; Widowski et al., 2013). The finding of 10% keel bone deformation in the free-range systems can be also related to reporting of Gauly et al. (2007) and Whitehead (2004), who found that helminth infections decreased the locomotor's activity and increased the prevalence of bone damage in infected hens compared to non-infected hens. According to

McCoy et al. (1996) and Nasr et al. (2012), keel bone deformation or a fracture has been shown to be associated with pain, decrease egg production and elevate mortality. Vits et al. (2005) found more keel bone deviations in Lohmann Brown compared to Lohmann Selected Leghorn hens. In a study by Eusemann et al. (2018), brown layer lines showed significantly more keel bone fractures than white layer lines in the 51st and 72nd week of age, indicate genetic effects on keel bone damage.

Skin lesions also depend on the environmental conditions in which the laying hens are raised (Blatchford et al., 2016). Comb wounds and lesions around the cloaca are often the results of pecking. Keutgen et al., (1999) found a higher number of lesions in the cloacal region in laying hens raised on the floor of the free-range system, while Elson and Croxall (2006) observed a higher number of comb wounds in the alternative housing system, as an indicator of aggressive hen pecking. In this study, there were significantly more body lesions ($p < 0.001$) in hens in the conventional cage (27%) and floor free-range system (15%) compared to the furnished cage and aviary system which is in agreement with those recorded by Abrahamsson and Tauson (1997), who found that hens in the furnished cage had fewer body lesions compared to the conventional cage.

According to European Food Safety Authority (EFSA) (2005), foot disorders and damage can be found in all types of housing systems but the type and severity differ from one system to another and are influenced by genetic strain and perch design. The most common foot disorders of laying hens are hyperkeratosis, foot pad dermatitis, and bumblefoot, which are thought to be more painful and of greater welfare significance (Tauson and Abrahamsson 1996). In the present study, there higher prevalence of laying hens with feet necrosis and proliferation of epithelium with or no swelling in the aviary (32%) and free-range system (40%) compared with cage systems while swollen feet was found only in non-cage systems (5% and 7%). Wang et al. (1998) reported that foot pad dermatitis (necrosis and ulceration of the epidermis) and bumblefoot (a localized bulbous swollen lesion in the ball of the feet) are caused by wet litter and high ammonia content of the litter, as well as feed and genetic components. Previous studies regarding foot pad disorders in non-cage systems reported similar and even higher prevalence to those found in our study ranged from zero to 39% for mild foot pad disorders such as

hyperkeratosis and foot pad dermatitis, and 24% for bumblefoot (Abrahamsson and Tauson 1995; Wang et al., 1998; Røongen et al., 2008; Heerkens et al., 2016; Jofran et al., 2019). According to many authors the housing system, perching behavior, wet litter, scratching, perch and flooring material, poor foot hygiene, and managing system have been identified as the cause of foot pad disorders (Tauson and Abrahamsson 1996; Wang et al., 1998; Blokhuis et al., 2007; Røongen et al., 2008; Shimmura et al., 2010; Lay et al., 2011).

In addition to infectious diseases of viral and bacterial etiologies, endo-parasites and ecto-parasites (coccidia, helminthes, and mites) are also extremely important (Widowski et al., 2013). Prevention and control of parasitic diseases are the basis for improving and protecting animal welfare (Fraser et al., 2013). Endoparasitic infections represent a challenge to the welfare of free-range and cage-free flocks (Groves 2021).

In this study, parasitic infections were found only in the free-range system. These results can be ascribed to the fact that biosecurity measures in other housing systems were implemented, which was confirmed based on inquiry in which managers from the observed laying hen housing systems participated. Biosecurity plays a critical role in lowering the risk for infectious diseases to develop and spread (Robertson 2020). The main risks for their occurrence are management, and inadequate implementation of sanitary measures.

According to Heckendorn et al. (2009) and Maurer et al. (2009), free-range system and a littered area enhance the risk of endoparasitic infections, as they are potential infection sources. In cage-free systems, poultry is freely exposed to wild birds and rodents allowing for transmission of internal parasites (Fraser et al., 2013).

When pasture is not periodically rested, such as a case in the observed free-range system, this can reduce access to fresh grass for the birds and increase the risk of diseases and parasitic infestations building up in the soil (Sossidou et al., 2011). In this study, mono-infection with *Capillaria* spp. and co-infections with *Coccidia* - *Trichostrongylidae*, and *Heterakis* spp. - *Capillaria* spp; *Trichostrongylidae* was found in laying hens in the free-range system. The results of this study are in line with the results of other authors who found that in the free-range system

the most common parasites were *Heterakis* spp. and *Capillaria* spp (Thapa et al., 2015; Grafl et al., 2017). Also, the results of this research indicate a seasonal effect, which affects the occurrence and intensity of infections caused by helminths, with regard to a higher prevalence of nematodes compared to cestodes. This finding can be explained by the fact that during the winter the transitional hosts of cestodes (water shrimps, earthworms, ants, terrestrial snails, flies, and Coleoptera) are not active, so the cestodes cannot complete their evolving cycle (Norton and Ruff 2003). Helminthiasis affects the welfare of animals because it can cause mortality, as well as morbidity since sub-clinical infections can predispose to the development of other diseases and increase their severity. Also, animal welfare will be impaired when clinical signs are present or the level of endoparasitic infection is high and causes an intestinal obstruction with consequent pain, suffering, and death of the laying hen (Sharma et al., 2019; McDougald 2020). Parasites can be vectors and cause secondary infections with *Escherichia coli* (Permin et al., 2006), and can also affect the growth as a result of reduced food conversion ratio and weight gain (Gauly et al., 2007).

