In-silico evaluation of aspartate therapy for lactic acidosis in *Alligator mississippiensis*

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Highlights:

- **Alligators maintain blood pH during lactic acidosis**
- **Ammonium bicarbonate secretion stabilises the pH**
- **Aspartate therapy favours aerobic respiration**
- **Clinical trials are needed**

Abstract. Hyperlactatemia, and/or lactic acidosis, is a common complication in wildlife due to the sensitivity of these species to capture induce complications. The treatment of lactic acidosis in humans is equally as controversial as in veterinary medicine. Stabilisation of blood pH during lactic acidosis is difficult to achieve. Crocodilians, such as the American Alligator (*Alligator mississippiensis*), are particularly prone to hyperlactatemia and are reasonably established models for metabolic studies. These studies prompted further investigation. The aims of the study are to mathematically model hyperlactatemia in the American Alligator. Three hundred simulations were performed per experiment, focused on the *in-vitro* and *in-vivo* derived mean and variance for metabolic data and enzymatic activities. The first experiment examined the rate of ammonium bicarbonate excretion and its potentially beneficial effect on pH stabilisation during states of hyperlactatemia. The second and third experiments compared the effects of intravenous aspartate administration upon the pH of alligators experiencing hyperlactatemia, with and without the addition of a metabolic feedback loop. The preliminary results support the hypothesis that the ammonium bicarbonate clearance from the kidneys is vital to the regulation and survival of the American alligator lactic acidosis. Computational administration of intravenous aspartate is also predicted to be beneficial although these results are not as conclusive. Clinical studies are needed to determine if aspartate therapy is helpful for the management of clinical lactic acidosis in the American Alligator and other animals.

Key words – Lactate, Metabolism, Crocodilian, Simulations

Main. Sensitivity of wildlife species to capture predisposes wildlife to hyperlactatemia and/or lactic acidosis (Molinaro et al, 2022). Management of lactic acidosis in humans and veterinary species alike is controversial – with the administration of a basic/ buffer solution of sodium bicarbonate associated with worsening outcomes (Yan et al, 2024). There are no established treatments for lactic acidosis.

During periods of exertion, glucose and other energetic metabolites are depleted to produce pyruvate. Pyruvate is then shuffled into other pathways, such lactate production. Lactate dehydrogenase reversibly catalyses the production of lactate from pyruvate in response to an oxygen deficit. During periods of rest, pyruvate is metabolised back into glucose and other metabolic derivatives (Lemieux et al, 1984). Capture related stress can shift metabolic activity towards anaerobic respiration. Lactate production amongst crocodilians has thus been reported to be an effective welfare metric following capture (Molinaro et al, 2022).

In states of extreme exertion – with lactate titres of 20mmol/L or higher and a pH lower than 7.0 – crocodiles can endure lactic acidosis in the absence of noticeable pathology (Seymour et al, 1985). Crocodilians are also capable of experiencing fatal complications from anethesia – closely related to hyperlactatemia (Molinaro et al, 2022).Ammonium producing animals, crocodilians excrete this metabolic byproduct from the renal tubules as ammonium bicarbonate. Carbonic anhydrase produces the bicarbonate, acid-base buffer within the kidneys to assist in excretion of ammonium (Coulson and Hernandez, 1959). It was therefore hypothesised that ammonium serves as a metabolic 'sink' for the management of acidosis by American Alligators (*Alligator mississippiensis*). Monte Carlo simulations were developed with a self-tuning hyperparameter for the optimisation of NH_4^+ excretion rate. To determine this rate, the pH, lactate and NH_4^+ of alligator blood at four tiers of exertion (rest, mild, burst and extreme anaerobic states – representing 5%, 10%, 50% and 100% anaerobic metabolism) was modelled to remain at the baseline blood measurement reported by Lemieux et al (1984).

Self-tuned hyperparameters generated a rate of 0.0339µmol/g/min which proved much higher than the experimentally observed rate of NH_4HCO_3 excretion 0.0039µmol/g/min. This discrepancy can be explained through blood lactate levels. The experimentally observed rate was obtained from an Alligator with a blood lactate of ~0.0037µmol/g (Lemieux et al, 1985). While Lemieux et al (1984) measured an average blood lactate of 6.095µmol/g.

