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Waste nitrogen dynamics and an alkaline tide in the gut and of freshwater rainbow trout after voluntary consumption of a single satiating meal

Chris M. Wood

woodcm@zoology.ubc.ca

University of British Columbia

Jonathan M. Wilson

Wilfrid Laurier University

Research Article

Keywords: Ammonia, Urea-N, Blood Plasma, Stomach, Intestine, Ion-Poor Water

Posted Date: November 11th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11515054/v1

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Additional Declarations: No competing interests reported.

Abstract

We examined sequential changes in ammonia, urea-N, and acid-base parameters in four gut sections (stomach, anterior, mid, and posterior intestine) over a prolonged period of digestion (175 h) following a satiating meal (4.54% body weight) of commercial trout pellets given to a homogeneous batch of fasted rainbow trout (~ 75g) held in ion-poor fresh water at 10ºC. We related these to simultaneous changes in some of these same parameters in the blood plasma. Initial post-feeding concentrations of ammonia and urea-N in the stomach chyme could be explained by leaching of these pre-formed N-products from the consumed food pellets. Chyme ammonia-N concentrations dropped progressively in all parts of the intestine through to 80 h post-feeding, while urea-N concentrations increased slightly. Ammonia appeared to be scavenged from the tract, whereas urea-N appeared to be synthesized in the tract. Yet the plasma concentrations of both N-products increased simultaneously by more than 3-fold up to 54 h post-feeding, before declining thereafter. The initial very acidic pre-feeding pH of the stomach (2.0) in fasted trout increased by more than 3 pH units as the pellets were digested by elevated gastric acid secretion, reflecting the high acid-neutralizing capacity of the pellet food. A marked long-lasting (> 80 h) alkaline tide in the blood plasma was associated with this acid secretion into the stomach. There was also a distinct alkaline tide in all parts of the intestine, manifested as substantial and prolonged elevations in chyme pH and HCO_3^- concentrations. These lagged slightly behind the alkaline tide in the blood plasma, suggesting that HCO_3^- secretion into the intestine helped compensate the systemic alkaline tide. We discuss the possible transport mechanisms responsible for these responses, and the influence of the ion-poor nature of the fresh water.

Introduction

In freshwater fish, information on the functioning of the gastrointestinal tract in nitrogenous waste and acid-base regulation is sparse relative to that on the well-studied gills (Bucking 2017). For the latter, it is well established that the chemical conditions in the external environment (e.g. pH, alkalinity, ammonia and CO_2 levels) have marked effects on the transport processes for nitrogenous waste excretion and acid-base exchange at the branchial epithelium (Evans et al. 2005; Zimmer et al. 2021; Zimmer and Perry,2022). For the gastrointestinal epithelia, the comparable external environment is the chyme, but there is only limited information on these same chemical components in gut chyme, and how they vary among different parts of the tract and with different times after eating. To date, the best-studied species in this regard is the freshwater-adapted rainbow trout (*Oncorhynchus mykiss*). Bucking et al. (2024) have summarized data from several different studies on this species where the fish were fed with commercial trout pellets, and since then, another relevant investigation on trout has been published (Wood and Eom 2025). From these, it is evident that the gut chyme presents a chemically extreme environment, one which would likely be very toxic to the gills. In different parts of the tract, the pH may range from 2.2 to 9.2, HCO_3^- concentration (equivalent to alkalinity) from 0 to 55 mmol L^{-1}, the partial pressure of carbon dioxide (PCO_2) from 0 to over 100 Torr, total ammonia from 200 to 7775 µmol L^{-1}, the partial pressure of

ammonia (PNH_3) from 0 to 1760 μTorr, urea-N from 1000 to 13,500 μmol L^{-1}, in addition to almost complete anoxia in all sections.

In the survey of freshwater trout studies presented by Bucking et al. (2024), there is a clear indication that the gut fluid chemistry changes both with feeding and across different sections. However, with the exception of the chyme pH study of Bucking and Wood (2009) and the chyme ammonia study of Bucking and Wood (2012), there have been no investigations of how these parameters change with time in the different gut sections after a meal or how they might relate to simultaneous changes in the blood plasma. For example, it is known that the blood plasma exhibits transient increases in pH and HCO_3^- concentrations (the "alkaline tide") (Bucking and Wood 2008; Cooper and Wilson 2008) after a meal. Bucking and Wood (2008) also reported marked but transient elevation of total ammonia concentration but not urea-N concentration in the blood plasma after the same meal. However, it is not known whether these plasma responses were reflected in time-dependent patterns of the same parameters in the gastrointestinal tract.

With this background in mind, the goal of the present study was to measure simultaneous changes in these parameters in both blood plasma and chyme from four gut sections (stomach, anterior, mid, and posterior intestine) over a prolonged period of digestion (175 h) following a satiatiating meal of commercial trout pellets in a homogeneous batch of freshwater rainbow trout. Our working hypothesis was that there would be marked changes in chyme parameters associated with digestion of the meal and that these would be reflected in simultaneous changes in blood plasma parameters.

Materials and Methods

Experimental animals and feeding.

Juvenile rainbow trout (*Oncorhynchus mykiss*) were obtained from the Freshwater Fisheries Society of British Columbia (Duncan, BC, Canada) and held in the aquatic facility of the Dept. of Zoology of the University of British Columbia for approximately 4 months prior to experimentation. At the time of this study, there were 250 juvenile trout in a single large 1000-L circular tank served with aeration and flowing dechlorinated Vancouver tap-water at 10ºC with a 12h:12h photoperiod. Based on a subsample, the mean weight was 74.8 ± 6.1 g (SEM, N = 13). Vancouver tap-water is very soft and low in ions (Na^+ ~ 0.09, Cl^- ~ 0.10, Ca^{2+} ~ 0.10, Mg^{2+} ~ 0.011, K^+ ~ 0.004 mmol L^{-1}, hardness ~ 3.3 mg $CaCO_3$ L^{-1}, pH ~ 7.0). These conditions were maintained throughout the experiment. All procedures were approved by the University of British Columbia Animal Care Committee (AUP A22-0195) under the guidelines of the Canadian Council on Animal Care.

For one month prior to experiments, the trout had been fed a maintenance ration of 1% body weight of commercial trout pellets (4.0-mm Oregon Biotrout™, pellets, Bio-Oregon, Longview, WA, USA) every 3 days. They were then fasted for 9 days prior to the "Fasted" (pre-feeding) sampling point in this study. The following day, a single large meal of the same pellets was administered over a 1 h period from 07:00

to 08:00, by hand-feeding from a pre-weighed bag. Food was administered in diminishing amounts at 10 min intervals until feeding ceased entirely. The consumed satiating ration was 4.94% of body weight.

Sampling

Trout (N = 12 at each time) were sampled ("Fasted" sampling point), on the day before feeding, then at 4 h, 10 h, 30 h, 54 h, 80 h, and 175 h after the end of feeding. These times are nominal, representing the midpoints of the sampling periods, as it took approximately 4 h to sample 12 animals. Fish were netted individually into a lethal concentration of MS-222 (0.2 g L^{-1}; Syndel, Parksville, Canada) which had been neutralized to pH 7.0 using NaOH. Weight and fork length were measured, then a blood sample (approximately 500 µL) was taken by caudal puncture using a 1-ml disposable syringe fitted with a 22G needle. The dead-space of the syringe was filled with 1000 i.u. mL^{-1} of lithium heparin (Sigma-Aldrich, St. Louis, MO, USA) in Cortland salmonid saline (Wolf, 1963). The blood sample was centrifuged at 5000 x g for 1 min, then the plasma was decanted, flash-frozen in liquid N_2, and stored at -80ºC for later analysis. Subsequently, two types of sampling were performed, with the procedures applied alternately. Half of the fish were used for sampling of chyme and for preservation of gastrointestinal tissues for another study The other half were used for a different type of preservation of tissues for this other study Therefore, the N number was 12 at each time for plasma analyses, and 6 at each time for chyme analyses.

For collection of chyme, the fish was placed on ice, the peritoneal cavity was opened, and the gastrointestinal tract was clamped at the esophagus and the anus. The tract was then excised, and cut sequentially into 4 sections starting at the caudal end. For each section, the chyme was extruded in the caudal direction into a 1.5-ml centrifuge tube, then mixed by vortexing. After measurement of the chyme pH, the sample was flash-frozen in liquid nitrogen, and then stored at -80ºC for later analysis. While it was possible to measure pH in all gut sections, it was not possible to collect gastric fluid for any other analyses from fasting trout because the fluid content of the stomach was negligible.

