

A neurochemical biogeography of the broiler chicken intestinal tract

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ABSTRACT The study of neurochemical-based interkingdom signaling and its impact on host-microbe interaction is called microbial endocrinology. Neurochemicals play a recognized role in determining bacterial colonization and interaction with the gut epithelium. While much attention has been devoted to the determination of neurochemical concentrations in the mammalian gut to better understand tissue and region-specific microbial endocrinology-based mechanisms of host-microbe interaction, little is known regarding the biogeography of neurochemicals in the avian gut. Greater resolution of avian gut neurochemical concentrations is needed especially as recent microbial endocrinology-based investigations into bacterial foodborne pathogen colonization of the chicken gut have demonstrated neurochemicals to affect *Campylobacter jejuni* and *Salmonella* spp. in vivo and in vitro. The aim of the present study was to determine the concentrations of stress-related neurochemicals in the tissue and luminal content of the duodenum, jejunum, ileum, cecum, and colon of the broiler intestinal tract, and to investigate if this biogeography changes with age of the bird. While all neurochemicals measured

were detected in the intestinal tract, many displayed differences in regional concentrations. Whereas the catecholamine norepinephrine was detected in each region of the intestinal tract, epinephrine was present only in the cecum and colon. Likewise, dopamine, and its metabolite 3,4-dihydroxyphenylacetic acid were found in the greatest quantities in the cecum and colon. Serotonin and histamine were identified in each gut region. Region-specific age-related changes were observed ($P < 0.05$) for serotonin, its metabolite 5-hydroxyindole acetic acid as well as for histamine. Several neurochemicals, including norepinephrine, were found in the contents of each gut region. Epinephrine was not detected in the gut content of any region. Salsolinol, a microbial-produced neuroactive compound was detected in the gut content but not in tissue. Together, our data establish a neurochemical biogeography of the broiler chicken intestinal tract. By providing researchers with a region-by-region map of in vivo gut neurochemical concentrations of a modern broiler chicken breed, this neurochemical map is expected to inform future investigations that seek to utilize avian enteric neurochemistry.

Key words: gut, biogeography, neurochemical, chicken, microbial endocrinology

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INTRODUCTION

Host-microbe bidirectional communication is mediated, in part, by neurochemicals. Comprising a diverse set of neuroendocrine signaling mechanisms, structurally identical neurochemicals are produced and recognized by both host and microbiota (Mittal et al., 2017). Since the founding of microbial endocrinology (Lyte and Ernst, 1992; Lyte, 1993), which is defined as the study of

interkingdom neurochemical crosstalk, neurochemical-based mechanisms have been demonstrated to mediate important aspects of host-microbe interaction ranging from the ability of the microbiota to influence host behavior and memory (Lyte, 2014) to the regulation of bacterial pathogenicity (Lyte, 2016). Norepinephrine, epinephrine, dopamine, serotonin, and other monoamine neurochemicals have been demonstrated to cause functional changes in the physiology of several bacterial species, including *Campylobacter jejuni*, *Salmonella* spp., avian pathogenic *Escherichia coli*, and other foodborne pathogens of particular importance in poultry (Villageliu and Lyte, 2017; Lee et al., 2021). Limited attention, however, has been directed towards defining the in vivo concentrations and regional distribution of

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these neurochemicals in the avian gut (Dennis, 2016). Considering the existence of regional differences in the composition and function of the gut microbiome as well as distinct bacterial colonization strategies of the chicken intestinal tract (Oakley et al., 2014), defining the in vivo enteric concentrations of neurochemicals known to mediate host-microbe interactions is needed to inform the design of microbial endocrinology-based strategies to improve poultry welfare and reduce food-borne pathogens in the avian gut.

Although commonly associated with the adrenal gland, initial investigation into the presence of catecholamine neurochemicals in the avian intestinal tract dates back nearly half a century (Komori et al., 1979; Konaka et al., 1979). That catecholamines are detectable in the gut should not be surprising as they play a variety of physiological roles important in intestinal function (Dennis, 2016). Despite ample investigation into the source of catecholamines in the mammalian gut including the local synthesis by intestinal epithelial (Vieira-Coelho and Soares-Da-Silva, 1998) and immune cells (Han et al., 2020), spillover from catecholaminergic nerve fibers (Aneman et al., 1996), and supply from the bloodstream (Marra et al., 2005), comparatively little work has been performed in birds. Nevertheless, microbial endocrinology-based investigations performed in poultry have revealed catecholamines to affect host-microbe interaction in the avian gut in vivo (Cogan et al., 2007). The first evidence that bacteria directly respond to catecholamines was reported using norepinephrine and *Escherichia coli* (Lyte and Ernst, 1992). Since that time, norepinephrine, epinephrine, or dopamine have been reported to affect a variety of microorganisms including *C. jejuni* (Xu et al., 2015; Truccollo et al., 2020), *Salmonella* spp. (Bailey et al., 1999), *Yersinia enterocolitica*, and others (Bansal et al., 2007; Nakano et al., 2007; Cambrone et al., 2019; Gao et al., 2019).

