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ORIGINAL PAPER

Do extreme postprandial levels of oxygen, carbon dioxide, and ammonia in the digestive tract equilibrate with the bloodstream in the freshwater rainbow trout (*Oncorhynchus mykiss*)?

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Abstract

The gastrointestinal tract (GIT) lumen of teleosts harbors extreme conditions, especially after feeding: high PCO_2 (20–115 Torr), total ammonia (415–3710 µM), PNH_3 (79–1760 µTorr in the intestine), and virtual anoxia ($PO_2 < 1$ Torr). These levels could be dangerous if they were to equilibrate with the bloodstream. Thus, we investigated the potential equilibration of O_2, CO_2, and ammonia across the GIT epithelia in freshwater rainbow trout by monitoring postprandial arterial and venous blood gases in vivo and in situ. In vivo blood was sampled from the indwelling catheters in the dorsal aorta (DA) and subintestinal vein (SIV) draining the posterior intestine in the fasting state and at 4 to 48 h following catheter-feeding. To investigate possible ammonia absorption in the anterior part of the GIT, blood was sampled from the DA, SIV and hepatic portal vein (HPV) from anaesthetized fish in situ following voluntary feeding. We found minimal equilibration of all three gases between the GIT lumen and the SIV blood, with the latter maintaining pre-feeding levels ($PO_2 = 25$–49 Torr, $PCO_2 = 6$–8 Torr, and total ammonia = 117–134 µM and $PNH_3 = 13$–30 µTorr at 48 h post-feeding). In contrast to the SIV, we found that the HPV total ammonia more than doubled 24 h after feeding (128 to 297 µM), indicative of absorption in the anterior GIT. Overall, the GIT epithelia of trout, although specialized for absorption, prevent dangerous levels of PO_2, PCO_2 and ammonia from equilibrating with the blood circulation.

Keywords Digestion · Dorsal aorta · Subintestinal vein · Hepatic portal vein · Lumen · Alkaline tide

Introduction

Conditions to improve fish health and maximize growth efficiency have been of growing interest in support of aquaculture, which is currently one of the fastest expanding industries globally (Food and Agriculture Organization 2020). Despite such interest, relatively little is known about the conditions in the gastrointestinal tract (GIT) and their significance to fish productivity. The GIT is an important multifunctioning organ in fish not only for digestion and nutrient absorption, but also for ionic and osmotic regulation, neuroendocrine regulation, immune responses, and even air breathing in some species (Grosell et al. 2011).

The GIT lumen is a unique extracorporeal environment containing a complex microbiome and lined with specialized GIT cells (e.g., oxynticopeptic cells, mucocytes, various enterocytes) interacting closely with other organ systems. In mammals, the lumen contains high carbon dioxide (CO_2) and low oxygen (O_2) levels derived from chemical reactions and bacterial fermentation of dietary substrates during digestion (reviewed by Kalantar-Zadeh et al. 2019). Recent research has shown that this is similar in fish. Direct measurements in the lumen of fasted freshwater rainbow trout (*Oncorhynchus mykiss*) and seawater English sole (*Parophrys vetulus*) have revealed low PO_2 (< 1 Torr) and high in PCO_2 (7–17 Torr) values (Wood and Eom 2019; Jung et al. 2020). Note that 1 Torr = 1 mm Hg = 0.13332 kPa. Feeding further elevates the luminal PCO_2 in fish (20–115 Torr), whereas PO_2 remains nearly anoxic (Wood and Eom 2019; Jung

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et al. 2020, 2022a). These luminal PO_2 and PCO_2 levels are much more extreme than normal arterial blood levels (PO_2 of ≥ 90 Torr; PCO_2 of ≤ 3 Torr, e.g. (Tetens and Lykkeboe 1985; Perry and Reid 1992; Thomas et al. 1994), and even more extreme than levels considered harmful in the natural environment (Alabaster et al. 1957; Ellis et al. 2017). Moreover, ammonia is also high in the lumen (415–3710 µM as total ammonia; 79–1760 µTorr in the intestine as PNH_3) as a result of breakdown of proteins in the feed (Bucking and Wood 2012; Rubino et al. 2014; Jung et al. 2022a). This also exceeds the water ammonia levels considered toxic for fish (Solbé and Shurben 1989; Randall and Tsui 2002).

Ultimately, there are significant gradients of both PCO_2 and ammonia from the lumen into the arterial blood perfusing the GIT, while the opposite is true for PO_2. Notably, plasma ammonia concentrations in mixed caudal blood (Kaushik and de Oliva Teles 1985; Bucking and Wood 2008), and in the hepatic portal vein (HPV) draining the GIT (Karlsson et al. 2006) are elevated significantly after feeding This excess ammonia is then excreted via the gills, and to a lesser extent via the kidney (Beamish and Thomas 1984; Wicks and Randall 2002; Karlsson et al. 2006; Bucking et al. 2010). However, transport of luminal ammonia into the systemic bloodstream must be carefully controlled as it can be a toxicant (Randall and Tsui 2002). Similarly, if the high luminal PCO_2 were to equilibrate with the blood, it would result in a very unfavourable blood acid–base disturbance (extreme respiratory acidosis) in fish (Perry and Gilmour 2006), though at the same time it potentially could help to release O_2 from haemoglobin by Bohr and Root effects (Nikinmaa 2006; Rummer and Brauner 2015). In mammals, some of the luminal CO_2 and other gases can be absorbed across the intestinal epithelia and enter the blood circulation, but they are also either removed by microbes or released as flatulence (Ohashi et al. 2007; Lacy et al. 2011). Whether or not high luminal PCO_2 and ammonia are also moving into the vascular system in fish is still largely unproven. A few calculated PCO_2 values in the venous blood draining from the GIT are reported to be low (Eliason et al. 2007; Cooper et al. 2014), but direct measurements in the subintestinal vein (SIV) have proven to be quite variable (Wood and Eom 2019; Jung et al. 2020, 2022a). This variability may reflect the fact that these direct measurements were taken from anaesthetized, artificially ventilated fish by direct micro-optode puncture of the SIV (in situ) rather than from conscious animals. Anaesthesia and/or blockade of blood flow may have confounded the results.

Therefore, the purpose of this study was to investigate the degree of equilibration of O_2, CO_2, and ammonia, between the GIT lumen and systemic bloodstream, using in vivo measurements on non-anaesthetized fish fitted with a stomach feeding tube and indwelling catheters for sampling inflowing blood from the dorsal aorta (DA) and outflowing blood from the subintestinal vein (SIV). We investigated this in freshwater-acclimated rainbow trout sampled in the fasting state and at 4, 8 12, 24, and 48 h after feeding (in vivo series; 1% body mass ration). We extended our in vivo ammonia findings with in situ experiments on anaesthetized trout that permitted sampling of blood from both the SIV that drains only the posterior tract and the hepatic portal vein (HPV) which additionally drains the entire GIT (in situ series; voluntarily fed to satiation). Plasma ammonia concentrations at the two sites were compared during the fasting state and at 24 and 48 h after voluntarily feeding (in situ series). In general, we hypothesized there would be minimal equilibration of these gases across the intestinal epithelia into the venous drainage. We also hypothesized that feeding would have little effect, so that SIV PO_2, PCO_2 and ammonia would remain largely unchanged during digestion. Finally, in light of the findings of Karlsson et al. (2006), we hypothesized that postprandial luminal absorption of ammonia would be seen in the anterior part of the GIT, and thus higher plasma ammonia concentrations in blood sampled from the HPV than from the SIV.

