



Article Research Regarding Correlation between the Assured Health State for Laying Hens and Their Productivity

Alexandru Usturoi ¹, Marius-Giorgi Usturoi ², Bogdan-Vlad Avarvarei ², Claudia Pânzaru ², Cristina Simeanu ², Mădălina-Iuliana Usturoi ³, Mihaela Spătaru ⁴, Răzvan-Mihail Radu-Rusu ², Marius-Gheorghe Doliș ^{2,*} and Daniel Simeanu ¹

- ¹ Department of Control, Expertise and Services, Faculty of Food and Animal Sciences, "Ion Ionescu de la Brad" University of Life Sciences, 8 Mihail Sadoveanu Alley, 700489 Iasi, Romania
- ² Department of Animal Resources and Technology, Faculty of Food and Animal Sciences, "Ion Ionescu de la Brad" University of Life Sciences, 8 Mihail Sadoveanu Alley, 700489 Iasi, Romania
 - Laboratory of Medical Analysis, Providenta Hospital, 115 Nicolina Avenue, 700714 Iasi, Romania
- ⁴ Department of Public Health, Faculty of Veterinary Medicine, "Ion Ionescu de la Brad" University of Life Sciences, 8 Mihail Sadoveanu Alley, 700489 Iasi, Romania
- * Correspondence: mariusdolis@uaiasi.ro

Abstract: Predictions show the possibility of banning birds' rearing in batteries. From this reason, we aimed to study the welfare conditions assured to birds accommodated in lofts in comparison with those reared in improved batteries. The research targeted ISA Brown hybrids monitored over a period of 25–55 weeks. The batches were represented by birds that were differently reared in halls provided with lofts compared to with improved batteries. The research was carried out in real production conditions. Biochemical indicators were determined, using a BA 400 analyzer produced by BioSystems, as well as quantitative ones using specific formulas based on productions, consumptions, and batch outputs. A cumulated production of 199.24 eggs/week/head was realized in the loft, versus 199.98 in the battery, at a mean laying intensity of 91.82% and 92.17%. Batch output was 4.14% (loft) and 2.98% (battery). Mean consumption registered a level of 122.20 g m.f./head/day for birds in the loft and 115.87 g for the ones from the battery, and feed conversion index was 133.09 g m.f./egg, compared to 125.69. The aviary system ensures optimal conditions to express the birds' natural behaviors, with a positive impact on the metabolic functions, resulting in a good state of health and high productive levels, comparable to those of birds exploited in batteries.

Keywords: rearing system; birds' welfare condition; biochemical analysis; productive parameters

1. Introduction

Both fowl welfare and the performance of laying hybrids are affected by environmental factors, and they are still to be tuned within the context of modern industrialized aviculture.

The adaptability of hybrids to housing conditions is crucial in reaching efficiency while welfare is observed. Fowl's response to environmental factors relies on breed particularities. Stress markers are lower in less active, lymphatic genotypes (Green-legged Partridge hen) compared to the sensitive, active, and quite excitable ones (Leghorn) that are less adapted in large-scale farms [1]. Such differences also occur between purebreds and commercial hybrids, i.e., Sussex hens have the best levels of welfare markers, while ISA Browns have the lowest ones among commercial lines. However, mortality and aggressiveness seem to be more intense in pure breeds, and lower welfare occurs, compared to commercial hybrids that easily adapt to closed housing [2].

The European Union Council regulates the minimum comfort for laying hens and has also prohibited (since 2012) classic cage batteries as fowl housing [3,4]. Starting from this premise, there were designed various alternative rearing solutions to allow for the expression of flow productive potential [5,6], and to ensure wellbeing [7]. Housing in



Citation: Usturoi, A.; Usturoi, M.-G.; Avarvarei, B.-V.; Pânzaru, C.; Simeanu, C.; Usturoi, M.-I.; Spătaru, M.; Radu-Rusu, R.-M.; Doliş, M.-G.; Simeanu, D. Research Regarding Correlation between the Assured Health State for Laying Hens and Their Productivity. *Agriculture* **2023**, *13*, 86. https://doi.org/10.3390/ agriculture13010086

Academic Editor: Shugeng Wu

Received: 17 December 2022 Accepted: 26 December 2022 Published: 28 December 2022



Copyright: © 2022 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). limited spaces (conventional cages) reduces resistance to diseases and decreases the fowl's comfort state markers [8].

Consequently, starting from the premise of ensuring welfare conditions, improved cage batteries (increased room) [9,10] and furniture endorsements (nesting, resting perches, sand bath) were initially designed to support high yields and superior egg quality [11–13]. The fowl that benefited from the enlarged vital space yielded more egg mass and eggs with better specific weight, Fe, Mg, and glucose level compared to those reared on narrower surfaces [14].

The second stage of the changes consisted in housing laying hens in aviaries (lofts allowing for the expression of natural instincts but also generating superior egg yields [15]. The major disadvantage of the system is the increased incidence of mechanical accidents (fractures) [16], but this was mitigated through covered edgy equipment parts with polyurethane [17]. Freedom of movement, however, raised the aggressive behavior, a problem solved by introducing males into the flocks of laying hens (egg yields also improved) [18]. In aviary housing, hens' serum immunoglobulin Y was higher and improved the specific antibody response in industrially reared fowl [19].

Serum biochemical traits, performance of production, and livability of fowl are traits depicting the fowl's wellbeing state [20,21]. The levels of plasmatic proteins, Mg, glucose, cholesterol, and P decrease as egg-laying intensity increases [22], while calcium decreases during the laying peak [23]. However, such changes are not strictly correlated with a certain housing system [24]. Various stressor factors induce fear [25] and generate changes in the concentration of basal plasma corticosterone and serotonin, with direct effects on eggs yield, weight, and flock livability [26]. The stress generated by fowl transportation affects the ratio of heterophiles/lymphocytes and plasmatic glycoprotein 1-acid [27]. Beak clipping, a procedure that limits the negative consequences of aggressive behavior and improves production performance [28], is tough, and therefore a stressor. Fasting applied to induce artificial molting induces a significant decrease in the serum glucose and an intense increase in plasmatic cholesterol and GSH-Px activity (poor plumage quality, tonic immobility reaction, etc.) and negatively affects the welfare condition [29,30].

Bird feeding has generated many studies in which, for example, direct correlations were detected between the level of essential amino acids and the incidence of feather plucking or mortality through cannibalism [31]. It has been shown that when fed in cage batteries, low dietary protein levels (13%) lead to egg yield drops and decreases in plasmatic uric acid, triglycerides, and albumin compared to fowl fed 16% crude protein diets [32,33]. Low dietary metabolizable energy and crude protein can cut feeding costs, whilst serum creatine kinase activity rises and serum triglyceride and cholesterol decrease [34]. A high proportion of cereals (wheat) in feed can induce poorer plumage quality and thermoregulation issues [35,36], while using nonconventional feedstuffs (dried olive pulp) induces improvement in the lipid profile of eggs (PUFA increases and SFA decreases, AI and TI decrease and h/H ratio increases) [37].

The welfare and performance of laying depend on many factors and the interactions between them, but especially on the applied rearing system and its particularities. Consequently, we must find these correlations as well as the way to implement the solutions in real production conditions.

2. Materials and Methods

2.1. Animals and Housing

The study took place in a private production unit in Galati County, Romania, starting in July. We used the commercial ISA Brown hybrid due to its adaptability to local conditions. The experimental groups were represented by birds from two production halls. The first batch was raised in a hall equipped with Natura Nova Twin model aviary (initial population of 35,950 heads), and the second in a hall with improved Eurovent batteries (initial population of 10,217 heads). The research started when the birds were 25 weeks old and ended when they were 55 weeks old (a total of 31 weeks).

The housing of the hens was carried out in accordance with the requirements of the technical manual of the hybrid [38] and of the equipment manufacturer [39]. In both cases, the particular density standards for integrating the farm under the welfare regime were followed.

