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# Estimation and Identifiability for a Dynamic Model of Maternal Nutrition and Fetal Growth in Sheep

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## **Abstract**

The optimal maternal nutrition intake is extremely important in the second half of pregnancy for fetal development in mammals. It affects the health and wellbeing of the offspring. The purpose of this study was to determine the optimal daily nutrition intake for sheep during the second half of their pregnancy, to achieve a pre-determined desirable birth weight for lambs. By achieving the optimal birth weight, the postnatal development of the animals is likely to be improved.

In this study, pregnant sheep carrying singletons or twins were considered. There were two levels of nutrition, low and high. Various dynamic mathematical models were proposed to obtain the optimal daily nutrition intake. The model parameters were estimated by weighted least-squares. Bootstrap simulations were used to check the reliability of each estimated parameter. Finally, the optimal daily nutrition intake was obtained by solving the boundary value problems, with pre-determined parameter values.

The results suggested that the optimal daily nutrition intake for sheep in the second half of their pregnancy was a constant. For the particular breed of sheep, with target weight 6.5 kg for singletons, the optimal nutrition intake was 1.36 kg of dry matter per day. For twins, with a target weight of 12 kg, the optimal nutrition intake was 1.93 kg of dry matter per day. In addition, a comprehensive and generic 'black-box' algorithm was produced using the software MATLAB. It could return the optimal daily nutrition intake for any type of mammals given a time series of fetal weight and maternal nutrition.

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## Glossary

BLUE	Best linear unbiased estimate	
BVP	Boundary value problems	
GLS	Generalised least squares	
ODE	Ordinary differential equation	
PCA	Principal component analysis	
QQ Plot	Quantile-Quantile plot	
SSE	Sum of squares of errors	
WLS	Weighted least squares	
$a$	A factor which influencing the ‘carrying capacity’ on historical intake	kg
$b$	The discount factor, which indicates that the cumulative intake is influenced by the past	day <sup>-1</sup>
$k_0$	The basic component of the ‘carrying capacity’	kg
$L$	The value of nutrition intake at which the per unit mass growth rate is half of the maximum	kg/day
$L_0$	The value of cumulative intake at which the influencing factor is half of its maximum	kg
$r$	The maximum per unit mass growth rate	day <sup>-1</sup>
$t$	Time	day
$u$	The daily maternal nutrition intake	kg/day
$x_0$	The initial fetal weight at the start of the second half of pregnancy	kg
$y$	A cumulative nutrition intake	kg

# Chapter 1

## 1. Introduction

### 1.1 Background

Pregnancy is one of the most important events in the lifetime of female mammals. It is a part of the reproduction process. Reproduction plays a vital role to maintain the species. Pregnancy refers to fertilising and developing one or more embryos or fetuses in the uterus of a female mammal (Kiataramkul et al. 2011). A key factor for fetal growth is the nutrient supply to the fetus (Harding & Johnston, 1995).

The relationship between fetal growth and maternal nutrition intake during pregnancy is crucial. The pregnancy success, and the offspring's lifetime health and productivity, can be determined by this relationship (Redmer, Wallace & Reynolds, 2004). Some animal studies showed that high nutrition intake during early pregnancy led to reduced fetal size (Godfrey et al. 1996); Parr et al. (1987) suggested that overfeeding in early stage of pregnancy reduced pregnancy rate in sheep. However, undernutrition would also impact the fetal growth, and usually resulted in low birth weight (Harding & Johnston, 1995). The possibility of early mortality and later morbidity is increased by low birth weight of the offspring (Osrin, 2000). In addition, Wu et al. (2004) suggested that placental-fetal blood flows would be reduced by either maternal overnutrition or undernutrition. It would inhibit growth of the fetus. They also stated that suboptimal nutrition intake during pregnancy remains a problem for many species globally.

Furthermore, the last third of pregnancy accounts for ninety per cent of fetal growth for sheep. This is approximately from 90 days of gestation to 145 days (Redmer, Wallace & Reynolds, 2004). Therefore, the maternal nutrition intake in the later stage of gestation is extremely important to health and wellbeing of the offspring.

## **1.2 Description of data**

The datasets used for this study are extracted from two journal articles written by Rattray et al. (1974a, 1974b). Both of the articles are concerned with the same experiment. The figure 1 from Rattray et al. (1974b) shows the relationship between the fetal weight (kg) and day of gestation in sheep. We read off the data points from this plot. The table 6 from Rattray et al. (1974a) provides us the summary statistics of daily nutrition intake (gram Dry Matter/day) with approximate day of gestation at 70, 100, 125 and 140 days. There are two levels of nutrition intake, level low and high, for pregnant sheep in the experiment after day 70 of gestation. The datasets associated with the pregnant sheep carrying singletons and twins in the second half of their pregnancy are considered. Thus, we have two sets of experimental data which are available in this study.

## **1.3 Objective of this study**

In this study, we consider the fetal development in the second half of pregnancy, from day 70 to day 147 (birth time). The birth weight of the lambs is an important indicator to show how healthy both the pregnant sheep and her offspring are. The ultimate goal is to find the optimal daily nutrition intake for sheep during the second half of their pregnancy, in order to achieve the pre-determined desirable birth weight for the lambs, and also with the minimum total nutrition intake.

In addition, we need to produce a 'black-box' algorithm. It is a very comprehensive algorithm, which requires minimum human intervention. It is also a generic algorithm, which can be used for most mammals. This algorithm should be designed for people without sophisticated statistical or mathematical background. As long as the people collect enough data of relevant time, fetal weight and daily nutrition intake for a specific mammal, the algorithm can provide them the optimal daily nutrition intake for this kind of animal.

## **1.4 Thesis outline**

The thesis has been structured into the following chapters. In chapter 2, we will present some basic knowledge from the literature on the topics of least squares estimation, identifiability, bootstrap resampling and boundary value problems. In chapter 3, the dynamic system of the original model will be introduced, and we will discuss each of the model parameters. The methodology of the parameter estimation will also be outlined. The detailed results and discussion on the parameter estimation of the original full model and alternative models will be shown in chapter 4. The results of bootstrap simulations will be discussed in chapter 5. In chapter 6, we will present the optimal numerical solutions for this study. The chapter 7 will summarise the main findings of this study, and we will also outline the future research direction.