The result is supportive of the expected protective factor from the production and excretion of NH_4^+ against lactic acidosis. The NH_4^+ metabolite is inherently linked to the metabolism of lactate both as a feature of biochemical pathways (Fig. 1A), and as a direct regulatory mechanism of the American alligator's lactate pathway (Lemieux et al, 1984). Fig. 2A supports these findings, pH remained stabilised at 50% anaerobic respiration. Blood lactate was independent of the anaerobic rate modelled (Fig. 2A). Suggestive of a theoretical maximum being reached in the model, regardless of the method of metabolism, such an explanation proves to be metabolically incomplete. Pyruvate should be metabolised from phosphoenolpyruvate as modelled in the third model (Fig. 1C).

All simulations considered blood, liver, kidneys and muscle to be independent 'compartments', each with independent enzymatic activities and metabolite levels. All data was derived from published tables produced by Lemieux et al (1984), Coulson and Hernandez (1959), Bogan and Mitchell (2014), Jensen et al (1998) and Lemieux et al (1985). Additional parameters were included for the model calibration and are detailed within the GitHub repository for the study (tsto3616/alligator-lactate: The repository provides code for the American alligator hyperlactatemia modelling.).

The central role of glutamate in the consumption and or production of NH_4^+ (Fig. 1A) was motivation for the investigation into therapeutic aspartate for removal of NH_4^+ (Lemieux et al, 1984). Intravenous aspartate, in theory, should drive the production of glutamate by

86 forming positive feedback, for which NH_4^+ is consumed (Fig. 1B, C), thus reducing blood pH
87 (Coulson and Hernandez, 1959; Lemieux et al, 1984). The second model produced
88 phenomena with ten hyperparameters. The first set determined the rate of H^+ production for
89 anaerobic metabolism (hyperparameter 1), the rate of NH_4^+ excretion (hyperparameter 2) and
90 the activity of carbonic anhydrase in its production of HCO_3^+ (hyperparameter 3). The second
91 set studied the strength of the inhibitory effect of blood pH and CO_2 upon aerobic respiration
92 (hyperparameter 4 and 5), the extent to which the pH and CO_2 are inhibitory (hyperparameter
93 6), and the midpoint at which the H^+/CO_2 balance is predictive of 50% anaerobic metabolism
94 (hyperparameter 7). The third set defined the ideal aspartate dose for the stabilisation of the
95 pH, as an absence of change in pH for 50% anaerobic metabolism (hyperparameter 8), the
96 effect of aspartate on NH_4^+ excretion (hyperparameter 9) and the significance of aspartate as
97 a suppressor of anaerobic metabolism (hyperparameter 10). The ten hyperparameters were
98 optimised following initial calculation to maintain a global set of self-tuned hyperparameters.
99 These were recalculated for both the minimisation of anaerobic respiration and maximisation
100 of aerobic metabolism, thus countering the effects of lactic acidosis directly and indirectly.

101 The results of second model were conclusive of a relatively small benefit from
102 aspartate, with the ideal dose of aspartate at 8.169μmol/g/min of the total circulating blood
103 for 60 minutes. There was a significant need for an increase in NH_4^+ excretion (6.4% for
104 every 1μmol/g of aspartate) and decrease in anaerobic respiration (69.9% for every 1μmol/g
105 of aspartate). The rate of NH_4^+ clearance by the kidneys was 0.0339 μmol/g/min – as
106 predicted in the previous modelling scenario. The effects of aspartate when visualised
107 demonstrate a significant stabilising and/or buffering effect on pH (Fig. 2B).

108 The second model was limited by inaccuracies presented in the pyruvate end point for
109 metabolism (Fig. 1B). To prevent this limitation, the artificially altered metabolic pathway
110 was changed to skip this metabolic step, unaccounted for in the data; this enabled the PEPCK

catabolism of oxaloacetate into pyruvate directly. Under a complete model, PEPCK would be conjoined with the metabolism of phosphoenolpyruvate into pyruvate – removing assumptions of enzymatic activity for the conversion of phosphoenolpyruvate into pyruvate. It theoretically produced a greater effect from aspartate as the altered assumptions enabled a positive feedback loop surrounding the production of glutamate (Fig. 1C) – this feedback loop was demonstrated by the third model.

The third model determined that the ideal dose of aspartate needed was within 1.042µmol/g/min, with both a necessary 9.3% increase in the rate of NH_4^+ excretion for every 1µmol/g of aspartate and with a decrease in anaerobic respiration of 79.2% for every 1µmol/g of aspartate. The optimised rate of NH_4^+ clearance by the kidneys was 0.0339µmol/g/min – as predicted in the previous two modelling scenarios. The effect of aspartate metabolism significantly reduced the pH of alligator blood in a similar manner to second model (Fig. 2B, C).

