

SHORT COMMUNICATION

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FIELD MEASUREMENTS OF GASTROINTESTINAL PH OF NEW WORLD VULTURES IN GUYANA

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Hydrogen ion concentration (pH) is a major driver of the gastrointestinal microbiome, the community of microorganisms residing in the gut (Flint et al. 2007, Walter and Ley 2011, The Human Microbiome Project Consortium 2012). Gastrointestinal pH has been measured in a relatively small number of captive and wild birds (Farner 1942, 1943, Herpol and Van Grembergen 1967, Ziswiler and Farner 1972, Duke et al. 1975, Rhoades and Duke 1975). Only a few pH measurements have been made in free-living birds (Gremillet et al. 2000, Thouzeau et al. 2004, Gremillet et al. 2012), and none have been reported for free-living raptors. The sole report of gastrointestinal pH in an obligate scavenging raptor was provided by Houston and Cooper (1975) for freshly-killed White-backed Vultures (*Gyps africanus*). This report also appears to be the sole empirical source for the aphorism that carrion-feeding vultures have unusually low gastric pH.

Gastrointestinal pH of the New World vultures (Cathartidae) is of particular interest because of their chronic exposure to pathogenic bacteria in carrion (Winsor et al. 1981, Carvalho et al. 2003, Roggenbuck et al. 2014). Roggenbuck et al. (2014) hypothesized that gastrointestinal acidity in Black Vulture (*Coragyps atratus*) and Turkey Vulture (*Cathartes aura*) acts as a strong filter that greatly reduces the bacterial diversity in food as it passes through the gut. However, there are no published data on gastrointestinal pH for vultures in this monophyletic clade (Johnson et al. 2016). Here I present the first measurements of gastrointestinal pH in Black Vulture, Turkey Vulture, and Lesser Yellow-headed Vulture (*Cathartes burrovianus*).

METHODS

Specimens of Black Vulture (seven females; six males), Lesser Yellow-headed Vulture (two females; three males), and Turkey Vulture (two males) were collected from 16 to 21 October 2015 at Dadanawa Ranch, Upper Takutu-Upper Essequibo, Guyana (2°49.28'N, 59°31.34'W; 127 masl) for investigations of the gastrointestinal microbiome (Roggenbuck et al. 2014), cranial integument (Graves 2016), and sensory anatomy (Lisney et al. 2013). Vultures were trapped or shot over carrion and necropsied within 15–30 min of death. Hydrogen ion concentration (pH) was measured with a portable pH meter (Fisher Scientific™ accumet™ portable AP110 pH meter S04971; Waltham, MA U.S.A.) equipped with micro glass combination electrode (13-620-850) with a glass body (3 × 38 mm) and a resolution of 0.01 pH (±0.01). The pH probe was inserted through small incisions in the stomach (ventriculus), small intestine (ansa duodeni), and large intestine (rectum)—anatomical terms in parentheses are from *Nomina Anatomica Avium* (McLeland 1993). Intestinal incisions were made in sections isolated with medical hemostats approximately 4–8 cm below the pyloric sphincter (small intestine) and 4–6 cm above the cloaca (large intestine). Two independent pH measurements were made in each incision (Appendix). The pH probe was calibrated before each specimen with standard buffers (pH 2.0, 4.0, 7.0) and cleaned and dried after each measurement. Duplicate pH measurements at each anatomical site were converted to hydrogen ion concentrations, averaged $[(m_1 + m_2)/2]$, and then converted back to pH (Appendix). Sample sizes were too small for meaningful statistical tests for differences among species. All stomachs contained variable amounts of carrion, bone, and inorganic material (e.g., glass fragments). We did not quantify stomach contents or measure the pH of individual food items. The small and large intestines contained a comparatively homogenous slurry. Ambient temperature during necropsies varied from 32° to 37° C. Voucher

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specimens were deposited in the National Museum of Natural History (Smithsonian Institution).

RESULTS

Postprandial (after feeding) stomach pH values exhibited considerable variation in Black Vulture (2.0–5.6), Lesser Yellow-headed Vulture (3.5–4.3), and Turkey Vulture (2.9–3.5). Mean pH values (\pm SD) were highly acidic in all species: Black Vulture ($= 3.9 \pm 1.3$; $n = 13$); Lesser Yellow-headed Vulture ($= 4.0 \pm 0.3$; $n = 5$); and Turkey Vulture ($= 3.2 \pm 0.4$; $n = 2$). Small intestine pH was circumneutral in Black Vulture ($= 6.5 \pm 0.5$), Lesser Yellow-headed Vulture ($= 6.5 \pm 0.4$), and Turkey Vulture ($= 6.1 \pm 0.0$). Large intestine pH also approached neutrality: Black Vulture ($= 6.4 \pm 0.3$); Lesser Yellow-headed Vulture ($= 6.6 \pm 0.6$); and Turkey Vulture ($= 6.5 \pm 0.0$).

