Absorptive surface of the small intestine of three species of passerine birds with different dietary regimen: A comparative stereological study

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Abstract— *Morphology of the absorptive surface reflects the diet. Comparative stereology was done on the absorptive surface of the small intestine of similar sized, nectarivorous, omnivorous and granivorous species of passerine birds using light and electron microscopy. The aim was to determine differences that can be attributed to their disparate diets. The dimension of absorptive cells, the microvillous length, diameter and packing density were estimated. The absorptive cells are the same in the three species. The remarkable difference was the height and packing density of the microvilli. The significantly, shortest, microvilli were in the granivorous species which measured 1.11 ± 0.58 µm, intermediate in the omnivorous species, measuring 4.30 ± 0.23 µm and the nectarivorous species had significantly, tallest microvilli with a mean length of 8.025 ± 1.66 µm. The packing density was significantly lower (≤ 52.0 ± 4 per µm^2) in the granivore, intermediate in the omnivore (95.0 ± 8 per µm^2) and significantly larger (≤ 109.0 ± per µm^2) in the nectarivore. The absorptive surface in the small intestine of the nectarivore is greatly enhanced by the tall and densely packed microvilli and this type of absorptive surface is suitable for maximal absorption of liquid diet. Thus this species is more adapted to its liquid diet. The granivore has very short microvilli and a longest intestine allowing for thorough digestion and absorption and this suggests that the granivores are more adapted to their granivorous diet. The omnivore combines the qualities of the other two species.*

Keywords: Stereology, Small Intestine, Absorptive Surface, Microvillous.

I. INTRODUCTION

Stereology, a quantitative anatomical method was developed to correlate structure and function of various organs[1] by extrapolating three dimensional structural quantities from simple counts made on two dimensional slice images. The images may take various forms: physical, optical sections; magnetic resonance imaging (MRI) and computer tomographic scans (CT scans). Mayhew[2] wrote that the primary mucosal surface is anisotropic i.e. it is essentially cylindrical and has a preferred longitudinal orientation however the overall villus surface is isotropic (shows no preferred direction or orientation in space). The morphology of the alimentary tracts of mammals reflects their diets.[3] Avian gastrointestinal anatomy may vary due to differences in the diet between species. Some birds have a varied diet while others, such as honeyeaters and granivores, have restricted diets. Nectarivores (Brown honeyeaters, Hummingbirds, Sunbirds) feed exclusively on one diet, nectar[4,5] granivores (Finches) feed mainly on grass seeds. Omnivores (Silvereyes, White lateral eyes, Yellow white eyes) can feed on either nectar or

fruit-pulp.[4] The innermost layer of the avian and mammalian small intestinal mucosa projects into the lumen to form finger-like, leaf-like, or tongue-like structures referred to as villi. The villi are lined by enterocytes.[6,7] It has been known that absorptive enterocytes on the surfaces of villi possess a microvillous–glycocalyx brush border complex subserving the function of terminal digestion and absorption of ingested food.[8,9] The baso-apical location of enterocytes along villi reflects the state of cell maturity and this correlates with the levels of enzymatic activities.[10,11]

A villus does not function as a uniform unit, for example water absorption mainly occurs in the apical part of the villus[12] and the level of brush enzymes varies at different levels of the villus.[13] A component of the brush border, the microvillous membrane forms the digestive absorptive surface. The functions of this membrane are complimentary to and integrated with those of the basolateral membrane of the enterocyte and ensures effective transfer of food products across the enteocyte from the intestinal lumen to the interstitial fluid and blood.[14] The maximal absorptive efficiency of the microvilli is reflected by their surface area which is determined by their height and packing density.[7, 15] The longer and more densely packed microvilli serve to increase the surface area.[16] Mayhew et al.[17] noted that an increase in microvillous surface area is a dietary morphological adaptation.

Gut size is a functional trait rather than phylogenetic trait.[18] Wide variations in diet dictate the anatomy of the digestive tract according to the ingesta. For example, nectar is a liquid diet with a large amount of water and oligosaccharides which need little or no digestion before absorption.[4] and in contrast to grains which contain large amounts of polysaccharide that require extensive digestion before absorption. Thus, the surfaces digesting and absorbing a liquid nectarivorous diet may be different from those utilizing solid granivorous diets. Based on the functional relevance of the absorptive surface and wide variations in diets of over 4,000 passerine species, it is astonishing that "there is little information on the absorptive surface and ultrastructure of the intestines of birds and most studies have concentrated on the domestic chicken.[3] This paper describes the ultrastructural stereology of the absorptive surface in the small intestine of three species of passerines that have different dietary regimen and correlates the diet to the absorptive surface in these species.

II. METHODOLOGY

A total of fourteen adult, small passerine birds of similar size (8.1 – 13 g) were used for this study. Six specimens of *nectarivore,* and four omnivorous specimens were captured with a mist net under license and four granivorous specimens were bought from a commercial aviary. The birds were weighed and euthanized with intraperitoneal barbiturate. Birds were doused with 70% alcohol to dampen their feathers and were placed on dorsal recumbency. A ventral, midline incision was made on the *linea alba* through the skin, abdominal muscles, sternum, parietal peritoneum and the air sacs. These structures were reflected laterally to expose the visceral organs in the body cavity. The body cavity with the viscera was infused with half strength Karnovsky fixative for thirty minutes. The pericardium was incised to expose the heart and the left femoral artery was also incised. Using a 20 ml syringe and a 23 gauge needle, half strength Karnovsky fixative was injected into the left ventricle with a light thumb pressure at a rate of approximately 6 ml per minute. Perfusion continued until blood ceased to flow through the incised femoral artery and the visceral organs were pale in color. The fixative was also injected directly through the esophagus into the lumen of the gut to help wash out the digesta as well as

fix the luminal structures. Post in-situ fixation, the gastrointestinal tract was dissected free of its mesenteric attachments together with liver, pancreas and spleen and cut at the esophago-proventricular junction and cloacal junction and fixed in half strength Karnovsky fixative overnight.

