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TECHNICAL COMMENT

Tissue Damage Prelues the Use of the Everte Sleeve Tehniue to Measure Nutrient Utake in a Small Migratory Shorebir, the Western Sanier (Calidris mauri)

R. Will Ste*in
Tony D. Williams
Centre for Wildlife Ecology, Department of Biological Sciences, Simon Fraser University, Burnaby, British Columbia V5A 1S6, Canada

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Introution

In vitro measurement of nutrient (e.g., glucose and proline)

correcting for unabsorbed L-proline, passive and combined uptake values were normalized to intestinal sleeve mass (pmol mg^{-1} min^{-1}) and were expressed relative to absolute combined uptake at 50 mM in the same intestinal region of the same individual. Using relative uptake minimizes the effect of positional and interindividual variation (Karasov and Diamond 1983). The apparent passive permeability coefficient of L-proline was determined by simple linear regression of relative passive uptake on L-proline concentration:

$$\text{Relative passive uptake} = (A \times [\text{P}]).$$

The apparent passive permeability coefficient of proline is estimated by A, while [P] is the proline concentration (mmol) of the incubation solution. The analysis of relative combined uptake provides a second estimate of the apparent passive permeability coefficient of L-proline. The apparent carrier-mediated components of relative L-proline uptake were determined by nonlinear regression of relative combined uptake on L-proline concentration. The model for relative combined uptake incorporates passive and mediated (one carrier) uptake:

$$\text{Relative combined uptake} = (A \times [\text{P}]) + [(J_{\max} \times [\text{P}]) \div (K_{\text{m}} + [\text{P}])^{-1}].$$

The apparent maximal mediated uptake rate was estimated by $J_{\max}$. The apparent Michaelis constant, that is, the proline concentration at which uptake is 0.5 × apparent $J_{\max}$, was estimated by K_{m}, which is uncorrected for the effects of unstirred layers.

Histology

To obtain descriptive data on the internal morphology of the small intestine, histological sections were prepared from the proximal and distal duodenum, jejunum, and proximal and distal ileum of recently captured juvenile migrants (= 4) and long-term captive adults (= 4). To evaluate the effects of tissue handling during nutrient uptake experiments, histological sections were prepared from the jejunum (1) before eversion, (2) after eversion but before incubation, and (3) after eversion, preincubation for 5 min, and incubation for 2 min in a proline solution that was being stirred at 1,200 rpm. As before, a mucous-like material was extruded from the small intestine during eversion. Tissue sections were fixed in 10% formalin in 0.1 M phosphate-buffered saline, pH 7.4, for 48–72 h at room temperature. Fixed tissue sections were divided into subsections with a razor blade. Tissue subsections were dehydrated in 70% ethanol and 30% xylene, followed by 100% xylene, and then embedded in paraffin. Cross sections of the tissue subsections were cut at 5 μm on a rotary paraffin microtome, mounted onto microscope slides, and stained with hematoxylin and eosin.

Digital images of individual cross sections were obtained at four-power magnification with an Olympus Vanox microscope connected to a computer. Images were analyzed with a PC-based image analysis software (Empix Imaging 2001). Before analysis, digital images were converted to 8 bit gray scale, which allows contrast manipulation. For each section, total cross-sectional area, inner cross-sectional area (determined by the distinction between the mucosa and smooth muscle [ ]), circumference, villus length (broken and intact villi were measured separately), and villi number were measured. These measurements were made on digitized images of eight to 10 cross sections from three to four tissue subsections for each tissue section. The thickness of the smooth muscle layer was not uniform. In order to determine the mean thickness of the muscle layer, the circular shape of the intestinal cross sections was used to calculate the lengths of the radii of circles with areas equal to the total cross sectional and inner cross-sectional areas. The difference between these calculated radii was reported as the mean muscle thickness. Lumen area of intact cross sections and mucosal area of everted sections were obtained by adjusting the contrast of the digital images and then employing threshold analysis. Threshold analysis calculates the area within a defined polygon and partitions this area based on pixel contrast. Subsequently, the percentages of smooth muscle, mucosa, and lumen were calculated based on cross-sectional area. To avoid pseudoreplication, statistical analyses were performed on the mean values for a single tissue section.

Body Size

As a measure of structural body size, we used the first component (PC1) from a single principal components analysis (PCA) of culmen, tarsus, and keel length (Rising and Somers 1989) performed on all of the birds included in this study. PC1 explained 57% of the variation in the univariate measures of structural body size, which all had large positive loadings (culmen = 0.60, tarsus = 0.64, and keel = 0.49) on PC1 (eigenvalue = 1.71). Within each age class, the two sample -test was used to detect annual differences in body size (univariate: culmen, tarsus, and keel; multivariate: PC1) and body mass (capture and dissection). ANCOVA was used to examine the influence of year and acclimation to captivity on small intestine length and mass. The time that elapsed between capture and dissection was used as a covariate in the analysis of intestine length, because intestine length is known to increase after feeding (Robel et al. 1990); it is expected to contract while fasting. Small intestine length was used as a covariate in analysis of small intestine mass; this resulted in an analysis of length-corrected mass. Relationships between variables were examined using linear and nonlinear regression. Repeated-measures ANOVA was used to examine changes in body mass of captive sandpipers, variation in morphometry along the length of the