The positive effect of the aspartate intravenous dosing – as demonstrated by the second and third model – was contradicted by the experiments of Coulson and Hernandez (1959). The intraperitoneal injection of aspartate was met with minimal increase in the renal excretion of NH_4HCO_3, but the rate of excretion may not be regulated by the blood pH of the American alligator (Coulson and Hernandez, 1959). The ability of aspartate to *in-silico* reduce blood pH is suggestive of a safe alternative to the regulation of lactic acidosis in place of currently precarious sodium bicarbonate therapy (Yan et al, 2024).

The absence of NH_4HCO_3 excretion from the kidneys of other herpetology species could further mean that aspartate therapy would be ineffective for reptile species broadly. Not all reptiles predominantly excrete nitrogenous waste as ammonia with reptiles known to have diverse nitrogenous waste excretion mechanisms (Khalil, 1951). Clinical trials are needed in

both domestic and wildlife to determine the pH alleviating effects of aspartate upon lactic acidosis.

The data is available in the GitHub repository (tsto3616/alligator-lactate: The repository provides code for the American alligator hyperlactatemia modelling.). The research did not receive any funding. During the preparation of this work, the author used Microsoft Copilot for editing of code and or the manuscript. The autho reviewed and edited the output as needed and take full responsibility for the content of the published article.