The small intestine and its constituent parts (duodenum, jejunum and ileum) were measured using a thread, vernier's caliper and a ruler graduated in millimeters. Each segment was cut into small cylinders of 1 mm^3 from which one was picked randomly by lottery. The pieces were transferred into fresh fixative for one hour, washed several times in buffer solution and postfixed in 1% buffered osmium tetroxide for 90 minutes at 4^OC, sequentially dehydrated in graded alcohol and embedded in Epon. All pieces of tissue were embedded with an orientation which allowed sections to be cut transversely to the intestinal long axis.[2,10,19]

Semi-thin sections, 1µm thick sections (complete transverse section of each segment were cut, stained with toluidine blue for light microscopic analysis on Olympus BH2 microscope. The slide images were viewed by a video camera (Sony DXC-151P; Sony Corporation, Japan) and the signals relayed to and projected onto a light resolution monitor (Sony Trinitron, Sony Corporation, Japan). Different stereology grids were superimposed onto the microscope image on the monitor, using stereology program $Grid^R$ (Graffiti Data Corporation, Denmark). The software was run on an Amiga A2000 personal computer (commodore Amiga Inc., Germany). The objective lenses used were x4, x10, x20 and the average total magnification were x102, x250, x501 respectively. The x4 objective lens was used in point counting for primary mucosal surface, volume estimation and on intersection counts on the primary mucosal tube S(p) without the villi, secondary mucosal surface area S(v) surface due to villi and the tertiary surface S(m) contributed by the microvilli was calculated at the ultrathin level[20]. Mayhew[21] wrote that three parameters must be calculated first in order to estimate the absolute surface area of villi S(v) excluding the microvilli, and these are (i) the circumference of the primary mucosal tube, (ii) the surface area S(p) of the primary mucosal tube and (iii) the villous amplification factor S(v)/S(p).

Gundersen test grid lines was superimposed on microscopic images of the different segments of the intestine. The sites where the lines intersected the images of the circumference (Ib) and on the villi (Iv) were counted. From this $C = \pi/4 \times Ib \times d$

Where d = the test line spacing (given on the grid scale)

Ib = the intersection sites of test grid lines on the complete transverse section of the mucosal tube.

Iv = intersection sites of test grid lines on the villi

Segmental $S(p) = C \times l$

$S(v)/S(p) = 4/\pi \times Iv/Ib$[5]

So the absolute surface of the villi per segment: $S(v) = S(p) \times S(v)/S(p)$

Superimposing the test grid lines on images of crypt and surface epithelia was also used to estimate the thickness of the epithelium which is equivalent to the mean height of the cells. The thickness of these cells were also estimated from TEM micrographs and these corresponded reasonably well with the semithin images. Indirect estimation was done by dividing the villous epithelium V(ve) by the surface area of the villous epithelium A(ve).

EM analysis was carried out on tissue blocks (14) from the three segments of the small intestine of the three species in this study. For ultrathin sections, the tissue block was trimmed until 30-45^{O} sectors of intestinal cross section remained[10]. Ultrathin sections of 60-90 nm were collected on 200 mesh copper grids, counterstained with uranyl acetate and lead citrate and viewed under Philips 301 and CM 100 Biotwin transmission electron microscopes operated at an accelerating voltage of 80 kV.The bars of the supporting copper grids were used as the local vertical windows, fields of view were recorded in a systematic random fashion, providing the enterocytes were sectioned longitudinally to the *lamina propria* and the long axis of the microvilli were sectioned. Micrographs of some transversely sectioned microvilli were taken to measure the diameter and this strategy ensured that all regions of the villous surface were given the same chance of being selected.[2]. Sets of 10-12 fields of view per segment were printed to a final magnification of x27, 132. Print magnifications were calibrated with the aid of a carbon grating replica bearing 2160 lines/mm. The length and number of microvilli per square micron (packing density) of favorably sectioned microvilli were measured on electron micrographs with a ruler. The length of twenty microvilli per micrograph were measured and their diameter was measured from profiles in transverse sections (20/micrograph). The transverse, circular profiles were measured to cross check the diameter obtained from the longitudinal profiles. There was no significant difference between the measurements obtained from these two profiles of microvilli. Estimates of mean length and diameter were unbiased given the adequate random sampling design of the method[10]. The packing density was estimated using the longitudinal and transverse profiles of the microvilli per square micron. The microvillus surface area was calculated using πld.

Where $\boldsymbol{\pi}$ = 3.17 or 22/7; **l** = mean length and **d** = mean diameter of the microvilli

Stereology at this level was used to calculate the length and diameter of microvilli, microvillous surface area, packing density and microvillous amplification factor. The total microvillous surface per segment (segmental brush border was also calculated.

In order to calculate absolute surface area of the brush border in the different segments of the small intestine, the microvillous amplification factor S(m,v) per segment was estimated from intersection counts I(mic) and intersections between the unmodified cell surface I(v) using the quadratic test lines which was superimposed onto the photomicrographs of a final magnification of x27,132.

The microvillous amplification factor was $S(m,v) = I(mic)/I(v)$[21]

The above formula applies to zero section. Thus there is an over-projection effect due to the overestimation of S(mic)/S(v) because of section thickness of 90-100 nm thick. The bias was corrected using Gundersen equation.[22]

$$S(m,v)^* = S(m,v) / \{l + t/d.\ 2/\pi + d/l\}$$

Where S(m,v)* was the true estimate

S(m,v) was the biased estimate

t was the average of the section thickness

d, the mean diameter and l, the mean length of the microvilli

The absolute surface area of the microvilli per region or segment was estimated by multiplying the villous surface per segment by microvillous amplification factor.

$$S(mv) = \{S(m,v)\}^*$$

Values of morphometric variables for each segment were used to calculate each segmental group mean together with standard error of mean and coefficients of variations (CV) = SD given per group mean). One way analyses of variance (Statistix 4.1) were undertaken to test for the presence of species, segmental differences (species versus segmental variables). The variable terms served to indicate whether or not there was any difference between the microvilli of the various segment of the small intestine (that is if the diet had any preferred impact on particular segment of the small intestine).

