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EFFECTS OF FEEDING ON ARTERIAL BLOOD GASES IN THE AMERICAN ALLIGATOR *ALLIGATOR MISSISSIPPIENSIS*

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Summary

Reptiles habitually ingest large meals at infrequent intervals, leading to changes in acid–base status as the net secretion of acid to the stomach causes a metabolic alkalosis (the alkaline tide). In chronically cannulated and undisturbed amphibians and reptiles, the pH changes in arterial blood are, nevertheless, reduced by a concomitant respiratory acidosis (increased P_{CO_2} caused by a relative hypoventilation). Alligators (*Alligator mississippiensis*) have been reported to exhibit exceptionally large increases in plasma [HCO_3^-] following feeding, but these studies were based on blood samples obtained by cardiac puncture, so stress and disturbance may have affected the blood gas levels. Furthermore, crocodilian haemoglobin is characterised by a unique binding of HCO_3^- that act to reduce blood oxygen-affinity, and it has been proposed that this feature safeguards oxygen offloading by counteracting pH effects on blood oxygen-affinity. Therefore, to study acid–base regulation and the interaction between the alkaline tide and oxygen transport in more detail, we describe the arterial blood gas composition of chronically cannulated and undisturbed alligators before and after voluntary feeding (meal size 7.5±1 % of body mass).

Digestion was associated with an approximately fourfold increase in metabolic rate (from 0.63±0.04 to 2.32±0.24 ml O_2 min^{-1} kg^{-1}) and was accompanied by a small increase in the respiratory gas exchange ratio. The arterial P_{O_2} of fasting alligators was 60.3±6.8 mmHg (1 mmHg=0.133 kPa) and reached a maximum of 81.3±2.7 mmHg at 96 h following feeding; there was only a small increase in lactate levels, so the increased metabolic rate seems to be entirely aerobic. Plasma [HCO_3^-] increased from 24.4±1.1 to 36.9±1.7 mmol l^{-1} (at 24 h), but since arterial P_{CO_2} increased from 29.0±1.1 to 36.8±1.3 mmHg, arterial pH remained virtually unaffected (changing from 7.51±0.01 to 7.58±0.01 at 24 h). The changes in plasma [HCO_3^-] were mirrored by equimolar reductions in plasma [Cl^-]. The *in vitro* blood oxygen-affinity was reduced during the post-prandial period, whereas the estimated *in vivo* blood oxygen-affinity remained virtually constant. This supports the view that the specific HCO_3^- effect prevents an increased blood oxygen-affinity during digestion in alligators.

Key words: reptile, alligator, *Alligator mississippiensis*, feeding, specific dynamic action, gas exchange, arterial blood gases, acid–base balance, ‘alkaline tide’, blood oxygen-binding.

Introduction

An increase in metabolic rate following feeding, commonly referred to as specific dynamic action (SDA), has been described for many animals (e.g. Jobling, 1981; Kalarani and Davies, 1994; Busk et al., 2000); the rate of oxygen consumption increases severalfold over several days during digestion in many reptiles (Benedict, 1932; Secor and Diamond, 1997). The post-prandial period is also characterised by an increased base excess in the blood caused by HCl secretion into the lumen of the stomach (Hills, 1973). This ‘alkaline tide’ has been reported to be exceptionally large in alligators, with plasma [HCO_3^-] increasing by as much as 70 mmol l^{-1} and pH by 0.4 units (Coulson et al., 1950). The alkaline tide was found to be much smaller in chronically cannulated snakes and frogs, in which the metabolic alkalosis (increased plasma [HCO_3^-]) is counterbalanced by a concomitant respiratory acidosis (increased arterial P_{CO_2}), so that arterial pH increases only slightly (Overgaard et al., 1999; Busk et al., 2000). The increased P_{CO_2} is probably caused by a relative hypoventilation (i.e. the increased CO_2 production during SDA is not proportionally matched by increased ventilation).

Previous studies on blood gas levels and acid–base balance during digestion in alligators have all been based on blood samples obtained by cardiac puncture. Because the animals

must be severely disturbed during this procedure, it remains to be determined whether they also respond to the alkaline tide with a respiratory acidosis. Because of the enormous changes in plasma [HCO_3^-] reported previously, a large increase in P_{CO_2} would be required to maintain a constant arterial pH during digestion. In this case, a complete compensation of pH would require a large reduction in lung P_{O_2} that could impair blood oxygenation at the same time as metabolic rate is increased. Thus, a principal goal of the present study was to characterise arterial blood gas levels and acid–base balance during digestion in chronically cannulated and undisturbed alligators. Further, crocodilian haemoglobin is unique because HCO_3^- acts as an allosteric modifier of oxygen affinity (Bauer et al., 1981). Weber and White (1986) suggested that this feature may represent an adaptation to avoid a large and inappropriate increase in blood oxygen-affinity during digestion. However, the effect of the alkaline tide on blood oxygen-binding has not been studied, and a second goal of the present study, therefore, was to measure blood oxygen-binding during the post-prandial period.