# Chapter 2

## 2. Literature Review

In this chapter, we present some basic knowledge from the literature on the topics of least squares estimation, identifiability, bootstrap resampling and boundary value problems. These topics will be discussed briefly in the following sections.

### 2.1 Least squares estimation

After the stages of model definition and data collection, the next step is to estimate the parameters of the model based on the data we have got. This process is referred to as model fitting or parameter estimation (Chatterjee & Hadi, 2006). It can also be visualised as a study of inverse problems (Zhang, 1997). The most common method for parameter estimation is the least squares method.

The history of the least squares method can be traced to 1795. This method was invented by Karl Friedrich Gauss, who formulated the basic concept and applied it in astronomical computations (Gibbs, 2011). It has been widely applied in estimating unknown parameters, as well as solving some technique problems. Identifying the parameters from a dynamic system by using the least squares method has received considerable attentions in recent decades and is widely used.

The optimisation criterion of the least squares method is to set a ‘cost’ or ‘loss’ function, which needs to be minimised. In the least squares estimation, the function to be minimised is the sum-of-squares of residuals (Gibbs, 2011).

The form of general model is (Seber & Wild, 1988)

$$y_i = f(X_i; \theta^*) + \varepsilon_i \quad (i = 1, 2, \dots, n), \quad (2.1)$$

where  $E[\varepsilon_i]=0$ ,  $X_i$  is a vector, and the true value of  $\theta^*$  of  $\theta$  is known to belong to  $\Theta$ . It is also noted that  $\theta$  is a vector of parameters, while  $\Theta$  is a parameter vector set. The least-squares estimate of  $\theta^*$ , denoted by  $\hat{\theta}$ , minimises the error sum of squares

$$S(\theta) = \sum_{i=1}^n [y_i - f(X_i; \theta)]^2. \quad (2.2)$$

When each  $f(X_i; \theta)$  is differentiable with respect to  $\theta$ , and  $\hat{\theta}$  is in the interior of  $\Theta$ ,  $\hat{\theta}$  will satisfy

$$\left. \frac{\partial S(\theta)}{\partial \theta_r} \right|_{\hat{\theta}} = 0 \quad (r = 1, 2, \dots, p) \quad (2.3)$$

In this way, we can easily calculate the least squares estimate of the unknown parameters from equation (2.3).

Johnson & Faunt (1992) stated that there are six inherent assumptions related to the parameter estimation by least squares method. Thus we assume that

- All of the experimental errors can be attributed to the dependent variables;
- The experimental errors of the data follow a Normal distribution;
- There is no systematic error in the data;
- The function form  $f(X_i; \theta)$  is correct;
- There should be sufficient data points available to provide a good sampling of the experimental errors;
- The observations are independent of each other.

In addition, the constant variance is another important assumption related to the ordinary least squares. If this assumption is violated, we cannot achieve accurate estimation of the unknown parameters, and the values may be unreliable.

The generalised least squares (GLS) estimator can provide a better solution to handle the problem of heteroscedasticity. GLS estimators have some excellent attributes, namely they are unbiased, consistent, efficient and also asymptotically normal. The weighted least squares (WLS) is a special case of GLS.

WLS is a way of accommodating non-constant variance, in order to get precise estimates of the model parameters. However, it is assumed that we know the weights exactly, which is a disadvantage. In real applications, it is hard to fulfil this assumption.

A form of the GLS is (Seber & Wild, 1988) shown in equation (2.4).

$$s(\theta) = [y - f(\theta)]'V^{-1}[y - f(\theta)] \quad (2.4)$$

where  $V$  is a known positive definite matrix.



Generally, the optimal weights are proportional to the reciprocal of the variance for the observations. It can be expressed as  $V \propto \frac{1}{\sigma^2}$ . The ordinary least-squares is a special case of the GLS, where  $V = I_n$  (Seber & Wild, 1988). WLS is the special case where  $V$  is a diagonal matrix. In other words, the errors are independent but non-constant. In GLS, the errors may be correlated as well.

Minimising the objective function  $s(\theta)$  in equation (2.4), we can get the GLS estimate, denoted as  $\hat{\theta}_G$ . As we discussed earlier, the GLS estimate is unbiased and efficient. Hence, the GLS estimates  $\hat{\theta}_G$  is called the best linear unbiased estimate (BLUE) (Gibbs, 2011).

## 2.2 Identifiability

In recent decades, parameter identifiability has received considerable attention in the problem of building mathematical models of systems biology. The mathematical models are usually associated with dynamic systems, especially using ordinary differential equations (ODE) to describe the systems (Raue et al., 2011). The process of parameter estimation of this kind of model faces challenges, since the internal behaviour of biological or physiological systems are only partially observable. Most of the model parameters are measured indirectly by parameter estimation (Saccomani, 2004).

Jacquez (1990) stated that model specification refers to using the differential equations to show the processes occurring in the biological or physiological systems. The general dynamic models can be shown as follows (Miao et al., 2011):

$$\dot{x}(t) = f(t, x(t), u(t), \theta), \quad (2.5)$$

$$y(t) = h(x(t), u(t), \theta), \quad (2.6)$$

where  $x(t)$  is a vector of dependent variables;  $y(t)$  is the measurement;  $u(t)$  is the known system input vector; and  $\theta$  is the parameter vector.

The equation (2.5) is the ODE model. For the problem of parameter estimation, the parameter  $\theta$  is unknown, and needs to be estimated based on the experimental data.

The concept of identifiability has been defined by Miao et al (2011, P5) and refers to the dynamic system given by equation (2.5) and (2.6)

*'is identifiable if  $\theta$  can be uniquely determined from the given system input  $u(t)$  and the measurable system output  $y(t)$ ; otherwise, it is said to be unidentifiable'.*

In the literature, there are some relevant concepts related to the idea of identifiability. Ljung and Glad (1994) introduced the concepts of global identifiability and local identifiability. Others distinguish between structural identifiability and practical identifiability (Bellman & Astrom, 1970).

The difference between global and local identifiability is based on the parameter space of  $\theta$ . The identifiability can be expressed as  $y(u, \theta_1) = y(u, \theta_2)$  if and only if  $\theta_1 = \theta_2$ , with any admissible input  $u(t)$ . If it is considered as globally identifiable, any two parameter vectors  $\theta_1$  and  $\theta_2$  should be in the parameter space  $\Theta$ . However, if this condition holds only for any  $\theta$  within an open neighbourhood of some point  $\theta^*$  in the parameter space, then it can be considered as locally identifiable (Miao et al., 2011).