The feeding of the birds respected the nutritional management strategy used in the work unit. An identical combined diet was given to the two groups of birds, but the nutritional values varied according to the quantity and quality of eggs laid [40]. The combination feed 21-5A (PB = 18.6%; EM = 2748.6 kcal/kg; Ca = 3.82%; P = 0.40%) was given between the ages of 25 and 40 weeks, and the combined feed 21-5B (PB = 16.43%; EM = 2786.0 kcal/kg; Ca = 3.8%; P = 0.36%) was given to animals between the ages of 41 and 55 weeks.

2.2. Data Collection and Data Processing

The technical data (feed consumption, egg production, batch outputs) necessary for elaborating the paper were taken from the farm registers and the computerized devices that served the rearing halls. The monitoring was carried out daily, with a weekly presentation of the data. Later, standardized formulas were applied to obtain the values for various indicators [41].

Mean flock (heads/week) = (heads at the beginning of the week - heads at the end of the week)/2

Laying intensity (%) = [total egg production (pieces/week/shelter) \times 100]/[mean flock (heads) \times 7 days]

Individual egg production (egg/week/head) = total egg production (pieces/week/shelter)/mean flock (heads)

Daily mean consumption (g/head/day) = consumed fodders (kg/period) \times 1000/mean flock (heads)/number of days

Feed conversion index (g m.f./egg) = consumed fodders (kg/period) \times 1000/egg production (pieces/period)

The situation of outputs from the batch and their causes were counted by the farm manager and the veterinarian of the unit, and later reported numerically and percentagewise to the total population (weekly situation and for the total period). Biochemistry samples were taken at 25, 35, 45, and 55 weeks by the veterinarian. They were harvested from 35 specimens from each growth hall, in accordance with the specific methods [42,43]. After harvesting, the samples were processed with the ByoSystems BA 400 analyzer.

The main experimental data were processed by calculating arithmetic mean, mean standard deviation, and variability coefficient (using an algorithm included in the Microsoft Excel software).

3. Results

3.1. Biochemical Indicators

In the case of hens from the improved batteries, the cholesterol level oscillated between a minimum of 153.72 mg/dL, the value recorded at the beginning of the research, and a maximum of 206.14 mg/dL in the 55th week. For hens reared in the aviary, the swing limits varied between 148.81 mg/d (25th week) and 195.21 mg/dL (55th week) (Table 1).

The level of blood triglycerides showed quantitative increases with the advancing age of birds, with the mention that higher values were detected in birds from improved batteries (189.56-206.19 mg/dL) and somewhat lower values were detected in those from aviaries (186.12-201.34 mg/dL).

			Birds' Age									
Specification		n	25 Weeks	;	35 Weeks	6	45 Weeks		55 Weeks			
			$\overline{X}\pm s_{\overline{x}}$	V%	$\overline{X}\pm s_{\overline{x}}$	V%	$\overline{X}\pm s_{\overline{x}}$	V%	$\overline{X}\pm s_{\overline{x}}$	V%		
Cholesterol	А	35	148.81 ± 8.36	19.36	162.35 ± 12.18	18.31	175.36 ± 15.22	23.16	195.21 ± 12.68	24.33		
(mg/dL)	В	35	153.72 ± 10.14	21.14	164.14 ± 16.23	20.19	178.12 ± 19.46	25.31	206.14 ± 17.33	25.14		
Trighteerides (mg/dL)	Α	35	186.12 ± 5.34	16.12	191.37 ± 8.38	15.68	197.15 ± 8.55	17.22	201.34 ± 8.46	16.97		
ingrycendes (ing/aL)	В	35	189.56 ± 7.22	17.22	194.26 ± 9.55	17.12	203.41 ± 9.31	19.14	206.19 ± 10.15	19.45		
Total protein	Α	35	3.78 ± 0.72	12.20	4.12 ± 0.95	13.26	4.35 ± 0.51	10.46	4.87 ± 0.93	13.12		
(g/dL)	В	35	3.54 ± 0.57	17.56	4.06 ± 0.78	18.21	4.19 ± 0.47	9.58	4.56 ± 0.77	10.68		
Calcium	Α	35	8.94 ± 0.30	16.32	8.75 ± 0.21	17.38	10.16 ± 0.24	15.31	12.14 ± 0.51	15.96		
(mg/dL)	В	35	8.36 ± 0.25	12.44	8.16 ± 0.32	15.44	9.85 ± 0.31	12.14	11.61 ± 0.38	14.32		
Phoenhorous (mg/dL)	Α	35	6.87 ± 0.69	20.15	6.42 ± 0.36	18.26	8.05 ± 0.93	22.17	9.02 ± 1.19	21.38		
Thospholous (hig/ uL)	В	35	6.31 ± 0.52	18.34	6.11 ± 0.25	15.17	7.81 ± 0.75	20.19	8.77 ± 0.92	20.71		
Glucose	Α	35	208.23 ± 14.96	15.19	219.14 ± 17.16	13.61	238.45 ± 20.02	18.14	252.19 ± 16.77	15.36		
(mg/dL)	В	35	211.10 ± 15.23	17.32	223.08 ± 18.17	15.48	256.12 ± 19.33	19.38	270.14 ± 17.16	17.81		
Uric acid	Α	35	8.97 ± 0.51	12.95	7.85 ± 0.38	15.11	7.39 ± 0.26	15.19	6.89 ± 0.21	20.62		
(mg/dL)	В	35	10.14 ± 0.48	13.34	9.43 ± 0.41	14.24	9.08 ± 0.21	17.26	7.24 ± 0.19	22.14		
Urea	Α	35	4.95 ± 0.41	15.31	5.19 ± 0.27	16.21	5.57 ± 0.55	23.15	5.72 ± 0.22	16.96		
(mg/dL)	В	35	5.12 ± 0.38	19.76	5.34 ± 0.32	17.49	5.82 ± 0.71	24.21	5.95 ± 0.24	17.21		
ÄLT	Α	35	87.86 ± 12.32	13.35	89.24 ± 15.93	10.16	94.19 ± 12.38	17.31	98.83 ± 15.46	23.94		
(U/l)	В	35	89.56 ± 14.16	14.26	91.31 ± 16.41	11.35	95.26 ± 14.12	17.16	101.11 ± 17.32	24.13		
AST	Α	35	231.22 ± 15.73	15.26	241.44 ± 15.27	15.77	267.85 ± 17.06	24.08	294.39 ± 12.08	24.31		
(U/l)	В	35	236.16 ± 16.52	18.19	246.19 ± 16.11	15.27	$\textbf{272.11} \pm \textbf{16.19}$	24.25	300.41 ± 19.55	25.14		

Table 1. Biochemical indicators for the studied birds.

A: rearing in loft. B: rearing in battery.

The amount of total protein had an average level of 3.54 g/dL at the beginning of the analyzed period (25th week) and 4.56 g/dL at its end (55th week) in the birds raised in battery. In the case of the livestock in the aviary, the total protein content increased from 3.78 g/dL, as it was in the 25th week, to 4.87 g/dL, as found at the end of the study.

Blood calcium recorded lower values at 35 weeks (8.16 g/dL for battery hens and 8.75 g/dL hens in the aviary) and higher at 55 weeks (11.61 g/dL-battery and 12.14 g/dL-aviary). Phosphorus had a similar evolution, with lower levels at 35 weeks (6.11 mg/dL for battery hens and 6.42 mg/dL in aviary birds) and higher in 55 weeks (8.77 mg/dL-battery and 9.02 mg/dL-aviary) (Table 1).

In birds aged 25 weeks, the amount of glucose was at a close level between the two groups (208.23 mg/dL-aviary and 211.10 mg/dL-improved batteries), after which progressive increases were registered towards the end of the investigations, when they were 252.19 mg/dL in aviary birds and 270.14 mg/dL in those in improved batteries.

