Integration of Multimodal Spectroscopy Data through Modelling

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Hypoxia-Ischaemia, a condition characterised by inadequate blood flow and oxygen concentration, is one of the major causes of brain injury in newborn infants [22]. One approach to study the encephalopathy following the hypoxia-ischaemia is mathematical modelling.

The BrainPiglet model is a simplified model of circulation and metabolism in the brain [16]. Although being successful in studying anoxic piglets, the model has failed to make accurate predictions of the changes in brain metabolism during hypoxia-ischaemia. It has been proposed that inclusion of pH in the model would help improve these predictions [16]. In the following essay, a novel method will be proposed to calculate the pH from ^{31}P MRS measurements. The BrainPiglet model will then be modified to accept the calculated pH as input. Finally, comparisons will be made among the results obtained from the experiments, the modified and the original model.

Contents

1 Introduction

Hypoxia is insufficiency of oxygen concentration in the tissue. Ischaemia refers to inadequate blood flow in an organ or tissue [12]. Perinatal hypoxia-ischaemia is the major cause of brain injury in neonates [22]. It is a frequent event [21], resulting from premature births, difficult parturitions and mechanical injuries to head during labour [12]. Studies have shown that the injuries arise from the "metabolic perturbations" [22] following the hypoxia-ischaemia insult. It has been demonstrated that the encephalopathy following hypoxia-ischaemia is comprised of two energy failures [20, 21]. Understanding the condition of the brain between these injuries and the severity of the first injury would enable prevention, or at least, minimization of the damage [20].

The BrainPiglet model [14] has been previously used to study anoxia, correctly predicting the changes in metabolites' concentrations. However, the model has some limitations [16]. To be more specific, some metabolites including pH were assumed to be constant and thus, not modelled. This prevented the model to be applied to hypoxia-ischaemia, where a fall in pH was observed following the insult [11, 13, 20].

In the following essay, I will give a brief description of the brain physiology, focusing on the metabolic pathways of the brain (Section 2) and will explain the different measurements used in studying hypoxia-ischaemia, as well as the experiment done to obtain the data used in this study (Section 3). I will continue with introducing the BrainPiglet model and discussing its limitations to study hypoxia-ischaemia (Section 4.1). I will then propose a model which enables the estimation of pH from ^{31}P MRS measurements (Section 4.2). The pH, estimated by this model, would be given to the BrainPiglet model as input to simulate the changes in the brain during hypoxia-ischaemia. Next, the results of these simulations will be presented (Section 5). The essay will end with a discussion on the simplifications which may cause the contradictions between the model predictions and the actual data (Section 6).

2 Brain: The Physiology

In order to perform their responsibility, the cells need energy. This energy is supplied by a series of processes called the "energy metabolism", which use oxygen and different substrates (e.g. glucose) to produce energy [18]. In the brain, oxygenated blood is supplied by the internal carotid artery and the vertebral artery [15]. Due to its unusually high "energy requirement" [18] and thus "its high rate of oxygen consumption" [12], as well as "its lack of oxygen stores" [12], the brain is highly sensitive to the interruptions in the supply of its energy sources [12, 18]. Therefore, it is important for it to maintain a constant concentration of metabolites, and thus, a constant blood flow. This is done by cerebral autoregulation, which keeps the blood flow constant by changing the radius of blood vessels [15]. In disorders such as hypoxia-ischaemia, changes in the concentration of metabolites due to disruptions in blood circulation and metabolism cause instantaneous damage to the brain [12]. In the following section, the mechanisms in the brain, which assure sufficient production of energy in the cells, will be explained.

2.1 Metabolism

Energy metabolism is a series of reactions during which, glucose, the main fuel molecule in the brain [15, 18], is oxidised into carbon dioxide and water, producing adenosine triphosphate (ATP). The brain metabolic pathways can be divided into three stages. The first stage occurs within the cytoplasm, while the further stages take place in the mitochondria [9, 15].

2.1.1 Glycolysis

Glycolysis, the first stage in metabolic processes, is constituted of a set of reactions, converting the glucose into pyruvate. These reactions can be summed up as:

$$Glucose + 2P_i + 2ADP + 2NAD^+ \rightarrow 2Pyruvate + 2ATP + 2NADH \quad (1)$$

Glycolysis is responsible for energy production under anaerobic conditions, such as hypoxia. In fact, in the lack of oxygen, pyruvate is converted into lactate. This also results in reoxidation of NADH, thus,

ensuring that enough NAD^+ is available for the continuation of glycolysis [18]. The oxidation of NADH also leaves two H^+ ions. The excess production of hydrogen ions, following the increase in lactate production during hypoxia or ischaemia, is responsible for acidosis that occurs under these conditions [5, 15].

2.1.2 Tricarboxylic Acid Cycle (TCA)

Under aerobic conditions, the glycolytically produced pyruvate, penetrates into the mitochondria to be further metabolised. The TCA cycle is responsible for this, oxidising pyruvate into CO_2 and water [18]. The reactions of the cycle can be summed up as:

$$Pyruvate + 4NAD^+ + FAD + GDP + P_i \rightarrow 3CO_2 + 4NADH + FADH_2 + GTP \quad (2)$$

It should be noted that high levels of ATP inhibit the TCA cycle [9].

2.1.3 Oxidative Phosphorylation

The main function of the last stage of the metabolic processes, known as oxidative phosphorylation, is to produce ATP from adenosine diphosphate (ADP) and inorganic phosphate (P_i). This is done by a membrane-bound enzyme called *ATP synthase*, which requires a gradient of protons between the mitochondrial intermembrane and matrix to function. The electron transport chain builds this gradient, as well as producing P_i for the synthesis of ATP.

In the electron transport chain, the electrons are passed through four main complexes. The first complex is NADH-ubiquinone reductase, which transfers electrons from NADH to ubiquinone (Q). Uniquinone then passes the electrons to cytochrome c, through the third complex, cytochrome bc_1. The reduced cytochrome c carries the electrons to the fourth complex, cytochrome c oxidase (CCO), which transfers the electrons to oxygen molecules, forming water. These reactions lead to the production of hydrogen ions at each of the three complexes, which are pumped out of the inner membrane to generate the proton gradient [9].

CCO has three redox centres. The electrons are first transferred to Cu_A centre, then to haeme a centre and from there to Cu_B-a_3 centre, which then passes the electrons to oxygen molecules. Since CCO is the terminal complex in the electron transfer chain, it has been suggested that its oxidation state could be an indicator of cellular oxygen availability [6, 8, 21]. Tsuji et al [21] have shown that there is a correlation between the reduction of oxidised cytochrome aa_3 and cerebral energy loss in hypoxic piglets. Thus, suggesting that changes in redox state of oxidised cytochrome aa_3 may be partially, if not entirely, reflect the cellular energy state of the brain. Other works also reveal a correlation between the redox state of CCO and cellular energy status of the brain [6]. It has also been suggested that the changes in the redox state of CCO may be used as a predictor of brain energy failure [21], and as a biomarker of dysoxia, i.e. the brain oxygen insufficiency [8].

3 Neonatal Piglet Brain: The Measurements

Due to the similarity of their brains, neonate piglets are usually used as experimental models of human newborns [16]. They are used in many studies (for example, [6, 7, 20, 21]), in particular those of hypoxia-ischaemia, to gain a better insight into the physiological processes of the brain.

3.1 The Experiment

The data used in this research were obtained from the experiments carried out on newborn piglets, which were subject to hypoxic and ischaemic insults. The experiments used to study hypoxia-ischaemia, for example those described in [6, 7, 20], as well as those from which the data for this research were collected, follow a common procedure.

First, the piglets are under a normal condition. The baseline data, including the heart rate, blood pressure, arterial oxygen saturation and CO_2 levels, as well as the baseline MRS and NIRS spectra are collected during this period. Then, the piglets are exposed to periods of hypoxia-ischamea by simultaneous occlusion of common carotid arteries and reduction of fractional inspired oxygen. The occlusion of the arteries makes this experiment different from those studying hypoxia [21], in which only the concentration of inspired O_2 is changed. In fact, this assures the induction of both hypoxia and ischaemia, and prevents the oxygen delivery to the brain from returning to its normal value by autoregulation.

The fractional inspired oxygen is reduced in several steps. Then the phase of titration starts, during which, the level of inspired oxygen is increased in steps to the baseline value. Lastly, the occluders that block the arteries are deflated and the level of fractional inspired oxygen is restored to its normal value.

3.2 The Measurements

Depending on the aim of the study, different kinds of measurements could be done. Two non-invasive techniques, near infrared spectroscopy and magnetic resonance spectroscopy, are of particular interest in studying perinatal hypoxic-ischaemic brain injury [20]. These two techniques are complementary to each other, providing information about "cerebral oxygenation and haemodynamics" [23].

3.2.1 Near Infrared Spectroscopy (NIRS)

This technique is based upon the absorption of light by different compounds in the brain tissue. Due to its high absorption by the water in the tissue, visible light cannot be used for studying the brain. This is not the case for near infrared (NIR) light. Hence, NIR light, with wavelengths between 650 and 950 nanometers, can be used to study "cerebral structure and function" [23]. In particular, it can be used to study the brain of infants, because their thin skull enables the further penetration of light through the brain [15].

Two important compounds, whose concentration changes can be measured by NIRS are oxyhaemoglobin and deoxyhaemoglobin. They provide information about the oxygen delivery to the tissue [23]. They can also be further processed to calculate the changes in cerebral blood volume and cerebral blood flow [15, 23].

Information about the cellular oxygen availability could be obtained using the NIRS signals of CCO. In fact, it has been shown that changes in the redox state of different centres of CCO could cause changes in the NIRS signals in the 780-900 nanometers region. Furthermore, there is very good evidence that over 80% of the spectral changes are due to the Cu_A centre [8].

The changes in the concentration of the chromophores are quantified using the modified Beer-Lambert law, which relates these changes to the changes in absorption of light in the tissue [15, 23].