The structural and practical identifiability has been defined by Chis, Banga and Balsa-Canto (2011, P1) as follows:

*'Structural identifiability is a theoretical property of the model structure depending only on the system dynamics, the observation and stimuli function. Practical identifiability is intimately related to the experimental data and the experimental noise'.*

There are three main categories of identifiability techniques used for general ODE models, which are structural identifiability analysis, practical identifiability analysis, and sensitivity-based identifiability analysis (Miao et al., 2011).

The structural identifiability technique is used to evaluate the identifiability of theoretical model structure. It can be done without any experimental data. Thus it is called prior identifiability analysis. There are two assumptions related to structural identifiability analysis. The first assumption is the absolute accuracy of model structure; the second assumption is no measurement error. However, those two assumptions are hardly ever fulfilled in practice. Generally, there are four techniques used in determining the structural identifiability. These techniques are

- Power series expansion and similarity transformation
- Direct test

- Differential algebra
- Implicit function theorem

Both model error and measurement error are considered in practical identifiability analysis. It is also known as posterior identifiability analysis. If the model is determined as structural non-identifiable, there is no need to conduct the practical identifiability analysis, since the structural non-identifiability must imply the practical non-identifiability. If the model is diagnosed as structural identifiable, then further analysis is required to examine the further propriety of the model. That is, it is necessary to conduct a practical identifiability analysis. For this, we require initial values for each parameter. Generally, there are two techniques available to determine the practical identifiability. These techniques are

- Monte Carlo simulation
- Correlation Matrix

Sensitivity-based analysis is another technique of identifiability analysis. To some extent, it is similar to structural identifiability analysis. It does not require actual experimental data. In addition, it does not account for model uncertainty and measurement error. However, the major difference between sensitivity-based analysis and structural identifiability analysis is that there is no requirement for model structure information. The sensitivity-based analysis is similar to practical identifiability analysis in terms of requiring pre-determined values for the parameters. The sensitivity-based analysis can be considered as a technique between the structural identifiability analysis and practical identifiability analysis. The structural identifiability analysis may not be successful for dealing with highly dimensional ODEs or complicated ODEs. Since the structural identifiability is unknown, the practical identifiability analysis may be not reliable. In this case, the sensitivity-based analysis can handle this kind of tough situation. Generally, there are four techniques considered as sensitivity-based analysis, which are

- Correlation Method
- Tuning importance method and Principal Component Analysis (PCA)
- Orthogonal method
- Eigenvalue method

The consequence of non-identifiability of the model is that it is not possible to obtain a reliable estimation of the parameters. In this case, we cannot utilise the model to do further

prediction and analysis of the dynamic system (Miao et al., 2011). Raue et al. (2011) suggested two ways to resolve the issue of non-identifiability. The first method is to refine the experimental design, in order to get more experimental data based on suitable experimental conditions. The second method is model reduction. In this way, the dynamic models can be reduced, and the model simplified. The size of the model can be altered based on the information available from experimental data. In addition, Chis, Banga and Balsacanto (2011) suggested fixing the values of some parameters. Those fixed parameters should be ones that are less relevant to the model prediction. These three methods can help to remedy the problem of non-identifiability.

### 2.3 Bootstrap resampling

Bootstrap is a resampling procedure, which is a method to resample data from the original dataset with replacement (Chernick, 1999). Efron and Tibshirani (1986) stated that bootstrap is a general methodology to get an estimation of the accuracy of a parameter. This technique is simple to implement. The bootstrap simulations can be used for almost any conceivable problems. Once we program the bootstrap procedures, then the computer will do all the work for us. However, it is computationally intensive. Usually, it takes at least a thousand bootstrap replications to get the estimator (Chernick, 1999). Initially, the idea of bootstrap resampling was used to calculate valid confidence limits for population parameters. It also has been widely used for hypothesis testing (Manly, 2007).

The formal definition of (non-parametric) bootstrap is defined as follows (Chernick, 1999, P7):

*Given a sample of  $n$  independent identically distributed random vectors  $X_1, X_2, \dots, X_n$  and a real-valued estimator  $\theta(X_1, X_2, \dots, X_n)$  (denoted by  $\hat{\theta}$ ) of the distribution parameter  $\theta$ , a procedure (the bootstrap) to assess the accuracy of  $\hat{\theta}$  is defined in terms of the empirical distribution function  $F_n$ . This empirical distribution function assigns probability mass  $1/n$  to each observed value of the random vectors  $X_i$  for  $i=1,2,\dots, n$ .*

There are three types of bootstrap resampling methods, which are non-parametric, parametric, and semi-parametric. Their properties will be discussed briefly (Carpenter and Bithell, 2000).

The non-parametric resampling method does not require assumptions associated with the model for, or the distribution of, the data. The general procedures can be described in three steps as follows:

1. Generate a sample with replacement from original sample, with original sample size of  $n$ .
2. Compute  $\theta^*$ , the parameter we are interested based on bootstrap sample.
3. Repeat steps 1 and 2,  $k$  times to obtain an estimate of the bootstrap distribution.

The value of  $k$  is dependent on the situations. Chernick (1999) stated  $k$  is recommended to be at least 100 for estimating standard errors. For the confidence interval or hypothesis testing, the value of  $k$  should be at least 1000.

The parametric resampling requires the assumption of a model distribution. Thus, the bootstrap resampling is under the particular model distribution. The procedure is shown below:

1. Let  $\hat{\theta}$  be the estimate of the parameter  $\theta$  from the original dataset. Sample  $m$  observations from the particular known distribution with attributes of  $\hat{\theta}$ .
2. Compute  $\theta^*$ , the parameter we are interested based on bootstrap sample.
3. Repeat steps 1 and 2,  $k$  times to obtain an estimate of the parametric bootstrap distribution.

Lastly, the semi-parametric resampling method combines the features of both non-parametric and parametric resampling methods. It uses non-parametric resampling of the residuals from the fitted parametric model (Carpenter and Bithell, 2000). The generic procedure is described below:

Let us suppose we have two vectors  $y = (y_1, \dots, y_n)$  and  $x = (x_1, \dots, x_n)$ , then we fit the model

$$y = g(\beta, X) + r \quad (2.7)$$

getting estimates  $\hat{\beta}$  of the parameters  $\beta$ , and a set of residuals  $r_i, i=1, 2, \dots, n$ .