Materials and methods

Experimental animals

Rainbow trout (220–620 g) were obtained from Little Cedar Falls Hatchery (Nanaimo, BC, Canada) and transferred to the University of British Columbia (UBC) where they were held for several months prior to experiments. Fish were held at 9°C in flowing dechlorinated Vancouver tap water (Na^+ = 0.09, Cl^- = 0.10, Ca^{2+} = 0.10, Mg^{2+} = 0.011, K^+ = 0.004 mmol l^{-1}, hardness as $CaCO_3$ = 3.3 mg l^{-1}, pH = 7.0). During this time, fish were fed to satiation daily with commercial pellet food (BioTrout 4.0 mm, Bio-Oregon™, Long-view, WA, USA). Food was withheld for 7 days prior to experimentation. For the in vivo series, fish were anaesthetized and surgery was performed as described below. For the in situ series, fish were sampled either at 7 days of fasting (control), or at 24 h or 48 h after voluntary feeding to satiation. For both series, we measured or calculated P_aO_2, haemoglobin-O_2 saturation (Hb-O_2 saturation), pH_a, $[HCO_3^-]_a$, P_aCO_2, total ammonia (Tamm), and/or P_aNH_3 from blood sampled from the dorsal aorta (DA). Venous drainage was sampled from the subintestinal vein (SIV) and measured for P_vO_2, Hb-O_2 saturation, pH_v, $[HCO_3^-]_v$, P_vCO_2, SIV Tamm, and/or P_vNH_3. All experiments were approved by the UBC Animal Care Committee (AUP 14–0251 and 18–0271) and conformed to national regulations of the Canada Council for Animal Care.

Cannulation and blood sampling

For both in vivo and in situ series, the same DA and SIV cannulation techniques were performed. Fish were anaesthetized with 0.1–0.2 g l^{-1} NaOH-neutralized MS-222 (Syndel Laboratories, Parksville, BC). Once anaesthetized, the fish were transferred to an operating table and their gills were continuously irrigated with temperature-controlled anesthetic water (~0.03 g l^{-1}) to achieve stage 5 anaesthesia (McFarland 1959). The DA was cannulated according to the procedure described in Soivio et al. (1972). The SIV was cannulated near the proximal end of the posterior intestine, as described by Cooper et al. (2014). In brief, a 3-cm incision in the midline anterior to the anus was made, the SIV was located, and then the most posterior end of the vessel was tied with 2–0 silk thread to occlude posterior blood flow. About 2 cm of polyethylene tubing-10 (PE10; Clay-Adams™, Becton and Dickinson Co., Franklin Lakes, NJ, USA) was fitted to stretched PE50 tubing and filled with heparinized (150 I.U. ml^{-1}; Sigma-Aldrich, St. Louis, MO, USA) Cortland saline (Wolf 1963). An opening for the cannula in the SIV was made with a hypodermal needle, then the PE10-tipped cannula was pushed forward anteriorly about 2 cm and tightly secured around the vessel using 2–0 surgical silk.

For in vivo series, blood flow through the cannula was checked, which was then flushed with heparinized saline. The cannula was secured to the anal fin and the incision was sutured closed with silk suture. In addition, flared PE240 was inserted down the oesophagus into the stomach and securely sutured through a hole next to the nostril to serve as a feeding tube. Fish were then transferred to black Plexiglas chambers and were left to recover overnight. The chambers were supplied with flow-through 9˚C freshwater and were aerated throughout the experiment. After overnight recovery, blood was sampled from both the DA and SIV cannulae while the fish was still in the fasting state, which will be referred to as the "control" from here on. Approximately 0.5 ml of blood was sampled from each cannula by blood pressure-driven and/or gravity driven flow into 0.6-ml heparinized microcentrifuge tubes, taking care to minimize air exposure. Equivalent volumes of saline were immediately injected to replace the blood removed by sampling. Immediately after, fish were fed a 1% body mass ration of pellet food (delivered as a slurry of homogenised food pellets in two volumes of water; Cooper and Wilson 2008; Bucking et al. 2010) via the oesophageal feeding tube. Postprandial blood samples were collected at 4, 8, 12, 24 and 48 h after feeding.

For the in situ series, fish were implanted with the same DA and SIV cannulae on a surgery table with anaesthesia and gill irrigation maintained at the same level throughout. Experiments lasted approximately 1 h. Blood samples were collected via the cannulae into 0.6-ml heparinized microcentrifuge tubes. In addition, the hepatic portal vein (HPV) blood was sampled by direct insertion of the needle of a heparinized gas-tight 100 µL-syringe (Hamilton, Reno, Nevada, USA) into the blood vessel.

Whole blood measurements

Collected blood samples were first measured for pH, PO_2 and PCO_2 by inserting the micro-electrode and micro-optodes to the bottom of the microcentrifuge tube, while kept at the experimental temperature. The pH was measured using an oesophageal pH microelectrode (MI-508; 1.4–1.6 mm OD) and a flexible micro-reference electrode (MI-402; Microelectrodes Inc., Bedford, NH, USA) connected to a model 220 pH meter (Corning Instruments, Corning, NY, USA). The electrodes were calibrated with precision buffers at the appropriate temperature (Fisher Scientific and Radiometer-Copenhagen, Copenhagen, Denmark).

The PO_2 and PCO_2 were measured using micro-optodes (PreSens Precision Sensing GmbH Regensburg, Germany) positioned into the collection tubes using micro-manipulators (World Precision Instruments, Sarasota, FL, USA). The PO_2 micro-optodes were calibrated with air-equilibrated and sodium sulfite saturated saline kept in gas-tight bottles. The PCO_2 micro-optodes were prototype devices (PreSens 200 001 368) connected to an electronic transmitter (PreSens 300 000 114), with the output displayed on a personal computer running prototype software (PreSens 200 001 488). These PCO_2 micro-optodes were prepared and calibrated as described in Jung et al. (2020), with salines equilibrated to 0.04, 0.5, 1, 3, and 5% CO_2 using CO_2/air mixtures created by a 301aF precision gas-mixing pump (Wösthoff Messtechnik GmbH, Bochum, Germany). All calibration solutions were kept at the experimental temperature of 9 °C.