III. RESULTS

3.1 Length of each segment of the small intestine:

The mean length of the duodenum in all species ranged from 22.8 ± 1.46 mm to 38.5 ± 2.18 mm (Table 1). The difference in length was highly significant (P< 0.0001). The longest duodenal segment was in the granivore and the shortest duodenum was in the nectarivore while it was intermediate in the omnivore**.**

Table 1
Morphometric variables of various species

Variables Units	Spp	Duodenum Means ± SEM	Jejunum Means ± SEM	Ileum Means ± SEM	Total Mean
Length mm	1	22.8 ± 1.46	43.75 ± 1.45	12.2 ± 1.12	78.8
	2	23.4 ± 2.02	34.13 ± 0.12	10.35 ±1.17	68.9
	3	38.5 ± 2.18	83.5 ± 2.84	18.00 ± 1.86	141
P value		**< 0.0001 S**	**< 0.0001 S**	**> 0.05 NS**	

The mean length of the jejunum was from 34.13 ± 0.12 mm to 83.5 ± 2.84 mm. The differences in length were highly significant (P< 0.0001). The granivore had the significantly longest length while the length was statistically similar in the other species (Table 1)

Amongst all species, the mean length of the ileum varied from 10.35 ± 1.17 mm to 18.0 ± 1.86 mm (Table 1). The differences in the mean length of the ileum between the species were of no statistical significance. The granivore had the longest ileum (18 mm), the omnivore had the shortest (10.35 mm) and that of the nectarivore was intermediate (12.2 mm)

3.2 Volume:

Amongst all species, the mean volume of the duodenum varied from 4.54± 0.49 mm^3 to 10.89 ± 1.35 mm^3 (Table 2). The differences in the volume of the duodenum was highly significant (P<0.0001). The significantly largest volume was in the granivore and the lowest volume was in the nectarivore. The

fractional volume of the muscular layer ranged from 0.91 ± 0.16 mm^3 to 1.78 ± 0.29 mm^3 in these species (Table 2). The differences in the volume of the muscular layer were not significant. The fractional volume of crypt epithelium varied between 1.36 ± 0.2 mm^3 to 3.9 ± 0.43 mm^3 in all species. These differences were highly significant (P<0.0001). The largest crypt volume was in the granivore and the least crypt volume was in the nectarivore. The fractional volume of the villous epithelium ranged from 2.27 ± 0.26 mm^3 to 5.21 ± 0.87 mm^3. The differences between these values was of statistical significance (P<0.01). The largest value was in the granivore and similar volumes were observed in the other two species**.**

Amongst all species, the mean volume of the jejunum ranged from 10.77 ± 1.59 mm3 to 24.5 ± 4.09 mm^3. The differences in volume was highly significant (P<0.0001). The granivore had the significantly largest volume when compared with the other two species (Table 2).

Amongst all species, there was no significant difference in the mean fractional volume of the muscular layer of the jejunum (Table 2). However, the differences in the mean fractional volume of the crypt was of high statistical significance (P<0.0001). The largest fractional crypt volume was in the granivore, smallest in the omnivore and intermediate in the nectarivore. The fractional volume of the villi ranged from 5.57 ± 1.43 mm^3 to 9.84 ± 2.03 mm^3. The differences in these values were of no statistical significance (P>0.05). The largest fractional volume of villi was in the granivore, intermediate in the omnivore and smallest in the nectarivore.

Amongst all species, the volume of the ileum ranged from 1.54 ± 0.55 mm^3 to 3.94 ± 0.31 mm^3 (table 2). The differences in the ileal volume was highly significant (P<0.0001). The granivore had the largest volume (3.94 mm^3), the nectarivore's was intermediate (1.7 mm^3) and the smallest volume (1.53 mm^3) was that of the omnivore. The largest fractional volume was in the granivore, intermediate in the omnivore and smallest in the nectarivore. In all species, the fractional crypt volume ranged from 0.25 ± 0.09 mm^3 to 1.24 ± 0.22 mm^3 (Table 2). The differences in the fractional volume of the crypt were highly significant (P<0.0001). The granivore had a significantly larger crypt volume when compared to the other two species. Amongst all species, the fractional volume of the villi was between 0.64 ± 0.15 mm^3 to 1.52 ± 0.26 mm^3 (Table 2). The differences in the fractional volume of the villi was of statistical significance (P<0.0001). The fractional volume of the villi was largest in the granivore (1.52 mm^3), smallest in omnivore (0.64 mm^3) and intermediate in the nectarivore (0.69 mm^3).

3.3 Percentages:

The mean percentage volume contributed by the muscular layer to the thickness of the wall of the duodenum ranged from 16.4 ± 0.81% to 34.8 ± 3.73% in all species. These differences were of no statistical significance. The crypt epithelium accounted for between 25.0 ± 1.84% to 35.8 ± 3.33%. There was no statistical difference in these percentages. The villous epithelium added from 47.5 ± 3.53% to 50.0 ± 2.04%. These values were of no statistical difference in all species **(**Table 2**).** The villous epithelium contributed the largest proportion to the duodenal wall.

Table 2
Summary of segmental differences as revealed by light microscopical morphometry.