1. Adjust the residuals of the model (2.7),  $r = (r_1, \dots, r_n)$ , to make them have approximately equal variances and means, denoted  $\tilde{r} = (\tilde{r}_1, \dots, \tilde{r}_n)$ .

2. Sample with replacement from the set of adjusted residuals  $(\tilde{r}_1, \dots, \tilde{r}_n)$ , to obtain a set of bootstrap errors,  $r^* = (\tilde{r}_1^*, \dots, \tilde{r}_n^*)$ .
3. Then bootstrap the data  $y^* = (y_1^*, \dots, y_n^*)$  with model,  $y^* = g(\hat{\beta}, X) + r^*$ .
4. Fit the model  $y^* = g(\beta, X)$ , to get the bootstrap estimate  $\hat{\beta}^*$ .
5. Repeat the steps 2-4,  $k$  times to obtain the bootstrap distribution.

There is an important assumption underlying to this semi-parametric bootstrap method. It requires the adjusted residuals  $\tilde{r}_i$  independently and identically distributed (i.i.d.).

Two common methods used for bootstrap confidence interval are standard bootstrap confidence limits, and simple percentile confidence limits (Manly, 2007). We introduce those two methods briefly here.

The simplest method for getting the confidence limit is the standard bootstrap method. It uses the estimate of parameter  $\theta$  from original dataset, and the bootstrap standard deviation of estimates of parameter  $\theta$  to formulate the confidence limit (Manly, 2007). It can be shown as

$$\hat{\theta} \pm Z_{\alpha/2} * \sigma_{bootstrap} \quad (2.8)$$

Note that  $Z_{\alpha/2}$  is the z-score from the normal distribution with significance level of  $\alpha$ .

In this method, it requires three underlying assumptions. Firstly,  $\hat{\theta}$  has to be approximately normally distributed. Secondly,  $\hat{\theta}$  has to be an unbiased estimator. Thirdly, bootstrap resampling method gives a good approximation to bootstrap standard deviation,  $\sigma_{bootstrap}$ . The advantage of this method is that it is simple to implement, and the result is quite accurate. It works well for estimating the sample median. However, if the parameter of interest has some constraints, such as the parameter  $\theta$  has to be positive, then the confidence interval may include some unexpected value (Carpenter and Bithell, 2000).

The obvious way to get a confidence interval is the simple percentile confidence limits. Estimate the parameter of interest,  $\hat{\theta}$ , from each bootstrapping dataset. We can see that the bootstrap estimate  $\hat{\theta}^*$  can form a bootstrap distribution, and then sorting bootstrapped  $\hat{\theta}^*$  in ascending order. Finally take the central  $100(1-\alpha)\%$  of this bootstrap distribution as its  $100(1-\alpha)\%$  confidence interval (Chernick, 1999). This method is very simple, and it is quite generic. It can be applied to almost any statistic (Carpenter and Bithell, 2000). However,

Chernick (1999) stated that this method does not work well for small samples, especially for asymmetric distributions.

## 2.4 Boundary value problems

Boundary value problems (BVP) are widely applied in physics, mathematics, optimisation and control process (Ha, 2000). Boundary value problems have been defined as a set of ordinary differential equations with dependent variable or its derivatives specified at more than one point (Zill, 2009). Two-point boundary value problems are quite common. Usually, the first boundary condition is the starting value of the independent variable, and the other boundary condition is the final value of the independent variable (Ha, 2000). In order to solve the differential equations with boundary value problems, the boundary conditions must be satisfied by the solutions.

The general form of boundary value problems can be shown as follows (Ascher & Russell, 1981)

$$\dot{x} = f(t, x(t)), \quad a \leq t \leq b, \quad (2.9)$$

$$g(x(a), x(b)) = 0 \quad (2.10)$$

where  $x$ ,  $f$  and  $g$  should have  $n$  components;  $\dot{x}$  is defined as  $\frac{dx}{dt}$ . In addition,  $f$  and  $g$  can be nonlinear functions.

The feature of boundary value problems is that they usually cannot be solved analytically (Ha, 2000). The difficulties of solving it analytically may be due to inappropriate boundary conditions, irregular regions, or coefficients of variables. In these cases, alternative numerical solutions are needed. With the development of technology, the accessibility of computers is extensive. The numerical methods can be completed by machine computation with cheap cost (Powers, 2010). In addition, the boundary value problems can have one solution, or infinite solutions, or even no solutions (Zill, 2009). Due to this problem, it is necessary to provide an initial guess of desirable solution for computation (Shampine, Kierzenka & Reichelt, 2000).

The common techniques used to solve the boundary value problems are the finite difference method, and shooting method (Zill, 2009). Both methods will be discussed briefly.

The finite difference method is considered as a numerical solution. It adopts the finite difference equations to approximate the solution to differential equations. The finite difference approximation is based on Taylor series expansion.

Let us set  $h = t - a$ , centered at point  $a$ , and then the function  $x(t)$  can be expressed as (Zill, 2009)

$$x(t) = x(a) + \dot{x}(a) \frac{h}{1!} + \ddot{x}(a) \frac{h^2}{2!} + \ddot{\ddot{x}}(a) \frac{h^3}{3!} + \dots, \quad (2.11)$$

If  $h$  is small, and we can ignore the terms involving  $h^2$  and higher order, then we get the following approximations to the first derivative of function (2.11):

$$\dot{x} \approx \frac{1}{h} [x(t+h) - x(t)] \quad (2.12)$$

$$\dot{x} \approx \frac{1}{h} [x(t) - x(t-h)] \quad (2.13)$$

and also

$$\dot{x} \approx \frac{1}{2h} [x(t+h) - x(t-h)] \quad (2.14)$$

If we ignore the terms involving  $h^3$  and higher order, and then we can get the approximation to the second derivative

$$\ddot{x} \approx \frac{1}{h^2} [x(t+h) - 2x(t) + x(t-h)] \quad (2.15)$$

Based on the theory of finite difference approximation described above, let us consider a linear second order boundary value problem, shown as below

$$\ddot{x} + P(t)\dot{x} + Q(t)x = f(t), \quad x(a) = \alpha, x(b) = \beta. \quad (2.16)$$

Suppose  $t_0 (= a) < t_1 < t_2 < \dots < t_{n-1} < t_n (= b)$ ,

Let  $t_i = a + ih$ , where  $i = 1, 2, \dots, n$ ;  $h = (b - a)/n$ .