The haemoglobin-O_2 saturation (%) was calculated from the measured blood O_2 content ($[O_2]$) and haemoglobin concentration ([Hb]) by taking 4 O_2 molecules per Hb (tetramer) into account. Blood $[O_2]$ was measured using the Tucker method (Tucker, 1967). During collection of the blood from cannulae into the microcentrifuge tubes, 10 µL of blood was taken using a gas-tight syringe and injected into a custom-made 2344 µL Tucker chamber with the PO_2 micro-optode inserted through a gas-tight septum. The chamber was filled with a potassium ferricyanide and saponin solution to release O_2 bound by haemoglobin. The [Hb] was measured as described by Kampen and Zijlstra (1961). After all measurements were completed, the remaining blood was centrifuged (2 min, 5000 g), and the plasma was removed and flash-frozen in liquid N_2 and stored at –70 ºC in an ultra-cold freezer.

Plasma and chyme measurements: Tamm and TCO_2

At the end of the experiment, fish were euthanized by an overdose of neutralized MS-222. The four segments of the GIT (stomach, anterior intestine, mid intestine, posterior intestine) were ligated and excised, and the chyme was collected into 2-ml centrifuge tubes. The chyme samples were centrifuged (2 min, 5000 g), and the pH values of the supernatants were measured. The samples were then flash-frozen in liquid N_2 for storage at –70 ºC in an ultra-cold freezer.

Plasma and chyme supernatant were later measured for total CO_2 content (TCO_2) using a Corning 965 CO_2 analyser (Ciba-Corning Diagnostics, Halstead, Essex, UK) calibrated with $NaHCO_3$ standards. The samples were also analyzed for total ammonia (Tamm; μM) by an enzymatic assay based on the glutamate dehydrogenase/NAD method using a commercial kit (Raichem; San Diego, CA, USA).

Calculations and statistical analyses

The [HCO_3^-] values in plasma and chyme, and the PCO_2 values in chyme were derived from measurements of pH and TCO_2 via rearrangements of the Henderson–Hasselbach equation (see Wood et al., 1983) using values for pK' and CO_2 solubility for teleost plasma (Boutilier et al., 1984). Partial pressures of ammonia (PNH_3) of both plasma and chyme were similarly calculated via the Henderson–Hasselbach equation using measured Tamm and pH values with the pK and solubility coefficients reported in Cameron and Heisler (1983).

Graphs were made and statistical analyses were performed using Graphpad Prism software (version 7.0a). Data have been expressed as means ± SEM (N = number of fish). For both series, comparisons between DA and SIV at each postprandial sampling time were made using two-way ANOVA with Sidak's post hoc test. Comparisons of the measured parameter of either DA or SIV from different postprandial sampling times to that of the control value were done using two-way ANOVA with Dunnett's post hoc test. Tamm and PNH_3 data were log transformed to meet assumptions of homogeneity of variance, and comparison between plasma and chyme Tamm or PNH_3 values were done using one-way ANOVA with Sidak's post hoc test. One-way ANOVA with Tukey's post hoc test were performed to compare chyme pH, [HCO_3^-], PCO_2 between GIT compartments. Comparison of pH, [HCO_3^-] or PCO_2 between posterior chyme and SIV were done using two-way ANOVA with Sidak's post hoc test. A significance level of $p < 0.05$ was used in all tests.

Results

Postprandial blood O_2 (in vivo)

Within 4 h of feeding, P_aO_2 in the DA increased significantly from about 100 to 140 mmHg, remained high for about 8 h, then gradually came back down to control level by 24 and 48 h after feeding (Fig. 1A). Feeding had no significant effect on the P_vO_2 in the SIV (25–49 Torr), which

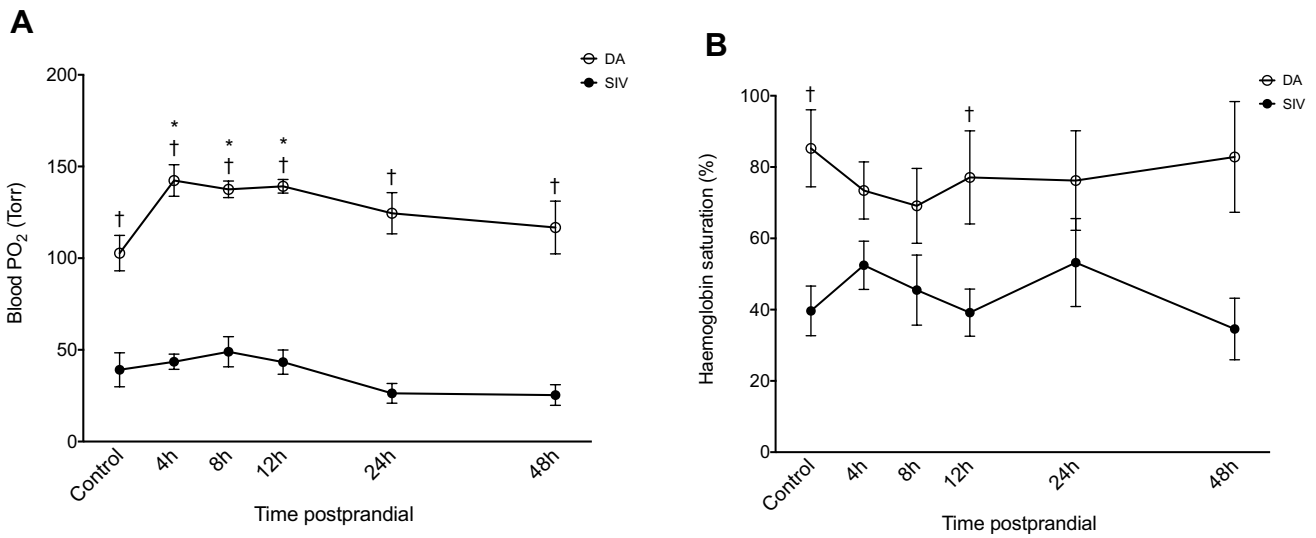

Fig. 1 Postprandial dorsal aorta (DA) and subintestinal vein (SIV) blood **A** PO_2 (DA parameters indicated with subscript "a" and SIV with subscript "v"; P_aO_2 and P_vO_2, respectively; Torr; N = 5–9) and **B** haemoglobin saturation (%), measured on rainbow trout in vivo. Control represents fasting state prior to feeding which occurred immediately thereafter. Values are means ± SEM (N = 4–6). †Indicates significant difference between DA and SIV values. All SIV PO_2 were significantly lower than DA PO_2. *Indicates a significant difference from control values for either DA or SIV

remained lower than P_aO_2 throughout the series. The difference between P_aO_2 and P_vO_2 was approximately 64 mmHg in fasting fish (control) then increased to 98 mmHg at 4 h following feeding. Despite these changes and the observed increase in P_aO_2 in response to feeding, the Hb-O_2 saturation did not change in either arterial (DA) or venous (SIV) blood, averaging 77.3 ± 2.4% and 44 ± 3.1%, respectively (Fig. 1B).