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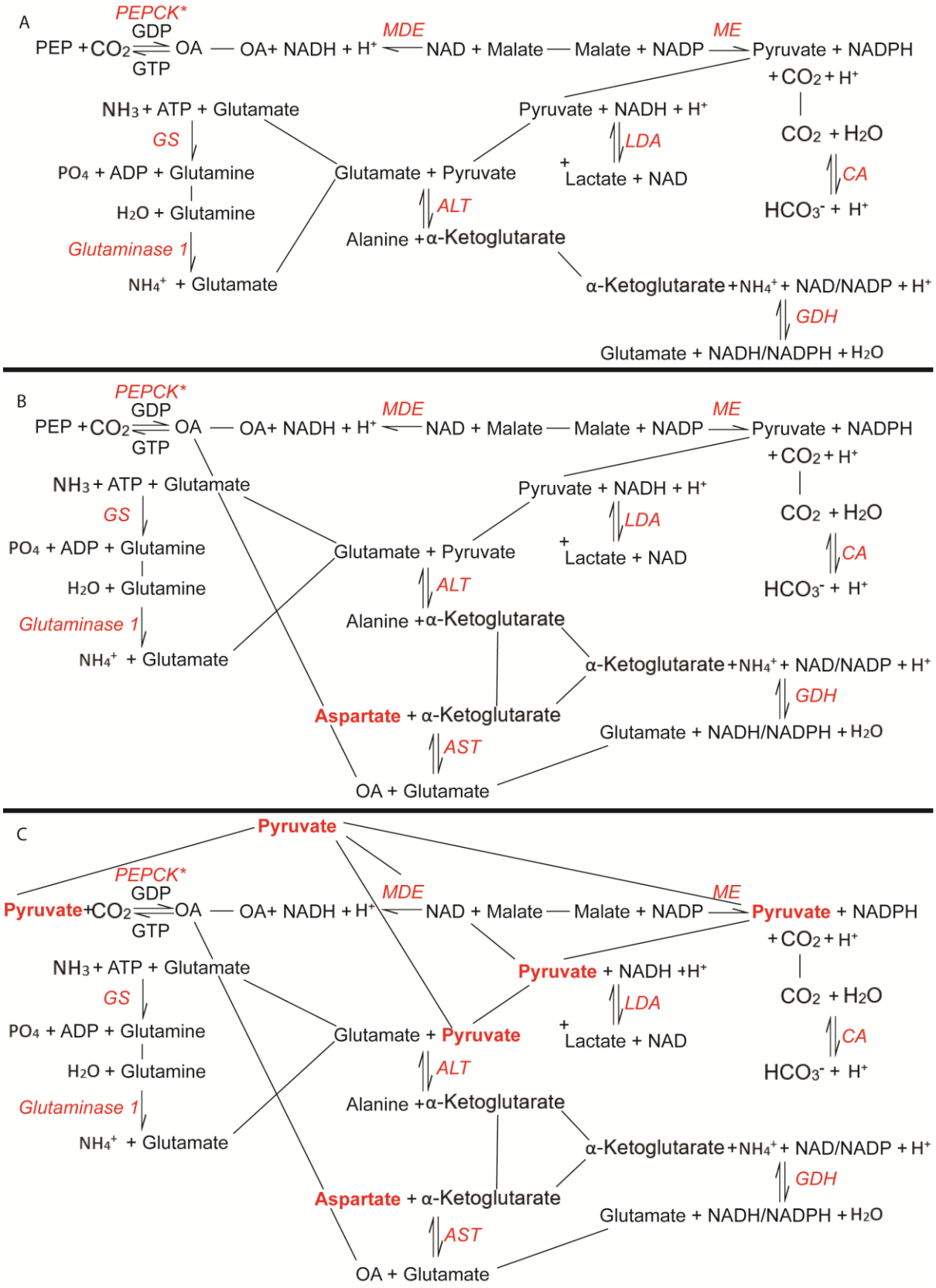
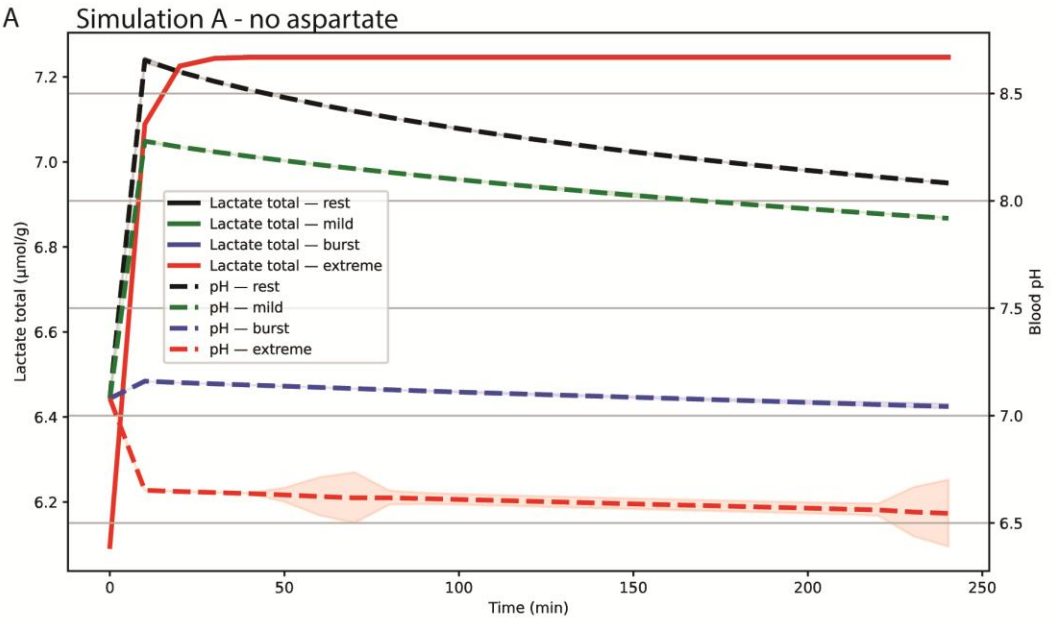
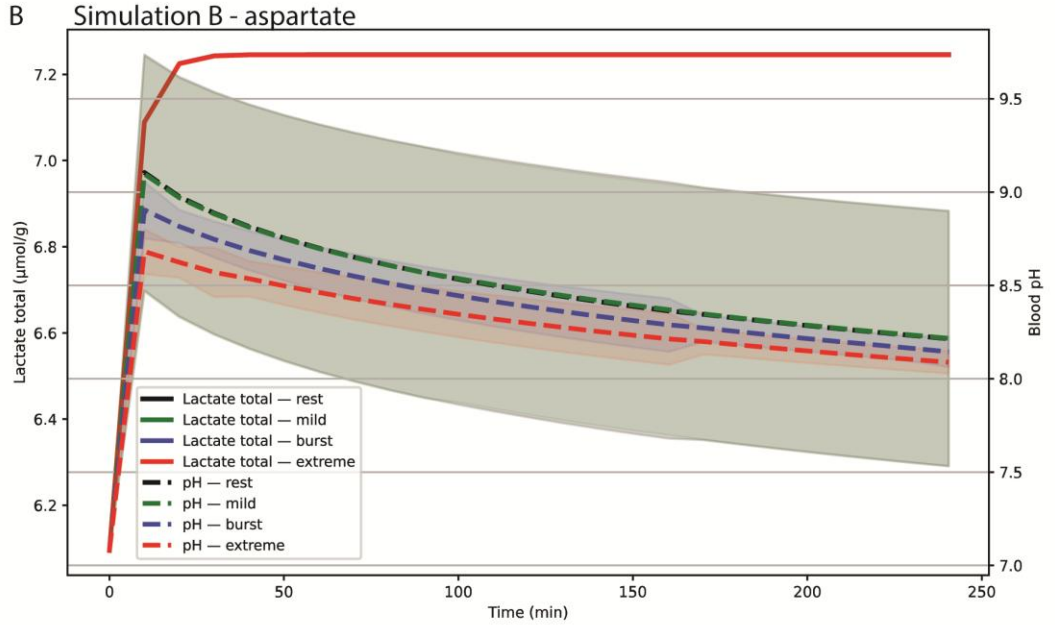
Figure 1: Experimental design of the simulations. The enzymes (featured in red italics next to arrows) are phosphoenolpyruvate carboxykinase (PEPCK), malate dehydrogenase (MDE), malic enzyme (ME), carbonic anhydrase (CA), lactate dehydrogenase (LDA), alanine aminotransferase (ALT), glutamine synthase (GS), glutaminase 1, glutamate dehydrogenase (GDH) and aspartate aminotransferase (AST). The asterix denotes the PEPCK reaction as the forward and reverse reaction independently to be consistent with the data by Lemieux et al (1984). The abbreviated OA metabolite represents oxaloacetate and PEP represents phosphoenolpyruvate. These enzymes and metabolites are not equally distributed throughout the body within each simulation as these are compartmentalised. In the first Model (A), devoid of aspartate, the data was solely sourced from Lemieux et al (1984) and Bogan and Mitchell (2014). Thus, oxaloacetate is an end point product due to the lack of data – this is not representative of fact. Phosphoenolpyruvate in real scenarios is converted into pyruvate, which is cleared by the model as lactose. In the second Model (B) the model has the effects of aspartate but still lacks the conversion of phosphoenolpyruvate to pyruvate. This model also considers pyruvate removed by aerobic respiration (not shown) at a rate determined by metabolic activity, pH and CO_2, with the elimination of pyruvate through this pathway resulting in the production of CO_2. In the third Model (C), the same metabolic processes remain but with the assumption that PEPCK converts oxaloacetate directly into pyruvate using the enzyme activity rate determined by Lemieux et al (1984).