Analytical methods

Extraction of Food Pellets

According to the manufacturer, 4.0 mm Oregon Biotrout™ pellets contain 45.0% protein, 24.0% lipid, 8.5% moisture, 12.0% ash, and 2.0% fibre. However, the possible ammonia and urea-N content of the food, which could greatly influence the concentrations of these substances in chyme, is not provided. Therefore, analyses identical to those of Wood and Eom (2025) were performed. In brief, pellets (approximately 0.9 g) were digested in exactly 0.57 mL g^{-1} of either Vancouver water (N = 6) or 0.57 mL g^{-1} of 0.1 M HCl (N = 6, to mimic gastric acid) in sealed 1.5 mL tubes at 10°C. This fluid to pellet ratio was chosen to represent the overall % water measured in stomach chyme sampled at 2–24 h post-feeding by Bucking and Wood (2006) in trout fed a similar diet. After both 1 h and 18 h incubation, the

tubes were vortexed, and subsamples were taken which were then centrifuged at 5000 x g for 2 min. Aliquots (30 µL) of the supernatant were taken for ammonia and urea-N analysis, so as to represent the concentrations that would occur in chyme by dissolution of the pellets. Half of incubations were performed with whole pellets, and half with pellets that had been ground to a fine powder with a mortar and pestle.

Ammonia and Urea-N Analyses

Ammonia and urea-N concentrations were measured in plasma, chyme, and food pellet supernatant using a commercial enzymatic assay kit (Raichem ammonia kit based on glutamate dehydrogenase, Cliniqa Corporation, San Marcos, CA, USA) and the colorimetric method of Rahmattulah and Boyde (1980) respectively. Plasma, chyme, and food supernatant samples were thawed on ice, vortexed, centrifuged at 10,000 x g at 4ºC for 15 min (chyme only), and then subsampled for either acid-base (see below) or N-product analyses. For the latter, the subsamples were first deproteinized as described by Wood et al. (2023). In brief, the subsamples were diluted with an equal volume of ice-cold 8% $HClO_3$, vortexed, and incubated on ice for 10 min in 0.5 ml centrifuge tubes. The tubes were then centrifuged at 10,000 x g at 4ºC for 15 min, and the supernatant was removed. For ammonia, an aliquot of the supernatant was neutralized with 1.5 volumes of saturated Tris buffer (pH 7.5), then analyzed with the Raichem kit against ammonia standards that had been put through the same procedure. For urea-N, an aliquot was appropriately diluted with 4% $HClO_3$ and assayed directly without neutralization by the method of Rahmattulah and Boyde (1980) against urea-N standards that had been put through the same procedure.

The ammonia assay measures total ammonia - i.e. the sum of NH_3 and NH_4^+. The partitioning of these in chyme depends on the pH, which was measured as described below. The partial pressure (PNH_3) of NH_3 in chyme was calculated from total ammonia and pH measurements using the Henderson -Hasselbalch equation (see Wright et al. 1988) and pK and NH_3 solubility values for teleosts from Cameron and Heisler (1983).

Acid-Base Analyses

The pH of chyme was measured immediately after collection (i.e. before freezing) using a combination glass micro-electrode (Biotrode™, Hamilton, Reno, Nevada, USA) and an Accumet pH meter (Fisher Scientific, Nepean, Ontario, Canada). The total CO_2 concentration of plasma and chyme were measured on the first thaw of the samples as described above, using a Corning 965 CO_2 analyzer (Corning Instruments, Corning, NY, USA). HCO_3^-concentrations and the partial pressure (PCO_2) of CO_2 in chyme were calculated from total ammonia and pH measurements using the Henderson-Hasselbalch equation (see Wood et al. 1983) and pK' and CO_2 solubility values for teleost fish from Boutilier et al. (1984).

Statistics

Data have been expressed as means ± 1 SEM (N). Prior to testing, all data were checked for normality of distribution and homogeneity of variance. If data failed, they were then subjected to standard transformations (log10, square root). If data still failed, the non-parametric Kruskal-Wallis ANOVA on ranks was applied, followed by Dunn's *post hoc* test. The plasma data, and gut data for each section were first analyzed using one-way ANOVA, followed by Tukey's *post hoc* tests to identify significant differences among means at different sample times. The gut data were then further analyzed, leveraging the repeated measures design (i.e. at each time, all intestinal sections were from the same fish). The fasting pre-feeding data were separated from the post-feeding data. A one-way repeated measures ANOVA was run on the fasted data to detect possible pre-existing differences among the three intestinal sections prior to feeding. In order to detect whether there were significant overall differences among anterior, mid, and posterior intestine associated with food ingestion, repeated measures two-way ANOVAs were applied to the post-feeding data using the factors intestinal section and time. Student's paired two-tailed t-tests were used for simple pair-wise comparisons for the food composition data. Statistical details have been reported in Figure and Table legends. Significance was accepted at $P \leq 0.05$.

Results

Ammonia and urea-N concentrations in food

The commercial trout pellets contained substantial concentrations of both ammonia and urea-N, with resultant high levels in the *in vitro* incubation media (Table 1). Concentrations are expressed as both dissolved values in the fluid phase, and as back-calculated values in the original pellets. There were no systematic or significant difference among the four extraction protocols (whole pellets with water, ground pellets with water, whole pellets with 0.1M HCl, ground pellets with 0.1 M HCl) so the results were combined. Ammonia concentrations were 1.5- to 3-fold higher than urea-N concentrations. There were no significant differences between the 1-h and 18-h incubations.

Table 1
Concentrations of total ammonia and urea-N in pellet food incubated for 1 h or 18 h at 10°C with 0.57 ml of fluid per g of pellet. The concentrations in the fluid phase (supernatant) are also reported. See text for details. Means ± SEM (N = 12).

Ammonia in original pellets (μmol kg^{-1})	**1-h Incubation**	**18-h Incubation**
	4698 ± 912	**6028 ± 1318**
Ammonia in fluid phase (μmol L^{-1})	2992 ± 581	3839 ± 840
Urea-N in original pellets (μmol kg^{-1})	2753 ± 585	2259 ± 356
Urea-N in fluid phase (μmol L^{-1})	1754 ± 372	1439 ± 227
There were no significant differences ($P > 0.05$, paired Student's t-tests) between comparable values for 1-h incubation and 18-h incubations.		

Progress of digestion

In the 9-day fasted trout, the stomach was essentially empty, but there were small amounts of chyme in all of the intestinal sections. After the satiation meal and right up to 50 h, the stomach was very full of pellets, which became progressively more hydrated and liquified over time. However, all sections exhibited a noticeable increase in chyme content as soon as the first sampling point 4 h after the meal, and the posteriorly directed wave of increased chyme content continued through the intestinal sections up to 80 h. By this time, the stomach was empty in three of the 12 fish, and by 175 h the stomach was devoid of solids in all but one of 12 fish. However, some gastric fluid remained, and all intestinal sections contained small amounts of chyme. There were no significant changes in body weight (81.6 ± 2.1 g, N = 84) or fork length (17.8 ± 0.2 cm, N = 84) over the experiment.

Ammonia and urea-N concentrations in plasma and chyme

Mean plasma total ammonia concentrations were about 100 μmol L^{-1} in fasting trout, and increased progressively after the meal, reaching almost 300 μmol L^{-1} at 54 h (Fig. 1A). By 80 h and 174 h, plasma ammonia had returned to a level not significantly different from the fasting value. Mean plasma urea-N concentrations in fasting fish were about 700 μmol L^{-1} - i.e. 7-fold higher than ammonia, but exhibited a similar pattern of progressive increase followed by decline after the meal. Plasma urea-N concentrations peaked at 2300 μmol L^{-1}, and thereafter fell sharply, though the fasting level was not re-established until 174 h (Fig. 1B).