Variables	Species	Duodenum Means ± SEM	Jejunum Means ± SEM	Ileum Means ± SEM
B(p) mm	1	0.198 ± 0.007	0.125 ± 0.02	0.089 ± 0.003
	2	0.161 ± 0.003	0.139 ± 0.002	0.086 ± 0.001
	3	0.184 ± 0.001	0.100 ± 0.008	0.099 ± 0.009
P value		**> 0.05 NS**	**> 0.05 NS**	**> 0.05 NS**
S(p) mm^2	1	4.31 ± 0.35	5.7 ± 0.8	1.2 ± 0.3
	2	3.59 ± 0.26	3.9 ± 0.5	1.09 ± 0.26
	3	7.00 ± 0.29	8.4 ± 0.75	1.83 ± 0.1
P value		**< 0.0001 s**	**< 0.05 s**	**< 0.05 s**
Ss(v,p) cm^0	1	2.37 ± 0.2	2.9 ± 0.45	2.8 ± 0.3
	2	4.50 ± 0.6	4.7 ± 0.41	3.7 ± 0.5
	3	3.39 ± 0.26	4.4 ± 0.6	3.1 ± 0.3
P value		**< 0.0001 S**	**> 0.05 NS**	**> 0.05 NS**
S(v) cm^2	1	10.0 ± 0.9	15.0 ±2.2	3.5 ± 1.0
	2	15.7 ± 1.4	19.0 ± 3.8	3.6 ±0.4
	3	23.8 ± 1.48	36.0 ± 4.0	5.7 ± 0.7
P value		**< 0.0001 S**	**< 0.05 S**	**< 0.05 S**
Vol(i) mm^3	1	4.54 ± 0.49	10.77 ± 1.59	1.7 ± 0.31
	2	5.72 ± 0.45	10.92 ± 0.22	1.54 ± 0.55
	3	10.89 ± 1.35	24.5 ± 4.09	3.94 ± 0.31
P value		**< 0.0001 S**	**< 0.0001 S**	**< 0.0001 S**
V(v) mm^3	1	2.27 ± 0.26	5.57 ± 1.43	0.69 ± 0.14
	2	2.30 ± 0.25	6.06 ± 0.47	0.64 ± 0.15
	3.	5.21 ± 0.87	9.84 ± 2.03	1.52 ± 0.26
P value		**< 0.01 S**	**> 0.05 NS**	**< 0.0001 S**
V(c) mm^3	1	1.36 ± 0.2	2.91 ± 0.41	0.46 ± 0.1
	2	1.43. ± 0.21	2.25 ± 0.13	0.25 ± 0.09
	3	3.9 ± 0.43	9.31 ± 1.1	1.24 ± 0.22
P value		**< 0.0001 S**	**< 0.0001 S**	**< 0.0001 S**
V(m) mm^3	1	0.91 ± 0.16	2.29 ± 0.46	0.55 ± 0.17
	2	1.99 ± 0.82	2.61 ± 0.55	0.65 ± 0.3
	3	1.78 ± 0.29	5.35 ± 1.58	1.18 ± 0.13
P value		**> 0.05 NS**	**> 0.05 NS**	**< 0.0001 S**

Where, B(p) is the circumference of the primary mucosal tube.
S(p) is the surface area of the primary mucosal tube.
S(v) is the surface area of the villi.
Ss(v,p) is the villous amplification factor.
V(c) is the volume density of crypt.
V(m) is the volume density of muscle layer.
Vol(i) is the volume of the intestinal segment i.
V(v) is the volume density of villi.

The mean percentage volume of the muscular wall ranged from 21.3 ± 3.41 to 23.9 ± 4.61. The omnivore had the highest percentage (23.9%), the nectarivore was lowest (21.3%) and the granivore was intermediate (21.8%). These differences were of no statistical significance. The mean percentage volume of the crypt layer varied from 20.6 ± 0.82 to 38.0 ± 3.05. The granivore had the highest percentage (38%), nectarivore was intermediate (27%) and omnivore had the smallest percentage (20.6%). Thus, the proportion of 38% seen in the granivore was significantly higher ($P<0.05$) than 20.6% seen in the omnivore. The mean percentage volume of the villi epithelium accounted for the largest percentage of the wall of the jejunum in all species and ranged from 40.2 ± 3.6 to 55.5 ± 5.4

(Table 2). However, there was no statistical difference in the percentage contributed by the villi epithelium (Table 2)

The mean percentage contributed by each layer to the wall of the ileal segment varied in each species. The muscular layer of the ileum added from 29.9 ± 4.2% to 42.2 ± 4.9%. The differences in the mean percentage volume of the muscular layer of the ileum were of no statistical significance in all species (Table 2). The highest percentage (42.2%) was in the omnivore and the lowest (29.9%) was in the granivore. The mean percentage volume of the crypt layer ranged from 16.2 ± 0.78% to 31.5 ± 4.6%. These differences were of no significance in all species. The highest (31.5%) percentage was in the granivore and the lowest (16.2%) was in the omnivore. The mean percentage volume contributed by the villi ranged from 38.6 ± 4.1% to 41.6 ± 5.8%. There was no significant difference in these values in all species. The highest value (41.6%) was in the omnivore while the lowest (38.6%) value was in the granivore.

3.4 Surface area of primary mucosal tube

The mean surface area of the primary mucosal tube ranged from 3.59 ± 0.26 cm^2 to 7.0 ± 0.29 cm^2 in all species (Table 2). The differences in the surface area of the primary mucosal tube were highly significant (P< 0.0001). The granivore had a significantly larger surface area of the primary mucosal tube when compared with the other two species. The other two species had similar values.

In all species, the mean surface area of the primary mucosal (jejunal) tube varied from 3.9 ± 0.5 cm^2 to 8.4 ± 0.75 (Table 2). There were significant differences (P<0.005) between these values. The surface area was significantly larger in the granivore than in the omnivore.