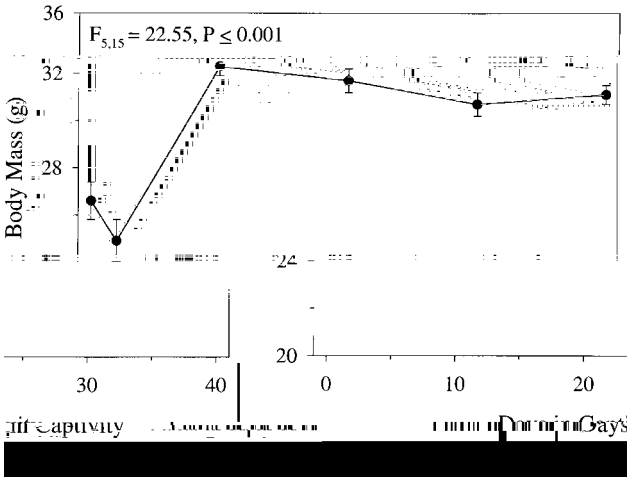
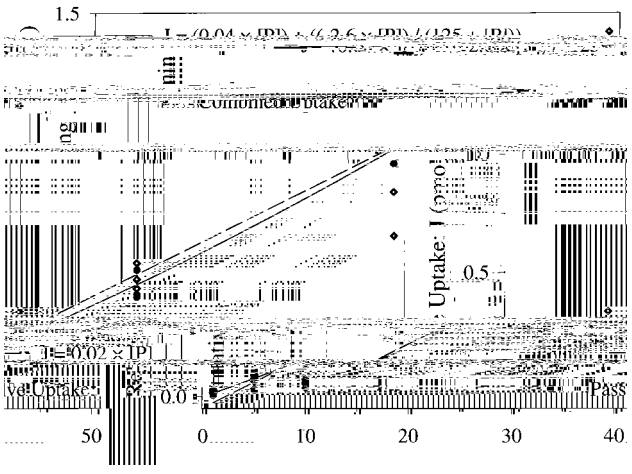


Figure 1. Changes in the body mass of female western sandpipers during acclimation to captivity (p 4). Values are means ± SEM.

Table 1: Body mass and small intestine size of recently captured migrant and long-term captive female western sandpipers (p 28) used in uptake experiments and histological studies

	Juveniles		Adults	
Parameter	1999 Migrants' Uptake	2000 Migrants' Histology	1999 Migrants' Uptake	2000 Captives' Histology
Capture mass (g)	29.1 ± .8[a]	25.4 ± .3[b]	25.8 ± 1.0[a]	26.6 ± .8[a]
Dissection mass (g)	26.2 ± .7			


1.5
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0.5
Pass
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50
0
10
20
30
40

Table 3: Small intestine morphometry of long-term captive adult female western sandpipers (p 4)

Morphometric Parameter	Proximal Duodenum	Distal Duodenum	Jejunum	Proximal Ileum	Distal Ileum
Linear measures:					
Circumference (mm)	6.0 ± .2[a]	5.9 ± .2[a]	5.0 ± .1[b]	4.6 ± .2[bc]	4.4 ± .0[c]
Muscle width (mm)	80 ± 2[a]	81 ± 3[a]	90 ± 4[ab]	112 ± 10[b]	124 ± 16[ab]
Villus length (mm)[a]	692 ± 51[a]	656 ± 51[a]	521 ± 59[ab]	436 ± 25[b]	441 ± 15[b]
Villi number	30 ± .9[a]	30 ± 1.0[a]	29 ± .2[a]	28 ± .7[a]	31 ± .5[a]
Area percentages:					
Muscle	16 ± 1[a]	17 ± 1[a]	22 ± 2[b]	28 ± 2[c]	32 ± 4[c]
Mucosa	70 ± 1[a]	66 ± 2[a]	62 ± 3[b]	56 ± 3[b]	52 ± 3[b]
Lumen	14 ± 1[a]	17 ± 1[a]	16 ± 2[a]	16 ± 2[a]	16 ± 3[ab]
	4	4	4	4	4

Note. Values are means ± SEM. Different superscripts indicate statistically significant differences, 0.05.
[a] Intact villus length.

formed by long, delicate villi. The connective tissue core (*a aa*) of the villi was characterized by the presence of smooth muscle cells and small blood vessels. The mucosal epithelium (*aaa*) was composed primarily of enterocytes with few goblet cells (characterized by large vesicles). Crypts of Lieberkhun opened at the base of the villi. The muscular wall (*aa*) was quite thick;