Due to the lack of gastric fluids in fasting fish total ammonia concentrations were not measured. After feeding, the mean levels in stomach chyme were rather variable in the 1000 to 3000 μmol L^{-1} range, with no significant differences from 4 h through 175 h (Fig. 2A). Mean total ammonia concentrations were similarly variable in the 2000 to 4000 μmol L^{-1} range in chyme in all three sections of the intestine in fasting trout, and were not significantly different among sections at this time point. However, after feeding, there was a pattern of decrease in chyme total ammonia concentration common to all three parts of the intestine (Figs. 2B, C, D) that roughly mimicked the inverse pattern of increase in plasma total ammonia concentration over the same time course (cf. Figure 1A). Total ammonia concentrations decreased significantly after feeding in the anterior (Fig. 2B), mid (Fig. 2C), and posterior intestine (Fig. 2D), reaching a minimum (500 to 1000 μmol L^{-1}) at 30–80 h post-feeding, then returned to levels not significantly different from the fasting values at 175 h. The changes in the posterior section were larger than in the other two sections. Two-way repeated measures ANOVA confirmed significant main effects of both section and time, as well as significant interaction between the two.

The partial pressure of ammonia (PNH_3) could not be calculated in the stomach of fasting trout, but it remained constant at close to 0 μTorr at all times after the meal (Fig. 3A). Mean fasting PNH_3 levels were similar at 60 to 90 μTorr in the anterior and mid intestine but significantly greater in the posterior intestine at about 380 μTorr (Figs. 3B, C, D). After the meal, the pattern of PNH_3 changes in all three segments of the intestine was opposite to that of total ammonia (cf. Figure 2). Rather than decreasing,

PNH_3 levels increased progressively by 3- to 5-fold in all three sections, peaking at 30 h. However, they had returned to levels not significantly different from fasting values by 54 h. This pattern of increase followed by decline in chyme PNH_3 was quite similar to but slightly preceded the comparable pattern in plasma total ammonia concentration (cf. Figure 1A). Two-way repeated measures ANOVA revealed significant overall effects of both time and gut section, but with no significant interaction. PNH_3 levels after feeding were the highest in the posterior intestine, lower in the mid intestine, and least in the anterior intestine, with all the differences being significant.

Urea-N concentrations was not measured in the stomach of fasting trout, but after feeding rose significantly by about 3-fold from 240 μmol L^{-1} at 4 h to 600–700 μmol L^{-1} at 54 h and 80 h before returning close to fasting values at 175 h (Fig. 4A). Mean fasting urea-N concentrations in the chyme of the anterior, mid, and posterior intestines were not significantly different from one another at 545, 345, and 815 μmol L^{-1} respectively. (Fig. 4B, C, D). After feeding, these values remained largely unchanged in the anterior intestine with the exception of a significant decrease between 54 and 80 h post feeding (Fig. 4B) but tended to rise slightly in the mid (Fig. 4C) and posterior intestine (Fig. D), but the only significant increase was at 4 h in the mid-intestine (Fig. 4C). Overall, the pattern of urea-N levels in chyme after feeding was unremarkable relative to the marked patterns of increasing urea-N concentration in blood plasma (cf. Figure 1B) and decreasing total ammonia concentrations in chyme (cf. Figure 2). Two-way repeated measures ANOVA revealed significant main effects of both time and section on chyme urea-N, with a significant interaction term. Mean urea-N levels were higher at most times in the posterior intestine than in the anterior intestine.

CO and acid-base parameters in plasma and chyme

Mean plasma total CO_2 concentration was about 5 mmol L^{-1}in fasting trout (Fig. 1C). After the meal, plasma total CO_2 increased quickly, reaching a peak of 8.3 mmol L^{-1} by 10 h, and remaining significantly elevated through 80 h before returning to fasting values at 175 h. For methodological reasons (see Discussion), pH was not measured in blood plasma, so HCO_3^- concentrations and PCO_2 could not be calculated. However, for any reasonable estimate of blood pH *in vivo*, greater than 90% of total plasma CO_2 would exist in the form of the HCO_3^- ion (see Discussion), so the post-feeding changes in plasma total CO_2 represent quantitatively similar changes in plasma HCO_3^- concentration.

In the gut, the mean pH in the gastric juice of fasted trout was extremely low, approximately 2.0 (Fig. 5A). After feeding, the mean pH of stomach chyme increased quickly with a significant elevation to 4.5–5.5 at 10 h through 80 h, before returning to the acidic fasting value at 175 h.

In fasting fish, the mean pH in the anterior intestine was about 6.95 (Fig. 5B), close to that in the mid intestine (7.12; Fig. 5C), and significantly lower than in the posterior intestine (7.37; Fig. 5D). After feeding, all three intestinal segments exhibited similar patterns, with progressive large increases in pH to

plateaus at 30 h and 54 h, followed by slow declines back towards fasting values thereafter. The plateau values were about 7.97 in the anterior intestine, 8.26 in the mid intestine, and 8.49 in the posterior intestine. Even at 175 h, chyme pH remained significantly elevated in the anterior and mid sections. By two-way repeated measures ANOVA there were significant main effects of both time and section, but the interaction term was not significant. Mean pH values were significantly different among all three intestinal sections (posterior > mid > anterior) at all times post feeding (Fig. 5B, C, D).

Mean PCO_2 values in gastric chyme did not vary significantly with time after feeding, ranging between 1 and 7 Torr at most times (Fig. 6A). The much higher mean at 10 h reflects inclusion of one unusually high value (102 Torr); omission of this value would not have affected the statistical outcome. Prior to feeding, there were no significant differences among the three parts of the intestine in fasting PCO_2 levels which averaged 2–4 Torr (Figs. 6B, C, D). After feeding, there was a tendency for chyme PCO_2 to rise at 10 h, but this was significant only in the anterior intestine where it reached 15 Torr. No other changes were significant. Repeated measures two-way ANOVA revealed significant main effects of both time and section, but not of interaction. Post feeding PCO_2 levels in the anterior intestine were significantly higher than those in the posterior intestine.

The mean concentration of HCO_3^- in the gastric juice was very close to 0 mmol L^{-1} with no significant variations over time (Fig. 7A). In the three intestinal sections, mean HCO_3^- concentrations were also low (1 to 2 mmol L^{-1}) prior to feeding, with no significant differences among the segments. However, following feeding, mean HCO_3^- levels started to rise in all three parts, with the increase being most rapid in the anterior section (significant by 10 h; Fig. 7B) and slowest in the posterior section (not significant until 30 h; Fig. 7D). By 30 h to 54 h, HCO_3^- concentrations had peaked at approximately 35 mmol L^{-1} in all three segments, followed by declines thereafter. By 175 h, the elevation remained significant only in the mid intestine (Fig. 7C). Overall, these time-dependent HCO_3^- patterns in chyme matched similar time-dependent patterns in intestinal pH (cf. Figure 5) but lagged behind the more rapid increase in plasma total CO_2 after feeding (cf. Figure 1C). Despite the slight differences in HCO_3^- patterns among the three segments of the intestine (Figs. 7C, D, E), two-way repeated measures ANOVA revealed only a significant main effect of time, with no significant effect of section or interaction.

Discussion

Overview

Overall, our working hypothesis was confirmed: there were marked changes in chyme parameters associated with digestion of the meal and these were reflected in changes in blood plasma parameters. These included substantial decreases in chyme total ammonia and large pH-driven increases in PNH_3 in all parts of the intestine, but only modest increases in urea-N concentrations. Ammonia appeared to be scavenged, whereas urea-N appeared to be synthesized in the tract. Yet the plasma concentrations of

both N-products increased simultaneously by more than 3-fold up to 54 h, before declining thereafter. A distinct long-lasting alkaline tide occurred in all parts of the intestine, manifested as persistent elevations in chyme pH and HCO_3^- concentrations. These lagged slightly behind the alkaline tide in the blood plasma. We speculate that HCO_3^- secretion into the intestine helped compensate the systemic alkaline tide, in the face of low ion concentrations in the water that would limit the capacity for branchial HCO_3^- excretion.