The mean surface area of the primary mucosal tube of the ileum (table 2) varied from 1.09 ± 0.26 cm^2 to 1.83 ± 0.1 cm2 in all species. The differences in these values were statistically significant (P<0.005). The surface area of ileal primary mucosal tube was largest in the granivore while these values were statistically similar in the other two species.

3.5 Villous amplification factor in each segment:

The mean villous amplification factor in the duodenum of all species was between 2.37 ± 0.2 times to 4.5 ± 0.6 (Table 2). The differences in the villous amplification factor were highly significant (P<0.0001) in all species. The largest amplification factor was in the omnivore and this was quite significant from that of the granivore and the least value was in the nectarivore. The value in the granivore was significantly different from that of the nectarivore.

Amongst all species, the mean villous amplification factor in the jejunum of all species varied from 2.9 ± 0.45 to 4.7 ± 0.41 (Table 2). The differences in the villous amplification factor were of no statistical significance (P>0.05). The omnivore had a slightly larger amplification factor than the granivore and the nectarivore was lowest.

In all species, the mean villous amplification factor ranged from 2.8 ± 0.3 times to 3.7 ± 0.5 times (Table 2). The differences in the villous amplification factor were of no significance (P>0.05).

3.6 Surface area of villi in the segments:

The mean surface area of villi in the duodenum ranged from 10.0 ± 0.9 cm^2 to 23.6 ± 1.48 cm2 (table 2). The differences in the villous surface area of the villi were of high statistical significance (P<0.0001).

The villous surface area in the duodenum of the granivore was larger than in the other two species. This value was statistically similar in the other two species. This value in the granivore was significantly different from that of the nectarivore and intermediate in the omnivore.

The mean surface area of villi in the jejunum varied from 15.0 ± 2.2 cm^2 to 36.0 ± 4.0 cm^2 (table 2) in all species. The differences in the villous surface area of the jejunum were very significant (P<0.005). The granivore had a significantly larger surface area of the villi in the jejunum while the other two species had similar values.

Amongst all species, the mean surface area of the villi in the ileum was from 3.5 ± 1.0 to 5.7 ± 0.7 cm^2. (Table 2). These differences were of significant value (P<0.005). The largest villous surface area was in the granivore, while these values were statistically comparable in the other two species.

3.7 Absorptive chief cells of the duodenum:

The height of duodenal absorptive chief cells ranged from 31.6± 1.47 µm to 36.6 ± 0.29 µm in all species (Table 3). There was no significant difference in the height of these cells in all species. The height of jejunal absorptive chief cells ranged from 32.3 ± 1.2 to 38.7 ± 1.4. In all species, the mean height of the absorptive chief cells of the ileum ranged from 25.7 ± 1.2 µm to 28.9 ± 1.4 µm. The differences in the height of the absorptive chief cells were of no statistical significance in all segments of the small intestine (Table 3).

3.8 Diameter of microvilli:

Amongst all species, the mean microvillous diameter in the duodenum ranged from 54 ± 20 nm to 119 ± 4 nm (Table 3). There was no significant difference in the diameter of the microvilli.

Amongst all species, the mean diameter of jejunal microvilli ranged from 92 ± 6 nm to 129 ± 2 nm (Table 3). There was a significant difference in the microvillous diameter of all species (P<0.05). The granivore had significantly wider microvilli. The diameter of the microvilli was widest in the granivore, intermediate in the omnivore and narrowest in the nectarivore.

In all species, the mean diameter of the ileal microvilli ranged from 75 ± 3 nm to 130 ± 15 nm (Table 3). There was no significant difference in the diameter of the ileal microvilli of all species.

3.9 Segmental microvillous height:

The height of microvilli on the duodenal absorptive chief cells ranged from 1.11 ± 0.58 to 5.95 ± 0.5 µm (Table 3). The height of the microvilli in the nectarivore was significantly taller than that of the other two species (P<0.0001). The tallest microvilli were in the nectarivore, the shortest were in the granivore while they were intermediate in the omnivore.

In all species, the mean height of microvilli of the jejunum was from 1.1 ± 0.133 µm to 8.025 ± 1.66 µm (Table 3). The tallest microvilli were in the nectarivore, shortest in the granivore and intermediate in the omnivore. The differences in the height of the microvilli in this segment were highly significant (P< 0.0001) in all species. The microvilli were significantly taller in the nectarivore than in the other two species.

Amongst all species, the mean height of the microvilli in the ileum varied from 1.6 ± 0.66 µm to 4.96 ± 0.88 µm (Table 3). The differences in the height of the microvilli was of statistical significance (P<0.01). The microvilli were significantly tallest in the nectarivore than the other two species. species.

3.10 Microvillous amplification factor in the duodenum:

The mean microvillous amplification factor in the duodenum was between 1.81 ± 0.64 times to 4.25 ± 1.45 times (Table 3). Although there were differences in the microvillous amplification factors, these differences were of no statistical significance.

The mean jejunal microvillous amplification factor varied from 2.08 ± 0.1 to 3.6 ± 0.68 times in all species. The amplification factor was highest in the omnivore, lowest in the granivore and intermediate in the nectarivore (Table 3). The differences in the amplification factors were of no statistical significance (P>0.05).

Amongst all species, the mean microvillous amplification factor in the ileal segment was from 2.2 ± 1.0 times 2.8 ± 0.2 times (Table 3). These differences were of no statistical significance in all species.

Table 3
Summary of segmental differences as revealed by electron microscopical morphometry.