Like the catecholamines, initial investigations into the presence of other monoamine neurochemicals in the avian gut, including serotonin and histamine, and their relationship to the microbiota date back several decades (Phillips et al., 1961; Beaver and Wostmann, 1962). While both serotonin and histamine have demonstrated roles in enteric neuroimmune signaling, it is the former that has received the most attention, mostly in mammalian models, as an interkingdom signaling molecule (Mittal et al., 2017). Yet, relatively few studies since have sought to determine differences in the concentrations of these neurochemicals among the different regions of the avian gut (Gross and Sturkie, 1975; Perez-Ruiz et al., 1988), or in intestinal content (Humer et al., 2015; Redweik et al., 2019; Lyte et al., 2021b). The latter aspect is of particular importance considering, for example, serotonin's extensive role in mediating microbiota composition (Kwon et al., 2019) as well as bacterial, including *C. jejuni*, colonization of the intestinal tract (Lyte et al., 2021b).

As such, we sought to determine the concentrations of monoamine neurochemicals and related metabolites in the tissue and content of each region of the broiler

chicken intestinal tract and to investigate whether these concentrations are affected by the age of the bird.

MATERIALS AND METHODS

Chickens and Tissue Collection

All procedures and management practices were approved by the University of Arkansas Institutional Animal Care and Use Committee (IACUC; protocol #21049) before the start of the study. One hundred fifty Cobb500 male chicks (Cobb-Vantress, 2013) were placed in 6 floor pens (1.5 × 1.5 m) (25 birds/pen) covered with wood-shaving reused litter material, and equipped with a 10-nipple water line and 1 hanging feeder. Birds were fed ad libitum mash diets under a 3-phase feeding program (Starter, 1 to 14 d; Grower, 15 to 29 d; Finisher 30 to 43 d) exceeding the National Research Council (1994) requirements and formulated according to the genetic line recommendations (Cobb-Vantress, 2018) as follows: Starter, 2,992 kcal metabolizable energy (ME) per kg diet, 22% crude protein (CP), 1.22% digestible lysine (dLYS), 0.90% calcium (Ca), 0.45 % non-phytic phosphorus (nPP); Grower, 3,075 kcal/kg ME, 20% CP, 1.149% dLYS, 0.80 % Ca, 0.41% nPP; and Finisher, 3,166 kcal/kg ME, 18.6% CP, 1.02% dLYS, 0.76% Ca, 0.38% nPP. The lighting program consisted of 24 h of light for the first 3 d and then 18 h thereafter. At all ages, the light intensity was between 27 and 44 lux. Ventilation and temperature were automatically controlled to follow a curve with 34, 32, 29, and 25°C at 0, 7, 14, and 42 d, respectively. Relative humidity was between 30 and 50% during the whole grow-out period. To examine age-based changes in enteric neurochemical concentrations, we randomly selected 12 male birds at 2, 4, and 6 wk of age (n = 12 birds per age group was chosen as this number was previously demonstrated as sufficient to detect a significant ($P < 0.05$) change in avian intestinal neurochemical concentrations [Lyte et al., 2021a]). Birds were euthanized by carbon dioxide inhalation.

The entire intestine was removed and manually dissected into the duodenum, jejunum, ileum, ceca, and colon. To avoid damage to the mucosa, a ball-tipped scissor was used to open each intestinal region. Luminal content from each section was collected, after which the tissue was gently rinsed with phosphate-buffered saline (PBS) to remove any remaining content. Full-thickness intestinal tissue immediately underlying the luminal content was then collected. Duodenal, jejunal, ileal, and colonic samples were collected from the proximal end of each intestinal region while the cecal sample was collected from the blind end of one randomly selected cecal pouch. Each sample was weighed, immediately acidified in individual 2-mL reinforced tubes each containing 1 mL of 0.2 N perchloric acid (0.2 N perchloric acid consisted of HPLC grade water [Catalog # 7732-18-5, VWR Life Science, Radnor, PA] and perchloric acid [Catalog #: AAA44464-AP, VWR Life Science]) and 6 ceramic beads (Tube and bead catalog #s: 19-648 and

19-646, Omni International, Kennesaw, GA), snap frozen on dry ice and then stored at -80°C until analysis.