Postprandial blood and chyme pH, $[HCO_3^-]$, PCO_2 (in vivo)

We observed a metabolic alkalosis (also known as an alkaline tide) at 24 h post-feeding where plasma pH_a and $[HCO_3^-]_a$ were elevated significantly relative to the control values (Fig. 2A, B). A 5.6 mM increase in $[HCO_3^-]_a$ at 24 h led to a 0.4 unit increase in pH_a. Both parameters then came back down to the control level by 48 h. In contrast, the pH_v and $[HCO_3^-]_v$ remained unchanged throughout digestion. DA and SIV values were similar except at 4 and 24 h for pH, and at 24 h for $[HCO_3^-]$. Throughout the experiment, the measured P_vCO_2 values in the SIV were generally higher than P_aCO_2 by approximately 1.5 Torr (Fig. 2C). Both P_aCO_2 and P_vCO_2 increased similarly at 12 h (by ~ 1.7 and 1.4 Torr, respectively), but came back down to control levels by 24 h. Interestingly, in both DA and SIV blood, the PCO_2 remained below 8 Torr throughout digestion.

The cannulated animals were killed for chyme sampling at 48 h post-feeding. Visual inspection showed that most of the chyme was in the posterior intestine at this time, with small amounts in the more anterior sections of the intestine and stomach. Levels of pH and $[HCO_3^-]$ in the chyme collected at 48 h increased from anterior to posterior sections

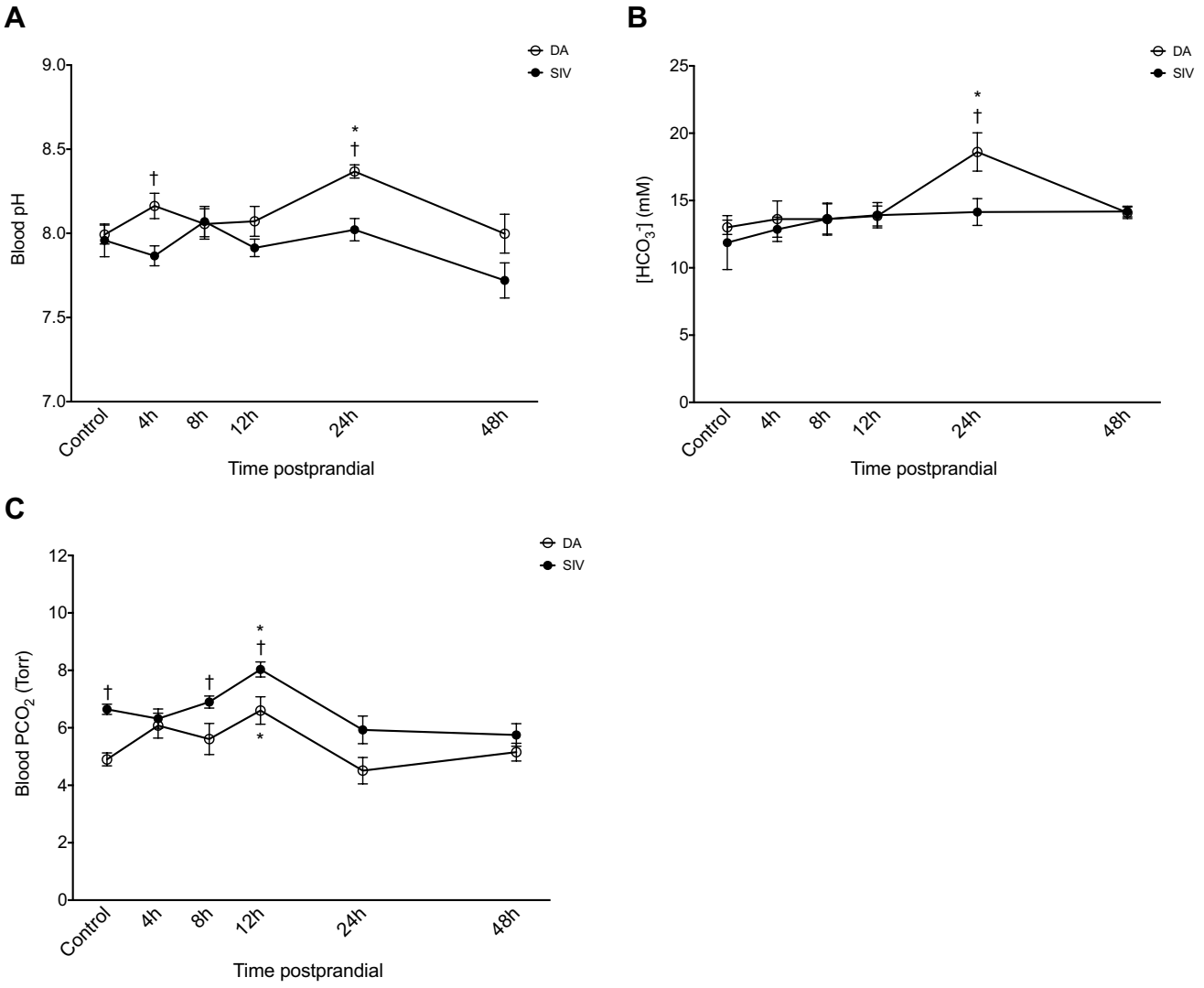

Fig. 2 Postprandial DA and SIV blood **A** pH (pH_a and pH_v, respectively; N = 5–9), **B** $[HCO_3^-]$ ($[HCO_3^-]_a$ and $[HCO_3^-]_v$ respectively; mM; N = 4–9), and **C** PCO_2 (P_aCO_2 and P_vCO_2 respectively; Torr; N = 4–9), measured on rainbow trout in vivo. See Fig. 1 legend for further details

of the GIT (Table 1). Consequently, the calculated PCO_2 was the highest in the stomach chyme despite the lowest $[HCO_3^-]$ due to its low pH. The intestinal chyme $[HCO_3^-]$ was much higher than that of the $[HCO_3^-]_v$ in the SIV blood plasma (Table 1 and Fig. 2B; $p = 0.005$), especially in the posterior intestine where the SIV blood was sampled. However, the pH (8.0 ± 0.1) and PCO_2 values (15.3 ± 7.7 Torr) in the posterior intestinal chyme were not significantly different from those (7.7 ± 0.1, 5.8 ± 0.4 Torr, respectively) in the SIV blood plasma (Table 1 and Fig. 2A, C; $p = 0.14$ and 0.31, respectively).

Postprandial plasma and chyme ammonia (in vivo and in situ)

In both the in vivo and in situ series, venous (SIV and/or HPV) plasma Tamm values were generally slightly higher than arterial (DA) plasma Tamm (Fig. 3 A&B; denoted by †). Interestingly, both series showed that DA and SIV Tamm did not increase with feeding. In fact, in the in situ series, SIV Tamm decreased by 52% at 48 h after feeding (Fig. 3B), a trend that was not seen in the in vivo series (Fig. 3A). Using the Henderson-Hasselbalch equation with measured Tamm and pH, we calculated PNH_3 in plasma and chyme in both series (Fig. 4). The DA and SIV plasma PNH_3 were not significantly different from each other, with values of 30–43 µTorr in the in vivo series and 8–13 µTorr in the in situ series. The SIV plasma Tamm and PNH_3 at 48 h were 79–96% lower than posterior chyme Tamm or PNH_3 collected immediately afterwards in both series (Fig. 4). In contrast, HPV Tamm increased by 132% at 24 h after feeding and remained high until 48 h (Fig. 3B).