Variables Units	Species	Duodenum Means ± SEM	Jejunum Means ± SEM	Ileum Means ± SEM
H(ac) um	1	32.2 ± 1.6	38.7 ± 1.4	25.7 ± 1.2
	2	31.6 ± 1.47	32.3 ± 1.2	26.8 ± 1.3
	3	36.6 ± 0.29	36.5 ± 1.3	28.9 ± 1.4
P value		**> 0.05 NS**	**> 0.05 NS**	**> 0.05 NS**
D(m) nm	1	110 ± 11	92.0 ± 6	75 ± 3
	2	54 ± 20	104 ± 12	110 ± 1
	3	119 ± 4	129 ± 2	130 ± 15
P value		**> 0.05 NS**	**< 0.05 S**	**> 0.05 NS**
H(m) µm	1	5.95 ± 0.5	8.025 ± 1.66	4.96 ± 0.88
	2	3.45 ± 0.28	4.3 ± 0.23	3.7 ± 0.1
	3	1.11 ± 0.58	1.1 ± 0.133	1.6 ± 0.66
P value		**< 0.0001 S**	**< 0.0001 S**	**< 0.0001 S**
Pd(m) #/µm^2	1	76.88 ± 8.4	109.0 ± 15	82.0 ± 11
	2	74.56 ± 4.1	95.0 ± 8.0	75.0 ± 11
	3	59.67 ± 2.63	52.0 ± 4.0	59.0 ± 7.0
P value		**> 0.05 NS**	**< 0.05 S**	**< 0.01 S**
Ss(m,v) cm^0	1	4.25 ± 1.45	2.5 ± 0.5	2.5 ± 0.4
	2	1.81 ± 0.64	3.6 ± 0.7	2.8 ± 0.2
	3	3.3 ± 0.58	2.08 ± 0.1	2.2 ± 1.0
P value		**> 0.05 NS**	**> 0.05 NS**	**> 0.05 NS**
S(m) cm^2	1	40.0 ± 11.7	42.0 ± 14.0	9.81 ± 0.4
	2	27.0 ± 9.8	61.0 ± 5.2	10.0 ± 1.4
	3	75.6 ± 12.1	75.0 ± 10.0	13.1 ± 1.5
P value		**> 0.05 NS**	**> 0.05 NS**	**> 0.05 NS**

Where, D(m) is the mean diameter of microvilli.
H(ac) is the mean height/length of absorptive chief cell.
H(m) is the mean height /length of the microvilli.
Pd(m) is the mean packing density of the microvilli.
S(m) is the mean surface area of the microvilli.
Ss(m,v) is the mean microvillous amplification factor.

3.11 Packing density:

In all species, the mean packing density of the microvilli in the duodenum was from 59.67 ± 2.63/µm^2 to 76.18 ± 8.4/µm2 (Table 3). The differences in the packing density per square micron were of no statistical significance.

Amongst all species, the mean packing density of microvilli in the jejunum was from 52/µm2 ± 4 to 109/µm^2± 15. The highest density was in the nectarivore, lowest in the granivore and intermediate in the omnivore. The packing density of the jejunal microvilli was significantly higher in the nectarivore (P< 0.05) than in the granivore (Table 3).

The mean packing density of the microvilli in the ileum ranged from 59/µm2 ± 7 to 82/µm2 ± 11. These differences were of statistical significance (P<0.01). The microvilli were more densely packed in the nectarivore than in the granivore.

3.12 Surface area of microvilli:

The mean microvilli surface area in the duodenal segment ranged from 40.05 ± 11.7 cm^2 to 75.6 ± 12.1 cm^2 (Table 3). The differences in the microvillous surface area were of no statistical significance (P>0.05).

The mean jejunal surface area of microvilli varied from 42.0 ± 14.0 to 75.0 ± 10.0 cm^2 (Table 3). There was no significant difference in the microvillous surface area (P>0.05) in this segment of all species.

In all species, the mean surface area of the microvilli in the ileum varied from 9.81 ± 0.4 cm^2. to 13.1 ± 1.5 cm^2 (Table 3). The differences in the surface area of the microvilli of this segment was of no statistical significance.

IV. DISCUSSION

The ultrastructural appearance of the enterocytes of the three species was similar. There was a proximo-distal variation in the segmental variables. The mid intestinal segment (jejunum) exhibited the largest value for all the variables (largest surface and largest volume while the least surface and volume was in the ileum of all species. The current results support the existence of parallel gradients in apico-basal variations in the internal structures of a normal villus: absolute villus surface area, villus amplification factor and villus mass.[7,19] The present results agree with the report that the microvillous amplification factor varies with the intestinal location.[10] The absolute surface due to microvilli also exhibited regional differences as shown by the microvillous amplification factor. The concept that regional differences in relative and absolute surface areas was due to local fluctuations in the length of the microvilli rather than the mean diameter and mean packing density[10] is consistent with the observations on the birds examined in the current study. The apparent segmental differences in the total complement of microvilli observed in this study did not attain statistical significance for these segments and this agrees with the conclusion of Mayhew.[10]

4.1 Interspecies variation:

It has long been known that diet dictates the morphology of the gut: fibrous diet requires a longer gut while energy rich diet needs a shorter gut.[23] The above statement is true to the current species of passerine birds, granivores had significantly longer intestine than the nectarivorous species. The current observations also support the report that birds fed on a high energy diet have the shortest small intestine and the smallest villi while those fed on an energy-dilute diet have the largest villus length, thickest lamina muscularis and greatest diameter of the small intestine.[23] The nectarivore, which feeds on an energy rich diet, had a significantly shorter intestine than the granivore feeding on an energy dilute diet and the omnivorous species combines the qualities of the other two species. The granivore had the significantly largest volume and absolute surface of intestinal villi in all three segments of the small

intestine, while the nectarivore had the smallest volume and smallest absolute surface area of villi. The villous amplification factor attained statistical significance only in the duodenal segment of the intestine of the granivore. These values were intermediate in the omnivorous species. The nectarivore had the significantly tallest microvilli in all the three segments of the small intestine. The microvilli were shortest in the granivore and intermediate in the omnivore. The diameter of the microvilli was widest in the granivorous species. The microvillous packing density attained statistical significance in the jejunal and ileal segments of the intestine, however the differences in the absolute microvillous surface did not attain statistical significance in all the intestinal segments of all species.