The level of uric acid recorded higher values at the beginning of laying (week 25), was 8.97 mg/dL in birds from the aviary and 10.14 mg/dL for those from modified batteries, after which the values decreased in parallel with the age of the birds until up to 6.89 mg/dL (aviary) and at 7.24 mg/dL (improved batteries). Urea did not fluctuate significantly between batches or between reference intervals, with the beginning of the research showing values of 4.95 mg/dL (birds from the aviary) and 5.12 mg/dL (those from the batteries), while in the last period it showed values of 5.72 mg/dL and d 5.95 mg/dL, respectively (Table 1).

The determined values for alanine aminotransferase (ALT) oscillated between 89.56 U/L (25-week-old birds) and 101.11 U/L (55-week-old chickens) in the flock from the improved batteries and between 87.86 U/L (25-week-old chicken's weeks) and 98.83 U/L (55-week-old hens) in those raised in the aviary. As for aspartate aminotransferase (AST), it showed limits of variation in the range of 236.16–300.41 U/L, as determined in hens from improved batteries and in the range of 231.22–294.39 U/L in those in the aviary.

3.2. Numerical Egg Production and Laying Intensity

Average weekly egg production ranged between 6.09 eggs/head (55th week of control) and 6.53 eggs/head (in weeks 31, 32, and 33 of control) in hens reared in the aviary and between 6.17 eggs/head (55th week of bird life) and 6.57 eggs/head (in weeks 31 and 32) in those in improved batteries. For the total studied period (25–55 weeks), the individual egg

production was 199.24 eggs/head for the specimens in the aviary and 199.98 eggs/head for those in the battery (Table 2).

			Rearing	g in Loft					Rearin	g in Batter	у	
Birds'		Total Egg	Individual Egg P (Egg/ (Egg/ Week/ Day/ Head) Head)		Production			Total	Individual Egg Production			
Age (Weeks)	Mean Flock (Head)	Production (Pieces/ Week/ Shelter)			Cumulated (Egg /Week/ Head)	Laying Inten- sity (%)	Mean Flock (Head)	Egg Pro- duction (Pieces/ Week/ Shelter)	(Egg/ Week/ Head)	(Egg/ Day/ Head)	Cumulated (Egg/ Week/ Head)	Laying Intensity (%)
25	35,927.5	229,512	6.39	0.91	6.39	91.26	10,214.5	65,445	6.41	0.91	6.41	91.53
26	35,884.5	229,639	6.40	0.91	12.79	91.42	10,209	65,717	6.44	0.91	12.85	91.96
27	35,838	230,671	6.44	0.91	19.23	91.95	10,202.5	66,261	6.49	0.92	19.34	92.78
28	35,787	230,767	6.45	0.92	25.68	92.12	10,198	66,367	6.51	0.92	25.85	92.97
29	35,742.5	231,032	6.46	0.92	32.14	92.34	10,194.5	66,409	6.51	0.93	32.36	93.06
30	35,705	231,815	6.49	0.92	38.63	92.75	10,189	66,573	6.53	0.93	38.89	93.34
31	35,670.5	232,564	6.52	0.93	45.15	93.14	10,182	66,940	6.57	0.93	45.46	93.92
32	35,635	232,657	6.53	0.93	51.68	93.27	10,174	66,838	6.57	0.93	52.03	93.85
33	35,600.5	232,482	6.53	0.93	58.21	93.29	10,164	66,573	6.55	0.93	58.58	93.57
34	35,567.5	232,142	6.53	0.93	64.74	93.24	10,154.5	66,404	6.54	0.93	65.12	93.42
35	35,535.5	231,958	6.53	0.93	71.27	93.25	10,146.5	66,274	6.53	0.93	71.65	93.31
36	35,498	231,340	6.52	0.93	77.79	93.10	10,137.5	66,151	6.52	0.93	78.17	93.22
37	35,455.5	230,766	6.51	0.92	84.30	92.98	10,127.5	65,973	6.51	0.93	84.68	93.06
38	35,415.5	230,158	6.50	0.92	90.80	92.84	10,117	65,826	6.51	0.92	91.19	92.95
39	35,371	229,696	6.49	0.92	97.29	92.77	10,108.5	65,686	6.50	0.92	97.69	92.83
40	35,323.5	229,214	6.49	0.92	103.78	92.70	10,099	65,723	6.49	0.92	104.18	92.79
41	35,275.5	228,829	6.49	0.92	110.27	92.67	10,087.5	65,394	6.48	0.92	110.66	92.61
42	35,232	228,497	6.48	0.92	116.75	92.65	10,077.5	65,308	6.48	0.92	117.14	92.58
43	35,188	228,187	6.48	0.92	123.23	92.64	10,069	65,218	6.48	0.92	123.62	92.53
44	35,137	227,709	6.48	0.92	129.71	92.58	10,061	65,159	6.48	0.92	130.10	92.52
45	35,084	227,120	6.47	0.92	136.18	92.48	10,053.5	65,096	6.47	0.92	136.57	92.50
46	35,028.5	225,902	6.45	0.92	142.63	92.13	10,045.5	64,939	6.46	0.92	143.09	92.35
47	34,970.5	224,794	6.43	0.91	149.06	91.83	10,035	64,653	6.44	0.92	149.47	92.04
48	34,916	224,175	6.42	0.91	155.48	91.72	10,023.5	64,460	6.43	0.91	155.90	91.87
49	34,863.5	223,179	6.40	0.91	161.88	91.45	10,011.5	63,997	6.39	0.91	162.29	91.32
50	34,806.5	222,107	6.38	0.91	168.26	91.16	9997.5	63,754	6.38	0.91	168.67	91.10
51	34,753	219,747	6.32	0.90	174.58	90.33	9983	63,403	6.35	0.90	175.02	90.73
52	34,703.5	216,494	6.24	0.89	180.82	89.12	9970	62,909	6.31	0.90	181.33	90.14
53	34,651	214,930	6.20	0.88	187.02	88.61	9956.5	62,454	6.27	0.89	187.60	89.61
54	34,597.5	212,007	6.13	0.87	193.15	87.54	9943.5	61,753	6.21	0.88	193.81	88.72
55	34,545	210,427	6.09	0.87	199.24	87.02	9929.5	61,277	6.17	0.88	199.98	88.16

Table 2. Numerical egg production and laying intensity.

At the beginning of the studied period (week 25), the laying rate recorded good levels, with 91.26% in hens reared in the aviary and 91.53% in those in modified batteries. The highest laying intensity (laying peak) was reached in the 33rd week of life by the birds raised in the aviary (93.29%) and in the 31st week by those in improved batteries (93.92%), after which it decreased progressively, so that at the end of the investigations (week 55), the levels achieved were 87.02% for hens in aviaries and 88.16% for those maintained in modified batteries (Table 2).

3.3. Analysis of Batch Outputs

The rate of exits from the batch, including the generative causes, represented another criterion based on which the welfare condition ensured to the laying hens operating in the two rearing systems tested (aviary vs. battery) was assessed (Table 3).

It was found that in the birds raised in the aviary, the number of specimens that left the batch in the first 5 weeks was 227 heads, corresponding to a mortality of 0.62%/week, while in the hens raised in improved batteries, only 25 cases were recorded, resulting in a mortality of only 0.25%/week. Next, the rate of exits from the herd showed fluctuating values, being 0.1% (36 heads) in the 30th week, 0.14% (49 heads) in the 40th week, 0.14% (48 heads) in the 50th week, and 0.15% (52 heads) in the 55th week for hens in the house equipped with an aviary and 0.06% (6 heads), 0.12% (12 heads), 0.15% (15 heads), and 0.17% (17 heads) for specimens from the hall equipped with batteries (Table 3).