(DOPAC), Catalog #: 102-32-9; for epinephrine, Catalog #: 329-63-5; for histamine, Catalog # 56-92-8).

Ultra-High Performance Liquid Chromatography

Samples were processed as previously described (Lyte et al., 2021b). In brief, samples were thawed and then homogenized in a Bead Ruptor (Catalog #: 19-040E, Omni International) for 2 cycles, each cycle consisted of 30 s at 5 m/s; a 10 s break separated each 30 s cycle. After homogenization, samples were immediately centrifuged at $3,000 \times g$ and 4°C for 15 min. The supernatant of each sample was collected and placed into individual 2 to 3 kDa molecular weight cut-off spin filters (Catalog #: 89132-006, VWR). Sample flow-through was stored at -80°C until ultra-high performance liquid chromatography (UHPLC) analysis with electrochemical detection was performed as previously described (Villageliu et al., 2018b). Histamine analysis was conducted as previously described (Nadeem et al., 2019) with minor modifications using UV/Vis detection. The UHPLC consisted of a Dionex Ultimate 3,000 autosampler and a Dionex Ultimate 3,000 pump, equipped with a Dionex Ultimate 3,000 RS electrochemical detector and VWD-3400 UV/Vis detector (Thermo Fisher Scientific, Sunnyvale, CA). Mobile phase consisted of buffered 10% acetonitrile (Catalog #: NC9777698, Thermo Fisher Scientific) and the flow rate was 0.6 mL/min on a 150 mm (length) 3 mm (internal diameter) $3 \mu\text{m}$ (particle size) Hypersil BDS C18 column (Catalog #: 28103-153030, Thermo Fisher Scientific). All samples were maintained at 4°C in the autosampler before injection. A 6041RS glassy carbon electrode set at 400 mV was used for electrochemical detection. The UV detection was set to 210nm. Data were analyzed using the Chromeleon software package (version 7.2, Thermo Fisher Scientific), and neurochemical identification was confirmed using the relative retention time of the corresponding analytical standard from Millipore-Sigma (Millipore-Sigma, St. Louis, MO) (for norepinephrine, Catalog #: 636-88-4; for serotonin, Catalog #: 61-47-2; for homovanillic acid (HVA), 306-08-1; for 5-hydroxyindoleacetic acid (5-HIAA), Catalog #: 54-16-0; for salsolinol, Catalog #: 59709-57-8; for dopamine, Catalog #: 62-31-7; for 3,4-dihydroxyphenylacetic acid

Statistical Analysis

Intestinal neurochemical data ($n = 12$ chickens per age group) were analyzed using two-way ANOVA followed by Tukey post hoc test. Differences were considered significant at the threshold of $P < 0.05$.

RESULTS

Norepinephrine Concentrations

Broiler chicken intestinal norepinephrine concentrations (μg of norepinephrine per g of tissue or content) are reported in Table 1. Norepinephrine was detected in the tissue of each intestinal region. Duodenal concentrations did not differ ($P > 0.05$) between chickens of different ages. The age of the chicken had a significant ($P < 0.05$) effect on norepinephrine concentrations in the jejunum where 6 wk/age birds had greater concentrations compared to 2 or 4 wk/age birds. Likewise, norepinephrine concentrations were higher ($P < 0.05$) in the ileum of 6 wk/age compared to 4 wk/age birds. Conversely, within the cecum, 6 wk/age birds had less ($P < 0.05$) norepinephrine compared to the 2 or 4 wk/age groups. Within the colon, norepinephrine concentrations decreased ($P < 0.05$) with bird age with 6 wk/age and 2 wk/age birds having the lowest and highest levels, respectively.

Intestinal content of each region had detectable concentrations of norepinephrine. The levels found in duodenal, jejunal, ileal, and colon contents were not affected ($P > 0.05$) by the age of the bird. Cecal content concentrations demonstrated an age-dependent decrease where 6 wk/age birds had less ($P < 0.05$) norepinephrine compared to 2 or 4 wk/age birds.