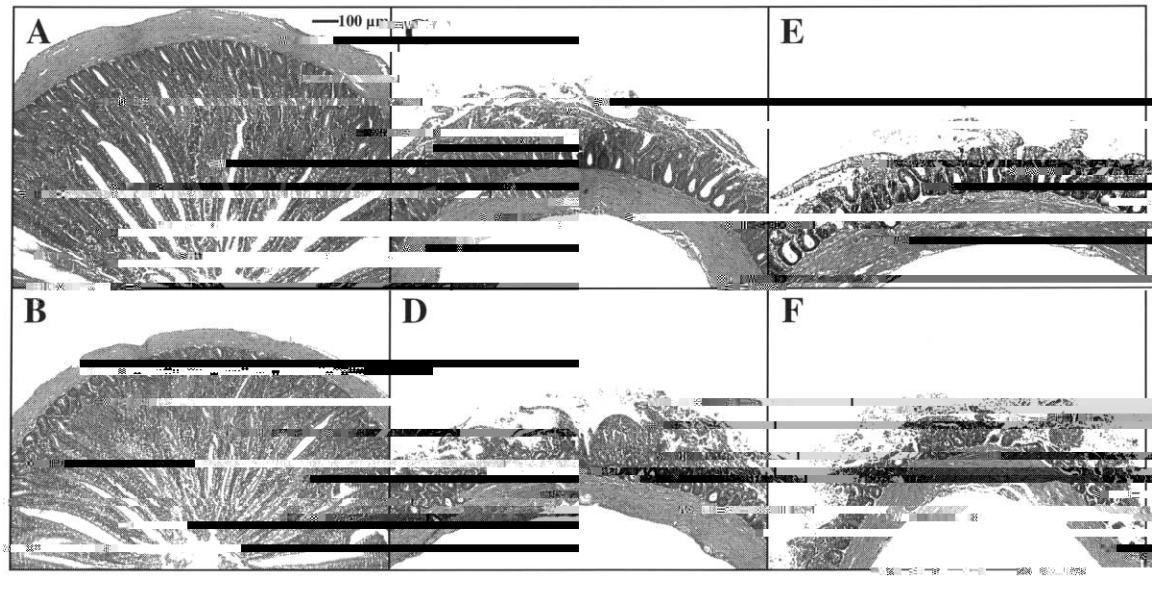

Figure 3. Histological cross sections of the jejunum of western sandpipers demonstrating damage due to eversion and subsequent incubation with the everted sleeve technique. *A*, *C*, and *E* are from recently captured juvenile migrants, while *B*, *D*, and *F* are from long-term captive adults. *A* and *B* depict intact tissue sections; *C* and *D* depict everted tissue sections; and *E* and *F* depict everted and subsequently incubated tissue sections.

histology of jejunal sections of migrants and captives (Fig. 3*C* and 3*D*, respectively). Many villi were missing or broken, and remaining villi often appeared as distorted structural artifacts. Following eversion, mucosal area decreased 67% in migrants ($F_{2,3}$ p 200.32, 0.001; Table 4) and 62% in captives ($F_{2,3}$ p 93.47, 0.01). Mean villus length decreased 44% in migrants ($F_{2,3}$ p 23.11, 0.01) and 51% in captives ($F_{2,3}$ p 80.94, 0.01). The percentage of broken villi increased threefold in migrants ($F_{2,3}$ p 34.31, 0.01) and in captives ($F_{2,3}$ p 939, 0.001). After eversion, the mucosal epithelium was no longer intact, as the majority of the villi had sustained extensive damage. Incubation of everted tissue sections in a

Table 4: Assessment of tissue damage to jejunal sections of the small intestine of recently captured juvenile migrant (p 4) and long-term captive adult (p 4) female western sandpipers due to the everted sleeve technique

	Jejunal Sections		
	Control	Everted	Everted and Incubated
Recently captured juveniles:			
Mucosal area (10^6 mm^2)	2.4 ± .2[a]	1.0 ± .2[b]	.8 ± .1[b]
Villus length (mm)[a]	569 ± 60[a]	367 ± 11[b]	318 ± 11[b]
Unbroken villi (%)	74 ± 10[a]	18 ± 6[b]	8 ± 3[b]
	4	4	4
Long-term captive adults:			
Mucosal area (10^6 mm^2)	1.3 ± .1[a]	.7 ± .1[b]	.5 ± .1[b]
Villus length (mm)[a]	452 ± 60[a]	288 ± 8[b]	223 ± 17[b]
Unbroken villi (%)	71 ± 7[a]	12 ± 3[b]	2 ± 1[b]
	4	4	4

Note. Values are means ± SEM. Different superscripts indicate statistically significant differences, 0.05.

[a] Weighted mean of broken and unbroken villi.

attempted to validate the everted sleeve technique for use on

bolic rate and its individual variation in cold-stressed mice. Physiol Zool 67:1186–1212.

Kristan D.M. and K.A. Hammond. 2000. Combined effects of cold exposure and sub-lethal intestinal parasites on host