Materials and methods

Experimental animals and implantation of the arterial catheter

This study was conducted on seven American alligators (*Alligator mississippiensis*, Daudin) of undetermined sex with body masses ranging from 0.9 to 9.0 kg (4.5±1.3 kg, mean ± S.E.M.). These animals were obtained from the Rockefeller Wildlife Refuge in Grand Chenier (Louisiana, USA) and had been kept at the University of California at Irvine for 1–2 years before experimentation. The alligators were maintained in large containers with free access to running water, dry land and a heating lamp that allowed for behavioural thermoregulation. They were maintained under a 12 h:12 h L:D photoperiod. All animals appeared healthy and had gained mass during captivity. They were fed on fish and chopped chicken several times a week, but were fasted for 3–4 weeks before surgery. The alligators were anaesthetised by placing a small plastic bin containing Halothane vapour over their nares. When ciliary reflexes disappeared, a 3–4 cm incision was made in the thigh, and a polyethylene catheter (PE60) containing heparinised saline was occlusively inserted into the femoral artery. The catheter was externalised through a small hole in the skin and secured with a few sutures. Bleeding from small vessels in the skin was stopped by cauterisation, and the incision was sealed with intermittent sutures. All animals received an intramuscular injection of enrofloxacin (Baytril; 2 mg kg^{-1}) to prevent infection. The glottis was intubated in three animals for artificial ventilation with room air until spontaneous breathing resumed.

Experimental protocol

After surgery, alligators were placed in individual plastic containers and left undisturbed for 36–48 h before measurements commenced. The containers were partly covered with a dark lid, to minimise visual contact, and placed in a climatic chamber kept at 30 °C, and with a 12 h:12 h L:D photoperiod. A 4 ml blood sample was removed anaerobically from fasting animals for analysis of blood gas levels, plasma acid–base variables, ion and metabolite levels (1 ml) and for the construction of blood oxygen equilibrium curves (3 ml). The alligators were then allowed to feed voluntarily on rats and mice *ad libitum* for 1–3 h. During this period, they consumed meals equivalent to 5.6–11.7 % (7.5±1 %, mean ± S.E.M.) of their body mass. Blood samples of 1 ml were removed at 6, 12, 24, 48, 72 and 96 h following feeding (two animals were also sampled at 120 h) for determination of post-prandial changes in blood respiratory variables and blood chemistry. After analysis, 0.4–0.5 ml of the blood was re-injected into the animals to reduce blood loss. At 24 h, an additional blood sample of 3 ml was taken for the construction of blood oxygen equilibrium curves. All blood samples were taken through the cannulae without handling or disturbing the alligators, which could not see the sample being taken. Animals remained quiet and at rest, except during the voluntary feeding bout.

Measurements of gas exchange

Rates of oxygen consumption ($\dot{V}_{O_2}$) and carbon dioxide production ($\dot{V}_{CO_2}$) were measured in fasting and digesting alligators using open-system respirometry. The animals were placed into an appropriate-sized metabolic chamber fashioned from plastic containers (19–190 l) and maintained in a temperature-controlled cabinet at 30±1 °C. Using a mass flow controller, air was continuously drawn through the chamber at a rate of 500–1000 ml min^{-1}. For a 15 min period every hour, a portion of the excurrent air was pumped through a column of water absorbent (Drierite) into an Ametek oxygen analyser (model S3A) and a Sensormedics (LB2) carbon dioxide analyser. Excurrent O_2 and CO_2 concentrations were recorded on a personal computer using data-acquisition software (Acknowledge, Biopac Systems, Goleta, CA, USA). $\dot{V}_{O_2}$ and $\dot{V}_{CO_2}$ were corrected to STPD.

Measurements of arterial blood gas levels and concentrations of plasma ions and metabolites

Arterial blood was analysed immediately for P_{O_2} (Pa_{O_2}) and pH using Radiometer O_2 and pH electrodes (E5046-0, PS-1 204, respectively) maintained and calibrated at 30 °C in a BMS Mk3 electrode assembly. Arterial O_2 content ($[O_2]_a$) was measured as described by Tucker (1967); the starting P_{O_2} was corrected for dilution of the sample as described by Bridges et al. (1979). Haematocrit was determined following a 3 min centrifugation at 12 000 revs min^{-1} in capillary tubes. The total CO_2 content of plasma was determined according to Cameron (1971). Arterial P_{CO_2} and plasma [HCO_3^-] were calculated from the Henderson–Hasselbalch equation on the basis of the pK′ provided by Jensen et al. (1998) and a CO_2 solubility of 0.0366 mmol l^{-1} mmHg (Heisler, 1986).

Plasma obtained by centrifugation of blood was immediately stored at −80 °C until analysis. Plasma lactate concentration was determined by Sigma Diagnostics procedure 735, and plasma

ammonium concentration was measured enzymatically using Sigma Bulletin no. 171-UV kit, while plasma protein was measured according to Lowry et al. (1951). Plasma chloride concentration was measured by coulometric titration (Radiometer CMT 10), while sodium levels were measured by flame photometry (Instrumentation Laboratory 243). Plasma osmolality was determined by vapour pressure osmometry (Wescor 5500).