3.2.2 Magnetic Resonance Spectroscopy (MRS)

Magnetic Resonance Spectroscopy (MRS) is based upon the Nuclear Magnetic Resonance (NMR) phenomenon. In NMR, magnetic properties and energies of the nuclei are examined [10]. Some atomic nuclei possess an intrinsic magnetic moment (spin). When placed in an external magnetic field, the spin axis of these nuclei precesses about the applied field. The precessional, or resonant, frequency depends on the strength of the applied field. The chemical structure of the environment, as well as the positioning of the neighbouring nuclei form a local magnetic field that changes the net magnetic field applied to the nuclei. Thus, instead of having a single resonant frequency, each nuclei has a range of resonant frequencies. Applying a broadband radio frequency pulse perturbs all these frequencies, letting the nuclei to produce a significant, measurable magnetic field. The Fourier transform of this magnetic field provides a spectrum containing the resonant frequencies [4]. Each peak in the spectrum is associated with the presence of a certain compound in the environment and the height of each peak corresponds to the concentration of that compound [15].

The ^{1}H MRS can be used to measure the concentrations of several compounds including N-Acetyl Asparate (NAA), lactate (Lac) and creatine [15]. In aerobic conditions, the lactate peak cannot be observed. However, under anaerobic conditions, such as hypoxia and ischaemia, the lactate peak becomes detectable [15].

Phosphorus can be found in some important metabolites, notably phosphocreatine (PCr), P_i, NTP and phosphomonoesters (PME). Thus, ^{31}P MRS is one of the most important techniques in investigations of the brain metabolism [4].

Different techniques can be used in ^{31}P MRS measurements. One method measures the ratio of metabolites, such as [PCr]/[P_i] and [PCr]/[NTP]. In is worth noting that the actual concentration of the metabolites cannot be derived from these ratios. Another method exists, which directly measures the concentration of metabolites. Using the concentrations obtained from this method, other parameters such as the intracellular pH (pH_i) can also be calculated. In fact, many phosphorus containing compounds exhibit a chemical shift, which is dependent on a number of parameters including the pH of their environment [4, 10]. This shift is a result of a change in the chemical environment due to "protonation of a compound" [10]. pH_i can be estimated from the chemical shift of most of the peaks present in the ^{31}P MRS spectra. However, the chemical shift of P_i relative to PCr is the most commonly used [4, 10]. It has been found that, compared with the other metabolites, this metabolite has a higher sensitivity to pH and in hypoxic-ischaemic conditions, its peak is increased, making it more useful for pH estimation [4]. The Henderson-Hasselbalch relationship is used to do the estimation [3, 4, 10]:

$$pH_i = 6.77 + log[(\delta_{Pi} - 3.29)/(5.68 - \delta_{Pi})] \tag{3}$$

where δ_{Pi} is the observed P_i chemical shift.

4 Modelling Brain Physiology

Below, the BrainPiglet model will be briefly explained. A more detailed description of the model could be found in [16]. Based on a previous model called BrainSignals [2], the model was developed to simulate the circulation and metabolism in the brain of neonate piglets. The model can also estimate the variables measured in NIRS and MRS [16]. The modifications I have made to the model, in order to improve it, will also be discussed.

4.1 The BrainPiglet Model

The BrainPiglet model takes arterial blood pressure and oxygen saturation as inputs and uses simplified equations to calculate the concentration of different compounds as outputs (Figure 1). These values can then be compared with NIRS and MRS measurements.

The model has two main parts: circulation and metabolism. The circulatory part is composed of blood vessels, i.e. arteries and arterioles, capillaries and veins. While the resistance of the venous compartment is constant, the resistance of the arterial compartment is considered to be variable, depending on a number of parameters and inputs. The total haemoglobin concentration is fixed in all compartments. A fraction of this is oxygenated in each compartment, depending on the arterial oxygen saturation and the rate of oxygen transport [16]. The changes in oxy and deoxyhaemoglobin can be derived from their concentration in the arteries and veins.

The metabolic part contains both cytoplasmic and mitochondrial processes. The cytoplasmic compartment contains glycolisis and conversion of pyruvate to lactate. The rate of glycolysis is dependent on the concentration of glucose, ADP and P_i. The conversion of NAD^+ to NADH is omitted from the glycolytic processes. Cytoplasmic NAD is also omitted from the reaction involving conversion of pyruvate to lactate. The rate of this reaction is sensitive to pH and adapts almost instantly to its changes [16].

The metabolic compartment contains TCA cycle, oxidation phosphorylation, and ATP consumption and production processes. The TCA cycle is simplified into a single reaction, involving consumption of

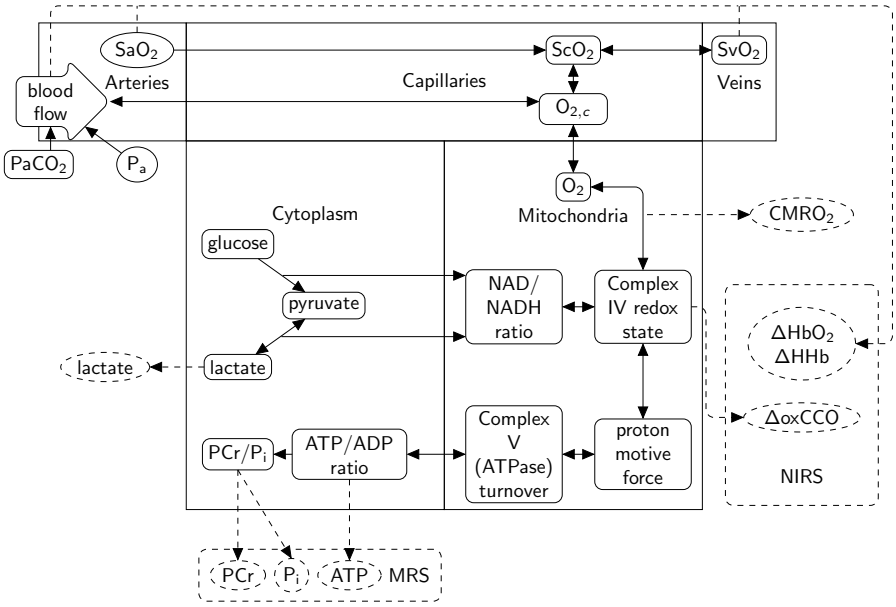


Figure 1: The schematic diagram of the BrainPiglet model taken from [14]

1 mole of pyruvate and 6 moles of NAD. The products of this cycle are not taken into account. The rate is mainly controlled by the NAD/NADH ratio and is only sensitive to the concentration of pyruvate when it falls below a certain limit [16].

The electron transfer chain pathways have been simplified into three reactions: one involving the transfer of electrons from NADH to Cu_A centre, one involving the electron transfer from there to $Cu_B - aa_3$ and one involving production of water from oxygen. Again, NADH is overlooked in these reactions.

This model has successfully simulated the oxygenation and metabolic changes in the brain of neonate piglets during anoxia [16]. However, when applied to hypoxic-ischaemic piglets, the model failed to make accurate predictions of the metabolic changes. It was noted in [16] that NAD, NADH and pH were not modelled in the cytoplasm. In particular, pH was taken as a settable parameter with an initial value of 7 [14]. In the study of anoxia, it was suggested that the hyperoxidation of Cu_A may be partly due to a fall in the levels of pH. However the authors stated that there was no mechanism in the model to give rise to this effect [16]. Since the model was able to predict this hyperoxidation to a good extent, it seems that the inclusion of pH was not necessary in that study. However, a fall in pH has been reported during hypoxia-ischaemia, both in lambs [11] and piglets [13, 20]. Thus, it is believed that in order for the model to be applied to piglets suffering hypoxia-ischaemia, the changes in pH should be taken into account.

4.2 Modelling the pH in Cytoplasm

In order to improve the model, the effect of including the pH in the model was investigated. Eight input files, containing ^{31}P MRS and NIRS measurements, as well as arterial pressure and oxygen saturation were provided. The method of doing the MRS measurements were such that only the ratios of the metabolites' concentrations were provided. Since the actual concentrations could not be derived from these ratios, it was impossible to use the Henderson-Hasselbalch relationship to calculate the pH.

Thus, another set of data was provided, containing the ^{31}P MRS measurements of five piglets during hypoxia-ischaemia. In these data, the pH, directly computed from the concentration of phosphorus containing compounds, was provided. The ratios of metabolites were also calculated. These pH data were used to find a function relating the pH to the metabolites' ratios.

First, to observe the temporal changes, the pH and the six measured ratios, i.e. [PCr]/[P_i], [PCr]/[EPP][1], [PME]/[EPP], [P_i]/[EPP] and [NTP]/[EPP], were plotted. The plots of [PCr]/[P_i], [PCr]/[EPP] and [NTP]/[EPP] exhibited changes similar to that of pH. [P_i]/[EPP] changed inversely with pH, meaning that there was a rise in the ratio during the insult, when the pH dropped. One possible explanation for this similar response to the insult was that a linear relationship may exist between the pH and the ratio of metabolites. In order to investigate this, the pH was plotted versus each of the ratios. Since none of these plots could be fitted by a line, it was concluded that pH could not be derived using just one of the parameters. In other words, it was probable that pH could be estimated by a linear function, having some or all of the ratios as variables.

To find this function, a statistical approach was taken, in which, *multiple linear regression method* was used to find the linear relationship between pH and the metabolites' ratios. The aim of linear regression is to investigate the existence and the nature of the relationship between a dependent variable and one or more independent variables [17]. It uses the method of least squares to fit a line to the data. The equation derived from line fitting could then be used to predict value of the dependent variable from the independent variables .