The plumage condition is one of the most important indicators of laying hens welfare (Bilcik and Keeling 1999; Whay et al., 2007; Welfare Quality 2009; Savory and Hughes 2010; Main et al., 2012). In this research, there was higher prevalence of plumage damage in cage systems regarding non-cage systems, with the highest prevalence of feather damage on neck/rump. Savory (1995) reported that poor condition of feathers can be caused by infectious diseases, ectoparasites, lack of nutrients, as well as pecking of feathers. According to the Welfare Quality (2009), damage to feathers of the head and rump usually indicate feather pecking, and behavioral disorders. In the present study, stocking densities in conventional cage system was 491.62 cm²/hens which are not in accordance with Regulation (RS Regulation 2010; 2014). These results can be ascribed to the fact that higher stocking densities have been associated with higher levels of feather pecking in laying hens, in different systems (Bilçik and Keeling 1999; Nicol et al., 1999; Zimmerman et al., 2005). Also, an increase in the number of birds per flock makes them more aggressive (Bilçik and Keeling 1999) which leads to feather pecking. The finding in this study was in agreement with the report made by Nicol et al. (1999), who found a positive relationship between feather pecking and high stocking density. According to Widows-

ki et al. (2017), higher stocking density in furnished cages influenced feather condition and cleanliness of birds. The available literatures suggested that the occurrence of these behavior disorders of laying hens is influenced by many factors such as environmental condition (Lambton et al., 2010; Collins et al., 2011), stress (El-Lethey et al., 2000), lighting (Kjaer et al., 2002; Riedstra et al., 2004), stocking density (Zimmerman et al., 2006; Zepp et al., 2018), genetics (De Haas et al., 2014; Van der Eijk et al., 2019; Iffland et al., 2019), nutrition (Van Hierden et al., 2004), immune status (Parmentier et al., 2009), neurobiological status (Kops et al., 2013), as well as the behavior of laying hens, ie the mapping of this disorder among chickens (Cloutier et al., 2002). The negative effects of these behavioral disorders in addition to impairment of welfare is also economic, because of increased food consumption due to higher energy demand, as well as stress that can affect egg production and mortality (El-Lethey et al., 2000; Janczak and Riber 2015). Poor feather condition will affect hens welfare because of the loss of body heat, and feed energy intake to maintain homeostasis in cold weather (Sarica et al., 2008).

In this study, the higher prevalence of hens with less than three comb pecking wounds was found in the free-range system while more than three pecking wounds in a conventional cage. This result is in agreement with those recorded by Tauson and Holm (2001) who found comb wounds of hens in approximately 61% in litter system and 14% in furnished cages. However, Rodenburg et al. (2008) found no differences in comb wounds among different housing systems and serious wounds were rare. Webster (2003) found that a higher incidence of comb pecking wounds occurs because of aggressive behavior during the establishment of a social hierarchy, lack of food, and periods of molting. In a free-range system, hens express foraging, exercising, exploration, and locomotion which can lead to comb wounds due to dragging through plants and shrubs.

Innate or 'normal' behaviors are those which are inherent to animals, and typically, which animals are motivated to carry out. Bracke and Hopster (2006) reported that the performance of these behaviors is thought to be a component of biological functioning, is pleasurable, and necessary to avoid stress.

In this work, it was determined that laying hens in cage systems have a higher expression of negative emotions in relation to the aviary and free-range sys-

tem. Depressed, bored, distressed and fearful, tense, and unsure were the most prevalent and manifested in hens in conventional and furnished cage systems, retrospectively. Hens in the aviary and free-range the system was the most expressed happy, energetic, positively occupied, and comfortable. On the other hand, positive emotions such as happy, energetic, positively occupied, and comfortable were expressed in the aviary and free-range system. The results of this study are in agreement with those recorded by Rodenberg et al. (2008) and Shimmura et al. (2010), who reported that laying hens are more fearful in cages compared to the non-cage system. The ability to express natural behaviors is crucial to achieving a positive emotional state, animal health, and welfare (Webster 2003). These results suggest that expressions of natural behaviors in hens are limited in cages, and with a relatively unstimulating environment compared to the non-cage systems.

CONCLUSION

It is clear from this study that all systems, offer both positive and negative welfare aspects for layer

hens: the non-free-range systems offer increased protection from predators as well as parasite infections, but also a reduced opportunity for extensive locomotion. The most affected, both emotional and physical hens welfare was in a conventional cage. These results indicate the need for replacement conventional with furnished or non-cage systems. The physical welfare of the hens in the furnished cage system was better than that of birds in the other systems while emotional welfare was in non-cage systems. This raises an ethical question, what is more important, to reduce the prevalence of welfare issues in these four housing systems or sustain production requirements.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest to disclose.

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