Ammonia and urea-N concentrations in food

To our knowledge, Table 1 presents only the second published measurements of ammonia and urea-N concentrations in commercial trout pellets. Wood and Eom (2025) used the same methods to analyze larger (6.0–mm) pellets that were produced by the same manufacturer but purchased at a different time. Overall, the two determinations agree that concentrations of both N-products are relatively high, with ammonia being approximately 1.5- to 3-fold greater than urea-N. The certified protein content of 4.0-mm Oregon Biotrout™ pellets used in the present study was 45%, with most of this likely originating from fish meal, so the ammonia and urea-N in the pellets probably also originated from fish meal. The concentrations of ammonia (~ 3000 μmol L^{-1}) and urea-N (~ 1500 μmol L^{-1}) in the fluid phase of our *in vitro* pellet incubations (Table 1) were certainly high enough to explain the initial post-feeding concentrations of both N-products in the chyme (Figs. 2 and 4). This agrees with the findings of Wood and Eom (2025) on much larger trout (~ 1.3 kg). Nevertheless, using the values in Table 1, the amount of pre-formed ammonia and urea-N in the single satiating meal (4.94% of fish body weight) would amount to less than 400 μmol-N per kg fish weight. This value is very small relative to the “excess” or exogenous N-waste that salmonids excrete for many hours after feeding, which may amount to 3,000 to 30,000 μmol-N per kg fish weight depending on fish size, temperature, and ration (reviewed by Wood, 1995 and Wood, 2001). Most of the exogenous N-waste is produced from metabolic breakdown of the protein content of the pellets. Using the same assumptions as Wood and Eom (2025), in the present study, this protein-N content amounted to 5,142,000 μmol-N per kg pellets, and to 201,000 μmol-N per kg fish weight for a trout consuming the 4.94% ration meal.

Ammonia dynamics in plasma and chyme

The rapid 3-fold rise and subsequent fall of plasma total ammonia concentration after the satiating meal was quantitatively very similar to the data reported by Bucking and Wood (2008) in 5-fold larger trout at slightly higher temperature (12ºC). Several other studies have reported qualitatively similar patterns in plasma following feeding in rainbow trout (Kaushik and de Oliva Teles, 1985; Wicks and Randall 2002; Zimmer et al. 2010; Bucking and Wood 2012). The meal is initially processed in the stomach, and absorption across the gastric mucosa likely explains the rapid rise in plasma total ammonia. We could not measure ammonia in gastric juice in fasted trout, but by 4 h to 10 h post-feeding, total ammonia concentrations in gastric chyme were in the 2000 to 3000 μmol L^{-1} range (Fig. 2A), levels probably attributable to ammonia leaching into the fluid phase out of the pellets (see above and Table 1). Ammonia production by microbial hydrolysis of protein in the food may explain the persistence of high

concentration at later times. Similarly high concentrations of total ammonia in gastric chyme have been reported in several previous studies on pellet-fed rainbow trout (Jung et al. 2022; Jung et al. 2023a; Jung et al. 2023b; Wood and Eom 2025).

Recently the stomach has been identified as the major site of ammonia absorption in the gastrointestinal tract of the rainbow trout based on *in vitro* experiments with gut sacs filled with either saline (Jung et al. 2023b) or endogenous chyme (Wood and Eom 2025). Additionally, using *in vivo* experiments, both Jung et al. (2023a) and Wood and Eom (2025) measured much higher plasma total ammonia concentrations in the hepatic portal vein (which drains both the stomach and parts of the anterior intestine) than in the sub-intestinal vein (which drains only the intestinal segments). Karlson et al. (2006) earlier reported marked increases in total ammonia in the hepatic portal vein of cannulated rainbow trout *in vivo* after a meal. The high volume of gastric chyme right up to 50 h post feeding and accompanying high surface area of the remarkably expansible stomach probably also contribute to its high ammonia absorption capacity. In the mammalian stomach, ammonia uptake (Summerskill et al. 1966) and secretion (Gips et al. 1973) have also been reported, where Rhesus glycoproteins are thought to play a role (Handlogten et al. 2005) in the face of low apical membrane permeability of gastric gland cells to ammonia (Waisbren et al. 1994).

The progressive decreases in chyme total ammonia levels up to 50 h post- feeding in all three intestinal sections (Figs. 2B, C, D) suggest that these sections scavenge ammonia as the meal passes down the digestive tract. The intestinal concentrations were in the general range of several previous studies surveyed by Bucking et al. (2024) but comparisons are difficult as those investigations did not follow time course. Nevertheless, it is notable that initial post-feeding levels in the posterior intestine was higher, and the subsequent decrease in total ammonia was greater in the posterior intestine relative to the other sections. The *in vivo* and *in vitro* experiments of Wood and Eom (2025) identified this section as the second most important site of ammonia absorption after the stomach, and the site of the highest rate of ammonia synthesis by microbial and/or enzymatic deamination. Experiments with saline-filled gut sac preparations demonstrated that intestinal ammonia absorption, as well as tissue ammonia production were upregulated after feeding (Rubino et al. 2014; Rubino et al. 2019, Jung et al.,2021).

The PNH_3 in stomach chyme was essentially zero throughout the experiment (Fig. 3A). This reflected the pH-dependent speciation of ammonia: there was almost no NH_3 present (≤ 0.05% of the total) at the pH values in the stomach, which remained below 5.4 (Fig. 5A), so NH_4^+ completely dominated. In the various parts of the intestine, even though total ammonia levels decreased to their lowest values at 30–80 h post-feeding, PNH_3 values exhibited an inverse pattern, with the highest levels at these times (Figs. 3B, C, D). This is because the pH values in the intestine were greatly elevated at these same times (Fig. 5B, C, D), especially in the posterior segment where chyme pH reached 8.5, so NH_3 comprised up to 4.3% of total ammonia and PNH_3 was at its highest.

The pH-dependent speciation of ammonia has implications for the transport mechanisms by which it is absorbed. In the gills, the Rh proteins serve as facilitated diffusion channels for ammonia in trout

(Nawata et al. 2007; Wright and Wood 2009; Weihrauch et al. 2009), and the gene expression of a putative apical transporter Rhcg2 (= Rhcgl1), is upregulated in the gills after feeding (Zimmer et al. 2010; Zhang et al. 2015). In the stomach of trout, mRNA of both Rhcg2 (Jung et al. 2023b) and a putative basolateral transporter Rhbg1 (Bucking and Wood 2012) have been detected, and the gene expression of the latter increased non-significantly after feeding. Notably, the mammalian analogues RHCG and RHBG have been localized to apical and basolateral membranes of gastric gland chief cells in mice (Handlogten et al. 2005). Thus, Rhbg1 could serve as the basolateral export pathway for the high ammonia absorption rate after feeding. However, oocyte expression analyses (Nawata et al. 2010) have indicated that the trout Rh proteins function as NH_3 rather than NH_4^+ transporters, and respond to PNH_3 gradients. The fact that the PNH_3 is zero in gastric chyme (Fig. 3a) would seem to negate any role for Rhcg2 in apical entry. Other possibilities for apical entry that deserve investigation in the future include active NH_4^+ entry in substitution for K^+ on the gastric H^+, K^+ ATPase (Sachs et al. 1988), as well as the mechanisms for NH_4^+ entry on apical mechanisms that have been identified in the intestine of rainbow trout, as discussed below.

The putative basolateral transporters Rhbg1 and Rhbg2 have been identified in trout intestine (Nawata et al. 2007; Nawata and Wood 2008). Bucking and Wood (2012) reported that the mRNA expression of Rhbg1 was upregulated by about 5-fold in both the anterior and posterior intestine after feeding, implicating the involvement of these channels in the basolateral export of ammonia. The putative apical transporter Rhcg2 is also expressed in all three sections of the trout intestine (Jung et al. 2023b). Therefore, the marked increases in chyme PNH_3 (Figs. 3B, C, D) could drive increased apical uptake of NH_3 at this time. However, in isolated saline-filled gut sac preparations, Jung et al. (2023b) reported that ammonia transport was insensitive to manipulation of the driving PNH_3 gradient, casting doubt on this interpretation. Alternatively, Rubino et al. (2019) presented pharmacological evidence for two mechanisms of apical NH_4^+ uptake in the intestine: movement through barium-sensitive K^+ channels, and transport in substitution for K^+ on the bumetanide-sensitive Na^+, K^+, $2Cl^-$ cotransporter (NKCC2) (Bergeron et al. 2003) the latter largely restricted to the anterior intestine. NKCC was upregulated at gene and protein expression levels after feeding, with immunohistochemistry confirming apical expression (Rubino et al. 2019).