The chyme Tamm (Fig. 4A, B) was highest in the stomach in both the in vivo and the in situ series (2184 and 2024 µM, respectively), followed by posterior intestine (1027 and 567 µM, respectively), and anterior and mid intestines (495–722 and 114–160 µM, respectively). Reflecting the pH gradient along the GIT (Table 1); however, PNH_3 was the lowest in the stomach in both series, with 0.02–3.1 µTorr. The intestinal chyme PNH_3 gradually increased down the

Table 1 Measured pH, $[HCO_3^-]$, and calculated PCO_2 of chyme collected in different GIT segments 48 h post-feeding of the rainbow trout in the in vivo series

	Stomach	Anterior intestine	Mid intestine	Posterior intestine
pH	5.3 ± 0.3 ^{x}	7.6 ± 0.1 ^{y}	7.7 ± 0.1 ^{y}	8.0 ± 0.1 ^{y}
$[HCO_3^-]$ (mM)	1.9 ± 0.9 ^{x}	19.4 ± 4.9 ^{y}	21.0 ± 5.1 ^{y}	40.1 ± 5.8 ^{z}
PCO_2 (Torr)	125.5 ± 15.8 ^{x}	12.0 ± 2.5 ^{y}	15.5 ± 7.0 ^{y}	15.3 ± 7.7 ^{y}

Letters indicate significant difference between different segments for that particular variable. Values are means ± SEM (N = 7–9 for pH; N = 4–8 for $[HCO_3^-]$ and PCO_2)

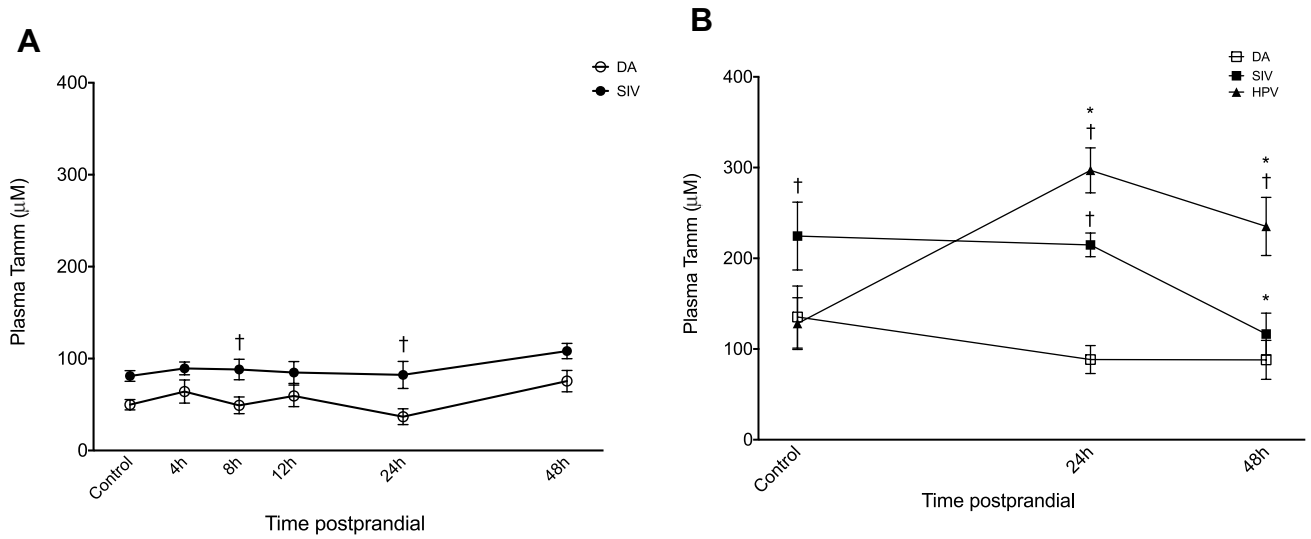

Fig. 3 Comparison of postprandial plasma Tamm (µM) from DA, SIV, and HPV blood sampled from rainbow trout **A** in vivo (N = 4–6) and **B** in situ (N = 5–7). Control represents fasting state, feeding occurred immediately after control sample was taken in **(A)**. See Fig. 1 legend for further details