Instead of applying linear regression to each dataset and then averaging the resulting functions, all five datasets were merged to form a single set of data. Performing the linear regression on this dataset led to the following function:

$$pH = 7.52617 - 0.479322\frac{[PME]}{[EPP]} - 1.02847\frac{[NTP]}{[EPP]} - 0.293162\frac{[PCr]}{[NTP]} \tag{4}$$

$$-0.0323199\frac{[PCr]}{[P_i]} + 2.27271\frac{[PCr]}{[EPP]} - 1.25259\frac{[P_i]}{[EPP]}$$

To check the goodness of this fit, pH was calculated from the given ratios using the above function. The modelled pH was then compared with the measured values (Figure 2). The R^2 was calculated as 84.27%. The normalised root mean square error (NRMSE)[2] [3] was 8.017%. It was concluded from these results that the measured data were well fit by the model. In fact, the function was able to make accurate predictions of the value of pH from about 1 hour before the insult to about 2 hours after the insult. This seemed contenting because the first aim was to model the pH throughout the insult. Thus, the function was applied to each of the eight input files to estimate the pH. Next, the calculated pH was given as input to the BrainPiglet model, which was then used to make simulations of the brain during hypoxia-ischaemia.

5 Results

In order to evaluate the changes made to the model due to including the pH, the input data, i.e. arterial pressure and oxygen saturation were fed into the original model. The outputs were then plotted and compared with those obtained from the modified model, as well as with the experimental data.

The devices used in making the measurements added noise to the signals. In order to remove this noise, a first order Butterworth lowpass filter was used. This filter had a cutoff frequency of 0.003π radians/sample. Although the cutoff frequency was chosen such that it would not change the overall shape of most of the signals, it did affect the amplitude of [NTP]/[EPP] and CCO signals. The filter was designed such that it would not cause phase distortion.

[1]EPP is the exchangeable phosphate pool and is defined as [EPP] = [P_i] + [PCr] + 2[γ NTP] + [βNTP].

[2]The NRMSE was calculated as the root mean square error (RMSE) devided by the range of measured values [19]:

$$NRMSE = \sqrt[2]{\frac{\sum_{i=1}^{n}(x_{measured,i} - x_{modelled,i})^2}{n}} / (x_{measured,max} - x_{measured,min})$$

[3]The data for all variables were interpolated before calculating the NRMSE.

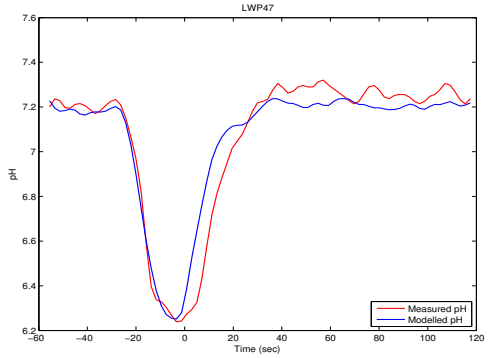


Figure 2: Comparison between the modelled pH and the pH calculated from ^{31}P MRS measurements using Henderson-Hasselbalch equation

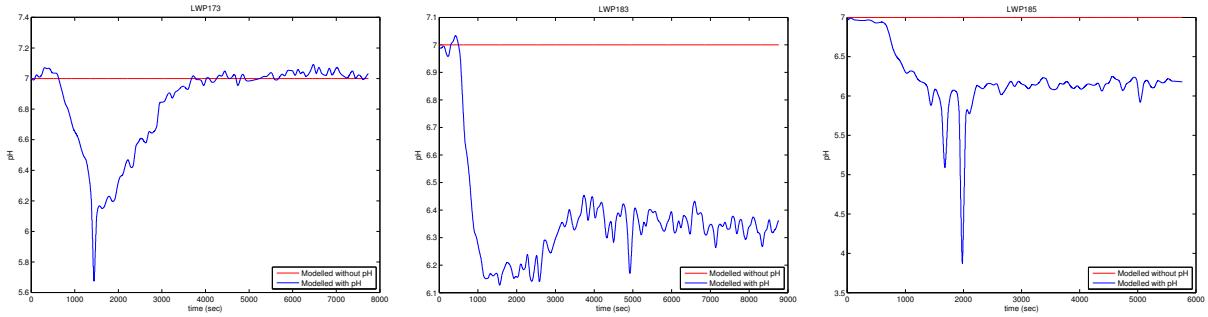


Figure 3: Changes in the pH, calculated from the ^{31}P MRS measurements using the proposed function

In all eight piglets, the pH dropped from around 7 to around 6, following the insult[4]. In five piglets, the pH increased during titration and returned to baseline at the end of the hypoxic-ischaemic period. In two of the remaining piglets, the pH did increase during titration. However, it did not reach the baseline, remaining constant at around 6.3. In one piglet, no increase in the pH was observed during titration.

No changes were observed in the blood oxygenation in the modified model, compared to the original model. This was expected, because there were no links in the model between the pH and the reactions involving oxy and deoxyhaemoglobin.

Since the model computed the concentrations of phosphate containing compounds, these values were divided to give the variables available from the MRS measurements. EPP was assumed to be constant. In addition, since the peak of NTP in MRS spectra mostly emerges from the concentration of ATP [16], a distinction has not been made between ATP and NTP. Figure 4 shows the modelled and measured values of these variables.

In all eight piglets, the predictions of $[\mathrm{PCr}]/[\mathrm{EPP}]$ and $[\mathrm{P_i}]/[\mathrm{EPP}]$ were improved in the modified model. There was a 52% decrease in the NRMSE for $[\mathrm{PCr}]/[\mathrm{EPP}]$, compared to the original model. This value was 37% for $[\mathrm{P_i}]/[\mathrm{EPP}]$. Despite having less error than the original model, the modified model failed to predict a correct fall (rise) in the concentration of $[\mathrm{PCr}]/[\mathrm{EPP}]$ ($[\mathrm{P_i}]/[\mathrm{EPP}]$). In the piglets whose pH returned to baseline after hypoxia-ischaemia, the model failed to predict the baseline value of these ratios. It also overestimated the changes in the values due to the insult. However, it was correct in predicting the recovery of the ratios to their baseline value after hypoxia-ischaemia. In the piglets whose pH did not return to normal after the insult, the modified model correctly predicted that $[\mathrm{PCr}]/[\mathrm{EPP}]$ and $[\mathrm{P_i}]/[\mathrm{EPP}]$ would not go back to their baseline value. The original model was not able to do this, predicting the return of these ratios to baseline after the insult.

[4]To be more specific, in two piglets, sudden drops in pH to below 5 (and in one piglet, below 0) were observed. These sudden drops are believed to be due to technical failures during measurements.

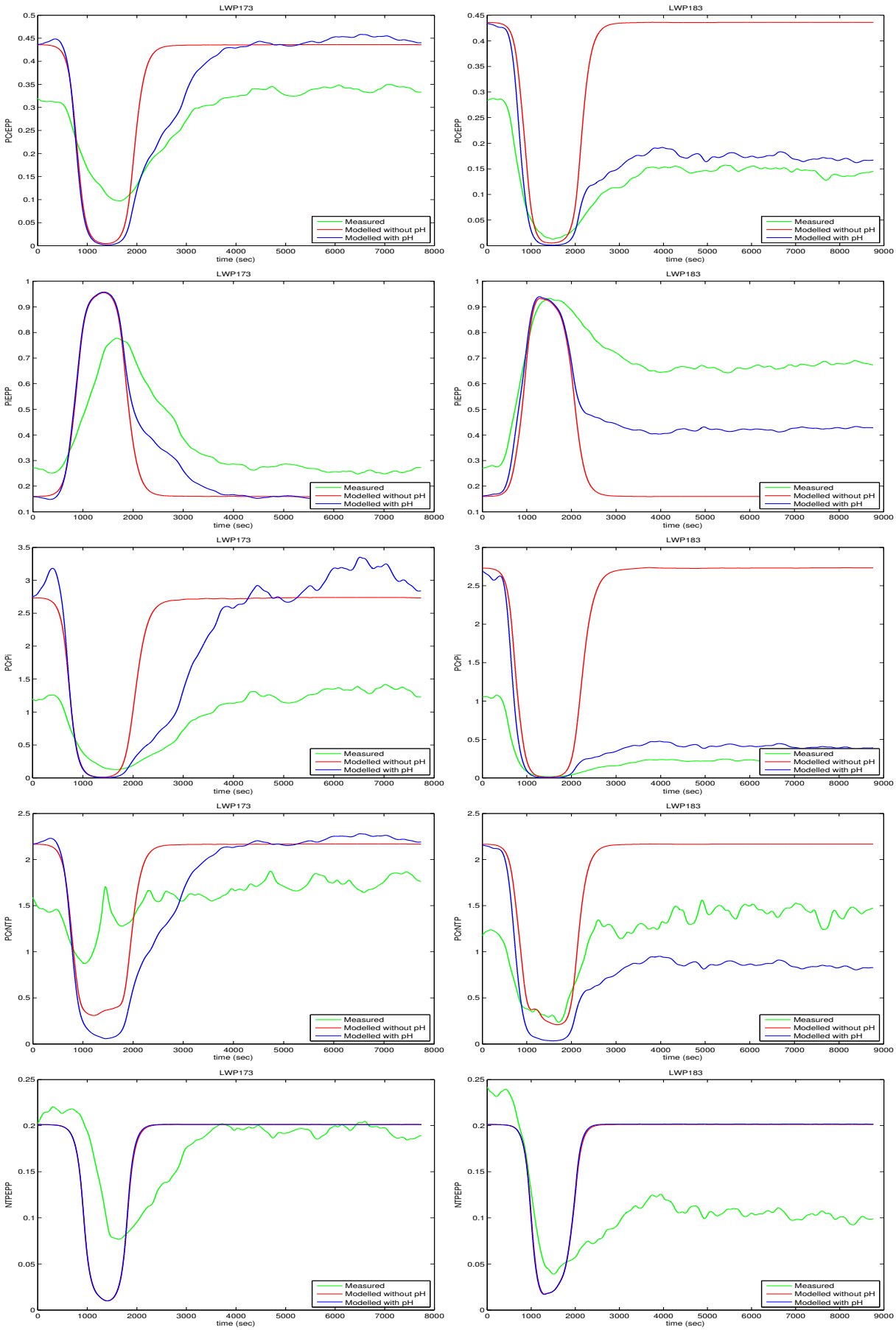


Figure 4: Comparisons between the model simulations (without and with pH) and measurements from ^{31}P MRS. The plots show the values when the pH did not return to baseline (right) and when it did (left).