Construction of in vitro *blood oxygen equilibrium curves*

Blood oxygen equilibrium curves were constructed in four alligators during fasting and during the post-prandial period. The equilibrium curves were constructed at constant P_{CO_2} from measurements of blood oxygen content at various P_{O_2} levels (Tucker, 1967) in rotating glass tonometers (Eschweiler, Germany) submerged in water thermostatted to 30 °C. Freshly drawn blood (3 ml) was divided equally between two tonometers that received a humidified gas mixture (prepared by a Cameron gas-mixing flow meter) with a P_{CO_2} of 14 or 35 mmHg. Initially, blood was equilibrated to a P_{O_2} of 291 mmHg for 35 min to obtain full haemoglobin oxygen-saturation (HbO_2sat). Blood oxygen content ($[O_2]$) was determined in triplicate as described above. The P_{O_2} of the gas mixture was then reduced sequentially to 55, 36 and 22 mmHg, and blood $[O_2]$ was determined in duplicate after equilibration for 35 min at each P_{O_2}. Data were plotted in Hill plots [$\log(HbO_2\text{sat}/(1-HbO_2\text{sat})$] *versus* $\log P_{O_2}$) that were linear in the range of haemoglobin oxygen-saturations between 20 and 80 %. Thus, P_{50} and the Hill coefficient (n_H) could be extrapolated from linear regressions. The pH of whole blood was determined at P_{50}; previous studies have shown that pH *in vitro* is not affected by haemoglobin oxygen-saturation (Jensen et al., 1998).

Statistical analyses

A one-way analysis of variance (ANOVA) for repeated measures was employed to determine whether feeding significantly affected the reported variables. A Bonferroni *post-hoc* test was used to identify mean values that were different from fasting values, applying a fiducial limit for significance of $P<0.05$. All results are presented as means ±1 S.E.M.

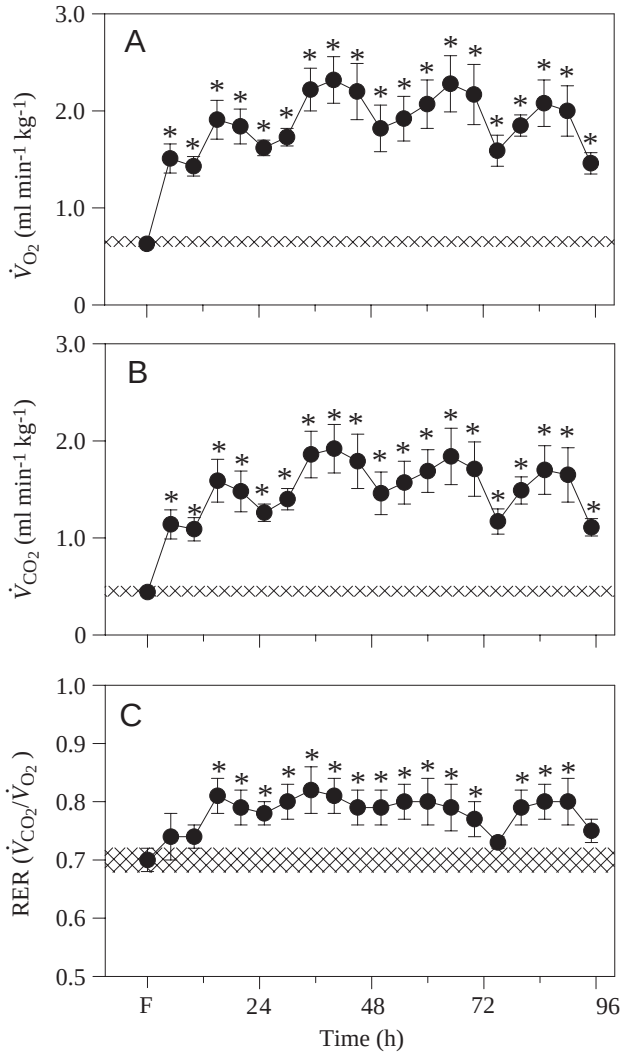

Fig. 1. Gas exchange before and following feeding in the American alligator (*Alligator mississippiensis*). $\dot{V}_{O_2}$, rate of oxygen uptake (A); $\dot{V}_{CO_2}$, rate of carbon dioxide release (B); and RER, the respiratory gas exchange ratio (C). The values obtained from fasting (F) animals are presented at 0 h, and the cross-hatched area highlights the means and S.E.M. of these values. All values are presented as means ±1 S.E.M. (N=5). Mean values that are significantly different ($P<0.05$) from the fasting value are marked with an asterisk.

Results

The metabolic response to food ingestion

The rate of oxygen uptake in fasting alligators was 0.63±0.04 ml min^{-1} kg^{-1} with a respiratory exchange ratio (RER) of 0.70±0.02 (N=5) (Fig. 1). The metabolic rate had increased significantly by 5 h following feeding, and peaked at approximately 36 h at a maximal value of 2.32±0.24 ml min^{-1} kg^{-1}. The respiratory exchange ratio increased significantly by 15 h to a level around 0.8 (the maximal value was 0.82±0.04 at 35 h), at which it remained throughout the duration of the experiment.