Insight on measurement repeatability was provided by the difference between the first measurement (m_1) and second measurement (m_2) of pH at each site in the gastrointestinal tract. The mean difference in pH units observed in the small intestine ($m_1 - m_2 = 0.05$; $n = 18$ individuals) barely exceeded the resolution of the pH electrode (0.01). Repeatability of pH measurement in the large intestine was similarly high ($m_1 - m_2 = 0.04$; $n = 18$ individuals). In contrast, pH measurements of the stomach contents were more variable ($m_1 - m_2 = 0.23$; $n = 18$ individuals). This was presumably caused by minor variation in probe placement coupled with significant acidity gradients in the marinated stomach contents.

DISCUSSION

Avian gastric juice is composed of water and lesser amounts of hydrochloric acid, salts, pepsin, and mucin (Sturkie 1976). The hydrogen ion concentration of gastric juice varies with the composition, texture, and volume of ingested dietary items and sampling location within the stomach (see Ziswiler and Farner 1972). Stomach pH rises rapidly after feeding (Van Dobben 1952, Smith and Richmond 1972, Duke et al. 1975, Gremillet et al. 2012) and after death (Farner 1943, Herpol and Van Grembergen 1967).

Few comparative data for stomach pH are available for falcons, owls, and accipitriform raptors, which include Old World and New World vultures (Jarvis et al. 2014, Prum et al. 2015). Houston and Cooper (1975) reported pH values of 1.0–1.5 for three White-backed Vultures that were said to be digesting food, although it is unclear whether the stomachs contained carrion. These measurements were made under field conditions with pH indicator papers which yield relatively imprecise readings compared to the high precision pH meters now widely available. In laboratory experiments employing an analytical pH meter, Duke et al. (1975) found a fairly narrow range (preprandial

to postprandial) pH values in Bald Eagle (*Haliaeetus leucocephalus*; pH = 1.3–1.5), Swainson's Hawk (*Buteo swainsonii*; pH = 1.6–2.1), and Red-tailed Hawk (*Buteo jamaicensis*; pH = 1.8–2.8). Preprandial-postprandial stomach pH in Peregrine Falcon (*Falco peregrinus*; pH = 1.8–3.5) and Gyrfalcon (*Falco rusticolus*; pH = 1.8–3.4) were similar to those observed in accipitriform raptors. Great Horned Owl (*Bubo virginianus*; pH = 2.2–2.7) and Snowy Owl (*Bubo scandiacus*; pH = 2.5–2.8) had somewhat less acidic preprandial stomach pH (Duke et al. 1975). Preprandial-postprandial data from Barn Owl (*Tyto alba*) showed a relatively large range in stomach pH (1.9–6.2; Smith and Richmond 1972).

Wide variation in postprandial stomach pH (2.0–5.6) observed in the New World vultures in this study was probably associated with stomach fullness, the concomitant dilution of gastric juices by ingested carrion, and time since feeding. Preprandial stomach pH in New World vultures remains to be investigated but is likely similar to that reported for non-scavenging birds that consume large vertebrate prey (Van Dobben 1952, Duke et al. 1975, Rhoades and Duke 1975).

Roggenbuck et al. (2014) hypothesized that gastric acidity was a strong filter for bacteria in Black and Turkey vultures based on the seven-fold decrease in bacterial diversity observed in the large intestine compared to fecal swabs. Beasley et al. (2015) went a step farther by proposing that carrion-feeding and carnivorous birds that feed on mammals and birds have lower stomach pH values than herbivorous species. Omnivorous, piscivorous, and insectivorous species were hypothesized to fall somewhere between the extremes. However, their supporting general linear model pooled mammals and birds and included a mixture of preprandial pH measurements for some species and postprandial measurements for others.

A review of avian literature shows that there is no consistent pattern in preprandial stomach pH among the small sample of carrion-feeding, carnivorous, piscivorous, and omnivorous birds that have been investigated thus far. Domestic fowl (Herpol and Van Grembergen 1961, 1967), Wandering Albatross (*Diomedea exulans*; Gremillet et al. 2012), American Bittern (*Botaurus lentiginosus*; Rhoades and Duke 1975), and Great Cormorant (*Phalacrocorax carbo*; Van Dobben 1952)—none of which can be classified as obligate carrion feeders—exhibit stomach pH as low or lower (pH = 0.5–1.8) than that reported for scavenging or carnivorous raptors (Duke et al. 1975, Houston and Cooper 1975). Ziswiler and Farner (1972) concluded, “The occurrence of hydrochloric acid in the gastric juice is apparently general in birds, although little is known about its actual concentration. The available data suggest, however, that pure gastric juice must have a hydrogen-ion concentration of the order of pH 0.2–1.2.”