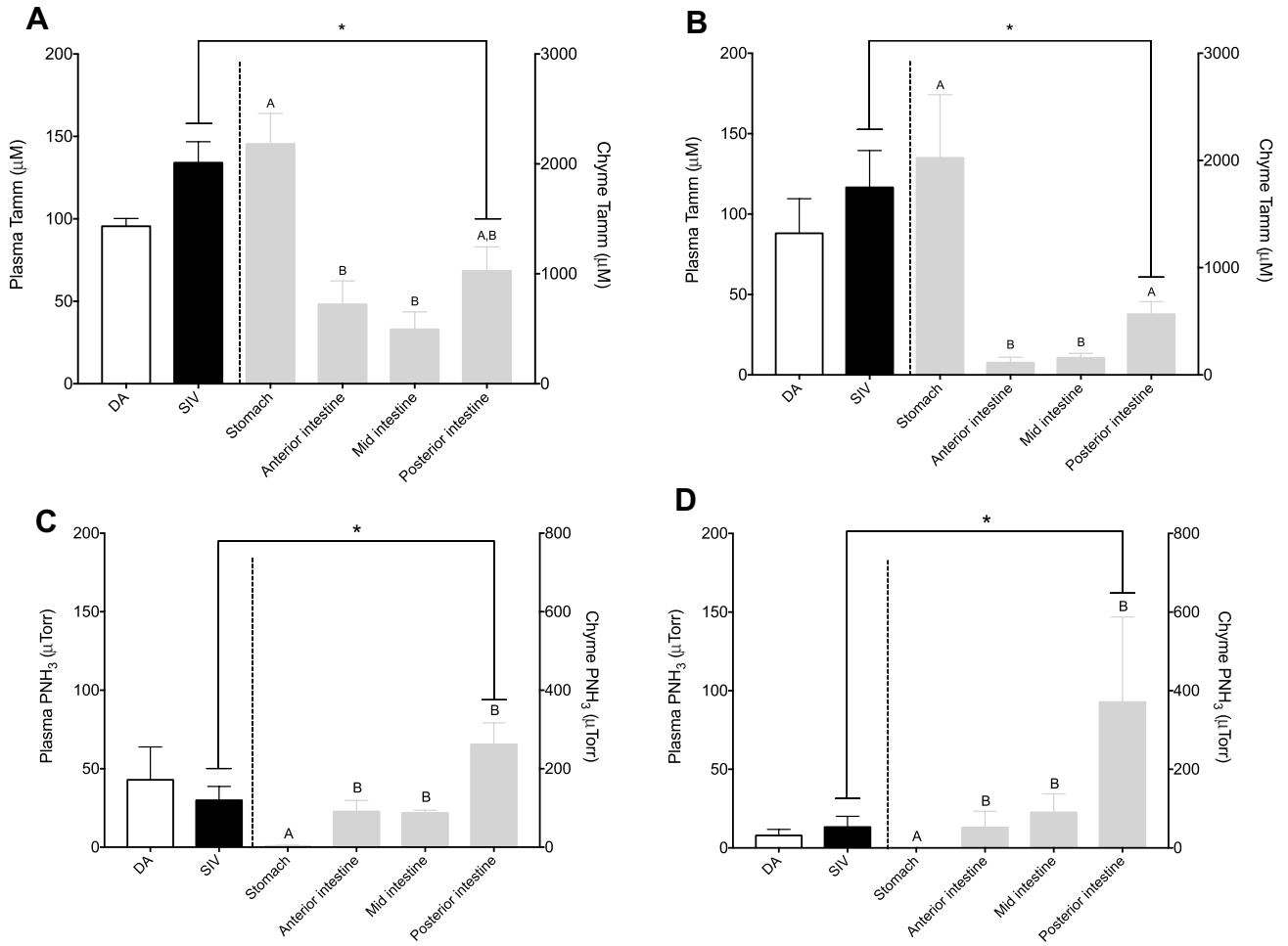

Fig. 4 Comparison of measured plasma and chyme (**A**, **B**) Tamm (μM) and calculated (**C**, **D**) PNH_3 (μTorr) sampled 48 h post feeding in rainbow trout in (**A**, **C**) the in vivo series (N = 4–7) and in (**B**, **D**) the in situ series (N = 5–7). Plasma Tamm values are reproduced from Fig. 3. In all graphs, left y-axis represents plasma values and right y-axis represents chyme values. Note different scales between left and right y-axes. Values are means ± SEM. DA and SIV plasma values were not significantly different from each other in all panels. Upper case letters indicate significant difference between chyme from different GIT compartments. *Indicates a significant difference between SIV plasma and posterior intestine chyme values

tract, with posterior intestine being significantly higher (in vivo 262; in situ 371 μTorr) than all other measurements including plasma values in both series.

Discussion

Here, we investigated potential equilibration of the two respiratory gases, O_2 and CO_2, and ammonia (total ammonia = $NH_3 + NH_4^+$) across the GIT epithelia in rainbow trout by monitoring arterial and venous blood status in vivo following feeding. Overall, feeding had no significant effect on subintestinal venous blood plasma PO_2, PCO_2, and ammonia sampled near the posterior intestine, and these were notably different from the posterior intestine chyme values measured in this study and those previously reported. Thus, our data support our overall hypotheses that there is minimal equilibration between the lumen and the blood circulation, and that blood gas levels in the venous drainage of the intestine remain largely unchanged after feeding. However, we did observe a significant increase in HPV plasma ammonia level after feeding, which agrees with our hypothesis that ammonia absorption occurs in the anterior part of the GIT.

Postprandial blood O_2 (in vivo)

The control P_aO_2 measured in this study is similar to previously reported values of fish starved for 48 h or more (Tetens and Lykkeboe 1985; Perry and Reid 1992). Feeding significantly increased this value within 4 h, which then gradually dropped back to the control level by 48 h (Fig. 1A; open circles). Interestingly, the arterial blood Hb-O_2 saturation (%)

was not affected by feeding (Fig. 1B) and remained similar to previously measured values of fasted animals (Milligan and Wood 1987) and within the typical range of rainbow trout blood (Rummer and Brauner 2015). This suggests a ventilatory response to feeding, rather than an increase in O_2 carrying capacity or blood-O_2 affinity. This is an opposite response to that known in air-breathing terrestrial vertebrates that reduce ventilation to retain CO_2, thereby triggering a rise in P_aCO_2 following feeding so as to minimize the rise in blood pH (reviewed by Wang et al. 2001). In water-breathing fish, for which O_2 solubility in the media is much less than CO_2 solubility, it has been understood that hypoventilation would detrimentally compromise O_2 uptake at a time when metabolic demand (MO_2) is elevated during digestion. Thus, hypoventilation is assumed to be an impractical response to feeding in fish, but a detailed study is lacking.

Although P_aO_2 increased after feeding, SIV P_vO_2 and Hb-O_2 saturation did not change throughout digestion (Fig. 1; closed circles). This agrees with our initial hypothesis where we expected to see no effect of low luminal PO_2 on P_vO_2. The lumen of the rainbow trout GIT remains nearly anoxic (< 1 Torr) even during digestion (Jung et al. 2022a). The lumen of the mammalian GIT also remains virtually anoxic (Kurbel et al. 2006; Bettinger 2015; Kalantar-Zadeh et al. 2019), due to the respiration of inhabiting microbes (Espey 2013) and the oxidative chemistry of the GIT fluids (Friedman et al. 2018). In humans, systemic hypoxemia can downregulate many of the GIT transporters (Ward et al. 2014), hindering its functions in assimilation and absorption. The multifunctional GIT of fish (Grosell et al. 2011) also possesses many of the same transporters as in the human GIT, and so may be maintaining its bloodstream PO_2 to avoid such challenges. Thus, mechanisms appear to be present to prevent hypoxia such as increases in blood flow rate. Indeed, to meet the postprandial increase in GIT O_2 requirement, blood flow is increased and redistributed to the GIT (Axelsson et al. 1989, 2000; Axelsson and Fritsche 1991; Thorarensen and Farrell 2006; Eliason et al. 2008). Human colonic cells are shown to survive in an anoxic environment for up to 96 h with a sign of acclimation to the condition such as lowering levels of oxidative stress (Vissenaekens et al. 2019), but similar mechanisms in teleost intestinal cells are as yet unknown. Feeding induces an increase in whole-animal MO_2 by ~ 96% by 27 h in adult rainbow trout (Eliason et al. 2008), which is likely accompanied by a simultaneous increase in GIT tissue MO_2 (Taylor and Grosell 2009). Although the difference between P_aO_2 and P_vO_2 was greater following feeding (63 Torr in control vs. 88–99 Torr during digestion), there was no change in SIV Hb-O_2 saturation (Fig. 1B). In fish, however, the GIT blood flow can be controlled and quickly increased to supply metabolically active organs at the time of need (Dupont-Prinet et al. 2009; Gräns et al. 2009a, b; Sundh et al. 2018). In various species including rainbow trout, feeding is shown to induce redistribution of blood flows to the stomach and intestine, with elevations of 72–136% (Axelsson et al. 1989, 2000; Axelsson and Fritsche 1991; Thorarensen and Farrell 2006; Eliason et al. 2008). Thus, the postprandial increase in GIT O_2 requirement could be presumably met by an increase in blood flow regardless of the luminal PO_2 status.

