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Does social position affect well-being in laying hens?

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ABSTRACT. The level of stress is undoubtedly related to the position occupied by an individual in a social group. Research shows that this relationship is complex and can be ambiguous. The study involved 53-week-old 80 Green-legged Partridge hens kept in four independent identical boxes divided by opaque partitions, 20 individuals each. Behaviour was recorded for the following activities: pecking and flight. Behavioural observations began when four flocks were established with combined birds (day 1, 2, 3). Subsequent observations were carried out after the flock structure was established (day 14, 15, 16). Counting was conducted for 8 h/day, 4 h in the morning (6:00–10:00), and 4 h in the afternoon (14:00–18:00). Stress level was assessed using physiological indicators such as steroid hormone levels (serotonin, dopamine, noradrenaline, and adrenaline), corticosterone level and intestinal microflora composition. Changes in group structure were observed and only a small percentage remained constant in terms of belonging to a dominant, subordinate or neutral group. Significant differences in hormone levels were found between birds belonging to the above groups. The results indicated that the lack of an established position in the group structure was a social stressor for laying hens.

Introduction

Domestic hens are social birds living naturally in a defined territory in groups composed of males and females (Quieroz and Cromberg, 2006). When kept in small flocks, laying hens exhibit behaviour similar to their wild ancestors when environmental conditions allow it (Campderrich et al., 2016; Carvahlo et al., 2018; Kozak et al., 2019). Behavioural disorders resulting from social stress can be observed in the case of excessive density (Cheng et al., 2002).

Chickens acquire social behaviour from each other by learning to identify visual and auditory stimuli (Wang et al., 2022). The flock structure is hierarchical with male dominance over hens, and the hierarchy determined by the pecking order. The competition is authoritarian in nature, implying dominance of the strongest individuals (Quieroz and Cromberg, 2006). Since behavioural synchronization occurs in social animals, resources should depend on the size and density of the flock (Du et al., 2022). Otherwise, a struggle for resources can ensue, which can already be observed in one-week-old chickens when food is restricted (Quieroz and Cromberg, 2006; Wang et al., 2022). Dominance-subordination interactions, maintained by means of aggressive behaviour, contribute to maintaining stability in the group (Bhanja and Bhadauria, 2018; Wang et al., 2022). Social relationships between members of a poultry group in which one (subordinate) avoids confrontation with another (dominant) are called the dominance hierarchy. Aggression is usually rare in a small and stable poultry group, because subordinates avoid dominants whenever possible (Shimmura et al., 2007). Aggression is directed primarily towards individuals with a lower social status or with specific phenotypic traits, e.g. low body weight or small comb size (Campderrich et al., 2016). The addition of unfamiliar hens to the flock disrupts the hierarchy, which needs to be re-established. Consequently, social disruption has been indicated as one of the greatest stressors for hens (Fahey and Cheng, 2008; Matur et al., 2015). In response to a social stressor, a reaction involving the release of glucocorticoids along with hormones secreted by the sympathetic nervous system is triggered. Long-term stress has a negative impact on reproduction and can reduce the performance of laying hens, which in turn results in economic losses (Matur et al., 2015; Carvahlo et al., 2018).

The level of stress is undoubtedly related to the position occupied by an individual in a social group, but as research shows, this is not a clear correlation. There is a complex relationship between the stability of the in-group structure, as well as the role played by the dominant and subordinated individual in the group (Wang et al., 2022). Increased activity of the hypothalamic-pituitary-adrenal axis (HPA) may be a consequence of subordination or represent a cost associated with dominance (Bortolotti et al., 2008). Interestingly, elevated stress hormone levels were observed in both dominant and subordinate birds (Carvahlo et al., 2018). A chronically activated stress response appears to be a low-rank indicator in many animal species. However, there are numerous other species in which social subordination is not associated with an overreaction to stress. A lower position in the group is not necessarily associated with the presence of physical or psychological stressors. Moreover, group instability and the need to re-establish the hierarchy may be more stressful for dominant individuals (Kotrschal et al., 1998).