Epinephrine Concentrations

Broiler chicken intestinal epinephrine concentrations (μg of epinephrine per g of tissue or content) are reported in Table 2. In each age group, epinephrine was detectable in cecal and colonic tissue. Epinephrine concentrations in the colon were lower ($P < 0.05$) in 6 wk/age compared to 2 or 4 wk/age birds. Epinephrine

Table 1. Norepinephrine concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	0.429 \pm 0.012	0.472 \pm 0.015 ^a	0.399 \pm 0.013	0.464 \pm 0.055 ^a	0.565 \pm 0.018 ^a
	4 wks/age:	0.373 \pm 0.016	0.431 \pm 0.031 ^a	0.343 \pm 0.024 ^a	0.420 \pm 0.016 ^a	0.438 \pm 0.030 ^b
	6 wks/age:	0.408 \pm 0.039	0.592 \pm 0.046 ^b	0.469 \pm 0.051 ^b	0.236 \pm 0.026 ^b	0.270 \pm 0.069 ^c
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	1.321 \pm 0.122	2.211 \pm 0.144	0.891 \pm 0.132	3.260 \pm 0.584 ^a	1.012 \pm 0.268
	4 wks/age:	0.960 \pm 0.082	2.320 \pm 0.273	1.017 \pm 0.150	2.996 \pm 0.622 ^a	0.809 \pm 0.108
	6 wks/age:	1.119 \pm 0.138	2.624 \pm 0.359	1.063 \pm 0.214	1.633 \pm 0.305 ^b	0.998 \pm 0.168

^{abc}Values with different superscript letters within columns denote significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. ND, not detectable. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

Table 2. Epinephrine concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	0.045 ± 0.030	ND	ND	0.024 ± 0.024	0.274 ± 0.059 ^a
	4 wks/age:	ND	ND	ND	0.078 ± 0.033	0.259 ± 0.037 ^a
	6 wks/age:	ND	ND	ND	0.028 ± 0.008	0.133 ± 0.033 ^b
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	ND	ND	ND	ND	ND
	4 wks/age:	ND	ND	ND	ND	ND
	6 wks/age:	ND	ND	ND	ND	ND

^{ab}Values with different letters within columns denotes significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. ND, not detectable. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

was not detectable in the content of any intestinal region at any age of the chicken.

Dopamine Concentrations

Broiler chicken intestinal dopamine concentrations (μg of dopamine per g of tissue or content) are reported in Table 3. Dopamine was detected in each region of the intestinal tract. Ileal tissue concentrations were lower ($P < 0.05$) in 6 wk/age birds compared to 2 or 4 wk/age birds. Cecal and colonic levels of dopamine were not affected ($P > 0.05$) by age of the bird. Dopamine was undetectable in the intestinal content of most regions, with low levels being identified in ileal and colonic content.

Serotonin Concentrations

Broiler chicken intestinal serotonin concentrations (μg of serotonin per g of tissue or content) are reported in Table 4. Serotonin was detected in each region of the intestinal tract. Jejunal and ileal serotonin concentrations were greater ($P < 0.05$) in 6 wk/age compared to 2 wk/age birds. Conversely, an age-dependent decrease ($P < 0.05$) in serotonin levels was identified in the cecal and colonic tissues. Serotonin levels in duodenal tissue were not affected ($P > 0.05$) by age of the bird.

The luminal content of each intestinal region had measurable levels of serotonin. Duodenal content serotonin was lower in 6 wk/age birds compared to those at 4 wk/age. Jejunal, ileal, and cecal content concentrations were not affected ($P > 0.05$) by age of the bird.

Table 3. Dopamine concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	0.013 ± 0.013 ^a	ND ^a	0.103 ± 0.022 ^a	0.092 ± 0.024	0.111 ± 0.025
	4 wks/age:	0.102 ± 0.018 ^b	0.105 ± 0.019 ^b	0.124 ± 0.017 ^a	0.130 ± 0.023	0.115 ± 0.016
	6 wks/age:	ND ^b	ND ^b	0.033 ± 0.018 ^b	0.127 ± 0.017	0.077 ± 0.012
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	ND	ND	0.008 ± 0.008	ND	0.045 ± 0.039
	4 wks/age:	ND	ND	ND	ND	ND
	6 wks/age:	ND	ND	ND	ND	ND

^{ab}Values with different letters within columns denote significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. ND, not detectable. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

Colonic concentrations were lowest in birds that were 4 wk/age compared to those that were 2 or 6 wk/age.

