



SEROTONIN AND DOPAMINE LEVELS IN PLASMA AND TISSUES OF PHEASANTS IN COMPARISON TO CHICKENS

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Summary

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The growing global demand for meat products requires optimisation of poultry farming practices to ensure efficient production and animal welfare. Neurotransmitters such as serotonin and dopamine are key indicators of physiological and environmental stress in birds. Although the levels of both neurotransmitters in broilers are reported in scientific literature, such information for pheasants (*Phasianus colchicus*) is scarce. Therefore, the current study aimed to investigate their concentrations in plasma, brain tissue, and jejunum of healthy pheasants (n=6) and broilers (n=6) using LC-MS/MS analysis. The effect of using heparinised versus non-heparinised (conventional method) needles on the levels of the investigated neurotransmitters in thrombocyte-rich plasma was evaluated. Higher plasma concentrations of serotonin (497.83 ng/mL and 103.5 ng/mL) and dopamine (1.30 ng/mL and 0.51 ng/mL) were obtained by the conventional method in broilers compared to pheasants ($P < 0.05$). In broilers, the plasma levels of both neurotransmitters were higher when blood was collected by the conventional method vs using a heparinised needle: 497.83 ng/mL and 39.32 ng/mL for serotonin, and 1.30 ng/mL and 0.553 ng/mL for dopamine, respectively. A similar difference was observed for plasma serotonin in pheasants: 103.5 ng/mL and 29.51 ng/mL. Significantly higher serotonin concentrations were observed in the cerebrum (45.61 ng/mL) and the jejunum (785.91 ng/mL) of broilers than in respective tissues of pheasants – 8.18 ng/mL and 461.36 ng/mL, respectively. Dopamine levels were significantly higher in the jejunum of broilers (131.73 ng/mL) compared to those of pheasants (2.88 ng/mL). These findings highlight the importance of the blood sampling method for obtaining thrombocyte-rich plasma and reveal species-specific differences in serotonin and dopamine levels between the two Galliformes species.

Key words: chickens, dopamine, pheasants, plasma and tissue levels, serotonin

INTRODUCTION

The health of broilers and closely related bird species as pheasants, reared in aviaries is highly influenced by factors such as

the dietary nutrients and stress (Kostál *et al.*, 1999; Calefi *et al.*, 2016; Nanto-Hara *et al.*, 2020; Cifuentes & Acosta, 2022;

Huang *et al.*, 2023). Based on the information available for broilers, these factors significantly affect gastrointestinal integrity and gut microbiota, which in turn impact neurotransmitter levels (Yan *et al.*, 2018; Johnson *et al.*, 2024). Recent literature highlights the complex relationship between the microbiome and the levels of serotonin and dopamine, not only within the gastrointestinal tract but also in relation to brain function, underscoring the role of the microbiota-gut-brain (MGB) axis (Johnson *et al.*, 2024).

Serotonin (5-hydroxytryptamine; 5-HT) is one of the most extensively studied neurotransmitters in mammals, whereas in avian species information remains limited, and further research is needed to determine its levels in different tissues and its systemic effects on the macroorganism (Olivier *et al.*, 2015; Lyte *et al.*, 2022). In chickens, serotonin is implicated as a modulator of the central nervous system, playing a role in cognitive and autonomic control, motor activity, sensory processing, and regulation of sexual and foraging behaviour (Matsunami *et al.*, 2012). It is also involved in the manifestation of various conditions, such as stress, anxiety, depression, and aggression (Olivier *et al.*, 2015). Significant concentrations of serotonin have been detected in plasma, brain structures, and the intestinal tract (Lyte *et al.*, 2022). Nearly 5% of the serotonin available in the body is found in the central nervous system (CNS), while the remaining 95% are distributed in the gastrointestinal tract, approximately 90% in the enterochromaffin cells that synthesise it, and the other 10% in enteric neurons (Kim & Camilleri, 2000; Banskota *et al.*, 2019). Production of serotonin and its function are influenced by a variety of factors, such as gene transcription modulators, neurotrophic peptides, steroids, and psycho-

tropic drugs (Lesch *et al.*, 2012). Investigations in chickens during post-hatching development show that serotonin levels depend on feed composition, negatively correlate with stress, and that disturbances in its turnover are a prerequisite for feather pecking, a pathological behaviour encountered in industrial poultry farming, often triggering cannibalism (Bacqué-Cazenave *et al.*, 2020; Fujita *et al.*, 2023; Huang *et al.*, 2023). While knowledge of the levels and role of serotonin in chickens and laying hens is increasing, little is known about its concentrations in closely related avian species, such as pheasants.