Urea-N dynamics in plasma and chyme

The progressive 3-fold increase in plasma urea-N concentration after feeding (Fig. 1B) paralleled that of plasma total ammonia (Fig. 1A). The concentrations in stomach chyme immediately after feeding (Fig. 4A) could be explained by leaching of pre-formed urea-N from the pellets into gastric juice (Table 1). There were modest increases in chyme urea-N concentrations after the meal throughout the tract, with the highest levels in the posterior intestine (Fig. 4). This pattern, together with the marked postprandial increase in plasma urea-N (Fig. 1B) suggests that urea-N was synthesized in the tract and absorbed into the bloodstream. To our knowledge, urea transport mechanisms have not yet been identified at a molecular level in the gastrointestinal tract of salmonids. However Bucking et al. (2013a) and Bucking et

al. (2013b) reported that the facilitated diffusion urea transporter UT is expressed in the gut of two Batrachoidid fish species, with increased expression after feeding.

In general plasma urea-N levels as well as whole animal urea-N excretion rates have been reported to rise after feeding but with great variability among studies (reviewed by Wood 2001). For example, Bucking and Wood (2008) reported an almost identical plasma ammonia profile but no change in plasma urea-N concentrations in 4-fold larger trout, whereas Karlsson et al. (2006) reported a modest increase in plasma urea-N levels but a large increase in plasma total ammonia in similarly large trout. We suspect that the source of this variability is the degree to which urea-N is synthesized in the gastro-intestinal tract. Jung et al. (2021) reported that intestinal tissues from trout 2-fold larger than used here synthesized urea-N by arginolysis, and that intestinal urea-N absorption rates in saline-filled gut sac preparations increased after feeding. Wood and Eom (2005) studied very large trout (~ 1.3 kg) kept on a continuous satiation feeding regime of commercial pellets. They found similar concentrations of urea-N in the stomach chyme as in the present study due to leaching from the pellets, but a massive 13-fold elevation in chyme sampled from the anterior intestine/pyloric caecae region. Urea-N absorption rates from chyme-filled gut preparations made from this region of the intestine were also very high. Together these indicated a high rate of urea-N synthesis by the pyloric caecae of large trout, which fits with an observed pattern of increasing plasma urea-N concentrations as size increases in rainbow trout (Guo et al. 2026). In our much smaller trout, the pyloric caecae were much less developed, so urea-N synthesis was likely distributed throughout the tract. The tract is anaerobic throughout (Jung et al. 2022), so a role for anaerobic urea-N production by microbes in the chyme (e.g. *Helicobacter pylori*; Mendz and Hazell, 1996), as well as for aerobic urea-N production by arginolysis or other processes in the gut tissue itself (Jung et al. 2021; Wood and Eom 2025), is suspected. Indeed, microbial urea-N synthesis from ammonia may have contributed to the apparent scavenging of ammonia from intestinal chyme (Fig. 2B, C, D).

Acid-base and CO_2 dynamics in plasma and chyme

We chose not to measure blood pH because of the well-known artifacts associated with caudal sampling of anaesthetized fish that depress blood pH, as discussed by Davison et al. (2023). Therefore, we could not calculate absolute plasma HCO_3^- concentrations with certainty. Nevertheless, our plasma total CO_2 measurements (Fig. 1C) still provide a useful representation of the plasma HCO_3^- response to feeding. At 10ºC, the real blood pH *in vivo* in a resting trout should be 7.8–8.0 (Cameron 1984) and according to Davison et al. (2023), caudal sampling depresses blood pH by about 0.5 pH units on average. Calculations using the Henderson-Hasselbalch equation and appropriate constants for trout blood from Boutilier et al. (1984) indicate that the percentage of total CO_2 present as the HCO_3^- ion would be 93.9% at pH 7.4 as opposed to 98.3% as pH 7.9. At the higher plasma total CO_2 levels observed after feeding, the discrepancy would less. Therefore, the marked rise in plasma total CO_2 concentration after the satiating meal (Fig. 1C) indicates the occurrence of a substantial alkaline tide. This is a well-known phenomenon in predatory fish (reviewed by Wood 2019, and Chiu et al. 2026) and has been documented in two previous studies on freshwater rainbow trout (Bucking and Wood 2008; Cooper and

Wilson 2008). Relative to these two previous studies, the response was similar in magnitude but longer-lasting, remaining significant through 80 h post-feeding (Fig. 1C), which likely reflected the larger meal size, lower temperature, and/or low availability of counter-ions for branchial acid-base regulation in dilute Vancouver tap water (Tovey and Brauner 2018; Brauner et al. 2019).

The alkaline tide is caused by extrusion of HCO_3^- ions into the blood in a 1:1 ratio to elevated rates of H^+ ion secreted into the gastric lumen by H^+, K^+ ATPase in oxynticopeptic cells during digestion (Hanke et al. 1931; Niv and Fraser,2002; Wood,2019). Counterintuitively, gastric pH increased by more than 3 pH units after feeding and stayed elevated through 80 h (Fig. 5A), in parallel to the alkaline tide in the blood plasma (Fig. 1C). This rise in gastric pH after feeding is in fact a well-established phenomenon in carnivorous fish (Wood 2019). In rainbow trout (Bucking and Wood 2009; Wood and Eom 2019), it reflects the high acid-neutralizing capacity of commercial trout pellets in the face of increased gastric H^+ secretion (Márquez et al. 2026).

Elevated PCO_2 levels (Fig. 6) in the gastrointestinal tract after feeding have now been observed in a number of fish species (summarized by Wood 2019), including the freshwater rainbow trout (Wood and Eom 2019). In the present study, they were relatively modest, and statistically significant only in the anterior intestine (Fig. 7B). This was likely due to CO_2 generation by the reaction of intestinal HCO_3 with the acidic gastric chyme discharged through the pyloric sphincter. The "CO_2 recycling" model presented by Wood (2019) would support further HCO_3^- secretion and prevent PCO_2 from rising too high in the tract.

More surprising were the marked increases and later decreases of pH of the chyme, which occurred more or less in parallel in all three parts of the intestine (Fig. 5B, C, D). These were explained by similarly marked increases and decreases in chyme HCO_3^- concentrations in these same sections (Fig. 7B, C, D). Elevated intestinal HCO_3^- concentrations associated with feeding have been reported in several different freshwater teleosts (Taylor et al. 2007; Goodrich et al. 2020), including the rainbow trout (Wood and Eom,2019; Wood and Eom 2025). However, to our knowledge, the present study is the first demonstration of an alkaline tide in the digestive tract (Fig. 7B, C, D), lagging slightly behind that in the blood (Fig. 1C).

Bucking and Wood (2008) showed that the major mechanism for compensation of the systemic alkaline tide in the bloodstream of trout was excretion of net "base" (i.e. HCO_3^-) to the external fresh water. This occurred mainly across the gills as renal excretion accounted for only about 10% of the total (Bucking et al., 2010). Presumably, the well-studied Cl^-/HCO_3^- exchange mechanism in the gills (De Renzis and Maetz 1973; reviewed by Brauner et al., 2019 and Zimmer and Perry 2022) was the primary mechanism involved. However, in the present study the extremely low concentration of Cl^- in Vancouver tap-water (~ 0.1 mml L^{-1}) compared to that (~ 0.7 mmol L^{-1}) used by Bucking and Wood (2008) may have severely limited the transport capacity of the branchial Cl^-/HCO_3^- exchanger (Brauner et al. 2019), necessitating

increased dependence on HCO_3^- secretion into the intestine as a mechanism for coping with the systemic HCO_3^- load.

In seawater teleosts, high rates of HCO_3^- secretion by the intestinal tissues serve to precipitate divalent cations in the lumen so as to improve the osmotic gradient for water absorption (e.g. Wilson et al. 1996; Wilson et al. 2002; Wilson and Grosell 2003; Grosell and Taylor 2007). In freshwater, fish face the opposite osmotic problem, so this function is not needed. Indeed, it remains unclear whether or not the intestinal tissues themselves are responsible for the secretion of HCO_3^- into the chyme after feeding in freshwater fish. In intestinal sac preparation from freshwater trout, HCO_3^- secretion rates were low, and did not increase after feeding, in contrast to seawater trout (Bucking et al. 2009). Furthermore, intestinal sac preparations from the freshwater climbing perch exhibited HCO_3^- absorption rather than secretion, and the absorption rate actually increased after feeding (Goodrich et al. 2020). These reports oppose but do not entirely eliminate a role for direct HCO_3^- secretion by the intestinal tissues, as saline-filled gut sacs may not duplicate *in vivo* conditions. Future research on this topic in freshwater fish should start by pharmacological and molecular searches for the complex HCO_3^- secretion mechanisms which have been well-characterized in seawater fish (Grosell 2006. Grosell 2011; Grosell and Oehlert 2023).