DOPAC Concentrations

Broiler chicken intestinal DOPAC concentrations (μg of DOPAC per g of tissue or content) are reported in Table 5. DOPAC was identified in the tissue and luminal content of each of region of the intestinal tract. Concentrations increased in each region of the small intestine with the age of the chicken. Cecal levels of DOPAC were greatest ($P < 0.05$) in 6 wk/age chickens compared to those at 2 or 4 wks/age. Likewise, colonic DOPAC was highest ($P < 0.05$) in 6 wk/age compared to 4 wk/age chickens. Cecal and colonic luminal content DOPAC levels were highest in 4 wk/age compared to 2 or 6 wk/age chickens.

Histamine Concentrations

Broiler chicken intestinal histamine concentrations (μg of histamine per g of tissue or content) are reported in Table 6. Histamine was detected in each region of the intestinal tract. Duodenal levels were greater ($P < 0.05$) in 4 wk/age compared to 2 wk/age birds. Jejunal, ileal, and colonic histamine concentrations increased ($P < 0.05$) with the age of the bird. Within ileal and colonic luminal contents but not in cecal content, histamine levels also increased ($P < 0.05$) with the age of the bird. Histamine was not detected in duodenal luminal content.

Table 4. Serotonin concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	16.881 ± 1.491	6.969 ± 0.489 ^a	9.630 ± 0.605 ^a	15.636 ± 2.017	24.313 ± 2.519 ^a
	4 wks/age:	21.556 ± 2.083	9.928 ± 1.081	11.950 ± 0.972	20.639 ± 1.508 ^a	15.685 ± 1.662 ^b
	6 wks/age:	18.615 ± 1.490	12.543 ± 1.266 ^b	15.025 ± 1.429 ^b	14.816 ± 1.404 ^b	6.798 ± 1.697 ^c
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	20.163 ± 2.676	5.405 ± 1.000	1.518 ± 0.427	4.211 ± 0.735	22.137 ± 8.615 ^a
	4 wks/age:	28.287 ± 4.049 ^a	6.063 ± 1.748	4.329 ± 2.410	5.643 ± 0.904	5.064 ± 2.178 ^b
	6 wks/age:	14.533 ± 2.927 ^b	5.713 ± 1.268	3.227 ± 0.589	3.530 ± 0.480	20.878 ± 8.876 ^a

^{abc}Values with different letters within columns denote significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. ND, not detectable. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

Table 5. DOPAC concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	ND ^a	ND ^a	ND ^a	0.133 ± 0.026 ^a	0.160 ± 0.013
	4 wks/age:	0.118 ± 0.005 ^b	0.105 ± 0.010	0.132 ± 0.006 ^b	0.183 ± 0.031 ^a	0.132 ± 0.005 ^a
	6 wks/age:	0.124 ± 0.007 ^b	0.125 ± 0.006 ^b	0.089 ± 0.021	0.588 ± 0.103 ^b	0.248 ± 0.053 ^b
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	ND	ND	0.216 ± 0.216	ND ^a	0.578 ± 0.349 ^a
	4 wks/age:	0.077 ± 0.053	0.063 ± 0.063	ND	0.899 ± 0.248 ^b	0.070 ± 0.070 ^b
	6 wks/age:	ND	0.063 ± 0.063	0.064 ± 0.064	0.238 ± 0.132 ^a	0.941 ± 0.227 ^a

DOPAC: 3,4-Dihydroxyphenylacetic acid; ND, not detectable.

^{ab}Values with different letters within columns denote significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

HVA Concentrations

Broiler chicken intestinal HVA concentrations (μg of HVA per g of tissue or content) are reported in Table 7. HVA was detected in the luminal content but not the tissue of each intestinal region. The HVA concentrations in luminal content were not affected ($P > 0.05$) by the age of the chicken.

and cecal tissue concentrations increased ($P < 0.05$) with age of the bird, whereas duodenal, ileal, and colonic levels were unaffected ($P > 0.05$) by age. Small intestinal luminal content concentrations did not increase with bird age. Cecal and colonic 5-HIAA concentrations were greatest ($P < 0.05$) in 6 wk/age compared to 2 or 4 wk/age birds.