In addition, the interaction between the serotonergic and dopaminergic systems in avian species is also of interest (Matsunami *et al.*, 2012; Fujita *et al.*, 2023). Huang *et al.* (2019) demonstrated a relationship between the serotonergic and dopaminergic systems throughout embryonic development in chicken embryos, observing a concurrent rise in the concentrations of both neurotransmitters between E16 and E19. Correlations in monoamine levels during embryogenesis and the post-hatching period provide evidence that the serotonergic system plays a key role in the formation of the serotonin-dopamine neural circuitry (Huang *et al.*, 2019; Fujita *et al.*, 2022). Dopamine has been detected in the avian brain and is primarily produced in the ventral tegmental area, substantia nigra, and the hypothalamus (Reiner *et al.*, 2004). Its presence has been reported in the cerebellum, midbrain, pons, hypothalamus, thalamus, neostriatum, paleostriatum, olfactory lobes, hyperstriatum, archistriatum, and ectostriatum of pheasants (*Phasianus colchicus*) (McIntyre & Chew, 1983). The role of dopamine in the modulation of behaviour, appetite regulation, gut motility, and voluntary movement in mammals and poultry has been described

in several studies (Dicks, 2022; Zarei *et al.*, 2025). Similarly to serotonin, dopamine levels depend on diet, the intestinal microbiota and have been linked to the frequency of feather pecking in bird species (Van Hierden *et al.*, 2002; de Haas & van der Eijk, 2018; Johnson *et al.*, 2024). The literature mainly reports investigations in chickens as farm animals of economic importance, while information about minor species, such as pheasants, is scarce (Omaliko *et al.*, 2024). While behavioural disorders in chickens have been partly explained by the microbiome-gut-brain axis and its impact on dopamine levels, little is known about dopamine levels in healthy pheasants, making it difficult to understand their role in health and disease in this avian species.

The aim of the current study was to evaluate serotonin and dopamine levels in thrombocyte-rich plasma from pheasants and broiler chickens and to compare the concentrations of these neurotransmitters across these avian species. Additionally, plasma levels were quantified using two blood sampling methods to assess the impact of thrombocyte activation, with and without a heparinised needle. The presence of both compounds in the jejunum and cerebrum was determined in pheasants and compared with that in chickens in order to characterise potential differences between the two bird species reared under similar conditions. It was hypothesised that broilers and pheasants would exhibit species-specific differences in plasma, cerebrum, and jejunum serotonin and dopamine concentrations. Also it was expected that plasma monoamine levels would be influenced by the blood collection method, with non-heparinised sampling resulting in higher detectable serotonin and dopamine due to increased thrombocyte activation.

MATERIALS AND METHODS

Reagents and chemicals

The reagents used in the current experiment were HPLC or LC-MS/MS grade. The following chemicals were used for LC-MS/MS analysis: formic acid (purity ~98%, LC-MS/MS grade, Honeywell, Seelze, Germany), methanol (CHROMASOLV®, LC-MS/MS grade, ≥99.9%, Honeywell, Seelze, Germany), water for chromatography (LC-MS grade, LiChrosolv®, Merck KGaA, Darmstadt, Germany), and acetonitrile (CHROMASOLV®, LC-MS purity ≥99.9%, Honeywell, Seelze, Germany). Serotonin hydrochloride (5-hydroxytryptamine, purity ≥98%, Sigma-Aldrich, Saint Louis, MO, USA) and dopamine hydrochloride (TLC, purity ≥98%, Sigma-Aldrich, Saint Louis, MO, USA) were used as reference materials for standard curves.

Experimental design

The experiment was conducted after obtaining approval from the Ethical Commission of the Bulgarian Food Safety Agency (No 386/18.04.2024).

The study involved six-day-old Ross hybrid broiler chickens (Cornish ♀ × Plymouth Rock ♂), with an average body weight of 0.043 ± 0.0013 kg originating from the Milenium 2000 Ltd. hatchery (Yambol, Bulgaria). The group comprised an equal number of males and females. The chickens were fed a commercial diet shown in Table 1 (Vladini Trading Ltd, Chirpan, Bulgaria). The feeds used in the experiment were free of additives, including antibiotics and coccidiostats (Table 1). Feed and water were provided *ad libitum*. Until the experimental phase, chickens were housed in cage batteries measuring 0.8 m² and 60 cm in height (Biobase of the Faculty of Veterinary Medicine, Tra-