Arterial oxygen levels, haematocrit and blood chemistry

In fasting alligators, the arterial P_{O_2} (Pa_{O_2}) was 60.3±6.8 mmHg (Fig. 2A). During the post-prandial period, Pa_{O_2} increased to a maximum value of 81.3±2.7 mmHg at 96 h post feeding, but this elevation was not statistically significant. $[O_2]_a$ was 3.89±0.21 mmol l^{-1} in fasting alligators and did not change significantly during digestion (Fig. 2B). Similarly, haematocrit did not change significantly from the fasting value of 22.2±0.7 (Fig. 2C).

The arterial acid–base status of fasting alligators was characterised by an arterial pH of 7.510±0.013, a plasma bicarbonate concentration ($[HCO_3^-]_{pl}$) of 24.4±1.1 mmol l^{-1} and a P_{CO_2} of 29.0±1.1 mmHg (Figs 3, 4). Feeding did not affect pHa significantly, but there was a small increase to 7.583±0.014 and 7.597±0.028 at 24 h and 72 h, respectively. The relatively stable pH resulted from a combination of a metabolic alkalosis

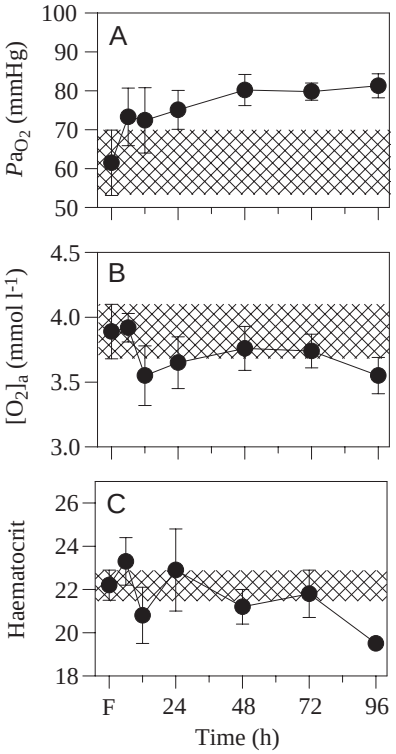

Fig. 2. Arterial blood gas levels before and following feeding in the American alligator (*Alligator mississippiensis*). $P_{a_{O_2}}$, arterial P_{O_2} (A); $[O_2]_a$, arterial O_2 content (B); and haematocrit (C). The values obtained from fasting (F) animals are presented at 0 h, and the cross-hatched area highlights the means and S.E.M. of these values. Values are means ±1 S.E.M. (N=4–7). 1 mmHg=0.133 kPa.

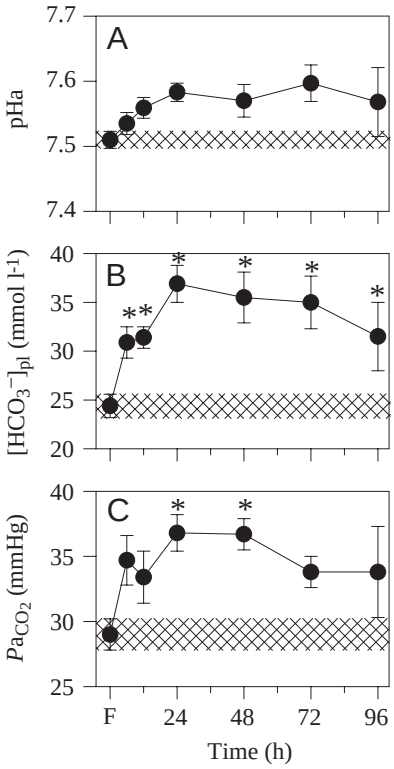

Fig. 3. Arterial blood gas levels before and following feeding in the American alligator (*Alligator mississippiensis*). pHa, arterial pH (A); $[HCO_3^-]_{pl}$, plasma $[HCO_3^-]$ (B); and $P_{a_{CO_2}}$, arterial P_{CO_2} (C). The values obtained from fasting (F) animals are presented at 0 h, and the cross-hatched area highlights the means and S.E.M. of these values. Values are means ±1 S.E.M. (N=4–7), and mean values that are significantly different (P<0.05) from the fasting value are marked with an asterisk. 1 mmHg=0.133 kPa.

(increased $[HCO_3^-]_{pl}$) and a respiratory acidosis (elevated arterial P_{CO_2}, $P_{a_{CO_2}}$), which developed within the first 6 h following ingestion. Thus, $[HCO_3^-]_{pl}$ increased significantly to a maximum value of 36.9±1.7 at 24 h, while $P_{a_{CO_2}}$ increased to a maximum value of 36.8±1.3 mmHg at 24 h. The combined metabolic alkalosis and respiratory acidosis persisted at 96 h, but acid–base status had nearly returned to fasting values at 120 h (Figs 3, 4).