We assumed that by assessing the levels of selected stress-related indicators, we would evaluate whether being a group leader or being a subordinate individual was more stressful for the hens. The aim of the study was to analyse the relationship between social position in the group and the stress level in laying hens, as determined by various biological indices.

Material and methods

Ethical statement

All experimental procedures were approved by the 2nd Local Ethics Committee for Animal Studies at the University of Life Sciences in Lublin, Poland (Approval no. 69/2017 of 28 September 2017).

Animals and housing

Before the study, the birds were examined by a veterinarian to confirm that their health status would not affect the results.

The research material consisted of 80 Green-legged Partridge hens, aged 53 weeks, kept at the Educational Research Centre in Lublin. The birds were provided with constant access to a complete diet and water in accordance with standard requirements for poultry rearing. Four independent identical boxes were divided by opaque partitions and each box was equipped with drip drinkers, a feeding tube, and nests lined with straw litter. The hens were maintained under a 16 h light/8 h darkness schedule. The birds were randomly allocated to one of four groups kept in the same farm building in boxes, 20 hens each, which ensured identical rearing conditions. The composition of each group did not change throughout the experiment. The density of birds in each box was 0.3 m^2/hen. For focal sampling, all 20 birds in a group were individually tagged on the wing. To record the hens' behaviour, a camera was mounted above covering the entire area of each cage. Stress level were assessed using physiological indicators such as steroid hormone level, corticosterone level and intestinal microflora composition.

Behavioural observation

Observations of the hens' behaviour at the time of grouping were used to assess group position. It is at this time when the hens' behavioural reactions are most vivid and easy to observe. Behaviour was recorded for the pecking and flight activities. Aggressive pecking included the head of the recipient, and excluded both severe feather pecking (forceful pecks, sometimes with feather pulling, and recipient bird moving away) and gentle feather pecking (cautious pecks, not resulting in feather pulling and usually without reaction from the recipient bird) (Shimmura et al., 2008). Flight was defined as an instinctive physiological response to a threatening situation that prepared a bird to flee from another one. Based on behavioural analysis, the hen's position in the group was classified as dominant (pecking had a higher frequency than flight), subordinate (fleeing had a higher frequency than pecking) or neutral (no pecking or flight). Counting was conducted separately for each hen during the first and last 3 days, and the results were summed for each period. The position of the hen was determined independently in the first study period (first 3 days after grouping) and in the last period (last 3 days). Behavioural observations began when four flocks were established (days 1, 2, 3), and subsequent observations were carried out after the flock structure was established (days 14, 15, 16). Counting was conducted for 8 h/day, 4 h in the morning (6:00–10:00), and 4 h in the afternoon (14:00–18:00). Analysis of all recordings was carried out by one observer.

Experimental procedures and sample collection

Concentrations of serotonin, dopamine, norepinephrine and adrenaline steroid hormones were measured to assess stress levels. To determine the level of hormones, blood was collected from the wing vein from all birds on day 14. Directly after sample collection, blood was aliquoted and transferred into tubes containing EDTA (MEDLAB-PRODUCTS, Sp. z o.o., Raszyn, Poland) as an anticoagulant. Blood samples were centrifuged for 10 min at 3000 *g* (MPW-260R, MPW MED. INSTRUMENTS, Warsaw, Poland) and plasma was stored at −80 °C until analysis. Quantitative determination of plasma serotonin levels was performed using the Eagle Biosciences Serotonin ELISA Assay Kit (catalog no.: SER39-K01; Eagle Biosciences, Inc., Nashua, NH, USA). Plasma levels of hormones (adrenaline, noradrenaline and dopamine) were determined using CAT ELISA assays (enzyme immunoassays for adrenaline, noradrenaline and dopamine quantification) in accordance with the manufacturer's procedure (DLD Diagnostika GMBH – catalog no.: EA603/288; Eagle Biosciences, Inc., Nashua, NH, USA). Determinations were performed using an Elisa reader (Sunrise™, Tecan Trading AG, Männedorf, Switzerland) and the absorbance was read at 450 nm.