Table 1. Composition of the feed for broiler chickens and pheasants

Nutrients	Unit	Starter		Grower	
		Broilers	Pheasants	Broilers	Pheasants
Crude protein	%	20.6	26.0	19.5	22.0
Total fat	%	3.7	4.36	4.7	3.10
Crude fiber	%	3.8	4.4	4.6	6.0
Crude ash	%	5.4	7.44	5.1	6.97
Available phosphorus	%	0.56	0.75	0.55	0.83
Sodium	%	0.22	0.15	0.18	0.166
Calcium	%	0.76	1.15	0.73	1.20
Metabolisable energy	kcal/kg	2959	2839	2981	2749
Lysine	%	1.27	1.6	1.11	1.3
Methionine	%	0.58	0.7	0.53	0.4986
Iron	mg/kg	164.00	146.44	147.00	–
Copper	mg/kg	20.00	16.485	19.00	24.653
Zinc	mg/kg	133.00	74.8	116.00	103.71
Manganese	mg/kg	154.00	96.77	130.00	118.17
Iodine	mg/kg	1.66	0.88	0.42	1.16
Selenium	mg/kg	0.46	0.29	1.34	0.37
<i>Technological additives</i>					
Butyl-hydroxyanisole (1b320)	mg/kg	10.49	–	10.39	–
Butyl-hydroxytoluene (1b321)	mg/kg	16.40	–	16.15	–
Propylgallate E310	mg/kg	0.15	–	0.14	–
Bentonite (1m558i)	mg/kg	–	300.00	–	300.00
<i>Nutritional supplements</i>					
Iron (ferrous (II) sulfate monohydrate) 3b103	mg/kg	50.00	50.00	40.00	–
Copper (copper (II) sulfate pentahydrate) 3b405	mg/kg	12.00	7.00	10.00	13.50
Zinc (zinc oxide)3b603	mg/kg	100.00	50.00	80.00	80.00
Manganese (manganese (II) oxide) 3b502	mg/kg	125.00	80.00	100.00	100.00
Iodine (calcium iodate) 3b203	mg/kg	1.56	0.8	1.25	1.00
Selenium (sodium selenite) 3b802	mg/kg	0.31	0.25	0.25	0.30
Vitamin A (retinyl acetate) 3a672a	IU/kg	12450.0	10000.0	10000.0	10000.0
Vitamin D3 (cholecalciferol) 3a671	IU/kg	4980.0	3200.0	4000.0	3000.0
Choline chloride 3a890	mg/kg	495.00	1656.22	402.00	1640.09
<i>Zootechnical additives</i>					
6-phytase EU 3.1.3.26 (4a18)	FYT/kg	1523.0	500.0	1500.0	600.0
1,4 beta xylanase (EU 3.2.1.8)	FXU/kg	150.0	150.0	150.0	150.0
<i>Coccidiostats and histomonostats</i>					
Avatec 150G (lasalocid A sodium) E763	mg/kg	–	75.00	–	75.00

kia University, Stara Zagora, Bulgaria). The total area provided met the requirements outlined in Table 7.1 of Regulation 20/01.11.2012, offering 0.133 m² per bird, with a minimum of 0.045 m², as well as the required length of the feeder. The planned enclosure area exceeded the minimum required area of 0.75 m². According to Regulation 26/05.08.2008, which establishes the minimal standards for the humane treatment and protection of broilers, the bird weight per square meter at the end of the experiment (maximum possible during the experiment: 7.2 kg/m²) did not exceed the regulated limit of 33 kg/m².

The broiler chickens were 42 days old and had an average body weight of 2.288 ± 0.09 kg. Blood samples (4 mL per chicken) were obtained from *v. subcutanea ulnaris* in vacutainers (2.5 mL lithium heparin, FL Medical, Italy): 2 mL were obtained with a heparinised needle and 2 mL with a non-heparinised needle immediately prior to euthanasia using T61 (MSD Animal Health, Rahway, NJ, USA). The heparinised blood was then centrifuged to separate the plasma (2000 × g for 10 minutes at 22 °C). Samples were stored at –80 °C until analysis.

Tissue samples from the jejunum and cerebrum were obtained immediately after euthanasia and snap frozen. They were stored under the same conditions as plasma samples.

The pheasants included in the study (*Phasianus colchicus*; n=6) at 45 days of age, with an average body weight of 0.387 ± 0.054 kg, were reared on the premises of the Philip Totyu Hunting and Fishing Association (Ruse, Bulgaria), in accordance with Regulation No. 2 of February 11, 2009, concerning the conditions for rearing furred and feathered game in livestock facilities, with consideration for the species' physiological and behavioural

characteristics. Birds were fed *ad libitum* with feeds supplemented with lasalocid as a coccidiostatic drug (Table 1) and had free access to water. Plasma, cerebrum, and jejunum samples were obtained from healthy pheasants from the same aviary, which had previously experienced an outbreak of ataxia in this species. Birds were delivered to the Unit of Pathology, Faculty of Veterinary Medicine, for pathological investigation to confirm their health status and to exclude pathological alterations previously observed in the aviary. Samples were obtained and stored as described for broiler chickens.