Feeding did not affect plasma osmolality, plasma protein concentration or plasma Na^+ levels (Fig. 5B,C,F). Although not statistically significant, the plasma Cl^- concentration ($[Cl^-]_{pl}$) decreased from 107.1±2.2 to 92.2±4.6 mmol l^{-1}, and there was a good agreement between the changes in $[Cl^-]_{pl}$ and $[HCO_3^-]_{pl}$ (Fig. 6). Plasma lactate and ammonia levels increased significantly following feeding (Fig. 5D,E); however, lactate levels remained quite low throughout the entire experiment, at values consistent with resting and undisturbed reptiles in general.

Blood oxygen equilibrium curves

The *in vitro* blood oxygen-affinity (P_{50}) and Hill coefficient (n_H) for fasting and post-prandial alligators are presented in Table 1 at two P_{CO_2} levels (14 and 35 mmHg). The Bohr

Table 1. *Blood oxygen-binding characteristics at two levels of* P_{CO_2} *for fasting and fed (24 h post-prandial) alligators*

Condition	P_{CO_2} (mmHg)	pH	$[HCO_3^-]_{pl}$ (mmol l^{-1})	P_{50} (mmHg)	n_H
Fasting	14	7.677±0.023	17.2±1.0	19.4±0.1	2.44±0.09
Fasting	35	7.371±0.009	19.9±0.5	37.4±0.3	2.13±0.07
Fed (24 h)	14	7.811±0.017	23.9±1.0	18.8±1.0	2.55±0.06
Fed (24 h)	35	7.477±0.039	26.3±2.8	36.2±1.8	2.37±0.09

$[HCO_3^-]_{pl}$ is calculated from pH and P_{CO_2}.
$[HCO_3^-]_{pl}$, plasma $[HCO_3^-]$; P_{50}, the P_{O_2} at 50 % haemoglobin oxygen-saturation; n_H, Hill coefficient.
Values are means ± 1 S.E.M. (N=4).
1 mmHg=0.133 kPa.

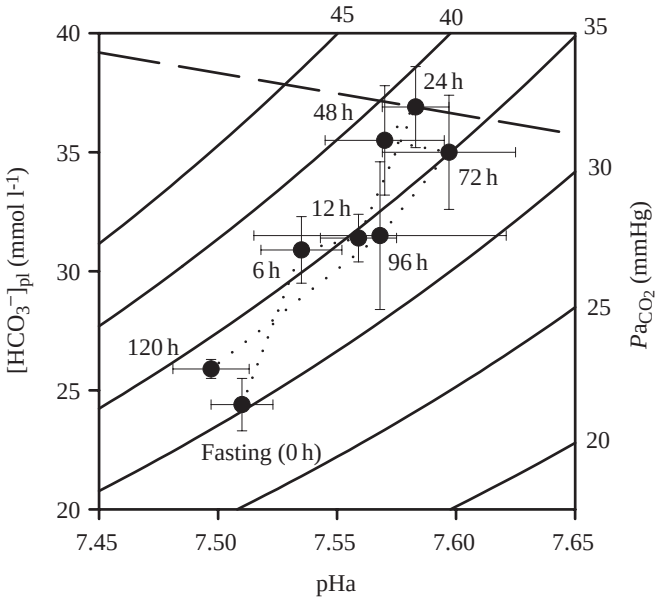

Fig. 4. Davenport diagram with calculated P_{CO_2} isopleths, showing plasma acid–base variables before and after feeding in the American alligator (*Alligator mississippiensis*). Dashed lines indicate expected *in vivo* changes. The thick broken line through 24 h is the non-bicarbonate buffer value from Jensen et al. (1998). pHa, arterial pH; $[HCO_3^-]_{pl}$, plasma $[HCO_3^-]$; and Pa_{CO_2}, arterial P_{CO_2}. Values are means ±1 S.E.M.

effects ($\Delta\log P_{50}/\Delta$pH) for fasting and post-prandial alligators were −0.94±0.05 and −0.87±0.08, respectively. Because of the post-prandial alkaline tide (see calculated plasma $[HCO_3^-]$ in Table 1), pH was significantly higher following feeding at a given P_{CO_2}. However, there was no significant difference in P_{50} between fasting and post-prandial alligators at a given P_{CO_2}. When all individual P_{50} values are presented as a function of pH measured *in vitro*, it becomes apparent that blood oxygen-affinity, at a given pH, is reduced during the post-prandial period (Fig. 7). For example, at the *in vivo* pH of 7.51 in fasting animals, the predicted *in vivo* P_{50} for fasting animals is 27.7 mmHg (filled square in Fig. 7), whereas the predicted value increased to 33.6 mmHg at the same pH 24 h after feeding (filled triangle in Fig. 7). This reduction in blood oxygen-affinity reflects the direct effect of HCO_3^- on haemoglobin oxygen-binding. However, because the alligators did not fully compensate for the rise in pH resulting from the $[HCO_3^-]$ elevation (Figs 3, 4), the predicted *in vivo* P_{50} at 24 h following feeding is 29.2 mmHg (filled square in Fig. 7). The cooperativity of blood oxygen-binding (n_H) was not significantly affected by feeding, but showed a pH dependency given as n_H=0.943pH–4.78 (r^2=0.61).