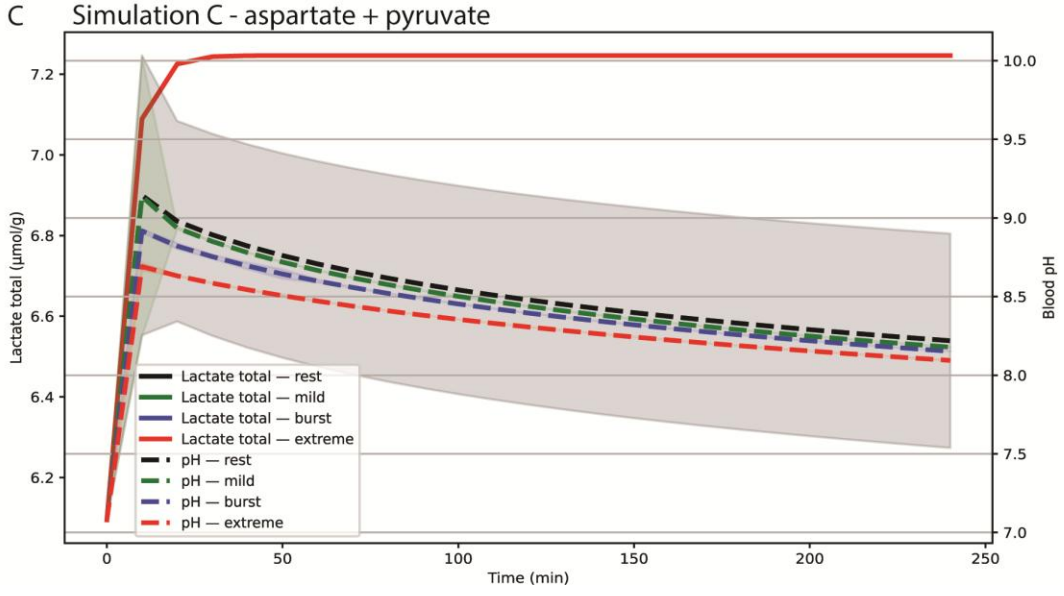

A
PEPCK*
GDP
PEP + CO2 OA
GTP
OA + NADH + H+
MDE
NAD + Malate
Malate + NADP
ME
Pyruvate + NADPH
+ CO2 + H+
NH3 + ATP + Glutamate
GS
PO4 + ADP + Glutamine
H2O + Glutamine
Glutaminase 1
NH4+ + Glutamate
Pyruvate + NADH + H+
LDA
+ Lactate + NAD
CO2 + H2O
CA
HCO3- + H+
Glutamate + Pyruvate
ALT
Alanine + α-Ketoglutarate
α-Ketoglutarate + NH4+ + NAD/NADP + H+
GDH
Glutamate + NADH/NADPH + H2O
B
PEPCK*
GDP
PEP + CO2 OA
GTP
OA + NADH + H+
MDE
NAD + Malate
Malate + NADP
ME
Pyruvate + NADPH
+ CO2 + H+
NH3 + ATP + Glutamate
GS
PO4 + ADP + Glutamine
H2O + Glutamine
Glutaminase 1
NH4+ + Glutamate
Pyruvate + NADH + H+
LDA
+ Lactate + NAD
CO2 + H2O
CA
HCO3- + H+
Glutamate + Pyruvate
ALT
Alanine + α-Ketoglutarate
α-Ketoglutarate + NH4+ + NAD/NADP + H+
GDH
Glutamate + NADH/NADPH + H2O
Aspartate + α-Ketoglutarate
AST
OA + Glutamate
C
Pyruvate
PEPCK*
GDP
Pyruvate + CO2 OA
GTP
OA + NADH + H+
MDE
NAD + Malate
Malate + NADP
ME
Pyruvate + NADPH
+ CO2 + H+
NH3 + ATP + Glutamate
GS
PO4 + ADP + Glutamine
H2O + Glutamine
Glutaminase 1
NH4+ + Glutamate
Pyruvate + NADH + H+
LDA
+ Lactate + NAD
CO2 + H2O
CA
HCO3- + H+
Glutamate + Pyruvate
ALT
Alanine + α-Ketoglutarate
α-Ketoglutarate + NH4+ + NAD/NADP + H+
GDH
Glutamate + NADH/NADPH + H2O
Aspartate + α-Ketoglutarate
AST
OA + Glutamate

167

Figure 2: Ammonium bicarbonate excretion from the kidneys into the urinary tract is a necessary regulator of blood pH under lactic acidosis in the American Alligator. The American alligator was examined three Models. In the first Model (A) the only hyperparameter was the rate of ammonium bicarbonate excretion by the kidneys. This rate was determined to be much higher for the stabilisation of the blood pH (0.0339μmol/g/min compared to 0.0037μmol/g//min). In later Models (B and C) the addition of multiple hyperparameters was conducted to add complexity to the model and determine the necessary dose of aspartate needed to minimise cellular respiration. Subfigure B was modelled under the assumption that PEPCK produces phosphoenolpyruvate, which is not recycled into the glutamate cycle. This reduced the effects of aspartate on a theoretical basis; the assumption was thus altered to skip the metabolic conversion of phosphoenolpyruvate into pyruvate enabling the third Model to recycle the PEPCK reaction into the glutamate pathway for regulation of NH_4HCO_3 excretion from the kidneys and the depletion of the lactate via the metabolism of pyruvate into α-ketoglutarate (Figure 1). The third Model is shown in subfigure C.


A
Simulation A - no aspartate
Lactate total — rest
Lactate total — mild
Lactate total — burst
Lactate total — extreme
pH — rest
pH — mild
pH — burst
pH — extreme
Lactate total (µmol/g)
Blood pH
Time (min)


B
Simulation B - aspartate
Lactate total — rest
Lactate total — mild
Lactate total — burst
Lactate total — extreme
pH — rest
pH — mild
pH — burst
pH — extreme
Lactate total (µmol/g)
Blood pH
Time (min)


C
Simulation C - aspartate + pyruvate
Lactate total — rest
Lactate total — mild
Lactate total — burst
Lactate total — extreme
pH — rest
pH — mild
pH — burst
pH — extreme
Lactate total (µmol/g)
Blood pH
Time (min)

183

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