5-HIAA Concentrations

Broiler chicken intestinal 5-HIAA concentrations (μg of 5-HIAA per g of tissue or content) are reported in Table 8. 5-HIAA was detected in the tissue and luminal content of each region of the intestinal tract. Jejunal

Salsolinol Concentrations

Broiler chicken intestinal salsolinol concentrations (μg of salsolinol per g of tissue or content) are reported in Table 9. Salsolinol was detected in the luminal content but not the tissue of each region of the intestinal

Table 6. Histamine concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	0.701 ± 0.351 ^a	0.275 ± 0.275 ^a	1.093 ± 0.599 ^a	0.079 ± 0.079	0.792 ± 0.575 ^a
	4 wks/age:	9.831 ± 2.510 ^b	5.255 ± 1.523 ^a	6.007 ± 1.499	0.233 ± 0.233	7.811 ± 2.047 ^b
	6 wks/age:	4.785 ± 1.607	13.180 ± 3.811 ^b	8.988 ± 2.533 ^b	4.528 ± 0.949	13.545 ± 1.742 ^c
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	ND	ND	0.320 ± 0.320 ^a	2.314 ± 2.314	ND ^a
	4 wks/age:	ND	3.885 ± 1.709	8.848 ± 1.883 ^b	4.138 ± 1.817	15.679 ± 2.128 ^b
	6 wks/age:	ND	0.873 ± 0.873	10.061 ± 1.566 ^b	4.699 ± 1.818	1.754 ± 1.190 ^a

^{abc}Values with different letters within columns denote significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. ND, not detectable. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

Table 7. Homovanillic acid concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	ND	ND	ND	ND	ND
	4 wks/age:	ND	ND	ND	ND	ND
	6 wks/age:	ND	ND	ND	ND	ND
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	0.191 ± 0.083	0.329 ± 0.052	0.684 ± 0.096	2.611 ± 2.208	0.753 ± 0.165
	4 wks/age:	0.153 ± 0.042	0.353 ± 0.037	0.510 ± 0.089	1.167 ± 0.209	1.200 ± 0.141
	6 wks/age:	0.307 ± 0.015	0.366 ± 0.059	0.618 ± 0.058	0.864 ± 0.231	1.586 ± 0.361

Values with different letters within columns denote significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region.

ND, not detectable.

Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group).

Data was analyzed using two-way ANOVA followed by Tukey's posthoc test as described in Methods.

Table 8. 5-HIAA concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	0.401 ± 0.026	0.294 ± 0.020 ^a	0.338 ± 0.027	0.655 ± 0.140 ^a	0.545 ± 0.052
	4 wks/age:	0.453 ± 0.033	0.412 ± 0.038 ^a	0.416 ± 0.043	1.071 ± 0.128 ^b	0.463 ± 0.032
	6 wks/age:	0.671 ± 0.120	0.736 ± 0.188 ^b	0.634 ± 0.038	1.218 ± 0.166 ^b	0.398 ± 0.091
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	0.483 ± 0.041	0.297 ± 0.013	0.317 ± 0.039	8.021 ± 2.221 ^a	1.102 ± 0.327 ^a
	4 wks/age:	0.550 ± 0.062	0.433 ± 0.028	0.304 ± 0.065	21.703 ± 4.173 ^b	1.142 ± 0.401 ^a
	6 wks/age:	0.539 ± 0.075	0.408 ± 0.063	0.394 ± 0.073	27.235 ± 4.318 ^b	8.123 ± 2.591 ^b

5-HIAA, 5-hydroxyindoleacetic acid; ND, not detectable.

^{ab}Values with different letters within columns denotes significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's posthoc test as described in Methods.

Table 9. Salsolinol concentrations of the broiler chicken intestinal tract.