Postprandial blood and chyme pH, $[HCO_3^-]$, and PCO_2 (in vivo)

Under control (fasting) conditions, pH_a, $[HCO_3^-]_a$, and P_aCO_2 were similar or slightly elevated relative to previous findings (e.g. Bucking and Wood 2008; Cooper and Wilson 2008; Milligan and Wood 1987; Perry and Reid 1992; Wood and Eom 2019). This study found a classic postprandial alkaline tide, where arterial pH and $[HCO_3^-]$ increased (Fig. 2A, B) presumably due to acid secretion by the stomach (reviewed by Hersey and Sachs 1995). In both voluntarily and catheter-fed rainbow trout, plasma $[HCO_3^-]$ level increases by ~ 3–4 mM, causing blood pH to rise by ~ 0.2–0.3 units (Bucking and Wood 2008; Cooper and Wilson 2008; Bucking et al. 2009). In this study, we found $[HCO_3^-]$ and pH values peaked at 24 h after feeding with elevations of 5.6 mM and 0.4 units, respectively (Fig. 2A, B). The delayed alkaline tide observed in this study (24 h *vs* 3–12 h; e.g. Bucking et al. 2009; Bucking and Wood 2008; Cooper and Wilson 2008) could be due to the relatively small ration (reviewed by Wood 2019) introduced via the feeding tube (Cooper and Wilson 2008) or delayed digestion due to stress from the surgery (Eliason et al. 2008). For example, a bigger ration size induces greater base excretion, likely to correct for greater blood-acid–base disturbance (Bucking and Wood 2008; Cooper and Wilson 2008; Wood 2019). Moreover, the lower acclimation temperature used here (9 ℃) can slow down digestion (Jobling 1981; McCue 2006) relative to the temperatures used in studies mentioned above (10–15.5 ℃). Nonetheless, the increase in pH_a and $[HCO_3^-]_a$ were indicative of animals undergoing digestion.

We found no change in P_aCO_2 following feeding (Figs. 1A and 2C), similar to the observations of Bucking and Wood (2008) in voluntarily fed rainbow trout. Our finding and other recent studies suggest fish have a compensatory mechanism for dealing with alkaline tide that is different from the hypoventilatory CO_2 retention strategy of air-breathing terrestrial vertebrates (e.g. Andrade et al. 2004; Busk et al. 2000; Higgins 1914; Wang et al. 2001). Nonetheless, fish are able to minimize blood pH disturbance relative to the postprandial metabolic base load (Wood et al. 2007; Bucking and Wood 2008; Bucking et al. 2010) by excreting HCO_3^- via branchial Cl^-/HCO_3^- exchange mechanisms (Wood et al. 2005, 2007; Tresguerres et al. 2007; Bucking and Wood 2008), as well as via the urine (Bucking

et al. 2010). However, it is interesting that the increase in [HCO_3^-] seen in DA plasma was not observed in SIV plasma (Fig. 2B). Some of the base load from the stomach is likely excreted on the first pass through the gills, and the remaining fraction is what was seen in our DA samples. This base load was not detected in the SIV sampling site, downstream of the gills and DA sampling site. In seawater fish, the intestine has the capacity to compensate the plasma base load by HCO_3^- secretion into the lumen where it is used for osmoregulation (Wilson et al. 2002; Wilson and Grosell 2003; Grosell and Genz 2006; Grosell et al. 2007; Taylor et al. 2010) and possibly even for postprandial HCO_3^- excretion in rectal fluid and feces (Wilson et al. 1996; Taylor and Grosell 2006; Bucking et al. 2009). Presumably, the intestine in freshwater fish may also be a potential site for postprandial base excretion to alleviate the alkaline tide in addition to export by the gills and kidney. However, an in vitro study found that freshwater rainbow trout intestine tissue had minimal involvement in removing a metabolic base load (Bucking et al. 2009). Additional studies are required to evaluate this possibility further.

Here, we have recorded the first in vivo measurements of PCO_2 in the SIV blood during digestion (Fig. 2C; closed circles). During the period of active digestion, luminal PCO_2 is found to be very high (calculated values of 25–111 Torr and direct measurements of 20–40 Torr; Jung et al. 2022a, b; Wood and Eom 2019). In mammals, microbial metabolic production, reaction of gastric HCl with food carbonates, and endogenous HCO_3^- secretion contribute to high luminal PCO_2 (Steggerda 1968; Altman 1986; Tomlin et al. 1991; Suarez et al. 1997; Kurbel et al. 2006). In fish, endogenous HCO_3^- secretion by the intestinal epithelium for neutralizing chyme (Bucking and Wood 2009; Wood and Eom 2019; Goodrich et al. 2020) and for osmoregulatory purposes, particularly in seawater fish, can contribute to the high luminal PCO_2. We found that luminal PCO_2 remained elevated even at 48 h after feeding, and at particularly high levels in the stomach in parallel to its low pH (Table 1).

Until now, it has been difficult to conclude whether such high luminal PCO_2 will equilibrate with the venous bloodstream draining the GIT. In seawater-acclimated rainbow trout (presumably) fasted for 72 h, calculated SIV PCO_2 was low at 4.7 Torr (Cooper et al. 2014). Moreover, in Atlantic salmon fed 24–48 h prior to measurement, calculated HPV PCO_2 was low at 4.8 Torr and remained low at 2.9–3.6 Torr for 7 days (Eliason et al. 2007), implying that PCO_2 may not be equilibrating into the blood draining the stomach as well. Both Jung et al. (2022a, b) and Wood and Eom (2019) found highly variable measured SIV PCO_2 levels (11–27 Torr, 2–34 Torr, respectively) using anaesthetized fed fish sampled in situ, even with direct insertion of the PCO_2 micro-optode into the SIV. The present study was able to measure SIV PCO_2 in non-anaesthetized fish and found low and constant levels during digestion, similar to the control fasting values. There was a slight elevation at 12 h, but in general, all measurements were below 8 mmHg, much lower than previously reported levels measured in the lumen. The calculated PCO_2 of chyme collected at 48 h following feeding was not significantly different from measured SIV plasma PCO_2, but we suspect the animals to be near completion of digestion at the time of sampling. In fact, our calculated intestinal chyme PCO_2 levels (12–15.5 Torr; Table 1) are similar to those directly measured in fasted rainbow trout (7–13 Torr; Wood and Eom 2019). Thus, despite the high luminal PCO_2 found during active digestion, this study and previous reported values support that minimal equilibration of PCO_2 is occurring across the GIT epithelium.