Next, the level of corticosterone in the feathers was determined using the method modified by Bortolotti et al. (2008). The second primary feather was collected from the left wing of each bird for analysis. Corticosterone levels were determined by immunoenzymatic assay using the Chicken CORT (Corticosterone) ELISA Kit (Biorbyt Ltd, Cambridge, United Kingdom) according to the manufacturer's procedure. At the end of the study, the birds were slaughtered for commercial purposes.

The composition of the intestinal microflora was also considered as a factor related to hormone levels, indirectly shaping the behaviour of individuals (Wlazło et al. 2021a; b). Intestinal contents from three sections of the gastrointestinal tract: small intestine, caecum, and large intestine were collected to sterile containers from each hen and transported in thermal bags to the laboratory. Samples of the birds' intestinal contents were homogenised, and 20 g of material was weighed and placed in containers with Ringer's solution (BTL Industries Ltd, Warsaw, Poland). Ten-fold dilutions were then prepared in Ringer's solution and plated onto prepared Petri dishes with the appropriate microbial medium (Table 1). Each sample was plated in triplicate. After incubation, colonies were counted using a Scan 300 counter (Interscience Laboratories, Saint Nom la Bretêche, France) and the abundance of each type was determined, expressed as the number of colony-forming units per g of intestinal content (CFU/g).

Statistical analyses

The recorded indices did not show a normal distribution, hence, the data were subjected to a rank transformation. Multiple comparisons with the Bonferroni correction of estimates of differences in the examined traits between the breeds were analysed in univariate models, taking into account the effect of the group to which the hen was assigned (attacking, fleeing, neutral). The number of the pen, in which the hens were kept was not a significant factor and was therefore excluded from the analyses. The GLIMMIX procedure (SAS Institute, 2018) was applied to evaluate the significance of differences between the study groups.

Results

More than 57% of the birds that mainly exhibited pecking behaviour on the day of the establishment of new flocks (day 1) showed the same response

Table 1. Microbiological assays

Parameter	Microbiological medium	Incubation
Total number of mesophilic aerobic bacteria	Agar medium	Incubation for 48 h at 37 °C in accordance with PN EN ISO 4833 2
Total number of fungi	Sabouraud agar medium	Incubation for 5–7 d at 25 °C in accordance with PN ISO 21527-1/2
Total number of coliform bacteria	Endo LES agar medium	Incubation for 24 h at 37 °C in accordance with PN-ISO 4832:2007
Total number of *Escherichia coli*	mFC agar medium	Incubation for 18–24 h at 44 °C in accordance with PN-ISO-16649-2
Total number of *Cloristidium perfringens*	Tryptose-sulphite-cycloserine (TSC) medium	Incubation for 48 h at 37 °C in anaerobic conditions using the GasPak Plus system (Anaerobic System Envelopes with Palladium Catalyst, BD BBL) in accordance with PN-EN ISO 7937
Number of lactic acid bacteria of the genus *Lactobacillus*	MRS agar medium	Incubation for 3–5 d at 30 °C
Salmonella presence	Buffered peptone water Rappa-port-Vassiliadis, XLD medium	Incubation for 24 h at 37 °C; final identification was carried out using biochemical tests and polyvalent sera in accordance with PN-EN ISO 6579:2003/A1:2007P

PN EN ISO 4833 2 – ISO 4833-2:2013 specifies a horizontal method for counting microorganisms that are able to grow and form colonies on the surface of a solid medium after aerobic incubation at 30 °C; PN ISO 21527-1/2 specifies a horizontal method for determining yeast and mould counts; PN-ISO-16649-2 specifies a horizontal method for determining the count of beta-glucuronidase-positive *Escherichia coli;* PN-EN ISO 6579:2003/A1:2007P specifies a horizontal detection method for *Salmonella* spp.

after flock hierarchy was established (day 14). Over 38% of the hens, whose primary behaviour was flight changed their strategy and showed aggressive behaviour. The behaviour of exactly the same percentage of hens changed to neutral. After establishing the hierarchy, almost 60% of neutral hens began to show aggression, and only less than 17% remained neutral (Table 2).