		Rearing in Loft			Rearing in Battery					
Birds' Age	Birds'	Flock	Cumula	ted Exits		Cumulated Exits				
(weeks)	at the Beginning of	at the End of the	from	Flock	at the Beginning of	at the End of the	from	Flock		
	the Week (Heads)	Week (Heads)	Heads	%	the Week (Heads)	Week (Heads)	Heads	%		
25	35,950	35,905	45	0.12	10,217	10,212	5	0.05		
26	35,905	35,864	86	0.23	10,212	10,206	11	0.11		
27	35,864	35,812	138	0.37	10,206	10,199	18	0.18		
28	35,812	35,762	188	0.51	10,199	10,197	20	0.20		
29	35,762	35,723	227	0.62	10,197	10,192	25	0.25		
30	35,723	35,687	263	0.72	10,192	10,186	31	0.31		
31	35,687	35,654	296	0.81	10,186	10,178	39	0.39		
32	35,654	35,616	334	0.92	10,178	10,170	47	0.49		
33	35,616	35,585	365	1.01	10,170	10,158	59	0.61		
34	35,585	35,550	400	1.11	10,158	10,151	66	0.68		
35	35,550	35,521	429	1.19	10,151	10,142	75	0.77		
36	35,521	35,475	475	1.32	10,142	10,133	84	0.86		
37	35,475	35,436	514	1.43	10,133	10,122	95	0.97		
38	35,436	35,395	555	1.54	10,122	10,112	105	1.07		
39	35,395	35,347	603	1.67	10,112	10,105	112	1.14		
40	35,347	35,300	650	1.80	10,105	10,093	124	1.26		
41	35,300	35,251	699	1.94	10,093	10,082	135	1.37		
42	35,251	35,213	737	2.05	10,082	10,073	144	1.46		
43	35,213	35,163	787	2.19	10,073	10,065	152	1.54		
44	35,163	35,111	839	2.34	10,065	10,057	160	1.62		
45	35,111	35,057	893	2.49	10,057	10,050	167	1.69		
46	35,057	35,000	950	2.65	10,050	10,041	176	1.78		
47	35,000	34,941	1009	2.82	10,041	10,029	188	1.90		
48	34,941	34,891	1059	2.96	10,029	10,018	199	2.01		
49	34,891	34,836	1114	3.12	10,018	10,005	212	2.14		
50	34,836	34,777	1173	3.29	10,005	9990	227	2.29		
51	34,777	34,729	1221	3.43	9990	9976	241	2.43		
52	34,729	34,678	1272	3.58	9976	9964	253	2.55		
53	34,678	34,624	1326	3.73	9964	9949	268	2.70		
54	34,624	34,571	1379	3.99	9949	9938	279	2.81		
55	34,571	34,519	1431	4.14	9938	9921	296	2.98		

Table 3. Situation of exits from flock at the studied hens.

During the entire study period (25–55 weeks), the total exits from the flock of hens raised in the aviary were at a level of 4.14% (1431 heads from an initial flock of 35,950 heads), while for those bred in battery this figure was only 2.98% (296 heads from an initial herd of 10,217 heads).

3.4. Causes of Batch Outputs

The main cause of batch exits was represented by mechanical accidents, but with large differences between the two growth systems used. Thus, for hens in the aviary, the mortality due to accidents was 49.27% (705 heads) with limits between 42.59% (45th week) and 55.55% (30th week) of total deaths (1431 heads), while in hens raised in improved batteries, batch losses due to accidents were only 39.53% (117 heads) with limits between 20.0% (38th week) and 100% (28th week) out of a total of 296 specimens withdrawn from the population (Table 4).

Another cause of exits from the batch referred to obstetric diseases, which, in the batch of birds raised in the aviary, represented 35.64% of the total losses (510 heads out of the total exits), with a minimum of 18.0% recorded in week 28 and a maximum of 50.0% in the 42nd week. In the batch grown in improved batteries, the average value of the output from the batch due to obstetrical diseases was 33.11% (98 heads of total losses), oscillating between 0.0% in the 28th week and 60.0% as they were in the 25th week.

The exits from the batch due to internal diseases were at much lower levels compared to the other causes, being only 15.09% in the case of specimens raised in the aviary (216 heads out of total losses) and 27.36% in those raised in improved batteries (81 chapters of total losses); in both cases, the analyzed parameter had an asymptotic evolution, with limits of 2.08–36.00%/week in hens from the aviary and 0.0–55.56%/week in those from improved batteries (Table 4).

	Rearing in Loft								Rearing in Battery						
Birds'				Ca	uses				Causes						
Age (Weeks)	Total Exits (Heads/Week)	Accidents		Obstetrical Diseases		Internal Diseases		Total Exits (Heads/Week)	Accidents		Obstetrical Diseases		Internal Diseases		
	(Ireaus, receir)	Head	%	Head	%	Head	%	- (1104405/110011)	Head	%	Head	%	Head	%	
25	45	21	46.67	11	24.44	13	28.89	5	2	40.00	3	60.00	-	-	
26	41	19	46.34	13	31.71	9	21.95	6	3	50.00	2	33.33	1	16.67	
27	52	26	50.00	12	23.08	14	26.92	7	3	42.86	3	42.86	1	14.28	
28	50	23	46.00	9	18.00	18	36.00	2	2	100	-	-	-	-	
29	39	19	48.72	16	41.03	4	10.26	5	3	60.00	2	40.00	-	-	
30	36	20	55.55	14	38.89	2	5.55	6	2	33.33	3	50.00	1	16.67	
31	33	16	48.48	13	39.39	4	12.12	8	2	25.00	3	37.50	3	37.50	
32	38	18	47.37	15	39.47	5	13.16	8	3	37.50	2	25.00	3	37.50	
33	31	17	54.83	11	35.48	3	9.68	12	5	41.67	4	33.33	3	25.00	
34	35	18	51.43	14	40.00	3	8.57	7	4	57.14	2	28.57	1	14.29	
35	29	16	55.17	11	37.93	2	6.90	9	4	44.44	3	33.33	2	22.22	
36	46	24	52.19	19	41.30	3	6.52	9	3	33.33	3	33.33	3	33.33	
37	39	19	48.72	17	43.59	3	7.69	11	3	27.27	4	36.36	4	36.33	
38	41	20	48.78	15	48.79	6	14.63	10	2	20.00	4	40.00	4	40.00	
39	48	25	52.08	22	45.83	1	2.08	7	3	42.86	2	28.57	2	28.57	
40	47	22	46.81	19	40.43	6	12.76	12	4	33.33	3	25.00	5	41.67	
41	49	23	46.94	14	28.57	12	24.48	11	4	36.36	4	36.36	3	27.27	
42	38	17	44.74	19	50.00	2	5.26	9	2	22.22	2	22.22	5	55.56	
43	50	27	54.00	17	34.00	6	12.00	8	3	37.5	4	50.00	1	12.50	
44	52	25	48.08	18	34.61	9	17.30	8	2	25.00	3	37.50	3	37.50	
45	54	23	42.59	19	35.18	12	22.22	7	2	28.57	2	28.57	3	42.86	
46	57	26	45.61	19	33.33	12	21.05	9	2	22.22	5	55.56	2	22.22	
47	59	31	52.54	23	38.98	5	8.47	12	3	25.00	5	41.67	4	33.33	
48	50	24	48.00	21	42.00	5	10.00	11	4	36.36	4	36.36	3	27.28	
49	55	28	50.91	19	34.54	8	14.54	13	4	30.77	6	46 15	3	23.08	
50	59	29	49 15	22	37 29	8	13.56	15	6	40.00	4	26.67	5	33.33	
51	48	26	54 17	19	39.58	3	6 25	14	7	50.00	3	21.43	4	28.57	
52	51	24	47.06	16	31.37	11	21.57	12	8	66.66	2	16.67	2	16.67	
53	54	29	53 70	15	27.78	10	18 52	15	8	53 33	4	26.67	3	20.00	
54	53	23	43.39	17	32.08	13	24.53	11	7	63.64	2	18 18	2	18 18	
55	52	27	51.92	21	40.38	4	7.69	17	, 7	41.18	5	29.41	5	29.41	
Total	1431	705	49.27	510	35.64	216	15.09	296	117	39.53	98	33.11	81	27.36	

Table 4. Causes of batch outputs.