However, the possibility of HCO_3^- secretion into the intestine by pancreatic and biliary system tissue should also be explored. In mammals, pancreatic HCO_3^- secretion is an important mechanism for compensating the postprandial alkaline tide as well as for neutralizing the acidic chyme entering from the stomach (Ashley et al. 1994; Rune 1966; Steward et al. 2005; Tampert et al. 2021). In teleost fish, the pancreatic tissue is diffuse, with numerous fine pancreatic ducts which variously discharge into the pyloric caecae, the bile ducts, and/or the main lumen of the anterior intestine (reviewed by Bakke et al. 2011). The daunting challenge here will be to collect this fluid, and measure its flow rate and HCO_3^- concentration after feeding.

Declarations

Acknowledgements

We thank Dr. Sunita Nadella for help with statistical analyses and figures.

Competing interests

The authors declare no competing or financial interests.

Author contributions

CMW and JMW designed the project, performed the experiments and analyzed the samples and data. CMW wrote the first draft, and JMW edited it.

Funding

Supported by Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery grants to CMW (RGPIN-2023-03714) and JMW (RGPIN-2019-06838).

Data Availability

From the authors upon reasonable request.

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Figures

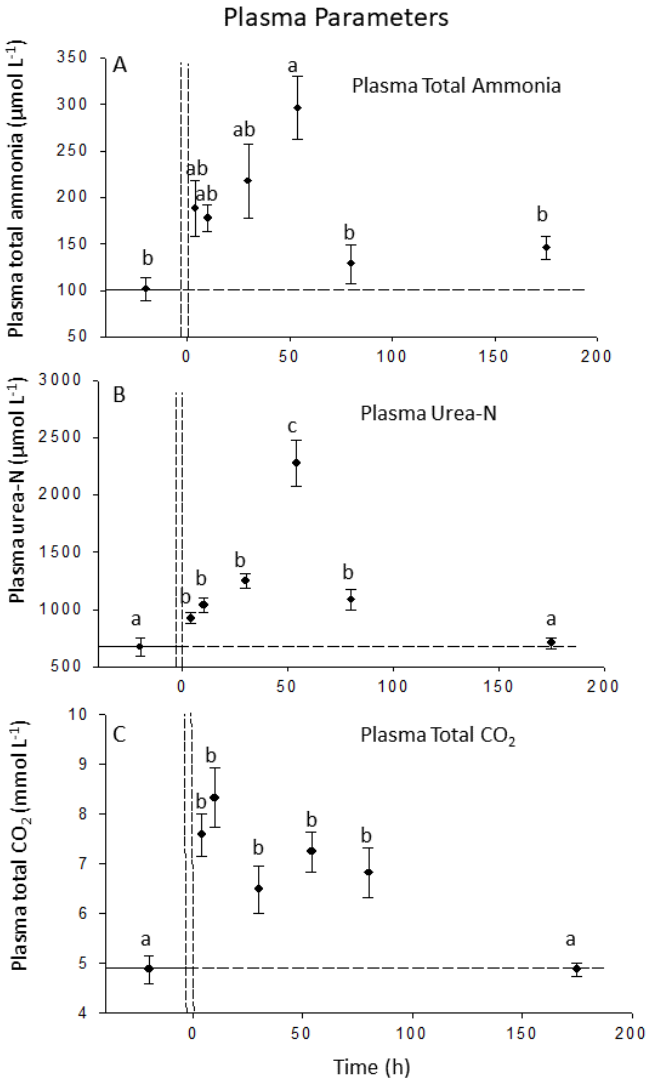


Figure 1

Figure 1

Time-dependent changes in (A) plasma total ammonia; (B) plasma urea-N; and (C) plasma total CO_2 concentrations over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 12) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. (A) plasma total ammonia: Kruskal-Wallis one-way non- parametric ANOVA $P < 0.001$; (B) plasma urea-N: one-way parametric ANOVA on log10 transformed data $P < 0.001$; and (C) plasma total CO_2 concentration: one-way parametric ANOVA on log10 transformed data $P < 0.001$. Means not sharing the same letter (a, b, c) are significantly different from one another by Dunn's non-parametric or Tukey's parametric *post hoc* tests respectively ($P \leq 0.05$).

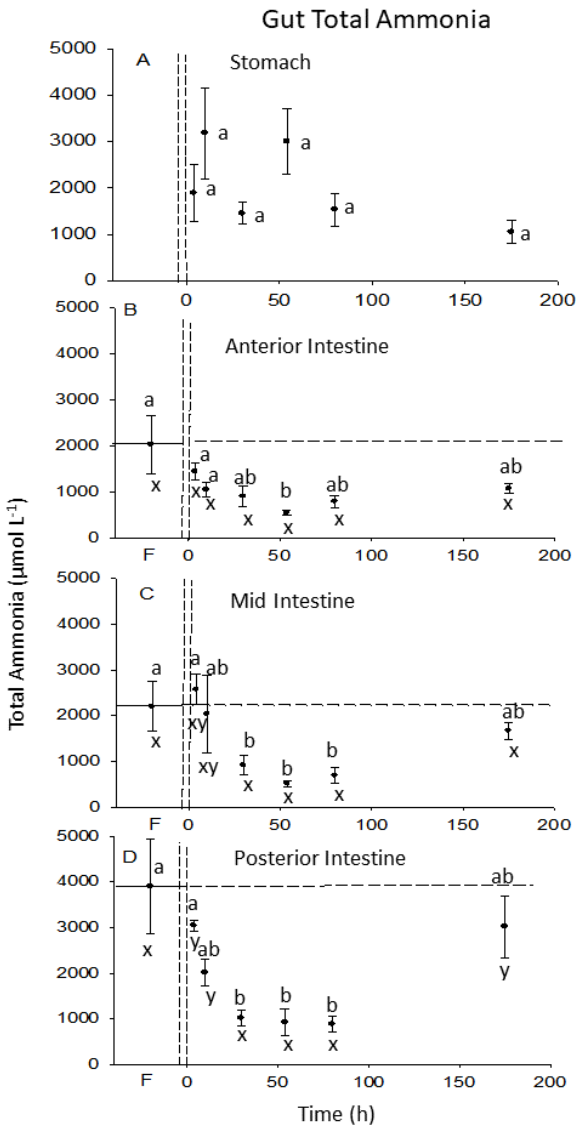


Figure 2

Figure 2

Time-dependent changes in total ammonia concentrations in chyme of (A) stomach; (B) anterior intestine; (C) mid-intestine; and (D) posterior intestine over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 6) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. For all values within a gut section (A) stomach: one-way parametric ANOVA on log10

transformed data $P < 0.001$; (B) anterior intestine: one-way non- parametric Kruskal -Wallis ANOVA $P < 0.001$; (C) mid intestine: one-way non-parametric Kruskal -Wallis ANOVA $P < 0.001$; and (D) posterior intestine: one-way non-parametric Kruskal -Wallis ANOVA $P < 0.001$. Within a gut section, means not sharing the same letter (a, b, c) are significantly different from one another by Dunn's non-parametric or Tukey's parametric *post hoc* tests respectively ($P \leq 0.05$). For the fasting values, a repeated measures one-way ANOVA revealed no significant differences among the three sections ($P = 0.184$). For the post-feeding values in the anterior, mid, and posterior intestine, two-way repeated measures ANOVA revealed significant main effects of time ($P < 0.001$), gut section ($P < 0.001$), and their interaction ($P = 0.019$). Within a time point, intestinal means not sharing the same letter (x, y, z) are significantly different from one another.