Discussion

The metabolic response to digestion in alligators and comparison with other species

The increased metabolic rate following feeding, specific dynamic action, observed in the present study is similar to that in previous studies on ectothermic vertebrates and invertebrates (e.g. Jobling, 1981; Kalarani and Davies, 1994; Secor and Diamond, 1997; Busk et al., 2000). Also, the increased respiratory gas exchange ratio is consistent with studies on toads and snakes (Wang *et al*., 1995; Overgaard et

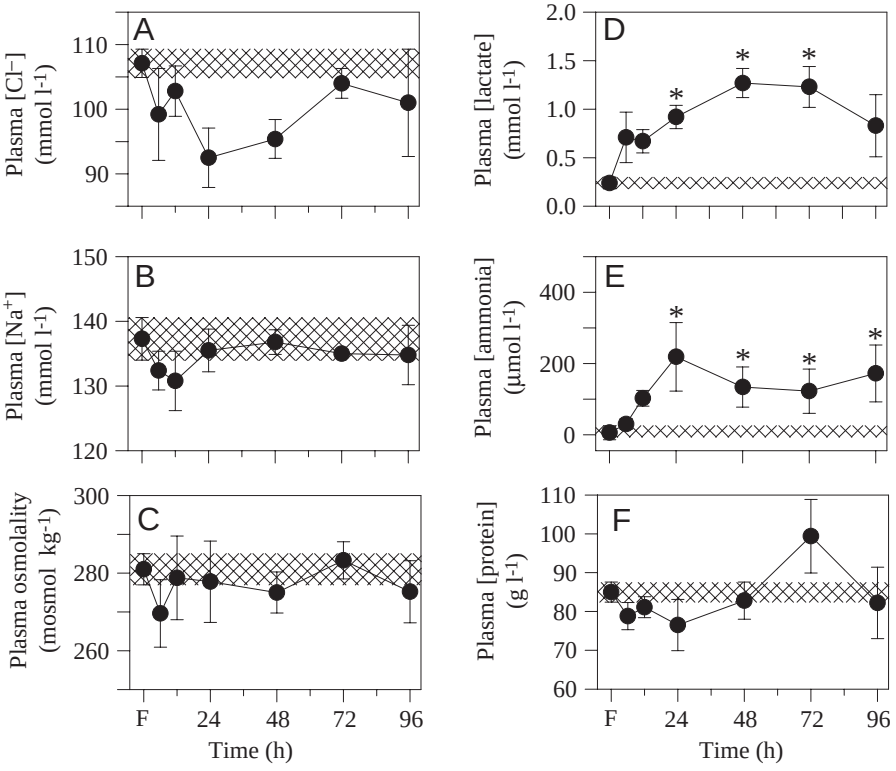

Fig. 5. Plasma ion and metabolite concentrations before and following feeding in the American alligator (*Alligator mississippiensis*); plasma $[Cl^-]$ (A); plasma $[Na^+]$ (B); plasma osmolality (C); plasma lactate concentration (D); plasma ammonia concentration (E); and plasma protein concentration (F). The values obtained from fasting (F) animals are presented at 0 h, and the cross-hatched area highlights the means and S.E.M. of these values. Values are means ±1 S.E.M. (N=4–7), and mean values that are significantly different (P<0.05) from the fasting value are marked with an asterisk.

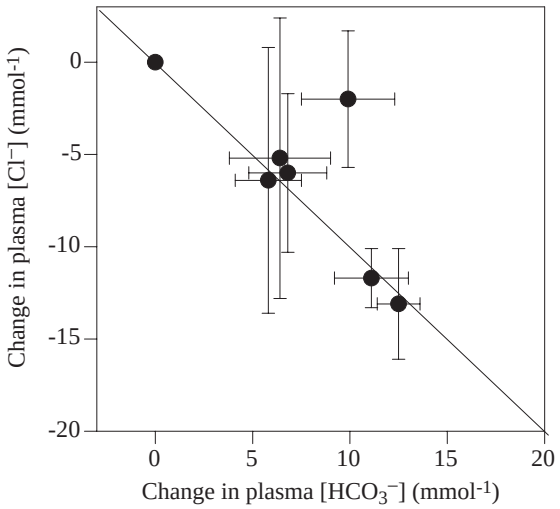

Fig. 6. Relationship between changes in plasma [Cl^-] and [HCO_3^-] after feeding in the American alligator (*Alligator mississippiensis*). Changes are calculated from the data presented in Figs 3 and 5. Values are means ±1 S.E.M. (N=4–7), and the line represents equimolar changes in [Cl^-] and [HCO_3^-].