This present study supports the concept that the combined effect of villi and microvilli along the vertebrate intestine is to increase the basic mucosal surface area.[19] Villi and microvilli jointly enlarge the basic mucosal tube more than 75-fold in the nectarivore, 83-fold in the granivore and 106-fold in the omnivore, confirming that the omnivore has combined qualities of the other two species. The observation in the present study agrees with the report that the more proximal stations of the small bowel have a greater surface area to subserve the functions of digestion and absorption of nutrients.[10]

4.2 Relation to diet:

It has long been recognized that absorptive enterocytes on the surfaces of villi possess a microvillous-glycocalyx or brush border complex subserving the function of terminal digestion and absorption of ingested food[8,13,24] and the enzymes involved in the breakdown of oligopeptides and oligosaccharides are embedded in the digestive-absorptive surface (microvillus glycocalyx) of enterocytes, particularly in the proximal regions.[9] The claim that the digestive and absorptive efficiency of these microvilli are portrayed by their density and surface area.[15] is reinforced by the present study.

An increase in surface area is characterized by an increase in height and density of the microvilli.[7] The longer and more densely packed microvilli serve to increase the surface area for absorption.[16,25] The above reports are consistent with the quantitative results of the intestinal tracts of the birds examined for this study particularly those of the nectarivorous and the omnivorous species. The nectarivorous species had the largest packing density and significantly tallest microvilli.

The report that an increase in microvillous surface area is a dietary morphological adaptation[17] concurs with the present observation in that the villous amplification factor and villous surface area of the intestinal tract of the nectarivorous species are significantly less than in the other two species. However, the surface area of the microvilli attained the same statistical significance as that of the granivore and the omnivore.

The liquid diet of the nectarivore has a very short transit time in comparison with the diet of the other two species. The maximum absorptive efficiency of microvilli is reflected by their density.[15] The maximal digestive and absorptive functions of this organelle are portrayed by their increase in surface area which is reflected by an increase in cell width as well as increase in length (height) and packing density of the microvilli.[7] Tedman and Hall[25] observed a relatively high density ($\leq 79/\mu m^2$) of very tall (< 5.7 μm) microvilli, resulting in an enormous increase in the absorptive surface area of small intestinal epithelial cell of frugivorous bat. These authors concluded that the relative high density and very tall microvilli are very important in maximizing the absorption of fluid digesta to compensate for the short exposure to absorptive cells because of the short food transit time. The longer and more densely packed microvilli serve to increase the surface area for absorption.[16,17] It is important to note that fruit bats,

which depend heavily on nectar and pollen[26] and makes them somewhat comparable to the nectarivorous avian species in the present study, exhibited the above characteristics.[16,25] These reports are consistent with the observation made on the intestinal tract of the nectarivores[honeyeaters) in that this species had a high packing density ($\leq 109.25/\mu m^2$) of very tall (8.02 μm) microvilli. Karasov et al.[27] noted that glucose extraction efficiency of hummingbirds (nectarivores) is 97-98%. The tall and densely packed microvilli observed in the nectarivorous species have greatly increased their absorptive surface area suggesting that they may have a high (97-99%) glucose extraction efficiency from nectar. The morphological adaptation to the liquid diet of nectar is reflected by the tall and densely packed microvilli observed in the nectarivorous species. The tall and densely packed microvilli are very important in maximizing the absorption of predominantly fluid digesta.[25] Thus the nectarivorous species has the adaptation of tall and densely packed microvilli to compensate for the short exposure of its liquid diet to the absorptive cells to maximize the absorption of glucose from nectar.

Nectar contains a large amount of water and hexoses with small quantities of lipids, amino acids and the essential ions, sodium and potassium.[28] The gastrointestinal tract of nectarivorous birds should be adapted to efficiently conserve the limited quantity of ions available from nectar. Thus their intestine should possess a high proportion of microvillous surface. Mayhew et al.[21] reported that the increase in microvillous surface leads to enhanced sodium absorption and such an event may be true in the nectarivorous species.

The significantly longer small intestine of the granivore with the shortest (1.9 μm) and less densely packed ($\leq 52/\mu m^2$) microvilli is suggestive of dietary morphological adaptation to their granivorous diets as the digesta stays longer in the relatively longer tract and is thereby thoroughly digested and absorbed.

Overall, the omnivorous species has values intermediate between the other two species. It has a larger microvilli packing density ($\leq 95/\mu m^2$) than the granivorous species and shorter microvilli (4.3 μm.) than the nectarivorous species. The omnivorous species is intermediate between the nectarivorous and the granivorous species. Thus the omnivorous species combines the qualities of the other two species. The shortest (1.09 μm) and less densely packed ($\leq 52.39/\mu m^2$) microvilli in the granivores is suggestive of a functional, morphological adaptation. The reduced packing density is compensated for by the fact that the digesta stays longer in the relatively longer tract to ensure thorough absorption.

The packing density of the microvilli in the omnivore was $\leq 95.25/\mu m^2$ and the height was 4.3 μm. The omnivorous species is intermediate between the nectarivorous and the granivorous species. This species combines the qualities of the other two species.

V. CONCLUSION

It can be concluded from this study that histologically, the cells are the same in all three species. The appearance of the absorptive chief cells is consistent with that of the mammalian and avian absorptive chief cells. The small intestine is longest in the granivore while the other two species have similar length. The villous surface is largest in the granivore than that of the nectarivore. The omnivorous species has the largest amplification factor. The microvilli are taller and more densely packed in the omnivore than those of the granivore while they are shorter when compared to that of the nectarivore. The absorptive surface area in the nectarivore is attributable to the very tall and densely packed microvilli and is more adapted to the liquid diet. The granivore has short microvilli and a long intestine

which are more adapted to its granivorous diet. The omnivore combines the qualities of the other two species.