In six piglets, including the pH in the model improved the predictions of $[PCr]/[P_i]$. In the remaining two, the changes were not significant and resulted in less than 5% increase in the error. Overall, there was a 44% decrease in NRMSE, following the inclusion of pH in the model. The improvements were particularly significant in those piglets whose pH did not recover. The original model could not distinguish between these three piglets and the other five, predicting the return of $[PCr]/[P_i]$ to baseline after the hypoxic-ischaemic period. The modified model on the other hand, correctly predicted that this ratio would not go back to normal, although overestimating the extent of the fall following the insult.

The changes in [PCr]/[NTP] were not significant in four piglets. In the others, there was a 24% decrease in NRMSE on average.

There was a 6% and 1% increase in the NRMSE for [CCO] and [NTP]/[EPP] on average, meaning that inclusion of pH in the model had made the predictions of these values slightly worse.

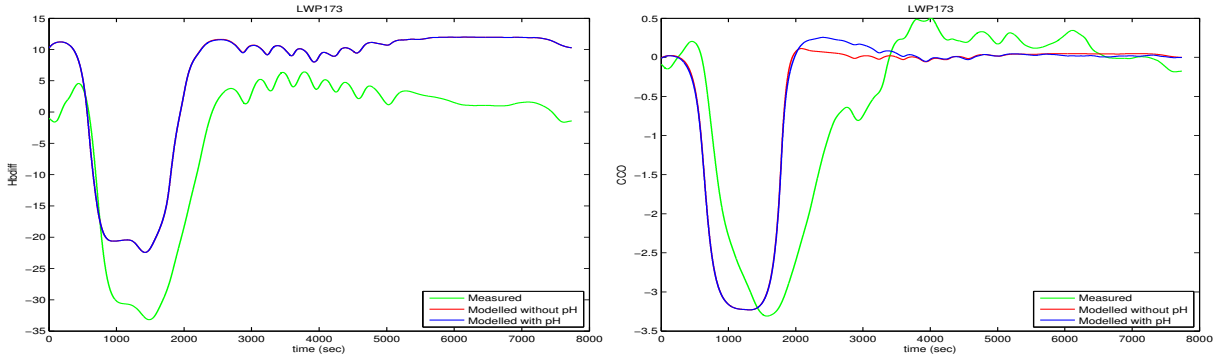


Figure 5: Comparisons between the model simulations (without and with pH) and measurements from NIRS, i.e. [Hbdiff] (left) and [CCO] (right)

The concentrations of lactate and pyruvate were also calculated (Figure 6). In all piglets, the pyruvate level dropped following the insult. In five of them, whose pH level was restored to baseline during recovery, a rise in the pyruvate level was observed. In four of them, the final pyruvate level was higher than the baseline. The pyruvate level was decreased to zero in the piglets whose pH did not recover to its initial value. Experimental data were not provided for these concentrations, thus, it was not possible to evaluate which model was making better predictions.

6 Discussion

Data from phosphorus MRS measurements were used to calculate the pH. A fall in pH was observed following the insult. This was consistent with previous experimental results provided in [13, 20]. It was demonstrated in those experiments that there will be a significant decrease in pH following hypoxia-ischaemia, which would return to baseline during recovery. The behaviour of the simulated pH was rather different. In fact, in three piglets, pH did not recover to baseline an the end of the hypoxia-ischaemic period. This may be due to the severity of the damage, as well as the individual resistance of the piglets to the injury. Since details of the experiments, from which the input data were obtained, were not provided, it was not possible to examine this statement.

One of the aims of the model was to increase insight into the physiological processes taking place during hypoxia-ischaemia and to help interpret the NIRS and MRS measurements [16]. The modification applied to the model enabled it to distinguish between piglets, based on the severity of injury caused to brain. The original model failed to do this distinction. While the original model predicted the restoration of the values of [PCr]/[EPP], $[P_i]/[EPP]$ and $[PCr]/[P_i]$ to the baseline after the insult, the modified model showed, in accordance with the experimental data, that this was dependent on the recovery of

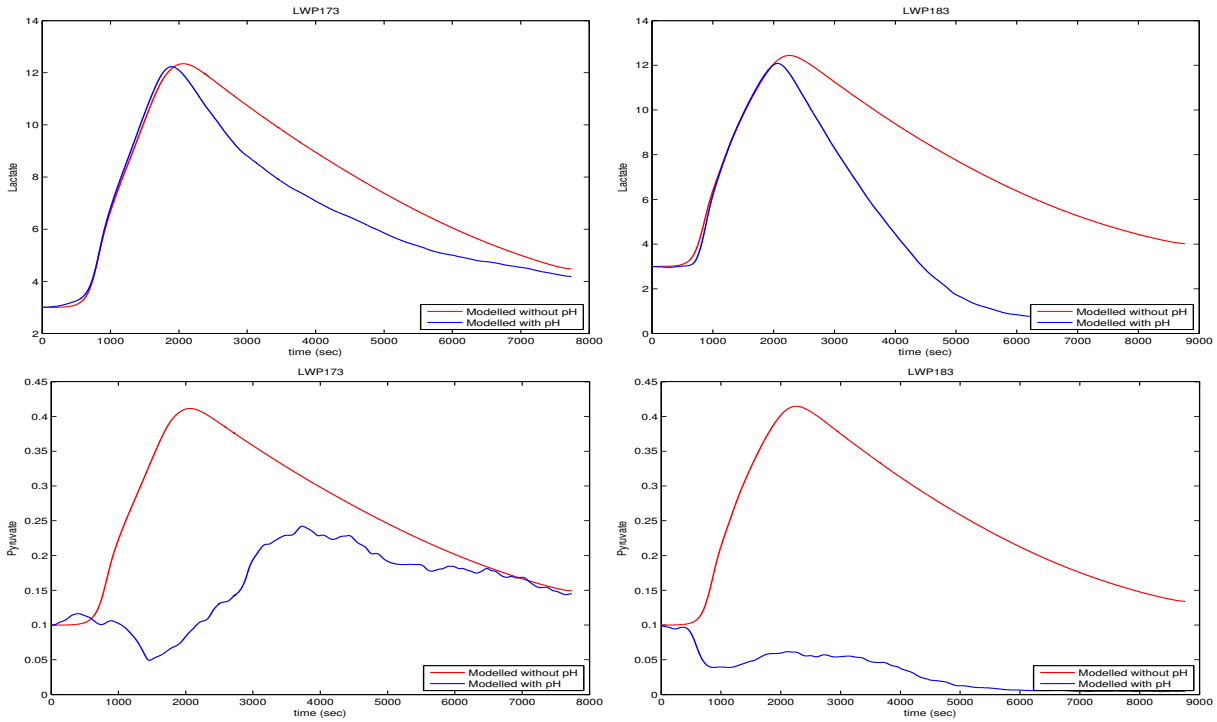


Figure 6: Comparisons between the modelled pyruvate and lactate concentrations, before and after including pH. The plots show the values when the pH did not return to baseline (right) and when it did (left).

pH to its baseline level. However, it should be noted that the modified model still failed to make accurate predictions of the actual values.

Similar to the original model, the modified model was unsuccessful in estimating the changes in [PCr]/[NTP] and [NTP]/[EPP]. This may partly be due to the fact that insertion of the pH into the model did not make any changes to its predictions of [ATP] (Figure 7). To find an explanation for the insensitivity of [ATP] to pH, I have looked for the links between these values in the model. It was found that the rate of change of [ATP] was dependent on the rate of five reactions. Two of these reactions, i.e. conversion of PCr to ATP and glycolysis were indirectly connected to pH. The rate of conversion of PCr to ATP was dependent on k_{PCr}, the forward rate of reaction in which phosphocreatine is combined with ADP to give ATP and creatine. k_{PCr} was dependent on the concentration of hydrogen ions in the cytoplasm, thus, linking [ATP] to pH. The rate of glycolysis was dependent on $\mathrm{P_i}$, which was connected to the concentration of hydrogen ions through the concentration of pyruvate. Despite the existence of these links, it seems that they were not strong enough to change the concentration of ATP following the insertion of pH into the model.

Inclusion of pH into the model did not make significant changes to the predicted values of [CCO]. In fact, the experiments showed a decrease in [CCO] following the insult, which would only return to baseline if the pH was recovered at the end of hypoxia-ischaemia. Both the original and modified models could predict the amount of fall due to the insult in four piglets, however, they predicted full recovery in all piglets after the insult. The only link I found between CCO and pH was through the proton motive force across the mitochondrial inner membrane, Δp. The value of this variable was calculated for both the original and modified models (Figure 7). It was concluded that the insertion of pH did not change Δp and thus, did not affect [CCO].