Substitute the central difference approximations (2.14) and (2.15) into equation (2.16). Then after simplifying the equation, we can get



$$\left(1 + \frac{h}{2}P(t_i)\right)x(t_{i+1}) + (-2 + h^2Q(t_i))x(t_i) + \left(1 - \frac{h}{2}P(t_i)\right)x(t_{i-1}) = h^2f(t_i) \quad (2.17)$$

Finally, the equation (2.17) provides us the numerical solution to the boundary value problems, with an approximation to the differential equation. This illustrates the process of finite difference method to solve the boundary value problems.

The other numerical technique for solving the boundary value problems is the shooting method. This method converts the boundary value problems into the initial value problem (Zill, 2009). Let us consider an example of boundary value problems:

$$\ddot{x} = f(t, x, \dot{x}), \quad x(a) = \alpha, \quad x(b) = \beta. \quad (2.18)$$

It can be reformed as initial value problem

$$\ddot{x} = f(t, x, \dot{x}), \quad x(a) = \alpha, \quad \dot{x}(a) = s \quad (2.19)$$

The value of  $s$  in equation (2.19) is unknown. We simply have an initial guess of  $s$ , and try to find the numerical approximation  $\beta^*$  for the value of  $x(b)$ . We repeat this process with different initial guesses of  $s$ , until  $\beta^*$  satisfies the given value  $x(b) = \beta$ , and also with a predetermined tolerance level. This method uses the approach of trial-and-error to get the appropriate value of  $s$ , in order to solve the boundary value problems numerically by shooting the boundary conditions,  $x(b) = \beta$  as a target.

There is an underlying assumption related to this numerical method. It is assumed that the boundary value problems should have a solution (Zill, 2009). In addition, the shooting method is a general approach to solve the boundary value problems. This method applies widely in differential equations. However, the shooting method may fail to converge in some cases. This may be due to sensitive initial conditions (Ha, 2000).

# Chapter 3

## 3. Parameter Estimation

### 3.1 Background

Kiataramkul et al. (2011) developed a model of biological systems with ordinary differential equations. The model is to describe an optimal control problem based on Pontryagin's Maximum Principle. The ultimate goal of this model is to figure out the optimal daily nutrition intake for pregnant sheep with a pre-determined desirable birth weight of lambs. The model consists of four ordinary differential equations shown as below:

$$\dot{x} = \frac{rux}{u+L} \left( 1 - \frac{x}{k_0 + \frac{ay}{y+L_0}} \right) \quad (3.1)$$

$$\dot{y} = u - by \quad (3.2)$$

$$\dot{u} = \frac{(u+L) \left[ \begin{array}{l} bL \left( k_0 + \frac{ay}{y+L_0} \right) \left\{ \left( k_0 + \frac{ay}{y+L_0} \right) - x \right\} (y+L_0)^2 \\ - (\lambda_2 + 1) \left\{ \begin{array}{l} axL_0 (bLy + u^2) \\ + bL \left( k_0 + \frac{ay}{y+L_0} \right) \left\{ \left( k_0 + \frac{ay}{y+L_0} \right) - x \right\} (y+L_0)^2 \end{array} \right\} \right]}{2L \left( k_0 + \frac{ay}{y+L_0} \right) \left\{ \left( k_0 + \frac{ay}{y+L_0} \right) - x \right\} (\lambda_2 + 1) (y+L_0)^2} \quad (3.3)$$

$$\dot{\lambda}_2 = \frac{aL_0 ux(u+L)(\lambda_2 + 1)}{L \left( k_0 + \frac{ay}{y+L_0} \right) \left\{ \left( k_0 + \frac{ay}{y+L_0} \right) - x \right\} (y+L_0)^2} + b\lambda_2 \quad (3.4)$$

where  $x(t)$  is the fetal weight in kilograms (kg) at time point  $t$ ;

$y(t)$  is the cumulative nutrition intake by the pregnant ewe in kg at time point  $t$ ;

$u(t)$  is the daily nutrition intake in  $\text{kg}\cdot\text{day}^{-1}$  at time point  $t$ ;

$\lambda_2(t)$  is an adjoint equation based on Pontryagin's Maximum Principle to achieve the condition of optimisation.

Kiataramkul et al. (2011) showed that the logistic model can fit experimental data well to describe the fetal development in the second half of pregnancy. The logistic model can be expressed in the form of an ODE as

$$\frac{dx}{dt} = rx \left( 1 - \frac{x}{k} \right) \quad (3.5)$$

where  $r (> 0)$  is the growth rate of the fetus;

$k (> 0)$  is the mythical ‘carrying capacity’.

The equation (3.5) can be solved by separating the variables, and then we can get

$$x(t) = \frac{x_0 k}{x_0 + (k - x_0)e^{-rt}}, \quad (3.6)$$

with initial condition  $x(t_0) = x_0$ ; where  $x_0$  is the initial fetal weight at the starting time point  $t_0$ .

Generally speaking, the ‘carrying capacity’ is always greater than initial fetal weight ( $k > x_0$ ). The equation (3.6) suggests that the fetal weight is asymptotically approaching to the ‘carrying capacity’ ( $k$ ) as the time goes to infinity. In reality, the time ( $t$ ) never goes to infinity, as the lamb will be born within the specific maternal period. Thus the fetal weight never reaches the ‘carrying capacity’. The ‘carrying capacity’ can be considered as the ‘mythical limit to growth’.

The physiological meaning on the rest of parameters in the model system will be discussed briefly. The equation (3.5) has been modified to apply the concept of Michaelis-Menten to describe the model systems explicitly (Kiataramkul et al., 2011).