3.5. Consumption of Mixed Fodders

In hens in the hall equipped with an aviary, the average daily consumption of mixed feeders recorded values of 120.45 g/head/day in the first studied period (25–40 weeks) and 124.10 g/head/day in the second period (41–55 weeks), while the feed conversion index was at levels of 130.0 g m.f./egg in the period of 25–40 weeks and 136.47 g m.f./egg in the interval of 46–55 weeks.

In the case of birds operating in improved batteries, the consumptions were better, both in the age period of 25–40 weeks (average daily consumption = 113.10 g/head/day; feed conversion index = 121.55 g m.f./egg), as well as during the period of 41–55 weeks (average daily consumption = 118.87 g/head/day; feed conversion index = 130.25 g m.f./egg).

Over the total period studied (25–55 weeks), the most convenient consumption of combined feed was achieved by the birds in the house equipped with improved batteries, which had an average daily consumption of 115.87 g/head/day (average effective = 10.092 head; feed consumed = 253.763 kg) and a feed conversion index of 125.69 g n.c./egg (egg production = 2.018.934 egg). Comparatively, hens reared in the aviary registered an average consumption of 122.20 g/head/day (average effective = 35.281 heads; feed consumed = 935.551 kg) and a feed conversion index of 133.07 g m.f./egg (egg production = 7.030.517 egg) (Table 5).

Age Period		Exploitation System			
(Weeks)	Specification	in Loft	in Battery		
	Mean flock (heads)	35.622	10.164		
25-40	Consumed fodders (kg/period)	480.553	128.747		
16 weeks	Daily mean consumption (g/head/day)	120.45	113.10		
(112 days)	Egg production (pieces/period)	3.696.413	1.059.160		
-	Feed conversion index (g m.f./egg)	130	121.55		
	Mean flock (heads)	34.917	10.016		
41–55	Consumed fodders (kg/period)	454.998	125.016		
15 weeks	Daily mean consumption (g/head/day)	124.10	118.87		
(105 days)	Egg production (pieces/period)	3.334.104	959.774		
	Feed conversion index (g m.f./egg)	136.47	130.25		
	Mean flock (heads)	35.281	10.092		
25–55	Consumed fodders (kg/period)	935.551	253.763		
31 weeks	Daily mean consumption (g/head/day)	122.20	115.87		
(217 days)	Egg production (pieces/period)	7.030.517	2.018.934		
	Feed conversion index (g m.f./egg)	133.07	125.69		

Table 5. Consumption of mixed fodders for studied hens.

4. Discussion

4.1. Biochemical Indicators

The determination of the main biochemical indicators was aimed at evaluating the comfort state provided to the studied hens, in correlation with the adopted breeding method (aviary vs. battery).

From the general analysis of the data regarding blood markers, it emerged that in most cases, their level was higher in hens raised in the aviary, regardless of age, a finding that reveals the fact that this production system ensures suitable wellbeing, as well as a good balance between neurohormonal function and metabolic activity.

The second finding was that the level of biochemical indicators was inversely related to the egg-laying intensity of the birds, in the sense that their level in the blood increased towards the end of egg laying, given the decrease in the number of nutrients necessary for egg formation (the rate of egg laying was increasingly reduced towards the end of the spawning period).

The high and even very high values of the coefficients of variation for each of the biochemical characters studied can be attributed to the physiological state in which the birds were at the time of sampling (with the egg in different stages of formation, with the egg prepared for oviposition, or with the egg already expelled).

For example, the level of cholesterol and triglycerides (components involved in the formation of lipids in the yolk) showed significant increases between the birds at the beginning of laying (week 25) and those at the end of laying (week 55), these being 31.18–34.10% for cholesterol and 8.17–8.77% for triglycerides, respectively. A similar situation was recorded for blood proteins (role in the formation of egg white), which increased by 28.84% in hens from the aviary and by 20.63% in those from the improved batteries.

In clinically healthy hens examined every five weeks of laying, Suchy et al. [22] found the following limits for biochemical parameters: protein = 47.43-60.45 g/L; glucose = 13.36-14.97 mmol/L; cholesterol = 2.73-6.18 mmol/L; calcium = 5.26-7.19 mmol/L; phosphorus = 1.25-1.90 mmol/L; magnesium = 1.08-1.42 mmol/L; sodium = 141.03-148.30 mmol/L; potassium = 3.40-5.00 mmol/L. The authors stated that the plasma levels of some biochemical parameters were directly influenced by the variations in laying capacity.

Triglyceride concentration determined in free-range Hy-Line hens was significantly lower than in those raised in battery cages (p < 0.05) [9].

The use of diets with reduced levels of crude protein in the Lohmann LSL hybrid significantly reduced the content of uric acid and albumin in the blood (variant with

13% CP) compared to the control group (16% CP); in contrast, blood triglyceride levels were higher (p < 0.05) in hens fed 14% and 13% CP than in those fed the control diet [32]. On the other hand, it was found that diets with low levels of metabolizable energy and crude protein reduced serum triglyceride and cholesterol concentrations in Gushi chickens, regardless of the density provided in the rearing cages (20–50 head/m²) [34].

Glucose used in energy metabolism was determined in smaller amounts in young birds (they were more active), and higher in birds aged 55 weeks (they were much quieter), with the differences between the two periods being 21.11% in the case of specimens from the aviary and 27.97% for those from the battery.

In an experiment that looked at the influence of fasting on blood parameters in different genotypes (Bovans, ISA Brown, and Ross-508), it was found that the lack of food caused a significant decrease in glucose levels and a significant increase in plasma cholesterol, which suggests a reduction in the welfare of the hens [29].

It is known that for the formation of the eggshell, a bird's blood must transport 100–150 mg Ca/h [44], and if calcium is not absorbed quickly at the intestinal level, calcemia can be established within only 10–12 min [37]. In the studied birds, the blood level of calcium was influenced only by the rate of egg formation (the lowest values were in hens in full production—week 35, and the highest were in hens at the end of laying—week 55). The amount of phosphorus in the blood evolved similarly, which had lower values at high laying intensities (35th week) and higher values at low laying intensities (55th week), with the mention that the oscillations recorded were generated by the different status of each specimen from which the sample was collected (phosphorus in the blood increases significantly during the formation of the egg shell in the uterus).

Comparing the blood plasma mineral profile (Ca, P, K, Mg, Zn, Cu, and Se) in ISA Brown hens from three different batteries (traditional batteries, improved batteries, and deep litter system) did not reveal a significant effect of the system of growth on the monitored indicators, with the obtained values falling within the physiological range [24].

In Leghorn hens, a direct correlation was found between the surface provided in the rearing battery and the level of some blood indicators; for example, in the variant with 500 cm² cage/head, significantly lower levels (p < 0.05) were found for plasma calcium and plasma uric acid, but significantly higher levels (p < 0.05) were found for iron, magnesium, and plasma glucose compared to specimens that benefited from 2000 cm²/head [14].

The uric acid level recorded higher values at the first determination performed (week 25), but it was significantly reduced at the last one, by 23.19% in the hens in the aviary and by 28.60% in those in the battery, while the amount of urea increased by 15.55% (aviary) and by 16.21% (battery) between the first harvest and the last harvest, respectively.

In Hisex Brown hens reared in different systems (battery and permanent litter) and with different diets (reduced levels of crude protein), a significant interaction was observed between the experimental factors and the level of serum protein, uric acid, and total blood cholesterol (p < 0.05). Diets with low CP levels decreased serum protein and cholesterol in litter-reared birds, as well as uric acid in battery birds, but without exceeding physiological limits [33].