Pyruvate was the only variable that exhibited completely different behaviour when pH was included in the model. In order to find the possible reasons, the reactions involving pyruvate were investigated. As mentioned in section 2.1, pyruvate is one of the products of glycolysis and it is consumed in the TCA cycle. Both of these cycles were simplified in the model. NADH has been omitted from both of these

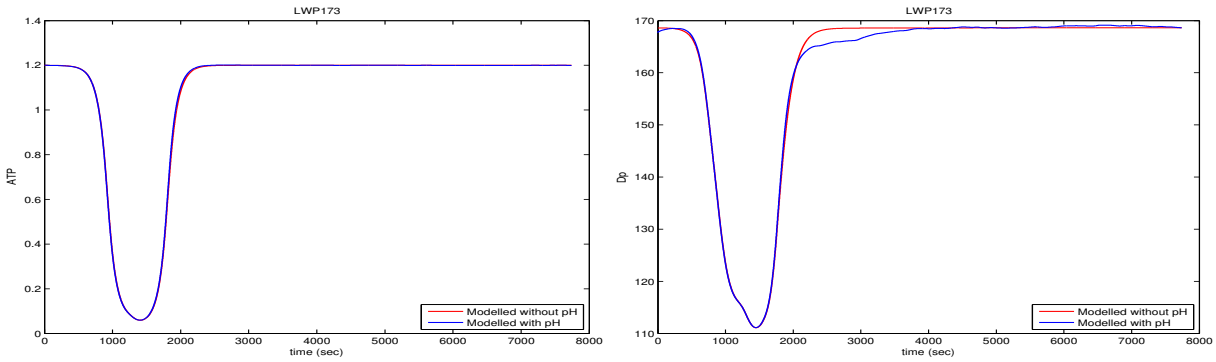


Figure 7: Comparison between the changes in modelled ATP (left) and Δp (right), before and after inserting pH.

reactions, as well as from the electron transfer chain. In the TCA cycle, $FADH_2$ has also been omitted. Both conversions of NAD to NADH and FAD to $FADH_2$ involve consumption of hydrogen ions, thus affecting the pH. Therefore, it was possible that excluding NADH and $FADH_2$ from the model affected the level of sensitivity of the reactions involved in glycolysis and TCA cycle to pH, and thus affected the changes in pyruvate concentration.

Finally, it is worth mentioning that the pH estimated from the experimental data is the pH in the tissue, a combination of pHs from different parts, e.g. cytoplasm, membrane, mithochondria. However, this pH was included in the model as the cytoplasmic pH. Furthermore, although different pHs are involved in the metabolic processes, e.g. pH in the mitochondrial matrix, inter membrane, the model does not distinguish between them. These may be responsible for some of the inconsistencies between the model outcomes and the experimental data.

To conclude, previous studies had shown the necessity of inclusion of pH in the model [16]. One possible approach was to study and modify the reactions in the model to enable the calculation of pH from the inputs. However, understanding the details of the brain physiology is not easy. Moreover, deciding how much detail to implement in the model to correctly replicate the experimental results, while keeping the model as simple as possible [16] is complicated. The advantage of the approach taken in this research is that it has given an insight of the changes induced in the model upon having a variable pH, without getting into details of physiological reactions. In fact, it was shown that upon including the pH in it, the model improved, making more accurate predictions of the changes in metabolites' concentrations. More importantly, the modified model was able to distinguish between the piglets based on the severity of the injury. This is of importance for clinicians, helping them to better interpret the measurements and estimate the amount of damage caused to the brain. Another advantage of the taken approach is that it has helped identify the reactions which require modification. In fact, some of the predictions of the modified model differed from the measurements. By comparing the original and the modified model, some of the simplifications which could be responsible for these inconsistencies were identified. Considering these advantages, I believe that in future studies, the pH should be calculated by the proposed function and be given to the BrainPiglet model as input to make more accurate simulations and to identity the oversimplifications in the model.

7 Acknowledgements

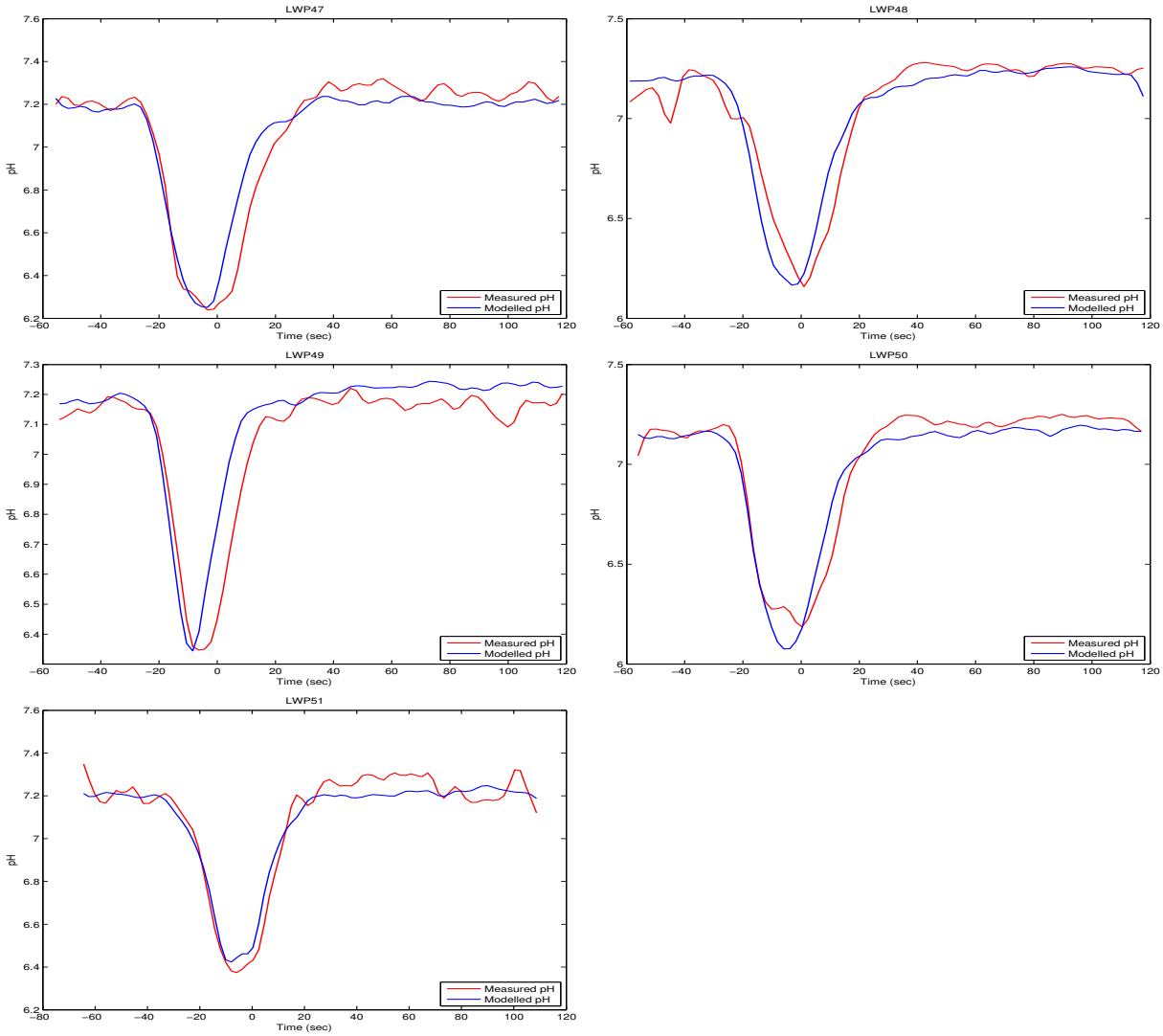
I would like to thank my supervisors for giving me the chance to experience the complications of data analysis. I am particularly grateful to Dr Ilias Tachtsidis for his continueing guidance, encouragement and support to overcome the difficulties of this research. I would also like to thank Tracy Moroz for sharing her work on the BrainPiglet model with me and for her patience in answering my endless questions.

Appendix

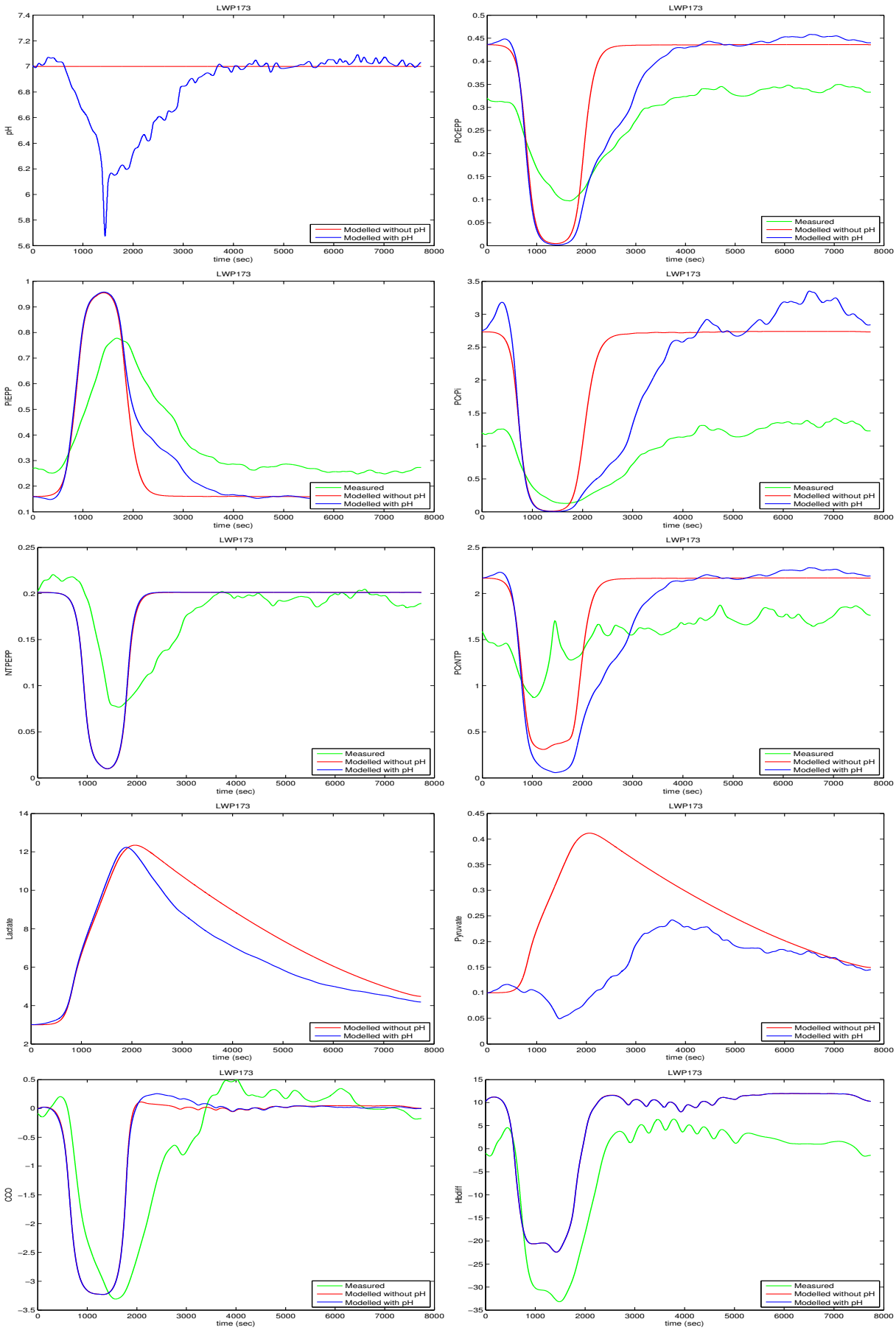
For five piglets, the pH data were provided. The plots comparing the modelled pH (using the function developed in this research) with the measured values are presented. A first order Butterworth lowpass filter with a cutoff frequency of 0.3π radians/sample was used to remove the noise from the signals.