The behaviour of the hens was assessed by recording the number of pecks and flee attempts. Analysis of the hens' behaviour after establishment of the hierarchy (day 14) showed that more than half of the birds classified as attackers (51.3%) exhibited aggressive behaviour on the first day of social structure formation. Hens that initially avoided conflicts constituted only 18% of the group of aggressive hens. Birds showing primarily flight responses at the beginning of hierarchy establishment employed both pecking and flight strategies. Birds that initially tried to remain neutral accounted for the lowest percentage. Particularly noteworthy was the group of neutral hens, which on day 1 constituted only 9%, and most of them, i.e. nearly 55%, demonstrated mainly the flight reaction (Table 3).

The mean levels of neurotransmitters (adrenaline, dopamine, noradrenaline, and serotonin), intestinal microflora composition (funglog, mesophlog, lactobaclog, colilog, ecolilog, clostridlog), and corticosterone levels are presented in Table 4. Statistically significant differences between the means are presented in Tables 5 and 6.

Table 2. Behavioural changes during flock hierarchy establishment

Behaviour		Percentage, %
Day 1	Day 14	
Attack	Attack	57.1
	Flight	20.0
	Neutral	22.9
Flight	Attack	38.7
	Flight	22.6
	Neutral	38.7
Neutral	Attack	58.3
	Flight	25.0
	Neutral	16.7

Table 3. Percentage of hens after hierarchy establishment relative to their behaviour on day 1

Behaviour		Percentage, %
Day 14	Day 1	
Attack	Attack	51.3
	Flight	30.8
	Neutral	18.0
Flight	Attack	41.2
	Flight	41.2
	Neutral	17.7
Neutral	Attack	36.4
	Flight	54.6
	Neutral	9.1

Table 4. Mean levels of the analysed indicators relative to the social status of hens

Parameter	Day 14	Mean	StdErr	Lower	Upper
Adrenaline, ng/ml	A	0.46	0.06	0.34	0.59
	F	0.42	0.10	0.23	0.62
	N	0.44	0.09	0.26	0.63
Dopamine, ng/ml	A	3.21	1.11	0.99	5.42
	F	1.54	1.78	−2.01	5.09
	N	8.96	1.66	5.64	12.28
Noradrenaline, ng/ml	A	0.60	0.19	0.22	0.98
	F	1.65	0.31	1.03	2.26
	N	0.88	0.29	0.30	1.45
Serotonin, ng/ml	A	117.60	2.98	111.65	123.55
	F	115.35	4.71	105.94	124.76
	N	98.81	4.38	90.06	107.55
Corticosterone	A	3.51	0.49	2.53	4.49
	F	4.64	0.79	3.07	6.21
	N	3.66	0.74	2.18	5.15
Total number of fungi log CFU/g	A	2.93	0.13	2.68	3.18
	F	2.64	0.20	2.24	3.03
	N	2.78	0.19	2.41	3.15
Total number of mesophilic aerobic bacteria log CFU/g	A	7.41	0.07	7.27	7.55
	F	7.31	0.11	7.08	7.53
	N	7.37	0.11	7.16	7.58
LAB log CFU/g	A	7.26	0.10	7.06	7.46
	F	7.16	0.16	6.84	7.48
	N	7.32	0.15	7.02	7.62
Total number of coliform bacteria log CFU/g	A	5.69	0.12	5.44	5.94
	F	5.70	0.20	5.30	6.09
	N	5.54	0.18	5.18	5.91
Total number of *Escherichia coli* log CFU/g	A	5.67	0.13	5.41	5.92
	F	5.64	0.20	5.24	6.05
	N	5.67	0.19	5.30	6.05
Total number of *Clostridium perfringens* log CFU/g	A	5.94	0.12	5.70	6.18
	F	6.16	0.19	5.79	6.54
	N	6,01	0.18	5.65	6.36