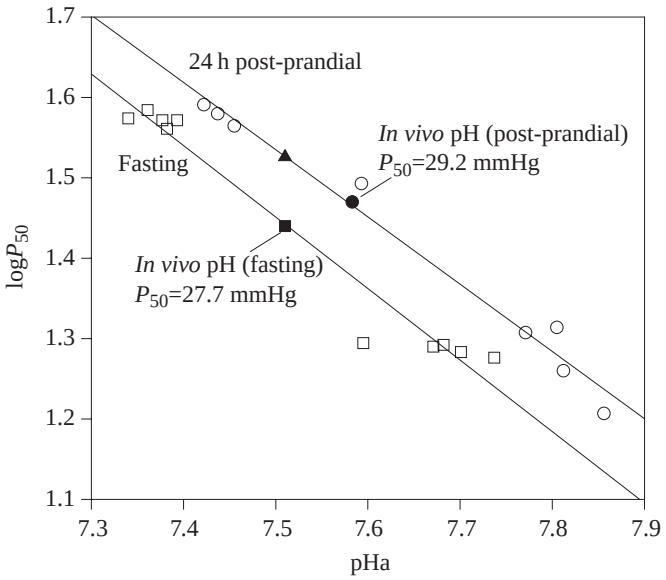

Fig. 7. Blood oxygen-affinity (P_{50}, mmHg) at various pH values in a pH/logP_{50} plot. Open symbols are pH and logP_{50} values determined from *in vitro* oxygen equilibrium curves constructed at two different P_{CO_2} levels with blood from fasting (squares) and fed (circles) alligators, and the lines represent linear regressions of these data (logP_{50}=–0.837pHa+7.812 (r^2=0.98) and logP_{50}=–0.889pHa+8.122 (r^2=0.96), before and after feeding, respectively, where pHa is arterial pH). Predicted *in vivo* logP_{50} values during fasting and at 24 h after feeding are shown by the filled square and circle, respectively. See text for further explanation. Filled triangle, predicted *in vivo* P_{50} of a post-prandial animal at the *in vivo* pH (7.51) of a fasting animal.

al., 1999; Busk et al., 2000) and may reflect a change from lipid metabolism during fasting to a mixed metabolism (i.e. protein, lipid and carbohydrates) during digestion. Nevertheless, these changes are difficult to interpret because the changes in strong ion difference associated with HCl transfer to the stomach and hypoventilation would lead to reductions in RER after feeding. In toads and varanid lizards, RER decreases transiently within the first few hours following feeding, an effect ascribed to secretion of gastric acid (Wang et al., 1995; Hicks et al., 2000).

Acid–base status and regulation

The post-prandial changes in plasma acid–base variables of alligators in the present study compares well in quantitative and temporal terms with values obtained from chronically cannulated snakes and frogs (Overgaard et al., 1999; Busk et al., 2000). In these studies, plasma [HCO_3^-] increased by 5–15 mmol l^{-1} concomitant with equimolar reductions in plasma [Cl^-] (Fig. 6), pointing to active H^+ secretion by the H^+/K^+-ATPase followed by passive diffussion of K^+ and Cl^- (Rabon et al., 1983). As in the present study (Figs 3, 4), the increase in plasma pH was greatly muted by an almost complete respiratory compensation *via* elevated P_{CO_2} throughout the post-prandial period. The increased plasma [HCO_3^-] of arterial blood in alligators following feeding also accord with most of the specimens studied by Coulson et al., (1950; Table 7.1, p. 139). However, in contrast, only a short-lived respiratory compensation was observed by Coulson et al. (1950) [Pa_{CO_2} calculated and presented by Weber and White (1986)], resulting in large post-prandial increases in plasma pH. This discrepancy may reflect the use of cardiac puncture for blood sampling since stress generally causes hyperventilation in alligators (T. Wang, personal observations).

The increased arterial P_{CO_2} during digestion in alligators, snakes and amphibians is probably due to a relative hypoventilation in comparison with resting fasted animals (i.e. the increased metabolic CO_2 production is not matched by an equal increase in ventilation) (Wang et al., 1995; Overgaard et al., 1999; Busk et al., 2000). Busk et al. (2000) suggested that the respiratory compensation of the metabolic alkalosis may play a role in safeguarding oxygen delivery to the tissues by limiting the alkalosis and thereby preventing the increased blood oxygen-affinity caused by an alkalosis. According to this paradigm, the allosteric decrease in oxygen affinity induced by bicarbonate in alligator haemoglobin should make the respiratory compensation of pH less important. The respiratory acidosis may also play a role in gastric acid secretion because an increase in serosal P_{CO_2} enhances acid secretion by the gastric mucosa *in vitro* (Kidder, 1976; Kidder and Montgomery, 1974).

Coulson et al. (1950) reported that some alligators experienced exceptionally large post-prandial increases in blood pH of up to 0.4 units, which was caused by a very large increase in plasma [HCO_3^-] from 25 to 90 mmol l^{-1}. Plasma [HCO_3^-] never exceeded 45 mmol l^{-1} in our study (Figs 3, 4). In both studies, the alligators were fed *ad libitum* and, although Coulson et al. (1950) did not report the meal sizes, they noted that ‘the amount was considerably below the alligator’s

capacity', suggesting that their alligators consumed relatively small meals. In our study, the magnitude of the alkaline tide did not correlate with meal size (5.6–11.7 % of body mass), and the very large alkaline tide reported by Coulson et al. (1950) compared with our values must be assigned to other factors. Coulson et al. (1950) obtained blood samples by repeated cardiac puncture, which is likely to cause lactacidosis and acidosis as a result of struggling during the stressful handling and the subsequent restlessness. In one alligator, we measured a plasma lactate concentration of 8 mmol l^{-1} 6 h following a failed attempt at force feeding (M. Busk, J. Overgaard, T. Wang, J. W. Hicks and A. F. Bennett, unpublished observation). It is possible that cardiac puncture and the associated stress and disturbance affected the responses observed by Coulson et al. (1950).