For eight piglets, pH was calculated from ^{31}P MRS measurements, using the proposed function, and was fed into the BrainPiglet model to run some simulations. In the following section, the outcomes of the simulations are also provided. The modelled values of [PCr]/[EPP] (PCrEPP), [P_i]/[EPP] (PiEPP), [PCr]/[P_i] (PCrPi), [NTP]/[EPP] (NTPEPP) and [PCr]/[NTP] (PCrNTP) are compared with the measurements from ^{31}P MRS. Changes in the concentrations of lactate and pyruvate are plotted for both the original and modified model. Changes in [CCO] and difference between the concentration of oxy and deoxyhaemoglobin (Hbdiff) are also plotted. A first order Butterworth lowpass filter with a cutoff frequency of 0.003π radians/sample was used to remove the noise from these signals.

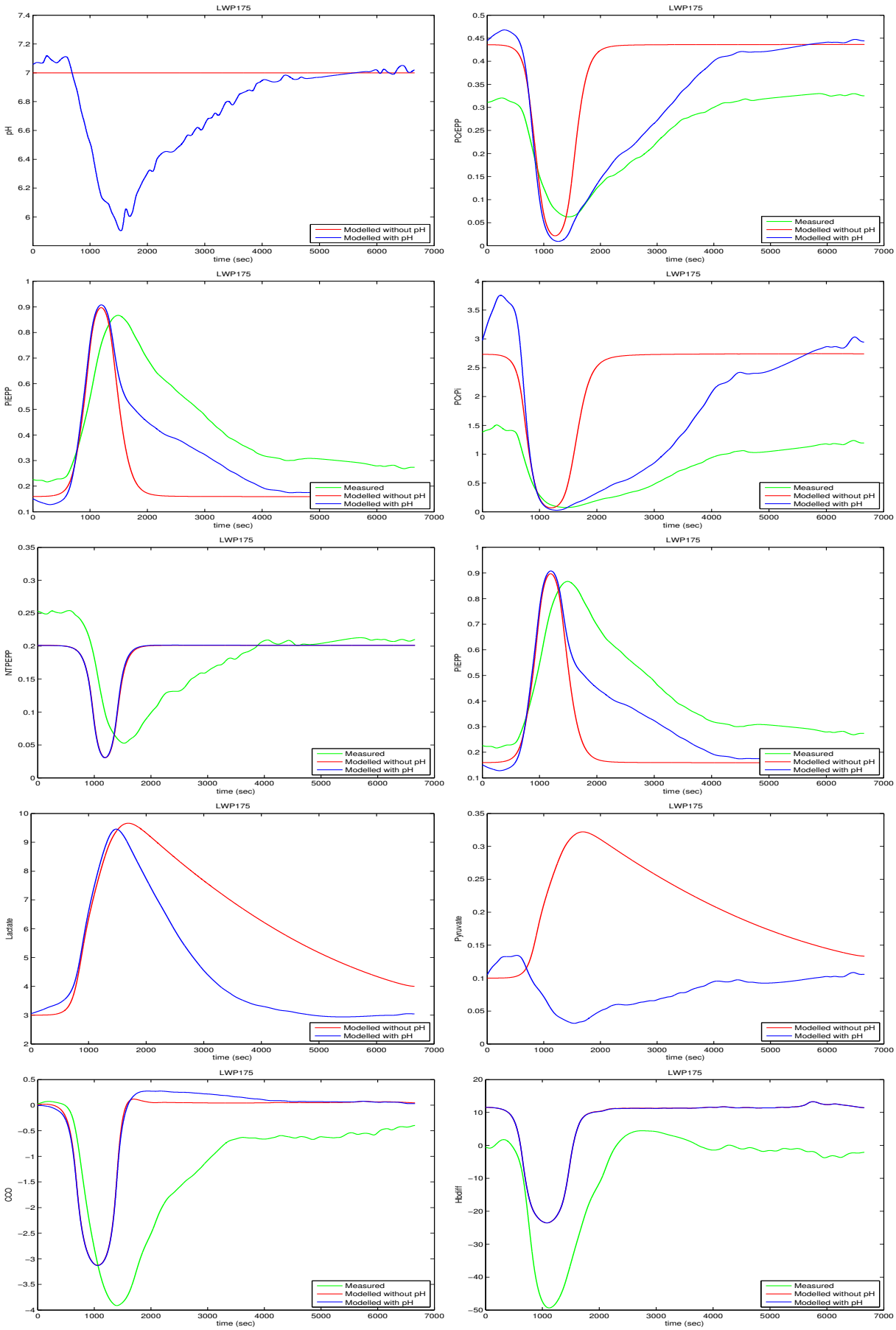
All simulations were run in the BRAINCIRC modelling environment [1]. All lowpass filters were designed in MATLAB.



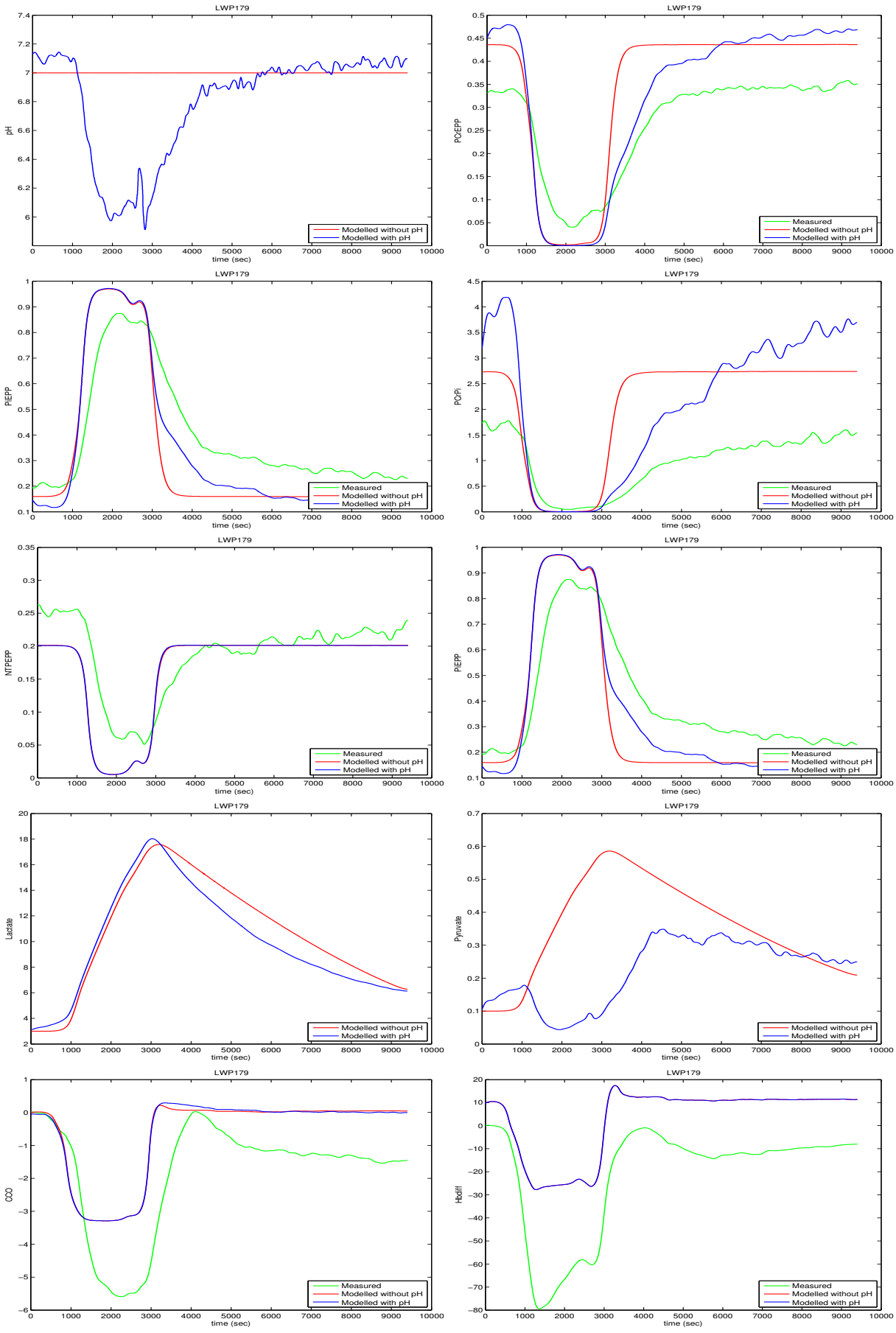
Comparison between the modelled pH and the pH provided from ^{31}P MRS measurements using Henderson-Hasselbalch equation