Formalin-induced fluorescence imaging for enterochromaffin cells detection in the jejunum

The jejunum was selected for analysis because it is an important site of enterochromaffin cell activity, closely associated with serotonin and dopamine activity in the gastrointestinal tract. Detection of enterochromaffin cells in the jejunum using autofluorescence has been previously described by Kaneko *et al.* (2016). Tissue samples were fixed in 10% neutral buffered formalin and embedded in paraffin blocks. Blocks were cut to 5 µm sections, deparaffinised, and hydrated. Serotonin presence was evaluated using an OPTIKA B-500TiFL trinocular fluorescence microscope equipped with standard objectives and an LED fluorescence illumination system (OPTIKA Microscopes, Italy). Images were captured with an OPTICAM PRO 5 digital camera mounted via C-mount and processed with OPTICAM VISION PRO software (OPTIKA Microscopes, Italy). Images were obtained with the blue filter.

LC-MS/MS analysis of serotonin and dopamine

Both compounds were extracted from plasma and tissues according to previously described procedures (Moriarty *et al.*, 2011; Thomas *et al.*, 2015). Shortly, to precipitate proteins in blood plasma, 100 μ L of trifluoroacetic acid (TFA) were added to 500 μ L plasma. The mixture was vortexed for 1 minute and centrifuged at $4000 \times g$ for 10 minutes at 4°C to obtain a clear supernatant. The supernatant was then filtered through 0.2 μm filters (Agilent Captiva Econo Filter PTFE 13 mm, Santa Clara, CA, USA). The filtrate (5 μL) was transferred to the analytical system for the determination of serotonin and dopamine.

Tissue samples (250 mg) were homogenised for 60 seconds (BioSpec Tissue-Tearor, Atkinson, NH, USA) in 250 μL methanol and vortexed for 2 minutes. Then, 250 μL of acetonitrile was added, and samples were vortexed for 2 minutes. After centrifugation at $18620 \times g$ for 20 minutes, the supernatant was filtered through 0.22 μm syringe filters. An amount of 5 μL of the clear supernatant was injected into the LC-MS/MS system.

LC-MS/MS analysis was performed according to previously described parameters for serotonin and dopamine determination in biological matrices (Moriarty *et al.*, 2011; Thomas *et al.*, 2015; Juck & Long, 2017). Separation was achieved using a Zorbax RRHD Eclipse Plus C18 Column (2.1×50 mm, 1.8 μm , Agilent, Santa Clara, CA, USA) with a 1260 Infinity II quaternary pump and a 1260 Infinity II Vial Sampler coupled to a QQQ LC-MS/MS Agilent 6460 equipped with Agilent Jet Stream technology. The mobile phase used for gradient elution consisted of mobile phase A: 0.1% formic

acid in water, and mobile phase B: 0.1% formic acid in methanol. The flow rate was set to 0.2 mL/min. The gradient was achieved by the following changes in the mobile phases: 0.0–3.00 min (80:20 %, A:B); 3.0–3.01 min (20:80 %, A:B); 3.01–5.50 min (50:50 %, A:B); 5.50–7.0 min (20:80 %, A:B); 7.0–8.0 min (20:80; A:B); 8.00–8.50 min (90:10; A:B) with 8 min post-run (90:10, A:B). The parameters for LC-MS/MS analysis were: 3500 V capillary voltage in positive mode, 10 L/min drying gas (nitrogen) with temperature of 350°C , 45 psi nebulizer gas (nitrogen), sheath gas temperature of 350°C and flow 11 L/min, 400 V nozzle voltage, and 200 V delta EMV. Dopamine and serotonin concentrations were quantified with Mass Hunter software (Agilent Technologies, Santa Clara, CA, USA). Literature data for m/z ratios of the precursor and product ions were used (Moriarty *et al.*, 2011; Thomas *et al.*, 2015; Juck & Long, 2017). For serotonin, the m/z values were 177 (precursor) and 160, 132, and 115 (product ions), respectively; and for dopamine they were 154 (precursor) and 137, 119, and 91 (product ions), respectively.