The first modification is to multiply the term  $\frac{u}{u+L}$  to the growth rate  $r$ , and then it becomes  $\frac{ru}{u+L}$ , where  $u$  represents the daily nutrition intake. The parameter  $L$  is the Michaelis constant (Raaijmakers, 1987). In this case, the parameter  $r$  refers to the maximum per unit mass growth rate; and the parameter  $L$  refers to the value of nutrition intake at which the per unit mass growth rate is half of the maximum. Thus the Michaelis-Menten equation  $\frac{ru}{u+L}$  suggests that the growth rate is dependent on the nutrition intake. If there is no nutrition intake ( $u=0$ ), then there is no fetal growth.

The second modification is to replace the ‘carrying capacity’  $k$  with  $(k_0 + \frac{ay}{y+L_0})$ . The term  $\frac{ay}{y+L_0}$  is also in the form of a Michaelis-Menten equation. The function  $y(t)$  refers to a cumulative nutrition intake. The function  $y(t)$  in the form of ODE is shown in equation (3.2). It can be solved analytically, as  $y(t) = \int_0^t e^{-b(t-s)} u(s) ds$ . The cumulative intake would be suitably discounted according to the history (Kiataramkul et al., 2011). Thus the parameter  $b$

is the discount factor, which indicates that the cumulative intake  $y(t)$  is more influenced by the recent past than by the distant past.

The Michaelis-Menten factor  $\frac{y}{y+L_0}$  is associated with parameter  $a$ . The parameter  $a$  refers to a factor which influencing the ‘carrying capacity’ on historical intake. To some extent, it suggests that the history of nutrition intake may have an effect on ‘mythical limit to growth’ of fetus. The parameter  $L_0$  refers to the value of cumulative intake at which the influencing factor is half of its maximum.

In summary, the ‘carrying capacity’  $k$  has two components. The parameter  $k_0$  represents the basic component; and Michaelis-Menten term  $\frac{ay}{y+L_0}$  represents the addition component which depends on the historical intake.

### 3.2 Methodology

At the stage of parameter estimation, we just need to focus on the equations (3.1) and (3.2) to determine the unknown parameters associated with this dynamic system. Those two equations are sufficient to estimate the unknown parameters, with initial conditions  $x(t_0) = x_0$ , and  $y(t_0) = 0$ ; where  $t_0$  is the starting time point of the second half of pregnancy for sheep. In addition, we assume the function  $u(t)$  is known. In this system, the seven unknown parameters are  $r, L, a, k_0, L_0, b$ , and  $x_0$ . The constraint on those unknown parameters is that all should be positive.

We use least-squares to estimate those unknown parameters. The error sum of squares,  $\sum_{i=1}^n [x_i - f(x_i; p)]^2$ , is the only criterion to assess the model fitting with experimental data. (Here  $p$  stands for the parameter vector). The smaller the value of sum of squares of errors (SSE), the better the model fits.

We use the computer software MATLAB to conduct all the computation and analysis. The built-in function ‘fminsearch’ is used to determine the optimal value of those unknown parameters with the best fit. The built-in function ‘fminsearch’ adopts the Nelder-Mead method. This technique minimises an objective function in a multi-dimensional space. It is a

widely used direct search method for an unconstrained optimisation process (MathWork, 2012). In this case, the least-squares SSE is used as our objective function. In addition, the initial guesses of each parameter need to be provided. The function ‘fminsearch’ will find the optimal value of each parameter to achieve the best model fit.

As discussed previously, the unknown parameters should be positive. However, the function ‘fminsearch’ uses an unconstrained optimisation process. We fix this conflict by conducting an appropriate transformation of those parameters. The exponential transformation ensures that the entire term after transformation always stays positive. In this case, we transform all the unknown parameters except  $x_0$ . The parameter  $x_0$  represents the initial fetal weight at the start of the second half of pregnancy ( $t_0$ ). The experimental data of fetal weight, at close to the time point  $t_0$ , are always positive. So we can ensure that  $x_0$  always stays positive without any form of transformation.

The exponential transformation can be expressed generally as

$$p = \exp(P).$$

where  $p$  = original parameter, and  $P$  = new parameter.

In this way, the new parameter,  $P$ , is unconstrained. It can take on a real value. After exponential transformation, the entire term  $\exp(P)$  is restricted to be positive values. This means that if new parameter ( $P$ ) is normal, then the original parameter ( $p$ ) will be log-normal. That means there is a difference in interpretation of the two model parameterisations.

Those new parameters are denoted as  $R$ ,  $Lt$ ,  $A$ ,  $k_1$ ,  $Lt_0$ , and  $B$ , corresponding to the original parameters  $r$ ,  $L$ ,  $a$ ,  $k_0$ ,  $L_0$  and  $b$  respectively. The equations (3.1) and (3.2) are modified to accommodate the property of unconstrained parameters with transformation, shown in the equations (3.7) and (3.8).

$$\dot{x} = \frac{e^{Rux}}{u+e^{Lt}} \left( 1 - \frac{x}{e^{k_1 + \frac{e^{Ay}}{y+e^{Lt_0}}}} \right) \quad (3.7)$$

$$\dot{y} = u - e^B y \quad (3.8)$$

In the next chapter, firstly we will show the results of parameter estimation with the equations (3.7) and (3.8) as our full model, and also with initial conditions  $x(t_0) = x_0$ , and  $y(t_0) = 0$ . Secondly, we will also discuss some results of parameter estimation on the modified models.

# Chapter 4

## 4. Results and Discussion

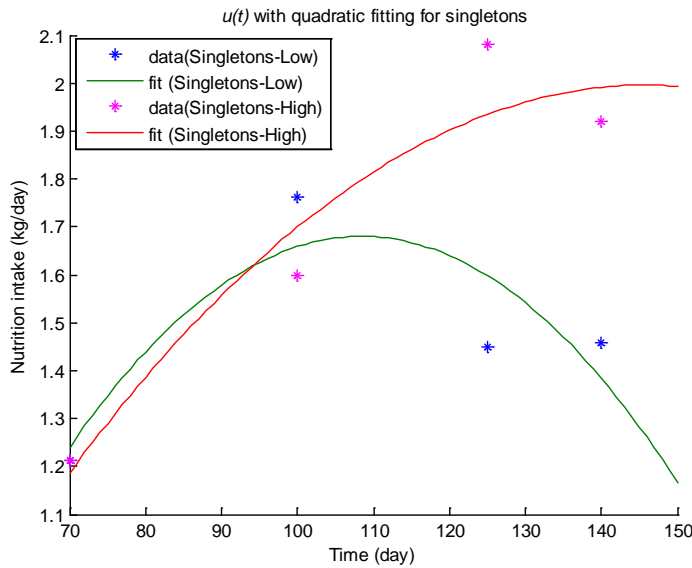
In this chapter, we are going to present the results of parameter estimation in the full model and alternative models. We will also introduce and discuss the alternative models with details. The results of each model will be discussed to assess the reliability of those estimated parameters.