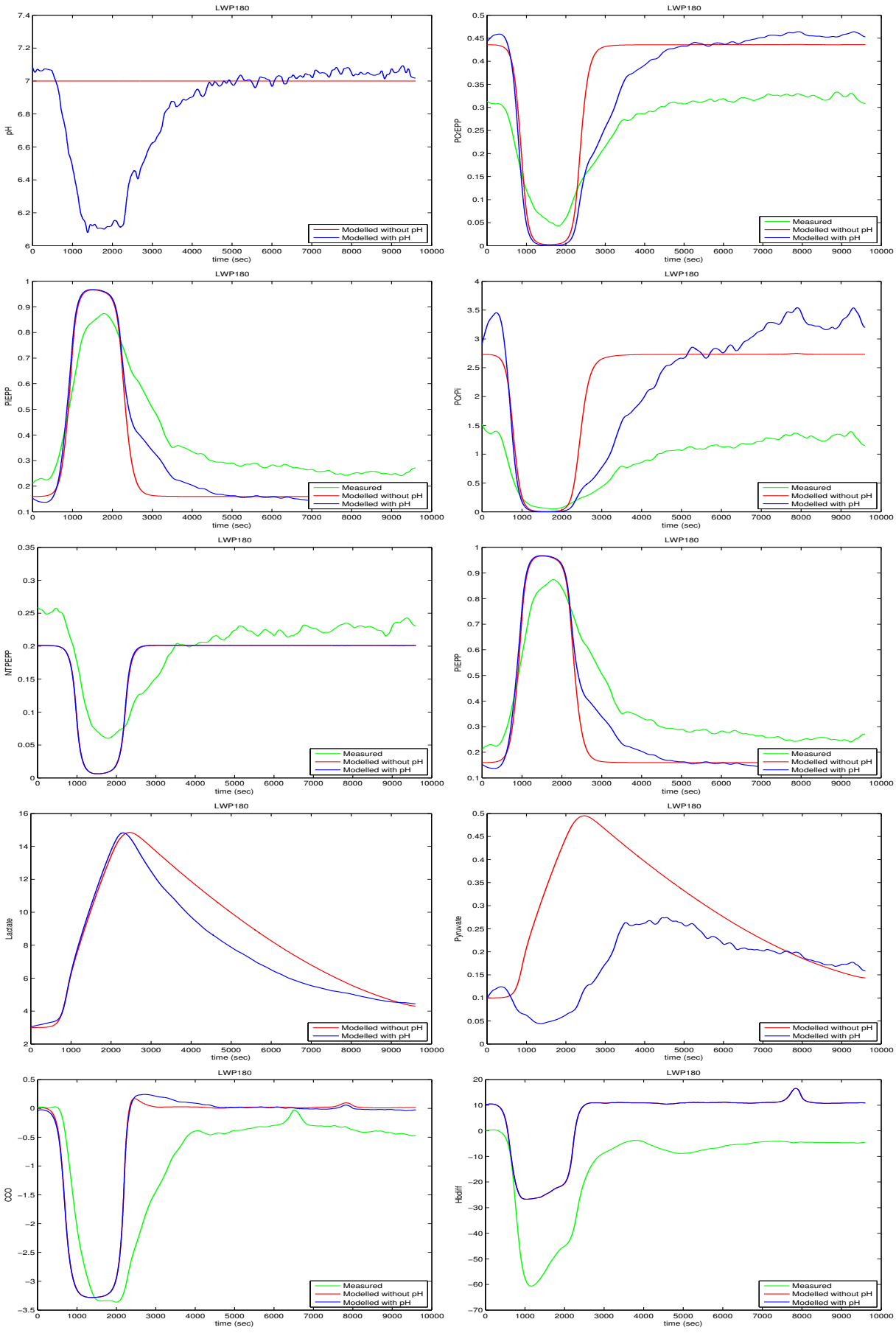
Outcomes of simulations from the BrainPiglet model for piglet LWP173.



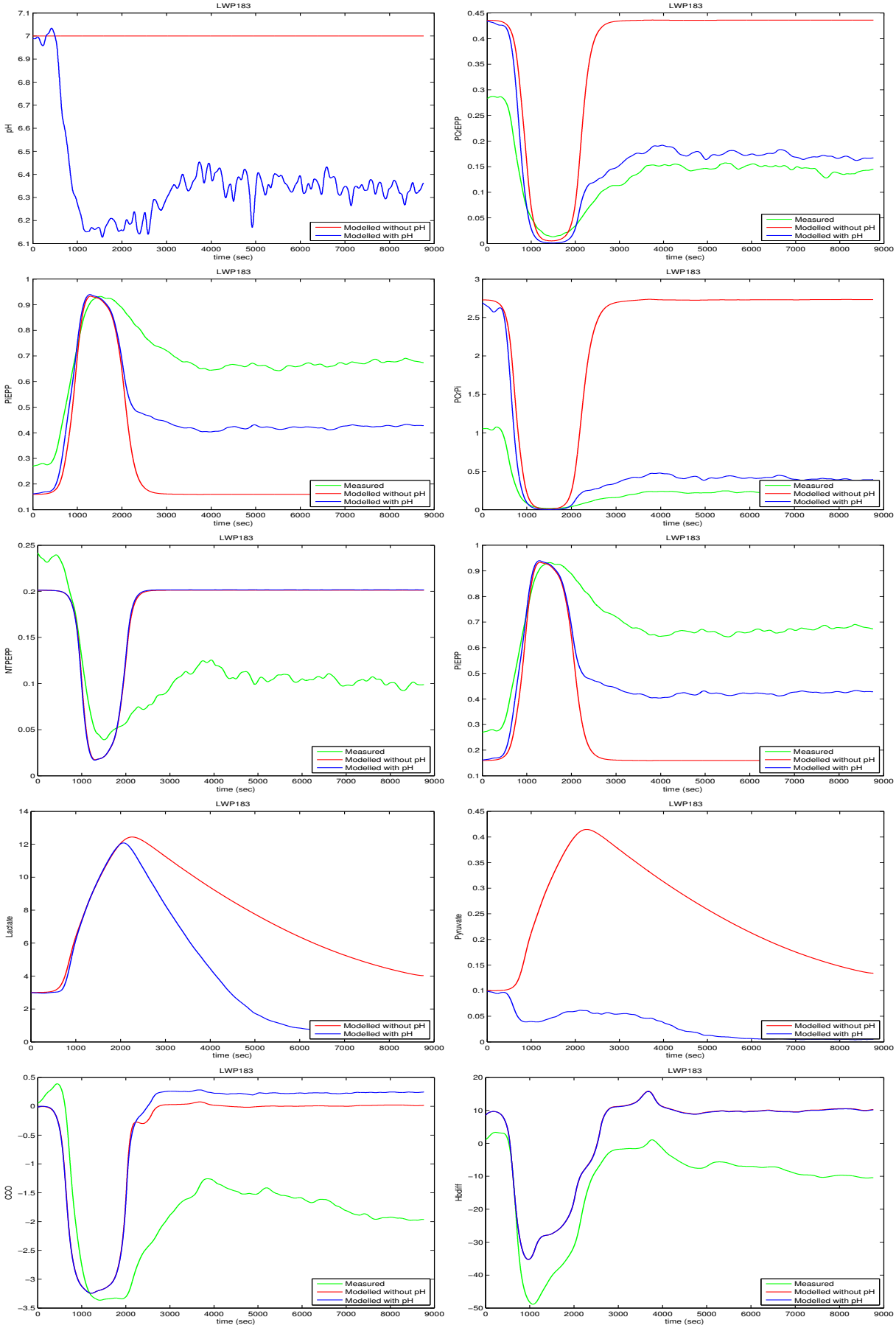
Outcomes of simulations from the BrainPiglet model for piglet LWP175



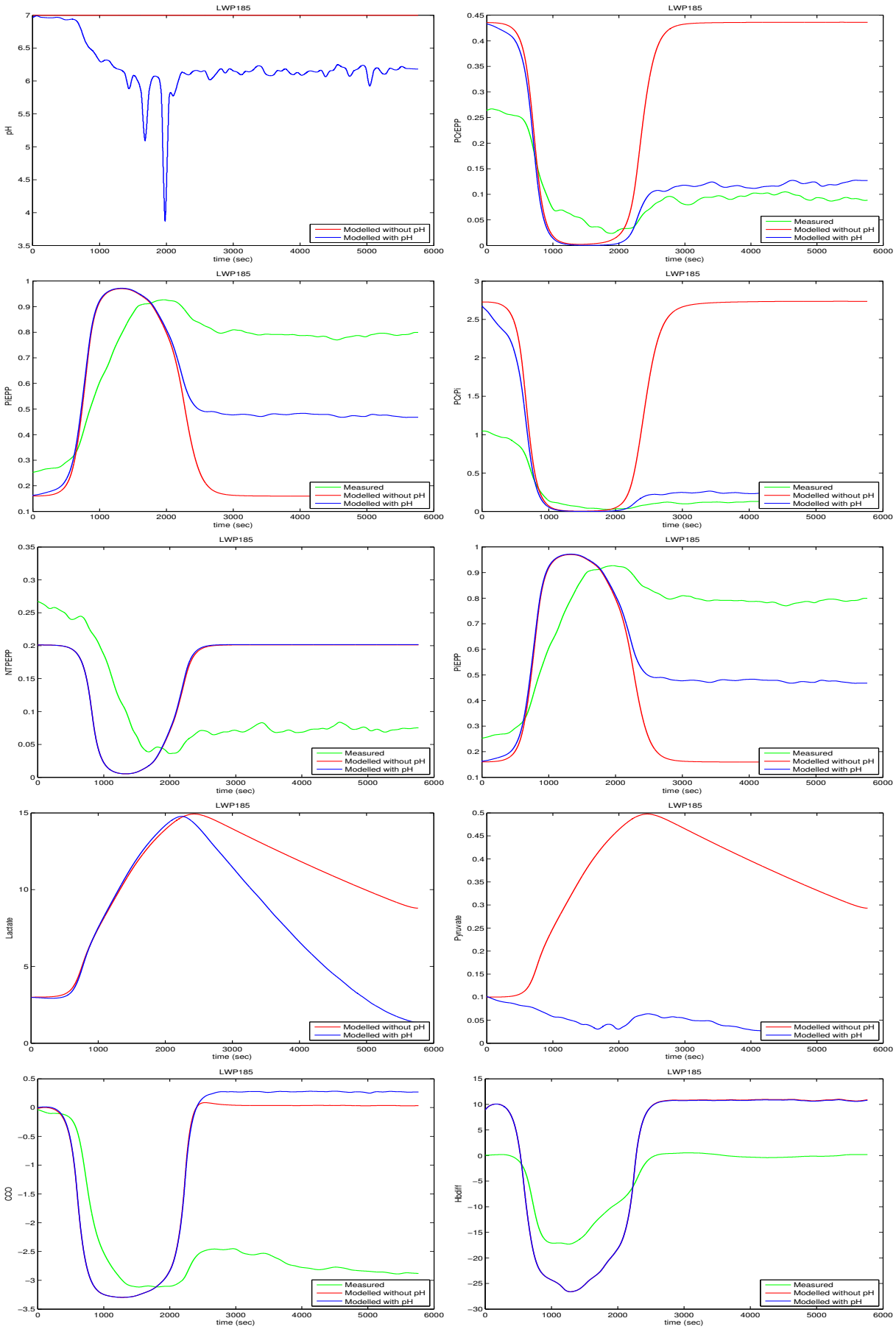
Outcomes of simulations from the BrainPiglet model for piglet LWP179