The determined values for blood enzymes indicated fluctuations concerning liver metabolism, generated by the different laying rhythms of the birds, but in both cases, higher values were recorded in young birds (aged 25 weeks) and lower values were recorded in old ones (week 55). Thus, the values for alanine aminotransferase (ALT) increased by 12.48% in hens from the aviary and by 12.89% in those from batteries, while aspartate aminotransferase (AST) showed quantitative increases of 27.32% (aviary) and 27.21% (battery), respectively, between the two mentioned age periods.

4.2. Numerical Egg Production

Between the two rearing systems tested (battery vs. aviary) there is a clear difference regarding the freedom of movement of the birds and what corresponds to the level of ensured wellbeing [45], but additional energy consumption is also implied, to the detriment of the productivity of the birds [46].

Contrary to expectations, the experimental factor applied in our research (the rearing system) did not generate significant differences in terms of individual egg production, these being only 0.35% in favor of those in the battery (199.24 eggs/head vs. 199.98 eggs/head); compared to the theoretical potential of the ISA Brown hybrid, the egg production of hens in the aviary was lower by 1.85%, and of the battery hens by only 1.49%.

This indicates that the functionality of the reproductive apparatus of the hens in the aviary was stimulated by the superior welfare conditions, materialized in a high and constant numerical egg production, similar to that of the battery-reared birds [47].

This aspect is also confirmed by the fact that both groups of birds benefited from the same technological parameters (light program, microclimate, and food), the only difference being that the hens in the aviary had the opportunity to manifest their instincts much more freely, with positive effects on the neurohormonal system.

4.3. Laying Intensity

At the beginning of the investigations (the 25th week of the birds' life), the egg-laying intensity recorded values lower than the theoretical potential of the hybrid used (lower by 4.74% in the hens in the aviary and by 4.47% in the ones in the batteries), the probable cause being not achieving optimal body weights at the time of spawning (the information comes from the data provided by the unit where the research was carried out).

This aspect also had an impact on the age at which the maximum egg-laying intensity was reached (the egg-laying peak), this being achieved in the 31st week of life for the specimens raised in the battery and in the 33rd week for the birds in the aviary; the delay in the laying peak in caged hens was due to both lower body weights and disruptions to the normal ovulatory cycle, amid the constant agitation of birds that benefited from large areas of movement.

During the 31 weeks of investigations, the studied hens achieved a good average egg-laying intensity (91.81% in the specimens raised in the aviary and 92.17% in those operated in improved batteries), very close to the potential of the hybrid used (lower by 0.82% and 0.06%, respectively) and similar to those presented in the specialized literature.

Thus, for ISA Brown hens, an average egg-laying intensity of 91.42% was reported for specimens raised in improved batteries and only 75.94% for those in classic batteries, in the age period of 37–45 weeks [21].

In an experiment that aimed at the effect of the presence of males on the productivity of ISA Brown hens raised on permanent litter (6.6 head/ m^2), an average egg-laying intensity of 76.21% was obtained in the version without males and of 84.4% in the version with introduced 1 male for 10 females; the control period was 18–31 weeks [18].

Egg production also depends on the technique of rearing replacement juveniles. Thus, in the case of adult ISA Brown hens raised in an aviary, an average egg-laying intensity of 85% was obtained when they came from chicks raised in a youth aviary and only 84% in specimens from chicks raised in a classic battery [15].

4.4. Batch Outputs Rate

The improved batteries limit the movement space of each bird [30], while the aviary is an equipment that respects the welfare condition, because it gives the birds the possibility to move on much larger surfaces, both vertically (between the floors of the same section) and horizontally (between two neighboring sections) [48].

Compared to the theoretical mortality rate specific to the ISA Brown hybrid for the age period of 25–55 weeks (3.1%), the birds studied by us had a 1.04% higher mortality rate in the specimens raised in the aviary (4.14%) and a 0.12% lower rate in those grown in the improved battery (2.98%).

Similar results were reported by Huneau-Salauen et al. [11], who found a mortality rate of 4.2% in ISA Brown hens at a density of 40 heads/cage in improved Zucami batteries (768 cm²/bird) and a mortality rate of only 2.4% in the version with 20 heads/cage.

From the direct observations of the two groups of birds, it turned out that the hens in the aviary had a much more active behavior, constantly moving between the areas of interest (feeders, waterers, and nests) and fully manifesting their instincts (perching, snorting, etc.). This freedom of movement generated a greater number of mechanical accidents (in contact with the hard parts of the equipment), but also of internal diseases, the temporal distribution of which was somewhat uniform throughout the entire period studied.

In an experiment that looked at the effect of genotype and the content of essential amino acids (methionine + cystine) on the feather chipping phenomenon, ISA Brown hens had a mortality rate of 17.5% (not attributed to the provided diet), compared to 2.4% in New Hampshire hens and 0% in White Leghorns [31].

4.5. Causes for Batch Outputs

For the exits from the batch studied by us, three main causes were identified (accidents, obstetric diseases, and internal diseases) whose weight was directly influenced by the breeding system used.

From this point of view, it was found that batch losses due to mechanical accidents (especially wing and leg fractures) were much more frequent in hens from the aviary, higher by 9.74% than those from the battery, the incriminating factor in this case being the movement of birds on much larger surfaces than in the battery; another factor generating accidental mortality is the effect of fights between birds to establish the social hierarchy, at least for the beginning of the investigations. The highest rate of mechanical accidents was recorded in the age period of 46–55 weeks (37.87% for hens in the aviary and 47.86% for those in the battery compared to total accidents), due to the increase in bone fragility (higher consumption of Ca for the shell eggs), increasing body weights, and quite possibly, the decrease in the agility of the birds as they get older (especially in the aviary).

In the growth conditions ensured by the aviary, ISA Brown hens recorded a 1.37% higher proportion of flock exits (due to fractures and injuries at the level of the sternal carina) compared to Dekalb White hens [17].

As for the rate of exits from the batch due to obstetrical diseases, it was higher by 2.53% in hens in the aviary, due to the fact that they moved much more in order to satisfy their natural instincts, thus affecting the regularity of the ovulatory cycle. It should be noted that the highest levels for obstetrical diseases were in the period of 25–45 weeks (62.35% for hens in the aviary and 59.18% for those in the battery out of total exits caused by obstetrical diseases), when an increased incidence of "peritonitis" was found "vitelline" with various forms of ovaritis due to the lack of correlation between the intense rhythm of ovulation and the insufficient amount of hormones stimulating the maturation of ovarian follicles.

There were also cases of birds with abdominal peritonitis (the oviduct can no longer capture the mature follicles and they fall into the abdominal cavity), and towards the end of the studied period, the number of birds with uterine prolapse, caused by the increased volume of the eggs, also increased quite a lot, and with both categories requiring the withdrawal of birds from the batch.

The exits from the batch caused by the manifestation of internal diseases registered a higher rate in the hens in batteries compared to those in the aviary, the difference between the batches being 12.27%. Among the internal diseases, a high incidence of mortality was due to the "fatty liver" syndrome detected only in the specimens in the battery (massive deposits of fat on the abdomen and mesentery due to lack of movement), especially after the age of 40 weeks. Another cause was the manifestation of necrotic enteritis, whose etiological agent is *Clostridium perfringens*, a bacterial species that colonizes the intestines of birds (the replacement chicks were probably not treated for this disease), but which can contaminate the administered chicks, the air admitted to the sheds, or even the bedding used [49].

4.6. Consumption of Mixed Fodders

This productive indicator was correlated with the egg-laying intensity of the birds but influenced by the freedom of movement granted to them by the state of ensured well-being.

For example, the numerical egg production from 25–40 weeks was 104.21 eggs/head (battery) compared to only 103.77 eggs/head (aviary), hence a lower feed conversion index by 6.95% in hens raised in improved batteries; in the age period of 41–55 weeks, the egg production of the birds was reduced (95.49 pcs./head in the aviary and 95.82 pcs./head in the batteries), which led to higher values of the feed conversion index, with the mention that hens reared in improved batteries still recorded more favorable consumption levels (4.77% lower than aviary hens).