Serotonin and dopamine were dissolved in water for LC-MS/MS chromatography. The concentrations of stock solutions of both compounds were 1 mg/mL and were prepared on the day of analysis. The working standard solutions were freshly prepared on the day of analysis. Standard curves for serotonin were linear over the concentration range ($R^2 > 0.99703$): 0.25, 0.5, 0.75, 1, 5, 10, 25, and 50 ng/mL; and respectively for dopamine ($R^2 > 0.99965$): 0.1, 0.25, 0.5, 0.75, 1, 5, 10, 25, and 50 ng/mL. The retention time for dopamine was 0.9709 min and for serotonin: 1.7431 min. The limit of detection (LOD) was calculated using the

standard deviation (SD) of the y-intercepts of the regression lines and the slope (S) of the calibration curve, according to the equation: $LOD = 3.3 \times (SD/S)$. Similarly, the limit of quantification (LOQ) was determined using the same parameters with the formula: $LOQ = 10 \times (SD/S)$ (Shabir *et al.*, 2003). For dopamine, the values of LOD were 0.10 ng/mL, and LOQ was 0.30 ng/mL. For serotonin, the LOD and LOQ values were 0.33 ng/mL and 0.99 ng/mL, respectively. The mean accuracy for serotonin was 92.76%, and for dopamine it was 98.52%. The inter-day and intra-day variability for serotonin and dopamine were below 9.69% and 7.51%, respectively.

Statistical analysis

Results are presented as geometric mean, minimum and maximum. Statistical analysis was performed using Statistica for Windows 10.0 (StatSoft LTD, USA). Parametric (*t*-test) or non-parametric (Mann-Whitney *U* test) methods were applied after the Shapiro-Wilk *W* test for normal distribution. Differences were considered statistically significant at $P < 0.05$.

RESULTS

Serotonin

The concentrations of serotonin and dopamine in the plasma of broiler chickens and pheasants are shown in Table 2. Plas-

Table 2. Serotonin and dopamine concentration (ng/mL) in plasma of 42-day-old broiler chickens (n=6) and in plasma of 45-day-old pheasants reared under aviary conditions (n=6)

Animal	Serotonin		Dopamine	
	Broiler chickens	Pheasants	Broiler chickens	Pheasants
<i>Plasma obtained after centrifugation at 400×g</i>				
1	167.95	159.84	0.93	0.79
2	1870.02	177.72	1.78	0.50
3	785.47	81.68	1.54	0.42
4	95.37	50.33	0.92	0.39
5	715.64	61.15	1.70	0.55
6	904.06	172.14	1.24	0.48
Geometric mean	497.83	103.5 *	1.30	0.51 *
(min-max)	(95.37–1870.02)	(50.33–177.72)	(0.92–1.78)	(0.39–0.79)
<i>Plasma obtained after blood sampling with a heparinised needle and centrifugation at 400×g</i>				
1	107.65	30.66	1.05	0.64
2	26.06	27.99	0.33	0.51
3	53.44	22.06	0.57	0.49
4	24.75	38.66	0.54	0.62
5	22.97	36.10	0.48	0.59
6	43.36	24.99	0.56	0.76
Geometric mean	39.32 ^	29.51 ^	0.553 ^	0.60
(min-max)	(22.97–107.65)	(22.06–38.66)	(0.33–1.05)	(0.49–0.76)

* Statistically significant differences between broilers and pheasants at $P < 0.05$; ^ Statistically significant differences between the of blood sampling methods at $P < 0.05$.

ma serotonin levels were almost fivefold higher in broiler chickens than in pheasants when using the non-heparinised needle method for blood sampling ($P<0.05$). Plasma obtained with a heparinised needle showed no significant differences in serotonin levels between broilers and pheasants (Table 2). Plasma serotonin levels in both avian species were significantly lower when blood samples were collected using a heparinised needle. The reduction was more pronounced in broilers than in pheasants, with approximately 10-fold and 3-fold decreases, respectively.

Serotonin levels in the cerebrum and jejunum of broilers were significantly higher than those of pheasants (Table 3).

The histological examination of the jejunum confirmed the presence of seroto-

nin in the enterochromaffin cells of both broilers and pheasants, localised at the periphery of the villi (Fig. 1).

Dopamine

Dopamine levels in thrombocyte-rich plasma were significantly higher in broilers compared to pheasants if a non-heparinised needle was used (Table 2). Twofold lower values of dopamine were observed in chicken plasma obtained with a heparinised needle ($P<0.05$). The amounts of dopamine in the plasma of pheasants were similar regardless of the blood sampling method used.