		Duodenum	Jejunum	Ileum	Cecum	Colon
Tissue	2 wks/age:	ND	ND	ND	ND	ND
	4 wks/age:	ND	ND	ND	ND	ND
	6 wks/age:	ND	ND	ND	ND	ND
		Duodenum	Jejunum	Ileum	Cecum	Colon
Content	2 wks/age:	0.191 ± 0.058	0.263 ± 0.041	0.448 ± 0.051	0.020 ± 0.020	0.430 ± 0.095 ^a
	4 wks/age:	0.104 ± 0.038 ^a	0.328 ± 0.014	0.342 ± 0.045	ND	0.465 ± 0.066 ^a
	6 wks/age:	0.264 ± 0.010 ^b	0.261 ± 0.048	0.358 ± 0.038	0.054 ± 0.054	0.065 ± 0.035 ^b

^{ab}Values with different letters within columns denotes significant difference ($P < 0.05$) compared between ages within tissue or luminal content of the same region. ND, not detectable. Values are μg of neurochemical per g of tissue or content. All values are expressed as mean \pm SEM ($n = 12$ chickens/age group). Data was analyzed using two-way ANOVA followed by Tukey's post-hoc test as described in Methods.

tract. Colonic luminal content salsolinol concentrations increased ($P < 0.05$) with age of the chicken.

DISCUSSION

The physical-chemical spatial heterogeneity of the intestinal tract underscores the need to consider region-specificity in determining mechanisms of host-microbe interaction. Neurochemicals, which are present in the avian gut and act as interkingdom signaling molecules, have been shown to affect the microbiota in poultry and influence bacterial pathogen colonization, thereby potentially serving as strategic targets to control site-specific host-microbe interactions (Villageliu and

Lyte, 2017). While a limited number of studies, some of which date back decades (Phillips et al., 1961; Konaka et al., 1979), have examined in vivo concentration distributions of different neurochemicals in the avian gut (Redweik et al., 2019), no investigation to date has reported neurochemical concentrations in the tissue and luminal content of each major region of the chicken intestinal tract (i.e., duodenum, jejunum, ileum, ceca, and colon). Such information is needed to inform the design of neurochemical-based strategies to target the microbiota or the tissue-specific colonization of a specific microbe, such as *C. jejuni*. As structurally identical neurochemicals are produced and recognized by both host and microbe, it is worth noting that neurochemical concentration heterogeneity in the gut is likely

due both to regional differences in host tissues types as well microbial functional capacities (Asano et al., 2012), therefore highlighting the need to examine neurochemical concentrations in both tissue and luminal content. We, therefore, sought to quantify in the tissue and luminal content of each region of the broiler chicken intestinal tract those neurochemicals which have demonstrated mechanistic roles in affecting host-microbe interactions.

In the present study, we specifically utilized the Cobb500 chicken, that despite being a highly crossbred broiler breed that is widely used in the modern poultry industry is understudied in terms of enteric neurochemistry. Major foodborne pathogen challenges faced in broiler chicken production include bacteria, such as *Salmonella* spp. and *Campylobacter* spp., that have long been demonstrated to be affected in vivo and in vitro by neurochemicals. For example, norepinephrine has been shown to affect *C. jejuni* colonization in chickens (Aroori et al., 2014) as well as increase *Salmonella* spp. virulence gene expression (Bailey et al., 1999). Norepinephrine was detected in the tissue and the luminal content of each region of the broiler chicken intestinal tract. Duodenal, jejunal, and cecal concentrations identified in the present study were in a similar range to those reported for the duodenum, jejunum (Konaka et al., 1979) as well as ceca (Redweik et al., 2019) of the white leghorn chicken. As norepinephrine concentrations displayed a region-dependent increase or decrease that coincided with aging of the bird, it would be warranted to explore tissue-specific noradrenergic plasticity in the context of susceptibility to infection. For example, in clinical medicine, catecholamine administration was reported nearly a century ago to reduce the dose of *C. perfringens* needed to cause infection in patients (Lyte and Freestone, 2010). Alterations in catecholamine distribution in the gut even under basal physiological conditions, such as the increase in jejunal norepinephrine concentrations seen here, may be hypothesized to play a role in *C. perfringens* infection in the chicken small intestine.

Dopamine, like norepinephrine, has been demonstrated to mediate the growth and function of several bacterial species (Freestone et al., 2007) including *E. coli* and *Salmonella enterica*. While dopamine was identified throughout the chicken intestinal tract, concentrations were primarily confined to the tissue. Previous reports have indicated extensive variability in gut microbial species to produce dopamine (Villageliu and Lyte, 2018), thereby suggesting the microbiota of the chickens in the present study may lack microbial consortia that can synthesize dopamine. The major metabolites of dopamine, DOPAC and HVA, were identified in tissue as well as luminal content; salsolinol, an exclusively microbial metabolite of dopamine was found only in the luminal content. While the presence of DOPAC has been reported in the cecal content of white leghorn chickens (Redweik et al., 2019), the role of the metabolite, if any, in affecting the microbiota is currently unclear. It should not be surprising that DOPAC was

identified in the luminal content as bacteria have been demonstrated to synthesize this neurochemical (Martin et al., 1991) independent of dopamine. Likewise, bacterial catabolism of DOPAC has been previously reported (Martin et al., 1991). HVA was detected solely in the luminal content suggesting potential microbial conversion of DOPAC to HVA.