### 4.1 Grouped data – ordinary least squares

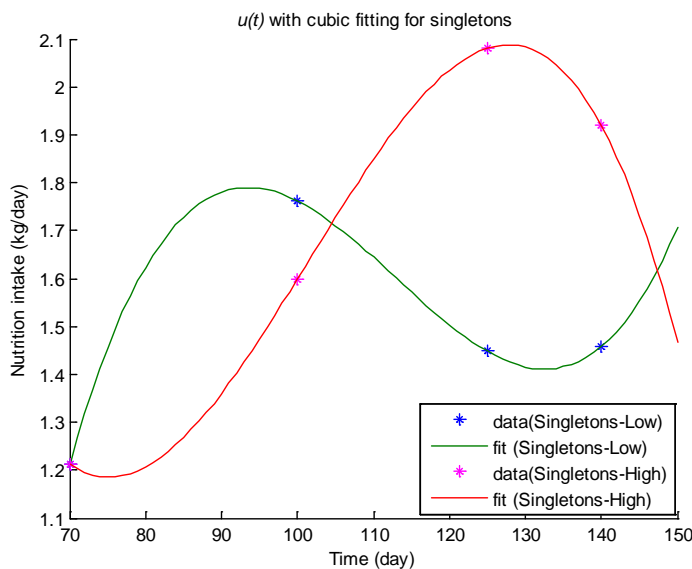
The available datasets from Rattray's studies are associated with two groups of nutritional schemes (1974a, 1974b), which are low and high levels. Initially, we continued the work on parameter estimation based on the full model developed by Kiataramkul et al. (2011). We set the starting time point  $t_0$  as day 70, based on the experiments in Rattray et al. (1974a, 1974b). After day 70 of gestation, the pregnant sheep were assigned into two different levels of nutrition. Day 70 is roughly close to the beginning of the second half of their pregnancy. Thus, day 70 has been considered as the starting time point for this study.

The available information related to the nutrition intake for those pregnant sheep is very limited. The data points of nutrition intake are only available at approximately 70, 100, 125 and 140 days of gestation (Rattray, 1974a).

The function of daily nutrition intake,  $u(t)$ , for each high and low level of nutrition can be modelled with a quadratic fit or a cubic fit separately. The fits are shown in plots (figure 4.1 – 4.4) of those models. We will discuss the suitability of the functions of daily nutrition intake for singletons and twins.



**Figure 4. 1:**  $u(t)$  function with quadratic fitting for singletons



**Figure 4. 2:**  $u(t)$  function with cubic fitting for singletons

For the quadratic fitting with singletons (figure 4.1), we notice that low nutrition group has mean slightly higher than that of high level for period from day 70 to day 95 of gestation. After day 95 of gestation, it is quite obvious that the fitting for high nutrition is higher than that of low level. It seems the function of daily nutrition intake with quadratic fitting for singleton is reasonable.

However, for the cubic fitting with singletons (figure 4.2), we notice that from day 70 to day 105 of gestation, low nutrition group is obviously higher than that of high level. For the rest of the period, the fitting for high level of nutrition is much higher than that of low level. In



this case, it does not really make sense for the period from day 70 to day105 of gestation, since the fitting for the high nutritional group is definitely lower than that of low nutritional group. This may due to the overfitting with limited nutritional data points available by using the cubic model. Hence we should not adopt the function of daily nutrition intake with cubic fitting for singletons.

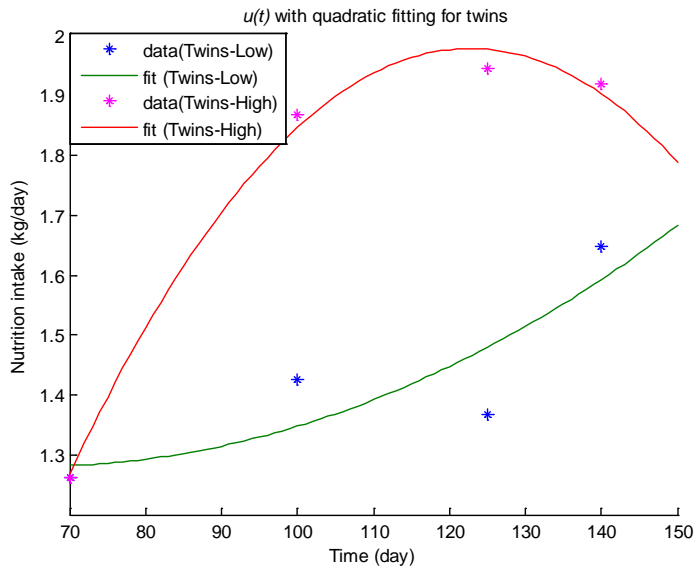


Figure 4. 3:  $u(t)$  function with quadratic fitting for twins

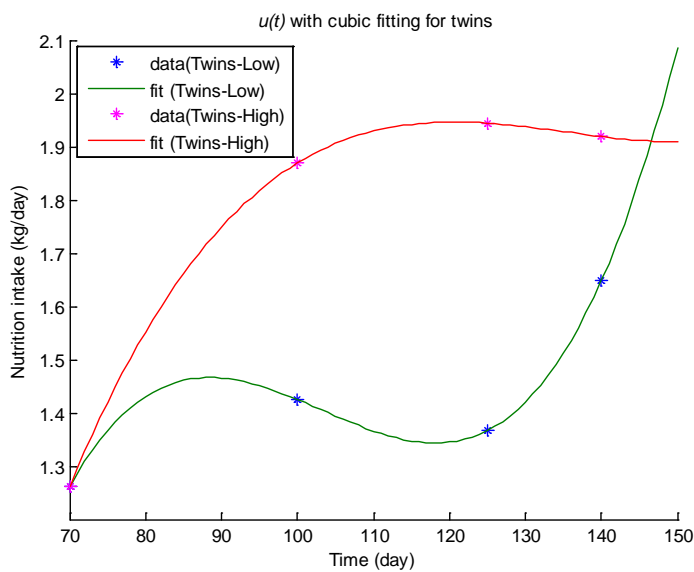


Figure 4. 4:  $u(t)$  function with cubic fitting for twins

The figure 4.3 and 4.4 show the function of daily nutrition intake with quadratic and cubic fitting for twins respectively. Both of these fits seem to be reasonable, since the fitting for high level of nutrition intake is always higher than that of low level of nutrition intake at most of the time. In this case, the function of daily nutrition intake with both of quadratic and cubic fitting will be considered to estimate the unknown parameters. We can justify the suitability of the fitting base on the results of parameter estimation in later sections.