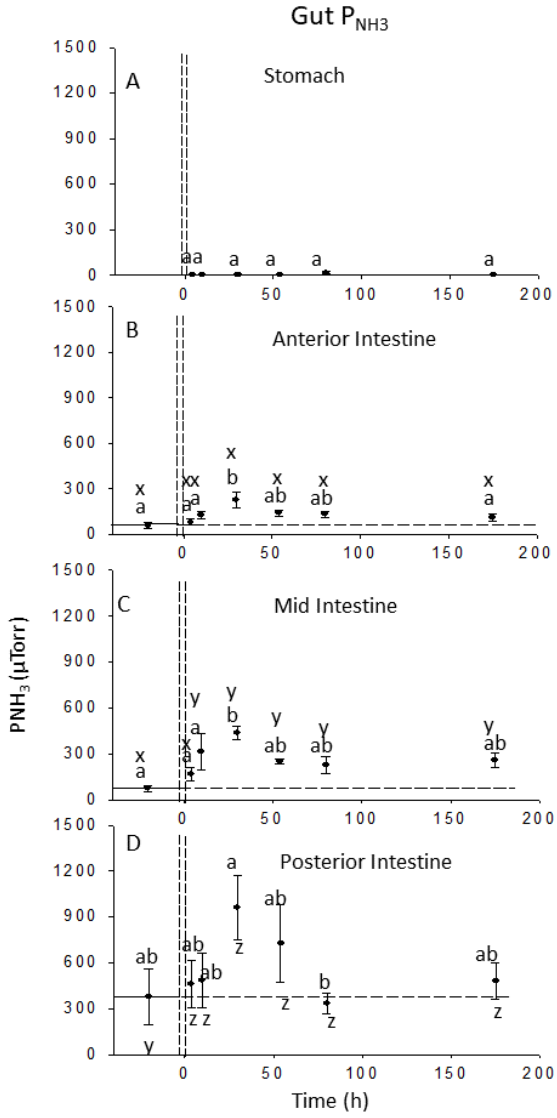


Figure 3

Figure 3

Time-dependent changes in the partial pressures of ammonia (PNH_3) in chyme of (A) stomach; (B) anterior intestine; (C) mid-intestine; and (D) posterior intestine over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 6) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. For all values within a gut section (A) stomach: one-way parametric ANOVA on

square root transformed data P = 0.794.; (B) anterior intestine: one-way parametric ANOVA P = 0.006; (C) mid intestine: one-way parametric ANOVA P = 0.007; and (D) posterior intestine: one-way parametric ANOVA on log10 transformed data P < 0.001. Within a gut section, means not sharing the same letter (a, b, c) at different times are significantly different from one another by Tukey's parametric *post hoc* tests ($P \leq 0.05$). For the fasting values, a repeated measures one-way ANOVA on the log10 transformed data (P = 0.0096) plus Tukey's test revealed that the posterior intestine value was higher than both the mid or anterior intestine value. For the post-feeding values in the anterior, mid, and posterior intestine, two-way repeated measures ANOVA on square root transformed data revealed significant main effects of time (P < 0.001), gut section (P < 0.001), but not their interaction (P = 0.794). Within a time point, intestinal means not sharing the same letter (x, y ,z) are significantly different from one another.

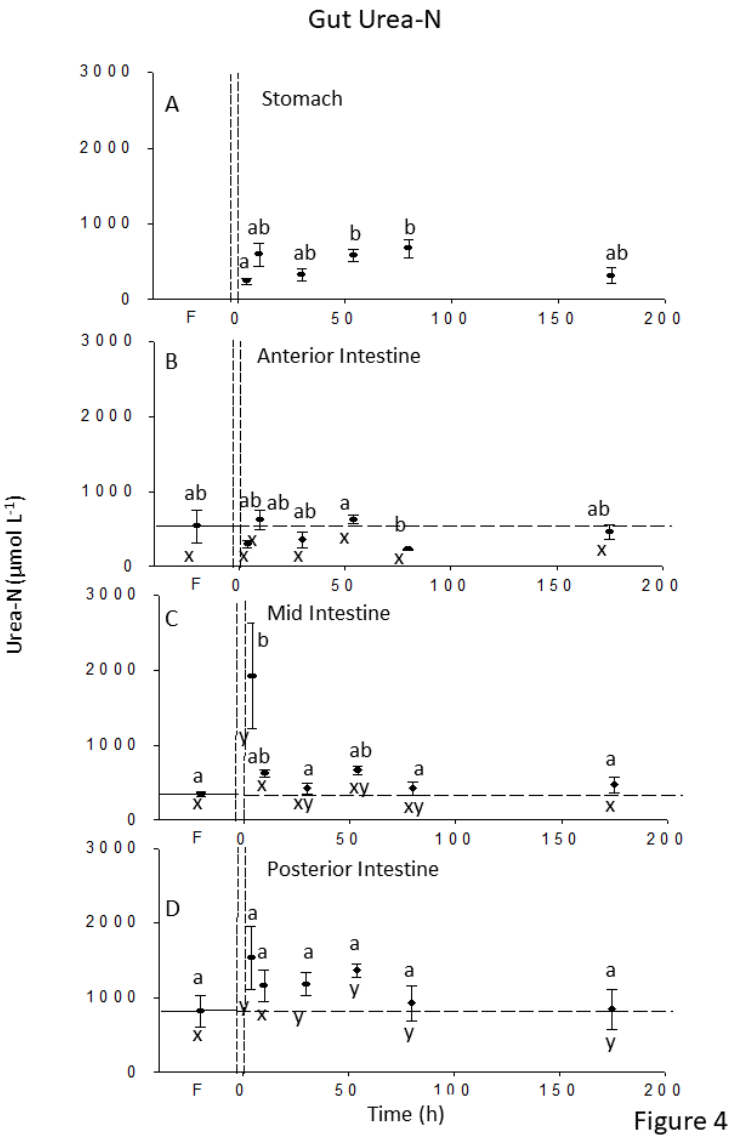


Figure 4

Figure 4

Time-dependent changes in urea-N concentrations in chyme of (A) stomach; (B) anterior intestine; (C) mid-intestine; and (D) posterior intestine over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 6) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. For all values within a gut section (A) stomach: one-way parametric ANOVA on log10

transformed data P = 0.494; (B) anterior intestine: one-way non-parametric Kruskal-Wallis ANOVA P = 0.011; (C) mid intestine: one-way parametric ANOVA on log10 transformed data P < 0.001; and (D) posterior intestine: one-way-ANOVA P = 0.312. Within a gut section. means not sharing the same letter (a ,b. c) at different times are significantly different from one another by Dunn's non-parametric or Tukey's parametric *post hoc* tests respectively ($P \leq 0.05$.). For the fasting values, a repeated measures one-way ANOVA revealed no significant differences among the three sections (P = 0.1566). For the post-feeding values in the anterior, mid, and posterior intestine, two-way repeated measures ANOVA on log10 transformed data revealed significant main effects of time (P < 0.001), and gut section (P < 0.001), and their interaction (P = 0.015). Within a time point, intestinal means not sharing the same letter (x, y, z) are significantly different from one another.

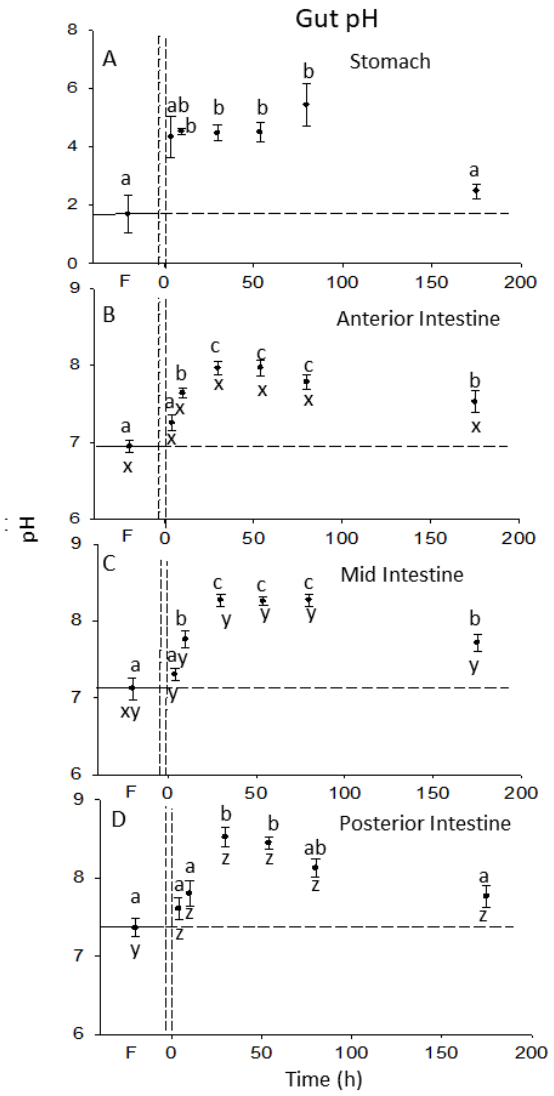


Figure 5

Figure 5

Time-dependent changes in the pH of chyme of (A) stomach; (B) anterior intestine; (C) mid-intestine; and (D) posterior intestine over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 6) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. For all values within a gut section (A) stomach: one-way parametric ANOVA P = 0.0027; (B) anterior intestine:

one-way parametric ANOVA $P < 0.001$; (C) mid intestine: one-way parametric ANOVA $P < 0.001$; and (D) posterior intestine: one-way parametric ANOVA $P < 0.001$. Within a gut section, means not sharing the same letter (a, b, c) at a different time are significantly different from one another by Tukey's parametric *post hoc* tests ($P \leq 0.05$). For the fasting values, a repeated measures one-way ANOVA on the log10 transformed fasting data ($P = 0.0276$) plus Tukey's test revealed that the posterior intestine value was higher than anterior intestine value. For the post-feeding values in the anterior, mid, and posterior intestine, two-way repeated measures ANOVA revealed significant main effects of time ($P < 0.001$) and gut section ($P < 0.001$), but not their interaction ($P = 0.898$). Within a time point, intestinal means not sharing the same letter (x, y, z) are significantly different from one another.

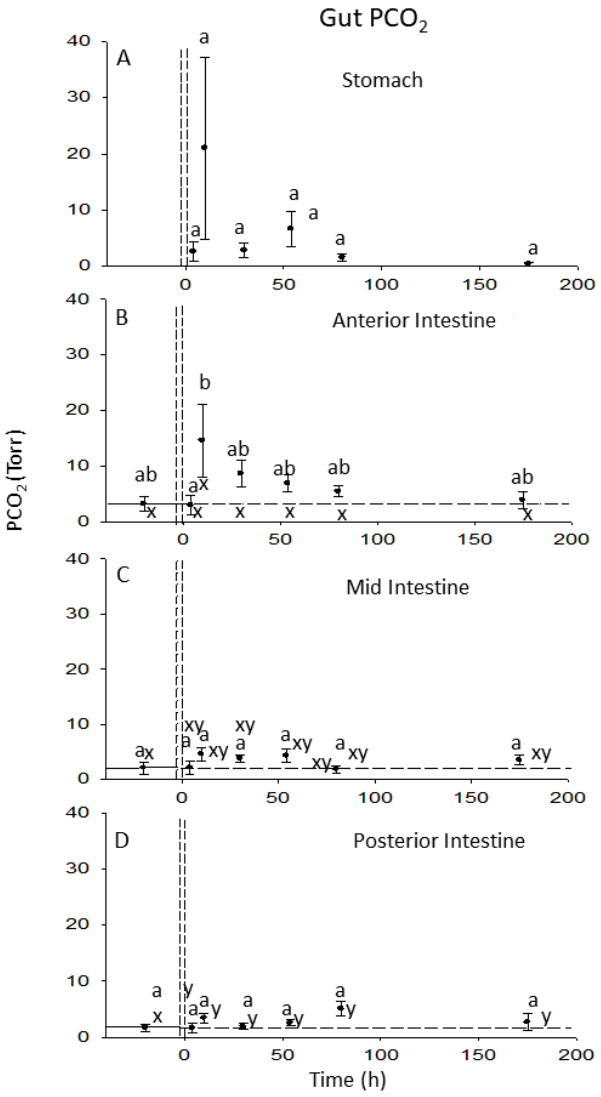


Figure 6

Figure 6

Time-dependent changes in the partial pressure of carbon dioxide (PCO_2) in chyme of (A) stomach; (B) anterior intestine; (C) mid-intestine; and (D) posterior intestine over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 6) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. For all values within a gut section (A) stomach: one-way parametric ANOVA on

log10 transformed data P = 0.100.; (B) anterior intestine: one-way parametric ANOVA on log10 transformed data P = 0.008; (C) mid intestine: one-way parametric ANOVA P = 0.316; and (D) posterior intestine: one-way parametric ANOVA on log10 transformed data P = 0.103. Within a gut section, means not sharing the same letter (a,b,c) at different times are significantly different from one another by Tukey's parametric *post hoc* tests ($P \leq 0.05$). For the fasting values, a repeated measures one-way ANOVA revealed no significant differences among the three sections (P = 0.086). For the post-feeding values in the anterior, mid, and posterior intestine, two-way repeated measures ANOVA on log10 transformed data revealed significant main effects of time ($P < 0.001$) and gut section ($P < 0.001$), but not their interaction (P = 0.160 . Within a time point, means not sharing the same letter (x, y, z) are significantly different from one another.

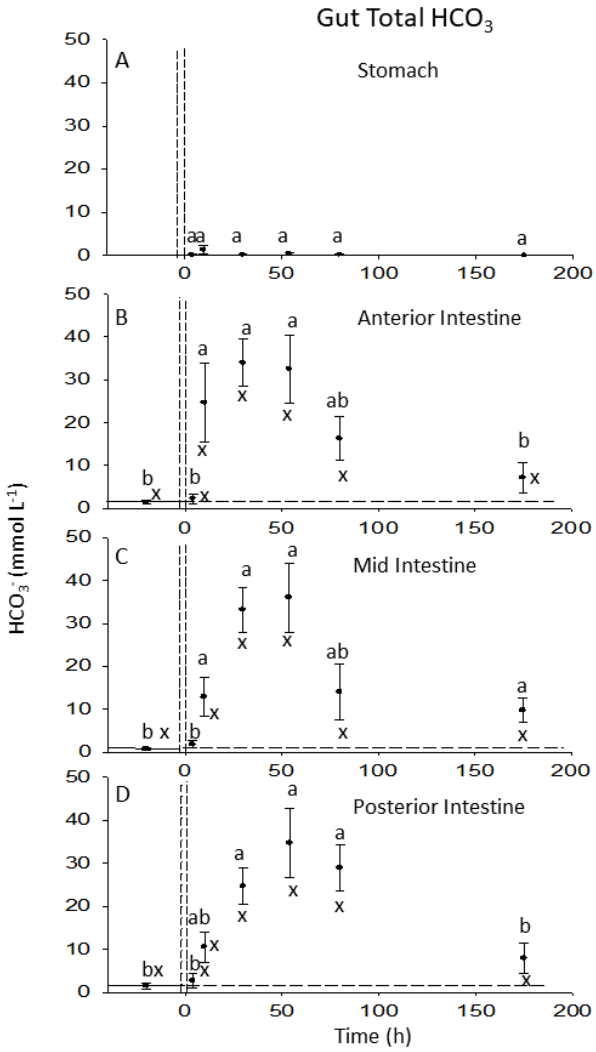


Figure 7

Figure 7

Time-dependent changes in the bicarbonate (HCO_3^-) concentrations in chyme of (A) stomach; (B) anterior intestine; (C) mid-intestine; and (D) posterior intestine over a 175-h period in response to a large meal (4.94% body weight) of commercial trout pellets in juvenile rainbow trout that had been fasted for 9 days previously. Means ± 1 SEM (N = 6) at each sampling time. F and dashed line indicate the fasting value, prior to feeding. For all values within a gut section (A) stomach: one-way parametric ANOVA P =

0.494; (B) anterior intestine: one-way parametric ANOVA on log10 transformed data $P < 0.001$; (C) mid intestine: one-way parametric ANOVA on log10 transformed data $P < 0.001$; and (D) posterior intestine: one-way non-parametric ANOVA on square root transformed data $P < 0.001$. Within a gut section, means not sharing the same letter (a,b,c) at different times are significantly different from one another by Tukey's parametric *post hoc* tests ($P \leq 0.05$). For the fasting values, a repeated measures one-way ANOVA revealed no significant differences among the three sections ($P = 0.285$). For the post-feeding values in the anterior, mid, and posterior intestine, repeated measures two-way ANOVA revealed significant main effects of time ($P < 0.001$) but not of gut section ($P = 0.853$), or their interaction ($P = 0.308$). Within a time point, intestinal means not sharing the same letter (x, y, z) are significantly different from one another.