Circumneutral pH values observed in the small intestine (pH = 5.6–7.3) and large intestine (pH = 5.9–7.3) of New World vultures were similar to those observed in the small intestine (pH = 6.0–6.5) of White-backed Vultures (Hous-

Appendix. Hydrogen ion concentration (pH) measurements from the stomach, small intestine, and large intestine of Black Vulture, Lesser Yellow-headed Vulture, and Turkey Vulture from Guyana. * = single measurement only.

USNM MUSEUM NUMBER	SPECIES	STOMACH pH	SMALL INTESTINE pH	LARGE INTESTINE pH
651997	Black Vulture	4.34	6.07	6.56
651999	Black Vulture	5.64	6.19	6.24
652001	Black Vulture	2.47	5.57	6.26
652002	Black Vulture	2.62	6.30	6.37
652003	Black Vulture	2.00	6.62	6.07
652007	Black Vulture	4.61	6.29	5.94
652010	Black Vulture	2.93	6.35	6.12
652011	Black Vulture	5.64	6.47	6.38
652012	Black Vulture	3.63	6.36	6.21
652014	Black Vulture	4.64	6.97	6.66
652015	Black Vulture	2.76	7.29	6.95
652016	Black Vulture	4.67	7.13	6.79
652017	Black Vulture	5.09	6.98	6.61
651996	Lesser Yellow-headed Vulture	3.79*	6.15*	6.10*
652000	Lesser Yellow-headed Vulture	3.53	6.33	6.27
652004	Lesser Yellow-headed Vulture	4.30	6.19	5.90
652018	Lesser Yellow-headed Vulture	4.19	6.54	7.26
652019	Lesser Yellow-headed Vulture	3.97	7.10	7.04
651995	Turkey Vulture	2.91*	6.07*	6.45*
652005	Turkey Vulture	3.49	6.06	6.51

ton and Cooper 1975). Small intestine pH measured in large samples of domestic fowl and domestic pigeon (pH = 5.2–7.5) likely brackets the intestinal pH values of all living birds (Farner 1942, 1943, Ziswiler and Farner 1972).

In summary, postprandial pH values observed in the stomach and intestines of New World vultures appear to be no more acidic than those reported for domestic fowl and non-scavenging birds that consume large animal prey. A full understanding of the role of gastrointestinal pH in the ecology and evolution of the avian microbiome will require a concerted effort to obtain new pH data from a broad taxonomic spectrum of avian species.

MEDICIONES EN CAMPO DEL PH GASTROINTESTINAL DE BUITRES DEL NUEVO MUNDO EN GUYANA

Resumen.—Los buitres del Nuevo Mundo (Cathartidae) están expuestos de manera crónica a bacterias patógenas debido a su dieta carroñera. Se ha hipotetizado que la fuerte acidez gastrointestinal actúa como un filtro robusto que reduce significativamente la diversidad bacteriana en el alimento a medida que progresa a lo largo del aparato digestivo. Sin embargo, no existen datos publicados sobre el pH gastrointestinal de los buitres de este clado monofilético. En este estudio, presento las primeras mediciones postprandiales (después de la alimentación) del pH gastrointestinal para *Coragyps atratus* ($n = 13$ individuos), *Cathartes burrovianus* ($n = 5$) y *Cathartes aura* ($n = 2$). La variación en el pH estomacal observado (2.0–5.6)

probablemente estuvo relacionada con el llenado estomacal, la dilución concomitante de los jugos gástricos por la ingesta de carroña y el tiempo desde la última alimentación. Los valores circumneutrales observados en el intestino delgado (pH 5.6–7.3) y el intestino grueso (pH 5.9–7.3) de los buitres fueron similares a los observados en la gallina doméstica y en otras aves. En resumen, los valores postprandiales del pH gastrointestinal en buitres del Nuevo Mundo no son más ácidos que los descritos para la gallina doméstica o aves no carroñeras que consumen presas animales grandes. Entender el rol del pH gastrointestinal en la ecología y la evolución del microbioma aviar va a requerir de un esfuerzo comunitario riguroso para obtener nuevos datos de un espectro taxonómico amplio de especies de aves.

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