CONFLICT OF INTEREST

None declared till now.

REFERENCES

[1] Mayhew, T. M. 1991. The new stereological methods for interpreting functional morphology from slices of cells and organs. Experimental Physiology 70, 639 – 665.

[2] Mayhew, T. M. 1984. Geometric model of the rat intestinal mucosa for stereological evaluation of villus amplification factors. Journal of Microscopy 135, 337-346.

[3] McLelland, J. 1979: Digestive system. In Form and Function in Birds. Vol.1, (Eds. A. S. King and J. McLelland) pp 69-181 , New York, Academic Press.

[4] Pyke, G. H. (1980). The foraging behaviour of Australian honeyeaters: a review and some comparisons with hummingbirds. Australian Journal of Ecology 5, 343-369.

[5] Martinez Del Rio, C. (1990). Sugar preferences in hummingbirds: the influence of subtle chemical differences on food choice. The Condor 92, 1022-1030.

[6] Hodges, R. D. 1974: The digestive system. In The Histology of the Fowl, pp 64-80. London Academic Press.

[7] Abbas, B. Hayes, T.L., Wilson, D. J. and Carr, K. E. 1989: Internal structure of the intestinal villus: morphological and morphometric observations at different levels of the mouse villus. J. Anatomy **162**, 263-273.

[8] Van Dongen, J. M., Visser, W. J., Daems, W. T. and Galjaard, H. 1976: The relation between cell proliferation, differentiation and ultrastructural development in rat intestinal epithelium. Cell and Tissue Research **174,**183-199.

[9] Mayhew, T. M., Middleton, C. & Ross, G. A. 1988. Dealing with oriented surfaces: studies on villi and microvilli of rat small intestine. In Stereology and Morphometry in Electron Microscopy: Problems and Solutions (eds. A. Reith & T. M. Mayhew), pp 85-100. New York, Hemisphere Publishing Corporation.

[10] Mayhew, T. M. (1987). Quantitative ultrastructural study on the responses of the microvilli along the small bowel to fasting. Journal of Anatomy 154, 237-243.

[11] Humphrey, C. D. and Turk, D. E. 1974: The ultrastructure of normal chick intestinal epithelium. Poultry Science **53,** 990-1000.

[12] McElligott, T F., Beck I. T., Dinda, P. K and Thompson, S. 1975: Correlation of structural changes at different levels of the jejunai villus with positive and negative net water transport in vivo and in vitro. Canadian Journal of Physiology and Pharmacology **53**, 439-450.

[13] Pothier, P. and Hugon, J. S. 1980: Characterisation of isolated villus and crypt cells from the small intestine of adult mouse. Cell and Tissue Research **211**, 405-418.

[14] Holmes, R. D., and Lobley, R. W. 1989: Intestinal brush border revisited. Gut **30**, 1667-1678.

[15] Bayer, R. C, Chawan, C. B., Bird, F. H. and Musgrave, S. D. 1975: Characteristics of the absorptive surface of the small intestine of the chicken from 1 day to 14 weeks of age. Poultry Science **54**, 155-169.

[16] Keagan, D. and Modinger, R. 1979: Microvilli of the intestinal mucosal cells of *Rousettus aegypttacus*. South African Journal of Zoology **14**, 220-223.

[17] Mayhew, T. M., Elbrond, V. S., Dantzer, V., & Skadhauge, E. (1992). Quantitative analysis of factors contributing to expansion of microvillous surface area in the coprodaeum of hens transferred to a low NaCl diet. Journal of Anatomy 181, 73-77.

[18] Richardson, K. C. and Wooller, R. D. 1986: The structure of gastrointestinal tracts of honeyeaters and other small birds in relation to their diets. Australian Journal of Zoology 34, 119-124.

[19] Mayhew, T. M. & Middleton, C. (1985). Crypts, villi and microvilli in the small intestine of the rat. A stereological study of their variability within and between animals. Journal of Anatomy 141, 1-17.

[20] Gundersen, H. J. G. & Jensen, E. B. (1985). Stereological estimation of the volume-weighted mean volume of arbitrary particles observed on random sections. Journal of Microscopy 138, 127-142.

[21] Mayhew, T. M. (1990). The striated brush border of intestinal absorptive epithelial cells: stereological studies on microvillous morphology in different adaptive states. Journal of Electron Microscopy Technique 16, 45-55.

[22] Gundersen, H. J. G. (1979). Estimation of tubule or cylinder Lv, Sv and Vv on thick sections. Journal of Microscopy 117, 333-345.

[23] Brugger, K. E. 1991. Anatomical adaptations of the gut to the diet in Red-winged Blackbirds, Agelaius phoeniceus. The Auk, 108, 562-567.

[24] Harrison, J. D. & Stather, J. W. 1996. The assessment of doses and effects from intakes of radioactive particles. Journal of Anatomy 189, 521-530.

[25] Tedman, R. A. and Hall, L. S. 1985: The absorptive surface of the small intestine of Pteropus poliocephalus (Megachiroptera: Pteropodidae): an important factor in rapid food transit? Australian Mammalogy 8, 271-278.

[26] Nelson, J. E. 1965: Movements of Australian flying foxes (Pteropodidae: Megachroptera) Australian Journal of Zoology **13**, 53-73.

[27] Karasov, W. H. Phan, D. Diamond, J. M., Carpenter, F. L., 1986: Food passage and intestinal nutrient absorption in hummingbirds. The Auk **103** (3), 453-464.

[28] Casotti, G. & Richardson, K. E. (1992). A stereological. analysis of kidney structure of honeyeater birds (Meliphagidae) inhabiting either arid or wet environments. Journal of Anatomy 180, 281-288.