laying hen behaviour: A – attack, F – flight, N – neutral; StdErr – standard error, CFU – colony-forming unit, LAB – *Lactobacillus*

In comparison to the attacking (dominant) and neutral hens, the fleeing (subordinate) birds had higher dopamine and lower serotonin levels. Neutral hens had higher noradrenaline levels than pecking individuals (Table 5). Initially neutral birds, which changed their strategy into fleeing after establishing the hierarchy, showed higher dopamine and lower serotonin and noradrenaline levels (Table 6). This was recorded in 25% of hens (Table 2). Almost 40% of hens that represented the fleeing type during the hierarchy setting, but whose social position changed to the dominant type, had lower levels of dopamine and noradrenaline and higher levels of serotonin. High serotonin and low noradrenaline and dopamine levels were determined in the blood of initially fleeing hens that retained this strategy (23%) or became neutral (39%) after the new social structure was established. Initially attacking birds that became subordinate (20%) or neutral (23%) after 14 days showed lower dopamine and norepinephrine levels and higher

Table 5. Estimators of differences in neurotransmitter levels relative to social status

Neurotransmitters	Behaviour		Estimate	StdErr	Probt	Lower	Upper
Dopamine	A	F	−5.76	2.00	0.005	−9.75	−1.76
	N	F	−7.43	2.44	0.003	−12.29	−2.57
Serotonin	A	F	18.80	5.30	0.001	8.22	29.38
	N	F	16.54	6.44	0.012	3.69	29.39
Noradrenaline	A	N	−1.05	0.36	0.005	−1.77	−0.32

laying hen behaviour: A – attack, F – flight; N – neutral, StdErr – standard error, Probt – probability

Table 6. Estimators of differences in selected indicators relative to social status of birds; standard error, significance of differences, and confidence interval

Parameter	Behaviour Day 1–14				Estimate	StdErr	Probt	Lower	Upper
Dopamine	A	A	N	F	−17.90	3.93	0.000	−25.74	−10.07
	A	N	N	F	−19.69	4.29	0.000	−28.26	−11.12
	A	F	N	F	−19.43	4.38	0.000	−28.16	−10.70
	N	A	N	F	−16.51	4.38	0.000	−25.25	−7.78
	N	N	N	F	−20.15	5.79	0.001	−31.71	−8.60
	N	F	F	A	20.11	4.09	0.000	11.95	28.28
	N	F	F	N	19.70	4.09	0.000	11.53	27.87
	N	F	F	F	17.83	4.38	0.000	9.10	26.56
Noradrenaline	A	A	N	N	−2.61	0.81	0.002	−4.23	−0.99
	A	N	N	N	−2.60	0.86	0.004	−4.33	−0.88
	A	F	N	N	−2.64	0.88	0.004	−4.39	−0.89
	N	A	N	N	−2.49	0.88	0.006	−4.24	−0.75
	N	N	N	F	2.32	1.00	0.023	0.33	4.31
	N	N	F	A	2.59	0.84	0.003	0.93	4.26
	N	N	F	N	1.96	0.84	0.022	0.29	3.62
	N	N	F	F	1.91	0.88	0.033	0.16	3.66
Serotonin	A	A	N	F	49.29	10.38	0.000	28.57	70.02
	A	N	N	F	50.62	11.31	0.000	28.04	73.21
	A	F	N	F	52.71	11.53	0.000	29.68	75.73
	N	A	N	F	52.79	11.53	0.000	29.77	75.81
	N	N	N	F	53.35	15.25	0.001	22.90	83.81
	N	F	F	A	−51.41	10.88	0.000	−73.14	−29.68
	N	F	F	N	−42.75	10.88	0.000	−64.48	−21.02
	N	F	F	F	−44.40	11.53	0.000	−67.42	−21.37
Total number of *Escherichia coli*	A	A	N	A	−0.61	0.32	0.058	−1.25	0.02
Total number of *Clostridium perfringens*	A	A	F	A	0.50	0.25	0.050	0.00	1.01
	A	N	F	A	0.79	0.31	0.014	0.16	1.41
	N	A	F	A	0.88	0.33	0.009	0.23	1.53
	F	A	F	N	−0.66	0.29	0.024	−1.23	−0.09