The values of dopamine concentrations in the cerebrum were almost equal in the investigated avian species (Table 3). The levels of this neurotransmitter were

Table 3. Serotonin and dopamine concentration (ng/mg) in cerebrum and jejunum of 42-day-old broiler chickens (n=6) and 45-day-old pheasants reared under aviary conditions (n=6)

Animal	Serotonin		Dopamine	
	Broiler chickens	Pheasants	Broiler chickens	Pheasants
<i>Cerebrum</i>				
1	37.33	7.98	0.88	1.75
2	42.90	7.97	1.47	1.84
3	48.76	7.44	1.89	1.77
4	47.41	7.44	2.81	2.07
5	43.59	9.32	3.09	2.36
6	55.75	9.09	2.72	2.29
Geometric mean (min-max)	45.61 (37.33-55.75)	8.18 * (7.44-9.32)	1.97 (0.88-3.09)	2.0 (1.75-2.36)
<i>Jejunum</i>				
1	889.60	684.09	118.82	4.20
2	878.31	740.59	134.64	2.37
3	672.98	149.58	138.10	2.53
4	868.57	374.26	150.39	3.15
5	807.47	553.50	120.48	2.91
6	712.46	614.35	130.55	2.49
Geometric mean (min-max)	785.91 (672.98-889.60)	461.36 * (149.58-740.59)	131.73 (118.82-150.39)	2.88 * (2.37-4.20)

* Statistically significant differences between broilers and pheasants at $P<0.05$.

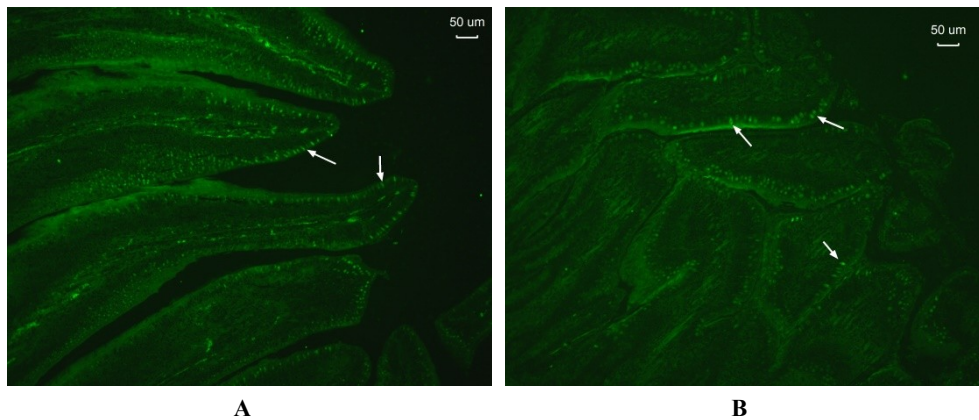


Fig. 1. Enterochromaffin cells (white arrows) containing serotonin (green autofluorescence) in the jejunum of 42-day-old chickens (A) and 45-day-old pheasants (B). Magnification $\times 100$.

significantly lower, almost 40-fold, in the jejunum of pheasants in comparison to broilers.

DISCUSSION

Scientific literature increasingly highlights the role of the neurotransmitters serotonin and dopamine in regulating nervous system function, particularly in relation to gastrointestinal health and maintaining a balanced microbiome (Montiel-Castro *et al.*, 2013; Jadhav *et al.*, 2022; Johnson *et al.*, 2024). Although many studies have been focused on chickens, knowledge regarding pheasants remains limited. This study aimed to address this gap, revealing some interspecies differences.

Our results for serotonin levels in the plasma of broilers sampled with a heparinised needle are similar to the concentrations in the blood obtained in EDTA-coated tubes via cardiac puncture of 43-day-old healthy broilers: 39.32 ng/mL and 46.00 ng/mL, respectively (Yan *et al.*, 2018). Higher plasma levels of this neurotransmitter, ranging from 98.81 ng/mL to 117.60 ng/mL, were found in 53-week-old

Green-legged Partridge hens with neutral and aggressive behaviour, respectively (Rozempolska-Rucińska *et al.*, 2023). In another experiment, the same group found much lower serotonin levels in 58-week-old laying hens: 8.14 ng/mL in Leghorn breed and 9.6 ng/mL in Green-legged Partridge breed (Kasperek *et al.*, 2023). Another study reported increasing plasma concentrations of serotonin with age in W36 Hy-Line laying hens: 142.36 ng/mL in 50-week-old birds and 172.26 ng/mL in 60-week-old birds (Johnson *et al.*, 2024). High variations of serotonin levels from 50 ng/mL to 2000 ng/mL in plasma were reported in 28-day-old and 56-day-old White Leghorn chickens from both genders that have been selected for high or low blood antibody response to intravenous sheep red blood cell injection (Lyte *et al.*, 2025). Altogether, the cited data reveal high variability in plasma serotonin concentrations depending on breed, age, feed composition, rearing conditions, and methods of blood sampling and analysis, which makes direct comparison difficult. The results obtained for the pheasants cannot be compared with published data