Blood oxygen levels during digestion

Using the measured respiratory gas exchange ratios and assuming that lung P_{CO_2} equals Pa_{CO_2}, the post-prandial period would be associated with a decrease in lung P_{O_2} from approximately 133 to 124 mmHg at 24 h (the right-to-left shunt increases Pa_{CO_2} and would, therefore, lead to an underestimation of lung P_{O_2}). It does not appear, therefore, that the post-prandial hypoventilation is sufficient to compromise oxygen loading in the pulmonary circulation. Similar changes in measured arterial oxygen levels and predicted lung P_{O_2} exist for frogs and snakes (Overgaard et al., 1999; Busk et al., 2000). Arterial oxygen levels were unchanged following feeding in alligators (Fig. 2) and, using our blood oxygen equilibrium curves, we estimate that haemoglobin oxygen-saturation of the arterial blood increased slightly from a fasting value of 86 % to approximately 90 % at 24 and 48 h following feeding.

In alligators, the left aortic arch originates from the right ventricle and oxygen-poor blood can, therefore, be shunted away from the lungs and re-enter the systemic circulation (Shelton and Jones, 1991). This right-to-left shunt normally occurs in resting undisturbed alligators whenever systemic blood pressure is low (Jones and Shelton, 1993) and leads to a decrease in systemic arterial oxygen levels. This shunt and the intermittent breathing pattern with the associated fluctuations in lung P_{O_2} (Glass and Johansen, 1979; Hicks and White, 1992) probably explain why arterial P_{O_2} is much lower than the predicted lung P_{O_2} in fasting animals (Fig. 2A). The left aorta supplies blood to the gastrointestinal system (Webb, 1979), which prompted Jones and Shelton (1993) to speculate that a right-to-left shunt during digestion may aid acid secretion by supplying the stomach with acidic and, hence, proton-rich blood (see Kidder, 1976; Kidder and Montgomery, 1974). If the right-to-left shunt increased during digestion, a larger lung–arterial P_{O_2} difference would be predicted, although it is uncertain that femoral arterial blood is identical to the blood supplying the gastrointestinal system (Webb, 1979; Jones and Shelton, 1993). We report a decrease in the lung–arterial P_{O_2} difference, indicating that the cardiac right-to-left shunt probably decreased during digestion, which is further supported by the slight increase in the arterial haemoglobin oxygen-saturation. On the basis of our previous models predicting arterial blood oxygen levels (Wang and Hicks, 1996), we suggest that a reduction in the right-to-left shunt is part of an appropriate response to ensure sufficient oxygen delivery to the gastrointestinal system during the increased metabolic rate associated with digestion.

Blood oxygen-binding

Crocodilian haemoglobin is unique in showing allosteric binding of HCO_3^-. Studies on stripped haemoglobin (Bauer et al., 1981) and whole blood (Jensen et al., 1998) reveal that two bicarbonate ions are bound upon deoxygenation. Furthermore, organic phosphates (i.e. ATP, GTP and IPP) affect blood oxygen-affinity only when $[Cl^-]$ is very low (Weber and White, 1994). However, the effects of *in vivo* changes in blood acid–base status during digestion have not previously been studied. Our values of blood oxygen-affinity for fasting alligators (Fig. 7; Table 1) are consistent with previous measurements on *Alligator mississippiensis* (Dill and Edwards, 1935; Weber and White, 1986) and other crocodilians (Grigg and Cairncross, 1980). Also, the CO_2 Bohr effect in the present study is similar to the value of –0.95 previously determined at 25 °C (Weber and White, 1986).

Our *in vitro* determinations of blood oxygen equilibrium curves allow for a quantification of the extent to which the haemoglobin bicarbonate-binding affects blood oxygen-affinity *in vivo* (Fig. 7). Using the regression line for fasting alligators, we predict that *in vivo* P_{50} is 27.7 mmHg (filled square in Fig. 7). If HCO_3^- did not modulate blood oxygen-affinity, the increase in arterial pH to 7.58 during digestion would reduce *in vivo* blood oxygen-affinity to a P_{50} of 23.8 mmHg. However, when applying the regression line for the data obtained on digesting alligators, the predicted *in vivo* P_{50} is 29.2 mmHg (filled circle in Fig. 7). This value is 1.5 mmHg higher than that in fasting conditions and is, therefore, in accord with the suggestion that the unique HCO_3^- effect prevents an increased blood oxygen-affinity during digestion in crocodilians and protects oxygen unloading in the tissue (Weber and White, 1986).

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