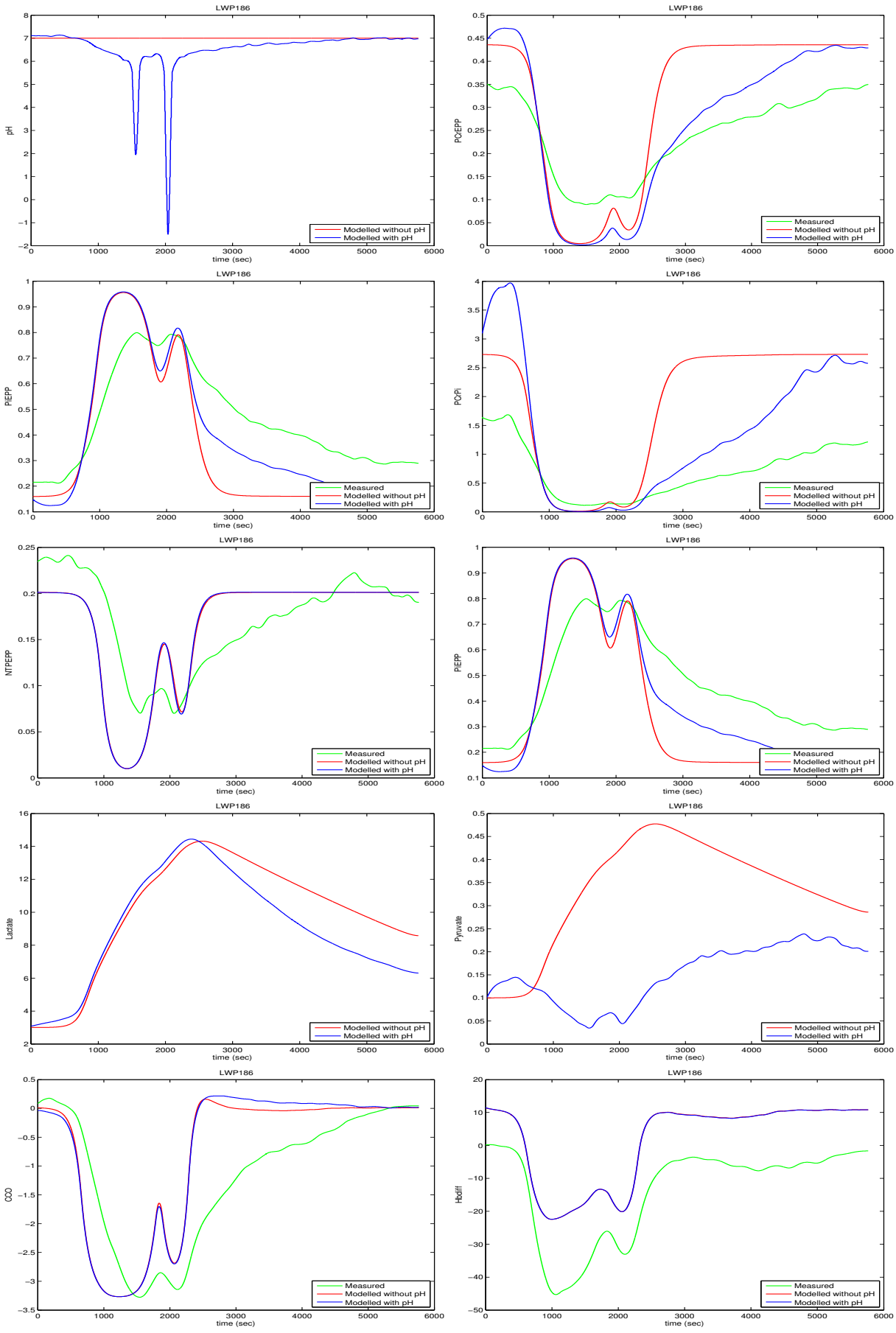
Outcomes of simulations from the BrainPiglet model for piglet LWP180



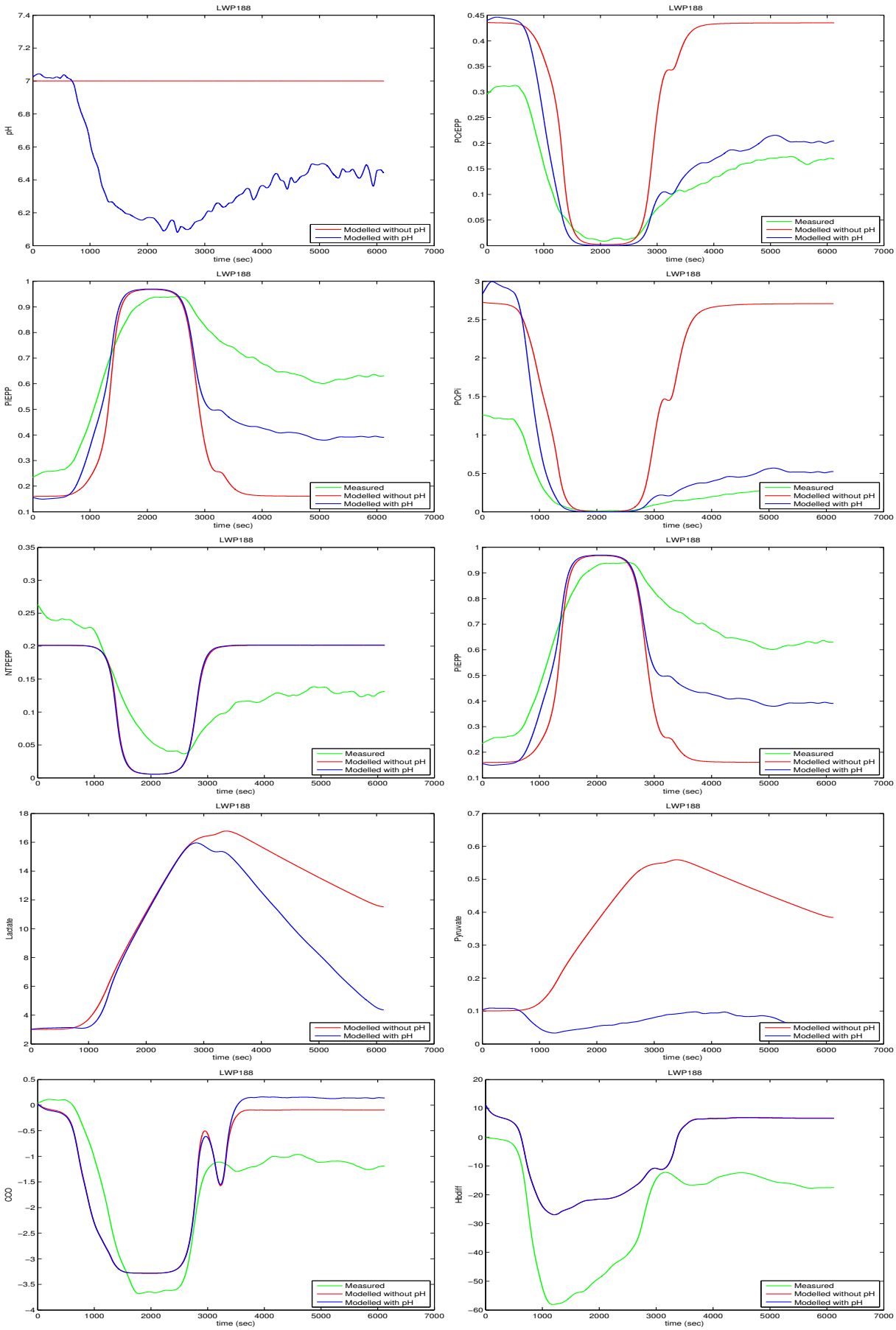
Outcomes of simulations from the BrainPiglet model for piglet LWP183



Outcomes of simulations from the BrainPiglet model for piglet LWP185



Outcomes of simulations from the BrainPiglet model for piglet LWP186



Outcomes of simulations from the BrainPiglet model for piglet LWP188

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