because of the lack of information in the available literature. Although the birds included in this study were from the same order, Galliformes, and were of similar age and rearing conditions, serotonin levels were lower in the thrombocyte-rich plasma of pheasants. In contrast, this difference between the two avian species was not observed when plasma was obtained with a heparinised needle, highlighting the importance of the blood sampling method used. It is well known that serotonin is stored in platelets and contributes to blood clotting by promoting platelet aggregation and inducing vasoconstriction upon vascular injury (Kim & Camilleri, 2000). The release of serotonin into plasma is directly linked to platelet activation, so differences in platelet activation during blood collection can affect measured serotonin levels. In this study, we compared two blood collection methods to assess their potential impact on plasma serotonin concentrations. Blood collected with non-heparinised needles showed significantly higher serotonin levels compared to blood collected with heparinised needles, suggesting that non-heparinised collection preserves thrombocyte integrity and allows greater serotonin release, whereas heparin may interfere with thrombocyte activation and aggregation. Different methods of plasma collection were used in the published studies, often without a precise description of the procedure, which may explain the discrepancies in the reported results. These data point to the importance of the blood sampling method when evaluating serotonin concentrations in plasma.

High variability in serotonin concentrations has been reported in brain tissues, mainly depending on the specific brain structures investigated. Its levels in raphe nuclei were 452.60 ng/g and nearly three-fold higher in the hypothalamus of broil-

ers – 1055 ng/g (Yan *et al.*, 2018). Considerably higher concentrations of serotonin were detected in the brain tissue (specific structure not described) of two breeds of laying hens exhibiting low (1705 ± 51 ng/mL) and high (1911 ± 59 ng/mL) feather pecking at 28 days of age (Van Hierden *et al.*, 2002). Lower levels were observed in the current study: 45.61 ng/mL in broilers and 8.18 ng/mL in pheasants. Kops *et al.* (2013) measured 16.36 ng/g serotonin in the dorsal thalamus, 28.12 ng/g in the medial striatum, and 31.23 ng/g in the hippocampus in healthy 33-week-old White Leghorn hens. In pigeons, serotonin concentrations in brain tissue vary widely, ranging from 25 ng/mL to 159 ng/mL depending on the racing breed, suggesting that genetic background and selective breeding may influence serotonergic regulation. Recent studies have shown that the development and proliferation of pathogenic microorganisms causing intestinal infection, as well as exposure to heat stress, affect the serotonergic system of the midbrain, rostral pallium, and hypothalamus, leading to elevated serotonin levels (Calefi *et al.*, 2016; Calefi *et al.*, 2019; Beldowska *et al.*, 2023). Such elevations in serotonin have been associated with depressive-like states, reduced feed and water intake, decreased locomotor activity, and increased expression of sleep-related behaviours. Meanwhile, aggressive behaviour is regulated by serotonin, with higher brain serotonin levels being associated with reduced aggression (Dennis & Cheng, 2011). The importance of the observed interspecies differences between the investigated Galliformes in the levels of this neurotransmitter in the brain is not well understood, and remains to be elucidated.

Serotonin was detected in the jejunum of the investigated avian species at higher

mean concentrations compared with plasma and brain tissues, which is consistent with recent literature describing the role of this neurotransmitter in gastrointestinal tract function (Lyte *et al.*, 2022). In the jejunum, serotonin is stored in enterochromaffin cells and released into the intestinal lumen. The localisation of serotonin in the enterochromaffin cells, demonstrated in Fig. 1A and 1B, supports these observations. Under stressful conditions, its synthesis and release can be increased (Lyte *et al.*, 2021). Similar to the results for serotonin levels in plasma and brain tissues, large differences in its intestinal concentrations were reported. The levels of serotonin in the jejunum in 2-, 4- and 6-week-old chickens indicated a linear increase in concentration with age: 6969 ng/g, 9928 ng/g, and 12543 ng/g, respectively (Lyte *et al.*, 2022). Lower concentrations were observed in the jejunum in the current study, significantly lower in pheasants compared to broilers. The corrected concentrations of the protein in the jejunal tissues were 5–6 ng/g protein (Xie *et al.*, 2020). Some of the observed variations can be partly explained by differences in the extraction procedure and the applied analytical methods, in addition to the impact of rearing conditions, feed, and health status of the animals.