StdErr – standard error, Probt – probability

serotonin concentrations (Tables 2, 6). Analysis of the intestinal microflora composition revealed significantly higher *Escherichia coli* counts in hens that were assessed as neutral on day 1 but attacked other hens on day 14. The abundance of these bacteria was lower in hens classified as dominant on day 1 and day 14. Lower levels of Gram-positive bacteria of the genus *Clostridium* were determined in the initially fleeing hens which became dominant during hierarchy establishment. In contrast,a significantly higher level of *Clostridium* bacteria was determined in fleeing birds which remained neutral after establishing the social structure in the flock (Table 6).

Discussion

Many studies have shown that social hierarchy is an important factor in the adaptation of an individual in a group (Bhanja and Bhadauria, 2018; Wang et al., 2022). Differences in behaviour are a form of communication that helps maintain balance and stability in the group (Queiroz and Cromberg, 2006). Our research has shown that flock structure is maintained through threatening or attacking behaviour, which is replaced by demonstrations of dominance and submission once structure is established. Therefore, hens were classified as dominant (pecking had a higher frequency than flight), submissive (fleeing had a higher frequency than pecking) and neutral (no pecking or flight) based on their behaviour.

Endocrine balance, reflected in the level of neurotransmitters, is the key factor necessary to ensure homeostasis (Karpiński et al., 2021). Differences were shown in the concentration of dopamine (DA) and dopaminergic receptors in dominant and subordinate hens (Cheng et al., 2002), which was confirmed by the present results. In comparison to the attacking (dominant) and neutral birds, the fleeing (subordinate) hens had higher levels of dopamine. As indicated by Favati et al. (2013) and Cheng and Fahley (2009), changes in dopamine and corticosteroid concentrations could stimulate harmful behaviours or result in the inability to cope with environmental stress. Therefore, it could be assumed that the dominant hens experienced higher levels of stress, which was probably due to the need to demonstrate their dominance. Analysis of DA levels and behaviour showed that the fleeing birds had a higher concentration of dopamine, which may have suggested that their behaviour was more impulsive than that of the other hens. The subordinate (fleeing) birds also exhibited aggressive behaviour in certain situations that depended on the confronted hen. Since these birds tried not only to flee but also to fight, their reactivity may have been high, as evidenced by higher blood dopamine levels compared to other hens. Since dopamine increases motivation and concentration, it could be assumed that its higher level in the fleeing birds was responsible for the high determination of birds to change their position. Therefore, it seemed that the fleeing birds showed the greatest need to change their position in the group.