Salsolinol, like the other dopamine metabolites, has received little attention in birds. While we recently reported the presence of salsolinol in the quail intestinal tract (Lyte et al., 2021a), investigations in the literature overwhelmingly focus on this molecule's role as a microbial produced neurotoxin, often in the context of Parkinson's disease (Kurnik-Lucka et al., 2018; Villageliu et al., 2018a). As salsolinol was detected in the present study of the broiler intestinal tract, a future role may be identified for this microbial-produced neurochemical in mediating effects on the avian brain via the microbiota-gut-brain axis. Indeed, salsolinol has been reported to have an effect in vitro on chicken muscle tissue (Rodger et al., 1979), thereby demonstrating that chicken physiology can respond to this microbial product.

Epinephrine was only detected in the ceca and colon of the intestinal tract. Similarly, in the white leghorn chicken intestinal tract epinephrine was identified in the ceca, but not a small intestinal wash (Redweik et al., 2019). Although adrenergic neuronal innervation of the rectum was previously reported as a source of epinephrine in the white leghorn chicken intestine (Komori et al., 1979), no other investigation to date has examined adrenergic innervation of other regions of the chicken gut. That epinephrine was identified in the ceca and colon of the intestinal tract may hold important consequences for region-specific host-bacterial interactions as epinephrine has been shown to increase the adherence and invasion of *C. jejuni* (Xu et al., 2015), which predominantly colonizes the cecal crypts, as well as serve as a chemoattractant for *E. coli* (Lopes and Sourjik, 2018).

Compared to the catecholamines, surprisingly few studies have investigated the ability of serotonin to mediate host-microbe interactions despite this neurochemical being widely distributed throughout the intestinal tract. In addition to affecting *C. jejuni* colonization in vitro (Lyte et al., 2021b), serotonin has been shown to affect *E. coli* (Oleskin et al., 1998), *Pseudomonas aeruginosa* (Knecht et al., 2016), and mediate compositional changes in the mammalian microbiota (Kwon et al., 2019). No study was found during the writing of this manuscript (PubMed, key terms used "5-HIAA," "5-hydroxyindoleacetic acid," "infection," "colonization," "bacteria," "microbiota", and/or "host-microbe") that investigated a role for 5-HIAA in affecting host-microbe interaction. Although we recently reported serotonin concentrations in the luminal content and tissue of the ileum, ceca, and colon of the broiler chicken (Lyte et al., 2021b), the present study provides additional insight into serotonin concentrations in the duodenum and jejunum, as well as 5-HIAA of every intestinal section. Serotonin concentrations reported here fall in the

same concentration range reported previously for the white leghorn duodenum (Phillips et al., 1961), ileum (Beaver and Wostmann, 1962), and ceca (Redweik et al., 2019).

Histamine was found in each section of the broiler chicken intestinal tract. That it was also identified in the luminal contents of the gut should not be surprising as histamine is produced by both host and bacteria (Pugin et al., 2017). Previous investigations into enteric histamine in the chicken have primarily focused on concentrations in small intestinal tissue, and the role of diet in determining these levels. Within the small intestine, we found similar histamine concentrations to those previously reported (Reimann et al., 1971; Liu et al., 2006). While the role of histamine as a neuroimmune mediator is well-established (Cacabelos et al., 2016), its role in host-microbe cross-communication is less clear. Food safety studies have long recognized microbial-produced histamine to present a consumer health risk (Tortorella et al., 2014), particularly in canned foods but broiler feed that contained histamine, for example, was not found to negatively affect bird performance (Bermudez and Firman, 1998).

Neurochemicals are one among many physiologic aspects that distinguish regions of the intestinal tract. The present study provides poultry researchers quantitative insight into the enteric neurochemical landscape of the broiler chicken intestinal tract. As neurochemicals act as interkingdom signaling molecules, this biogeography will inform future investigations that seek to utilize neurochemical-mediated host-microbe interactions to improve poultry gut health.

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DISCLOSURES

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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