The obtained data showed that hens in the aviary had a higher average daily consumption than those in the battery (by 6.50% in the period of 25–40 weeks and by 4.40% in the period of 41–55 weeks), due exclusively to the additional energy expenses caused by permanent movement in the much more generous space offered by the aviary.

The consumption of combined feed calculated for the entire period studied was at levels similar to those reported in different experiments, both for hens in the aviary (daily consumption = 122.20 g n.c./head/day; feed conversion index = 133.07 g n.c./egg) and especially for those in the shed with improved batteries (average consumption = 115.87 g n.c./head/day; conversion index = 125.69 g n.c./egg), which proves that both types of equipment tested by us ensure conditions of exteriorization of the productive potential of hybrids specialized in egg production.

When reared in the improved battery (768 cm²/bird) for 53 weeks, ISA Brown hens achieved an average daily consumption of 111.4–113.2 g d.c./head/day and a conversion index of 2.04–2.06 kg d.c./kg eggs, depending on the number of birds in the cage (20 vs. 40 heads/cage) [11].

ISA Brown hens weaned and in battery cages with different interior layouts (classic nest vs. nest lined with artificial turf or plastic mesh) had average daily intakes of 110.6–111.1 g n.c./head/day [28].

5. Conclusions

Our investigations concluded that the exploitation of laying hens in the aviary system ensures the most optimal conditions for the externalization of the birds' natural behaviors, with positive repercussions on the metabolic functions, resulting in a good state of health and high productive levels, comparable to those of birds raised in the battery. However, we believe that it is necessary to continue research in this direction by evaluating molecular, biochemical, or hormonal markers that accurately reflect the different types of stress to improve the productivity and quality of life of birds exploited for egg production.

Author Contributions: Conceptualization, A.U., M.-G.D., C.P., M.-G.U., R.-M.R.-R., M.-I.U. and D.S.; methodology, A.U., M.-I.U., M.S., C.P., B.-V.A. and D.S.; software, M.-G.D. and C.P.; validation, A.U., C.P. and M.-G.D.; formal analysis, M.-G.U., C.S. and R.-M.R.-R.; investigation, A.U., M.-G.D., C.P., M.-I.U. and M.S.; data curation, A.U. and M.-G.D.; writing—original draft preparation, A.U., B.-V.A., C.P., M.-I.U. and M.-G.U.; writing—review and editing, A.U., M.-G.D., M.S. and C.S.; supervision, A.U. and M.-G.D. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: We thank to the SC Condor Matca SRL, Galati County, Romania.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Rozempolska-Rucinska, I.; Czech, A.; Kasperek, K.; Zieba, G.; Ziemianska, A. Behaviour and stress in three breeds of laying hens kept in the same environment. *S. Afr. J. Anim. Sci.* **2020**, *50*, 272–280. [CrossRef]
- 2. Sosnowka-Czajka, E.; Herbut, E.; Skomorucha, I.; Muchacka, R. Welfare levels in heritage breed vs. Commercial laying hens in the litter system. *Ann. Anim. Sci.* 2011, *11*, 585–595. [CrossRef]
- 3. Craig, J.V.; Swanson, J.C. Welfare perspectives on hens kept for egg-production. Poult. Sci. 1994, 73, 921–938. [CrossRef] [PubMed]
- 4. Savory, C.J.; Jack, M.C.; Sandilands, V. Behavioural responses to different floor space allowances in small groups of laying hens. *Br. Poult. Sci.* **2006**, *47*, 120–124. [CrossRef]
- 5. Zotte, A.D.; Gottardo, F.; Ravarotto, L.; Bertuzzi, S. The welfare evaluation of laying hens reared with alternative housing systems. *Ital. J. Anim. Sci.* 2003, *2*, 462–464.
- 6. Appleby, M.C.; Walker, A.W.; Nicol, C.J. Development of furnished cages for laying hens. *Br. Poult. Sci.* 2002, 43, 489–500. [CrossRef]
- 7. Roberts, J. Welfare standards for laying hens. Achieving sustainable production of eggs. Anim. Welf. Sustain. 2017, 17, 85–97.
- 8. Bozkurt, Z.; Bayram, I.; Bulbul, A.; Aktepe, O.C. Effects of Strain, Cage Density and Position on Immune Response to Vaccines and Blood Parameters in Layer Pullets. *Kafkas Univ. Vet. Fak. Derg.* **2008**, *14*, 191–204.
- 9. Yang, H.M.; Yang, Z.; Wang, W.; Wang, Z.Y.; Sun, H.N.; Ju, X.J.; Qi, X.M. Effects of different housing systems on visceral organs, serum biochemical proportions, immune performance and egg quality of laying hens. *Eur. Poult. Sci.* **2014**, *78*, 48.
- 10. Blokhus, H.J.; Fiks van Niekerk, T.; Bessei, W. The LayWel projects: Welfare implications of changes in productions systems for laying hens. *Worlds Poult. Sci. J.* 2007, 63, 101–114. [CrossRef]
- Huneau-Salauen, A.; Guinebretiere, M.; Taktak, A.; Huonnic, D.; Michel, V. Furnished cages for laying hens: Study of the effects of group size and litter provision on laying location, zootechnical performance and egg quality. *Animals* 2011, 5, 911–917. [CrossRef] [PubMed]
- 12. Ozenturk, U.; Yildiz, A. Assessment of Egg Quality in Native and Foreign Laying Hybrids Reared in Different Cage Densities. *Braz. J. Poult. Sci.* 2021, 22, 85–97. [CrossRef]
- Guinebretiere, M.; Huneau-Salauen, A.; Huonnic, D.; Michel, V. Plumage condition, body weight, mortality, and zootechnical performances: The effects of linings and litter provision in furnished cages for laying hens. *Poult. Sci.* 2013, *92*, 51–59. [CrossRef] [PubMed]
- 14. Saki, A.A.; Zamani, P.; Rahmati, M.; Mahmoudi, H. The effect of cage density on laying hen performance, egg quality, and excreta minerals. *J. Appl. Poult. Res.* 2012, 21, 467–475. [CrossRef]
- 15. Colson, S.; Michel, V.; Arnould, C. Welfare of laying hens housed in cages and in aviaries: What about fearfulness? *Arch. Geflugelkd.* **2006**, *70*, 261–269.
- 16. Fossum, O.; Jansson, D.S.; Etterlin, P.E.; Vagsholm, I. Causes of mortality in laying hens in different housing systems in 2001 to 2004. *Acta Vet. Scand.* 2009, *51*, 3–11. [CrossRef]
- 17. Stratmann, A.; Froehlich, E.K.F.; Harlander-Matauschek, A.; Schrader, L.; Toscano, M.J.; Wuerbel, H.; Gebhardt-Henrich, S.G. Soft Perches in an Aviary System Reduce Incidence of Keel Bone Damage in Laying Hens. *PLoS ONE* **2015**, *10*, e0122568. [CrossRef]
- De Oliveira Pereira, D.C.; Da Silva Miranda, K.O.; Dematte Filho, L.C.; Pereira, G.V.; De Stefano Piedade, S.M.; Berno, P.R. Presence of roosters in an alternative egg production system aiming at animal welfare. *Rev. Bras. De Zootec.-Braz. J. Anim. Sci.* 2017, 46, 175–184. [CrossRef]
- 19. Marcq, C.; Marlier, D.; Beckers, Y. Improving adjuvant systems for polyclonal egg yolk antibody (IgY) production in laying hens in terms of productivity and animal welfare. *Vet. Immunol. Immunopathol.* **2015**, *165*, 54–63. [CrossRef]
- Sossidou, E.N.; Elson, H.A. Hens' welfare to egg quality: An European perspective. Worlds Poult. Sci. J. 2009, 65, 709–718. [CrossRef]
- 21. Corona, J.; Trompiz, J.; Jerez, N.; Gomez, A.; Rincon, H. Effect of warehouse type on productive variables and egg quality in laying hens Isa Brown. *Rev. Fac. Agron. Univ. Zulia* **2016**, *32*, 345–360.
- 22. Suchy, P.; Strakova, E.; Vecerek, V.; Sterc, P. Biochemical studies of blood in hens during the laying period. *Czech J. Anim. Sci.* 2001, 46, 383–387.
- 23. Pavlik, A.; Pokludova, M.; Zapletal, D.; Jelinek, P. Effects of housing systems on biochemical indicators of blood plasma in laying hens. *Acta Vet. Brno* 2007, *76*, 339–347. [CrossRef]
- 24. Pavlik, A.; Lichovnikova, M.; Jelinek, P. Blood Plasma Mineral Profile and Qualitative Indicators of the Eggshell in Laying Hens in Different Housing Systems. *Acta Vet. Brno* 2009, *78*, 419–429. [CrossRef]
- 25. Ito, S.; Tanka, T.; Yoshimoto, T. Effects of an 'enrichment feeder' on behavior and feather conditions of caged laying hens. *Anim. Sci. J.* **2002**, *73*, 149–153. [CrossRef]
- 26. De Haas, E.N.; Kemp, B.; Bolhuis, J.E.; Groothuis, T.; Rodenburg, T.B. Fear, stress, and feather pecking in commercial white and brown laying hen parent-stock flocks and their relationships with production parameters. *Poult. Sci.* **2013**, *92*, 2259–2269. [CrossRef]
- De Marco, M.; Miro, S.M.; Tarantola, M.; Bergagna, S.; Mellia, E.; Gennero, M.S.; Schiavone, A. Effect of genotype and transport on tonic immobility and heterophil/lymphocyte ratio in two local Italian breeds and Isa Brown hens kept under free-range conditions. *Ital. J. Anim. Sci.* 2014, 12, e78. [CrossRef]