It is widely believed that serotonin (5-HT) is the major neurotransmitter responsible for aggression. Reduced concentrations of 5-HT are associated with aggression; thus, it is considered a modulator of this type of behaviour (de Boer et al., 2015). In the central nervous system, serotonin is also involved in modulating stress responses, including social and environmental adaptation (Cheng and Fahey, 2009). The present analysis of serotonin levels in hens revealed that dominant birds had higher serotonin levels than subordinate hens. This could suggest that the emotion of anger/rage was not a motivator for such behaviour as pecking the other individual. Higher levels of this neurotransmitter in dominant birds could suggest that they experienced a lower level of stress because of their position in the group. Compared to the other hens, the fleeing birds had lower levels of serotonin, which is specifically involved in psychological comfort levels (increased fearfulness). Simultaneously, lower serotonin levels may contribute to increased dopamine concentration. The comparison of dopamine and serotonin levels with the strategy adopted by the birds yielded very interesting results. Higher dopamine and lower serotonin levels could indicate depression in birds. This could be related to their lower (subordinate) position after establishing the hierarchy compared to their initial (neutral) position. These birds lived in a state of constant alertness and showed symptoms of emotional arousal. In turn, lower dopamine and higher serotonin levels suggested a lower degree of arousal, accompanied by an elevated mood in laying hens. This was observed in initially fleeing birds that later became dominant.

Noradrenaline and adrenaline play an important role in maintaining behavioural and physiological balance (Cheng and Fahey, 2009; Dennis, 2016). Higher noradrenaline levels in neutral birds compared to pecking birds could mean that a lack of response did not indicate a lack of stress. Birds that responded with flight or pecking were characterised by lower concentration of noradrenaline, which could indicate lower levels of stress due to the opportunity to release emotional tension. Neutral birds could in fact experience greater discomfort, and the inability to peck or flee could result from a motivational conflict when the bird was unable to make a decision. Differences in behavioural patterns between dominant and subordinate individuals result not only from the different functioning of the neuroendocrine system, but also genetic factors, environmental conditions during the development, and previous experiences of birds (Cheng et al., 2002; Fahey and Cheng, 2008; Rozempolska-Rucińska, 2020).

There is ample evidence of the significant impact of the gut microbiota composition on physical and mental health (Villageliu and Lyte, 2017; Bryden et al., 2021). The barrier of the gastrointestinal system limits the negative effects of stressors, among others, by maintaining the correct pH or bacteriocin production (Truszczyński and Pejsak, 2012). In the present study, there were differences in the abundance of bacteria of the genus *Clostridium* and *E. coli.* Truszczyński and Pejsak (2012) reported that a prolonged stress response decreased the cellular response in birds, which could result in infections due to increased sensitivity to certain microorganisms, especially conditionally pathogenic organisms such as *E. coli*. Our results showed that birds that ranked higher after structure establishment had lower counts of *E. coli* and Gram-positive bacteria of the genus *Clostridium*. This suggested that due to their high rank, the birds did not experience stress, thus it was likely that their digestive tract barrier was not compromised, and the birds were not exposed to infections. The situation was different for birds that occupied a neutral position after the hierarchy was established, as they showed higher abundance of *Clostridium* bacteria. It can be concluded that birds whose position is uncertain experience more stress, which in turn has an adverse effect on their microflora. Such individuals become susceptible to certain microorganisms and are more likely to develop health problems and reduced productivity.

Summarizing the research results, it could be concluded that birds of different social position differed in the level of catecholamine neurotransmitters and microflora composition. The relationships between these indicators were associated with animal behaviour. Importantly, environmental and social effects also played a significant role in the ultimate type of behaviour. It is difficult to clearly define the role of these substances at the moment, and therefore research must be continued.

Conclusions

The results indicated that the lack of an established position in the group structure was a social stressor for laying hens. Hens with an undetermined position in the group, defined as neutral and subordinate hens, could have a strong motivation and need to re-establish their position, which was crucial for their emotional well-being. Subordinate birds appeared to be very impulsive as evidenced by dopamine levels. Thus, they seemed to show the greatest need to change position in the group. At this stage of the research, it is not yet possible to conclusively answer whether higher level of stress occur in dominant or subordinate birds, but the results have suggested that higher stress levels are associated with lower group position.

Conflict of interest

The Authors declare that there is no conflict of interest.

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