Dopamine, another neurotransmitter, plays a role in supporting adaptive responses to adverse conditions, and its levels can increase during stressful situations. Previous investigations demonstrated a wide range of dopamine concentrations in the plasma of chickens, from 1.54 ng/mL to 8.96 ng/mL (Rozempolska-Rucińska *et al.*, 2023) and even higher levels of 131.25 ng/mL (Johnson *et al.*, 2024). The results from the current study for dopamine levels in the plasma of broilers were close to the lowest cited value, and those

for pheasants were nearly threefold lower.

Dopamine levels have been measured in different regions of the poultry brain. In the raphe nuclei of chickens, reported concentrations were 118.16 ng/g, while in the hypothalamus they reached 354.70 ng/g (Yan *et al.*, 2018). Probiotic administration lowered these levels to 111.44 ± 1.79 ng/g in the raphe nuclei and to 267.35 ± 8.17 ng/g in the hypothalamus (Yan *et al.*, 2018). Kops *et al.* (2013) reported dopamine levels of 1.89 ng/g in the dorsal thalamus, 114.69 ng/g in the striatum, and 0.67 ng/g in the hippocampus. In the current study, dopamine concentrations in the cerebrum of broilers and pheasants were relatively low: 1.97 ng/g and 2.0 ng/g, respectively. McIntyre & Chew (1983) observed marked differences in dopamine concentrations within various cerebral regions between the most dominant and least dominant pheasants in a group. In the most dominant birds, dopamine levels were significantly higher in the neostriatum (6661 ± 361 ng/g) and in the paleostriatum and olfactory lobes (739 ± 60 ng/g), compared with 5262 ± 501 ng/g and 655 ± 42 ng/g, respectively, in the least dominant birds. Other authors reported comparable dopamine levels in the brain tissue of 28-day-old chickens with low or high feather pecking behaviour: 453 ± 36 ng/mL and 492 ± 27 ng/mL, respectively (Van Hardien *et al.*, 2002). These differences may be attributed to the specific brain regions examined and the analytical methods. Additional factors can also influence dopamine levels in the brain. For instance, poultry exposed to adverse conditions or stress demonstrated increased concentrations of this monoamine (Kostál *et al.*, 1999). Celefi *et al.* (2019) further suggested that alterations in brain dopamine levels during infection may result from modulatory interactions

between the serotonergic and dopaminergic systems.

Recent studies indicate that the role of dopamine is not limited to neurotransmission in the brain, as it also plays a significant role in the regulation of gastrointestinal function and in shaping the microbiome (Johnson *et al.*, 2024). Freestone *et al.* (2007) suggested that enteric pathogens may have evolved response systems related to the neurotransmitters found in the host's gut, exerting effect upon induced stress. This monoamine affects the host's intestinal microbiome, stimulates the bacterial growth of *Escherichia coli* O157:H7 and *Salmonella enterica* (Freestone *et al.*, 2007; Lyte *et al.*, 2022). Moreover, some microorganisms, such as *Enterococcus faecium*, are capable of producing dopamine in the gastrointestinal environment when supplied with dopamine precursors (Villageliú & Lyte, 2018). Lyte *et al.* (2022) reported the presence of neurotransmitters throughout the intestinal tract of chickens, noting that dopamine and its metabolites were distributed across all regions. In their study, dopamine concentrations in the jejunum of 4-week-old Cobb 500 male chickens (105 ± 19 ng/g) were comparable to those observed in broilers in the present investigation. In contrast, significantly lower jejunal dopamine levels were found in pheasants, suggesting an interspecies difference. In line with this observation, slightly lower dopamine levels, ranging from 0.253 ng/mL to 0.666 ng/mL, were reported in three pigeon breeds by El-Sayed *et al.* (2023), which are lower than the concentrations observed in the present study.

One limitation of this study is that serotonin and dopamine concentrations were measured in the whole cerebrum rather than in specific brain regions, and only a

single segment of the gastrointestinal tract was analysed. Additionally, the pheasants were reared with lasalocid supplementation, unlike the broiler chickens, which reflects standard practices but could potentially influence monoamine metabolism. Future studies should investigate receptor expression and distribution in different brain regions and intestinal segments to better understand species-specific differences.

CONCLUSIONS

In summary, this study reveals interspecies differences in serotonin and dopamine levels between broilers and pheasants. Broilers generally exhibited higher concentrations in the brain and jejunum, although no differences were observed in brain dopamine levels. Plasma monoamine levels were affected by the blood collection method, highlighting the importance of methodological considerations in such studies. These findings contribute to the understanding of avian monoamine physiology and provide a basis for optimising experimental protocols when comparing species.

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