- Guinebretiere, M.; Mika, A.; Michel, V.; Balaine, L.; Thomas, R.; Keita, A.; Pol, F. Effects of Management Strategies on Non-Beak-Trimmed Laying Hens in Furnished Cages that Were Reared in a Non-Cage System. *Animals* 2020, 10, 399. [CrossRef]
- Altan, O.; Oguz, I.; Erbayraktar, Z.; Yimaz, O.; Bayraktarl, H.; Sis, B. The effect of prolonged fasting and refeeding on GSH-Px activity. plasma glucose and cholesterol levels and welfare of older hens from different genotypes. *Arch. Geflugelkd.* 2005, 69, 185–191.
- 30. Lay, D.C.; Fluton, R.M.; Hester, P.Y. Hen welfare in different housing systems. Poult. Sci. 2011, 90, 278–294. [CrossRef]
- Kjaer, J.B.; Sorensen, P. Feather pecking and cannibalism in free-range laying hens as affected by genotype. dietary level of methionine plus cysteine, light intensity during rearing and age at first access to the range area. *Appl. Anim. Behav. Sci.* 2002, 76, 21–39. [CrossRef]
- 32. Torki, M.; Nasiroleslami, M.; Ghasemi, H.A. The effects of different protein levels in laying hens under hot summer conditions. *Anim. Prod. Sci.* 2017, *57*, 927–934. [CrossRef]
- Viana, E.F.; De Carvalho Mello, H.H.; Carvalho, F.B.; Café, M.B.; Mogyca Leandro, N.S.; Arnhold, E.; Stringhini, J.H. Blood biochemical parameters and organ development of brown layers fed reduced dietary protein levels in two rearing systems. *Anim. Biosci.* 2022, 35, 444–452. [CrossRef] [PubMed]
- 34. Wan, Y.I.; Ma, R.; Li, Y.; Liu, W.; Li, J.Y.; Zhan, K. Effect of a Large-sized Cage with a Low Metabolizable Energy and Low Crude Protein Diet on Growth Performance. Feed Cost and Blood Parameters of Growing Layers. J. Poult. Sci. 2021, 58, 70–77. [CrossRef]
- 35. Abrahamsson, P.; Tauson, R.; Elwinger, K. Effects on production, health and egg quality of varying proportions of wheat and barley in diets for two hybrids of laying hens kept in different housing systems. *Acta Agric. Scand. Sect. A-Anim. Sci.* **1996**, *46*, 173–182. [CrossRef]
- 36. Rodriguez-Aurrekoetxea, A.; Estevez, I. Use of space and its impact on the welfare of laying hens in a commercial free-range system. *Poult. Sci.* **2016**, *95*, 2503–2513.
- Dedousi, A.; Kritsa, M.Z.; Stojcic, M.D.; Sfetsas, T.; Sentas, A.; Sossidou, E. Production Performance, Egg Quality Characteristics, Fatty Acid Profile and Health Lipid Indices of Produced Eggs, Blood Biochemical Parameters and Welfare Indicators of Laying Hens Fed Dried Olive Pulp. Sustainability 2022, 14, 3157. [CrossRef]
- ISA Brown—Hendrix ISA. Available online: https://www.hendrix-isa.com/en/find-out-more-on-our-breeds-dekalb_whiteshaver_white-bovans_brown-isa_brown/isa-brown/ (accessed on 11 July 2021).
- Natura Nova—Big Dutchman. Available online: https://www.bigdutchman.de/de/legehennenhaltung/produkte/detail/ natura-nova/ (accessed on 11 June 2021).
- 40. Simeanu, D. Nutriția și Alimentația Animalelor; Ion Ionescu de la Brad: Iași, Romania, 2018; ISBN 978-973-147-303-1.
- 41. Usturoi, M.G. Creșterea Păsărilor; Ion Ionescu de la Brad: Iași, Romania, 2008; ISBN 978-973-147-012-2.
- 42. Burtis, C.A.; Ashwood, E.R.; Bruns, D.E. *Tielz Textbook of Clinical Chemistry and Molecular Diagnostics*, 4th ed.; WB Saunders Co.: Philadelphia, PA, USA, 2005.
- 43. Young, D.S.; Friedman, R.B. Effects of Disease on Clinical Laboratory Tests, 4th ed.; AACC Press: Washington, DC, USA, 2001.
- 44. Burtis, C.A.; Ashwood, E.R.; Bruns, D.E. *Tietz Textbook of Clinical Chemistry and Molecular Diagnostics*, 5th ed.; WB Saunders Co.: Philadelphia, PA, USA, 2012.
- 45. Tactacan, G.B.; Guenter, W.; Lewis, N.J.; Rodriquez-Lecompte, J.C.; House, J.D. Performance and welfare of laying hens in conventional and enriched cages. *Poult. Sci.* 2009, *88*, 698–707. [CrossRef]
- 46. Singh, R.; Cheng, K.; Silversides, F. Production performance and egg quality of four strains of laying hens kept in conventional cages and floor pens. *Poult. Sci.* **2009**, *88*, 256–264. [CrossRef]
- 47. Petričević, V.; Škrbić, Z.; Lukić, M.; Petričević, M.; Dosković, V.; Rakonjac, S.; Marinković, M. Effect of genotype and age of laying hens on the quality of eggs and egg shells. *Sci. Papers Ser. D Anim. Sci.* **2017**, *60*, 166–170.
- Widowski, T.; Hemsworth, P.J.; Barnett, J.; Rault, L. Laying hen welfare I. Social environment and space. Worlds Poult. Sci. J. 2016, 72, 333–342. [CrossRef]
- 49. Srinivasan, P.; Balasubramaniam, G.A.; Murthy, T.R.G.K.; Balachandran, P. Bacteriological and pathological studies of egg peritonitis in commercial layer chicken in Namakkal area. *Asian Pac. J. Trop. Biomed.* **2013**, *3*, 988–994. [CrossRef] [PubMed]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.