Studies on higher vertebrates provide some insights into possible reasons for this lack of PCO_2 equilibration. In mammals, some of the luminal CO_2 is absorbed to the blood, breathed out (Ghoos et al. 1993), removed by microbes, and/or released as flatulence (Ohashi et al. 2007; Lacy et al. 2011; Oliphant and Allen-Vercoe 2019). To large extent, the luminal epithelia in higher vertebrates appear to have limited permeability allowing only a small percentage of other diffusible gases such as H_2, He and CH_4 to diffuse from the lumen into the villus blood (Bond et al. 1977). As a result, feeding resulted in no change in arterial PCO_2 in humans (Mensink et al. 2006) and no difference between arterial and venous PCO_2 in chickens (*Gallus gallus domesticus*; Wideman et al. 2003). Moreover, mammals possess a countercurrent arrangement of the capillary bed in the intestinal villi and in the ruminant stomach that may possibly function to recirculate and trap the CO_2. While the rainbow trout do not have intestinal villi, they have larger epithelial folds that are more prominent in the posterior intestine (Harder 1975; Kapoor et al. 1975). If a similar system is present in the intestinal folds of trout as in the intestinal villi of mammals, the luminal PCO_2 could diffuse into and recirculate within the intestinal fold, without appearing in the venous drainage from the intestine. This locally high PCO_2 in the intestinal folds possibly could help with oxygen unloading to the enterocytes via the Bohr and Root effects (Nikinmaa 2006; Cooper et al. 2014; Rummer and Brauner 2015). Thus, it will be of great interest to further investigate the vascular micro-anatomy of the trout GIT in the future.

Postprandial plasma ammonia (in vivo and in situ)

In both the in vivo and the in situ series, control fish had DA plasma Tamm concentrations typical of those measured in other studies (Fig. 3A, B; Bucking et al. 2009; Karlsson et al. 2006; Knoph and MåsØval 1996). The Tamm levels in DA plasma sampled from fish in situ was higher than in vivo, which could be due to anaesthetic in the water reducing the ammonia excretion rate (Guo et al. 1995). More significantly,

SIV Tamm did not increase after feeding in both series, supporting our initial hypothesis. Our chyme measurements are in agreement with earlier studies (Bucking and Wood 2012; Rubino et al. 2014; Jung et al. 2022a) showing that chyme Tamm throughout the intestine is typically very high (Fig. 4A, B; 495–1027 µM in vivo; 114–566 µM in situ) following feeding in rainbow trout. This likely reflects the catabolism of amino acids within the GIT lumen after ingestion of protein. Importantly, these data also confirm our recent finding (Jung et al. 2022a) that chyme Tamm levels are even higher in the stomach (2024–2181 µM) than in the more posterior sections of the GIT (Fig. 4A, B). This suggests considerable absorption of ammonia in the anterior tract, perhaps in the stomach itself and/or anterior intestine. In fact, rainbow trout stomach tissue in vitro showed the greatest absorption and consumption of ammonia compared to the intestinal tissues (Jung et al. 2022b). And many studies have shown that plasma ammonia level increases up to threefold after feeding (Kaushik and de Oliva Teles 1985; Wicks and Randall 2002; Bucking and Wood 2008, 2012). These previous studies have measured ammonia levels in the mixed arterial–venous plasma samples collected from caudal punctures. With our localized blood sampling method, we were able to show that any postprandial ammonia absorption that occurred in the GIT in our study was likely metabolized in the liver and/or excreted to the water via the gills (Bucking and Wood 2008), thus resulting in no change in arterial plasma ammonia levels. However, we cannot eliminate the possibility that the small ration size used in our study contributed to this lack of elevation.

Previous studies found that feeding stimulates the endogenous ammonia production rate of the intestinal epithelia and increases the efflux rate of ammonia to the serosal solution in vitro (Rubino et al. 2014; Jung et al. 2021). Whole-body ammonia excretion rate increases after feeding (Alsop and Wood 1997; Taylor et al. 2007; Bucking et al. 2010), of which up to 47% can originate from the GIT, mainly by endogenous production (Rubino et al. 2014). However, we measured 5–sevenfold lower Tamm in venous drainage relative to the posterior intestine chyme (Fig. 4A, B). With measured Tamm and pH, we calculated PNH_3 of both plasma and chyme (Fig. 4C, D). Although our chyme PNH_3 values were lower than reported values at an earlier stage of the digestion (24 h after feeding; Jung et al. 2022a, b; Rubino et al. 2014), they were still significantly higher than SIV PNH_3 in both series. Thus, like PCO_2 (Sect. "Postprandial blood and chyme pH, [HCO3-], and PCO2 (in vivo)"), there was minimal equilibration of PNH_3 between the lumen and SIV.

In contrast, there was a clear increase in HPV Tamm sampled in situ (Fig. 3B), similar to findings by Karlsson et al. (2006). The HPV differs from the SIV by additionally collecting venous drainage from the stomach and anterior intestine, suggesting that the anterior part of the GIT could be a greater site of ammonia absorption. In fact, Rubino et al. (2019) have presented evidence that NKCC functions as an NH_4^+ transporter in the anterior intestine of trout, as it does in mammalian gastrointestinal tissues, probably by substitution of NH_4^+ at the K^+ site on the co-transporter (Abdoun et al. 2006; Worrell et al. 2008). Furthermore, Rubino et al. (2019) showed that NKCC is more highly expressed in the anterior intestine than in the mid and posterior intestine of fed rainbow trout. Another possible ammonia NH_3 transporter—rhesus glycoprotein (Rh protein)—is expressed along the mice GIT including the stomach; both apical (Rhcg) and basolateral (Rhbg) isoforms are present throughout the tract (Handlogten et al. 2005). In rainbow trout, the Rhbg is expressed along the whole GIT including the stomach, with feeding elevating the relative expression in both the anterior and posterior intestines (Bucking and Wood 2012). The Rhcg seems to be lacking in the intestine of rainbow trout (Nawata et al. 2007) and the situation is currently unknown in the stomach. However, two Rhcg isoforms, in addition to Rhbg, were expressed throughout the intestine in two marine Batrachoidid teleosts (Bucking et al. 2013). Nonetheless, our data suggest that the posterior intestine is not the major source of postprandial ammonia absorption, and that ammonia may be transported in the anterior intestine and/or even across the stomach (Jung et al. 2022b) into the portal bloodstream to be processed in the liver. NH_3 absorption across the stomach would be problematical, as it would mean moving against a large PNH_3 gradient (Fig. 4C, D; 0.02–3.1 µTorr in stomach *vs* 13–30 µTorr in SIV). More likely, ammonia is transported by the gastric epithelium in the ionic form NH_4^+ as there is now considerable evidence that the stomach plays an important role in the absorption of other cations (Na^+, K^+, Ca^{2+}, Mg^{2+}) in the rainbow trout (Bucking and Wood 2006, 2007; Bucking et al. 2011).

In summary, it is now clear that feeding and digestive processes establish a harsh environment in the GIT lumen of the rainbow trout: high PCO_2 (Wood and Eom 2019; Jung et al. 2020, 2022a), high ammonia (Jung et al. 2022a), and virtual anoxia (Jung et al. 2020, 2022a). This study found that despite such conditions and increased blood flow to the system after feeding (Eliason et al. 2008), the composition of the venous blood draining the posterior intestine remains relatively unchanged, indicating minimal equilibration of PO_2, PCO_2 and ammonia.

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Data availability The datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request.

Declarations

Conflict of interest We declare we have no competing interests.

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