The process flow chart is used to illustrate the process of parameter estimation for the full model, shown in equation (3.1) and (3.2).

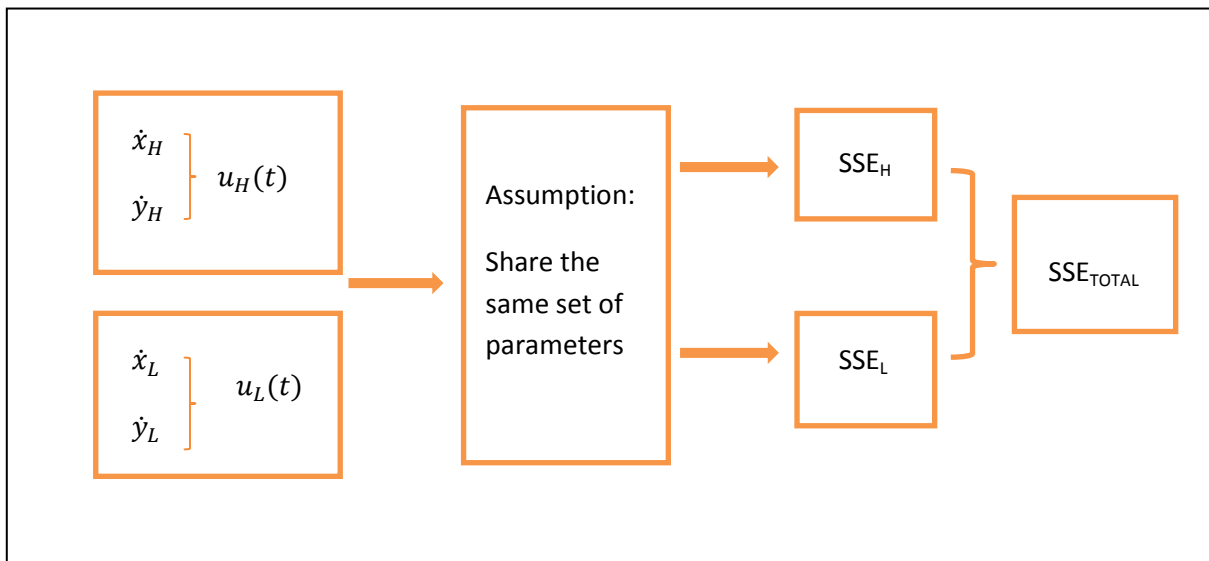


Figure 4. 5: The process flow chart for parameter estimation of full model

The process flow chart shows that the daily nutrition intake function should be modelled for low and high level separately. We should solve those four ODEs simultaneously with corresponding low and high level of nutrition intake. It is important to assume that those four ODEs share the same set of parameters. Using the criteria of ordinary least squares fit, we can get the minimum value of SSE for high and low level of nutrition separately. Finally, we sum them up to get the overall SSE value to assess the entire model fitting.

### 4.1.1 Full model

The equations (3.7) and (3.8) show the expression of the full model with appropriate transformation of parameters. The various initial guesses of each parameter are provided to estimate the final optimal value of each parameter.

#### 4.1.1.1 Singletons

The quadratic fitting is used to model the function of daily nutrition intake for singletons. Table 4.1 shows the final optimal value of each parameter based on various initial guesses provided.

Par	(1)		(2)		(3)		(4)		(5)		(6)		(7)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.5	-2.5854	-2.3	-2.5775	-2.0	-2.5681	-2.3	-2.5681	-2.3	-2.5681	-2.4	-2.5681	-2.6	-2.5681
$Lt$	-2.0	-0.9612	-1.5	-0.9118	-0.5	-0.8456	-1.8	-0.8456	-0.8	-0.8456	-1.3	-0.8459	-2.0	-0.8456
$A$	0.5	0.5709	0.2	0.1326	0.8	-11.113	0.5	-11.7502	0.3	-1.6364	0.9	-2.3302	2.0	-14.595
$k_1$	2.0	2.2551	1.5	2.3035	1.2	2.3498	2.3	2.3498	2.1	2.3311	1.8	2.3406	0.8	2.3498
$Lt_0$	0.2	0.3876	0.5	0.5611	0.3	-0.8055	0.3	6.7732	-0.3	-11.145	-0.5	-2.4653	-0.1	-0.7220
$B$	0.2	0.3032	0.5	0.6591	0.3	-0.1710	0.3	0.0485	-0.3	0.4298	-0.5	-1.2878	-0.1	-0.0595
$X_0$	0.2	0.1888	0.23	0.1893	0.18	0.1907	0.18	0.1907	0.22	0.1907	0.15	0.1907	0.2	0.1907
SSE		1.5950		1.5908		1.5860		1.5860		1.5860		1.5861		1.5860

Table 4. 1: Parameter estimation for full model of singletons

The global minimum SSE was not always reached. It depended on the starting values. In this case, the global minimum of SSE seems to be reached in four out of seven different sets of initial guesses. The lowest SSE is 1.5860 in this case. The sets of initial guesses (3), (4), (5) and (7) achieve the global minimum of SSE. The results of sets (3), (4) and (7) show that the final optimal value of parameter  $R$ ,  $Lt$  and  $x_0$  are always fixed at -2.5681, -0.8456 and 0.1907 respectively. Hence those parameters can be considered as identifiable. However, the final values of the other parameters show large variation.

From those sets which reached the global minimum of SSE, we notice that the parameter of  $A$  or  $Lt_0$  have quite large negative final optimal values. If we transform parameter  $A$  or  $Lt_0$  into the original scale of  $a$  and  $L_0$ , their values are approximately close to zero.

If the value of original parameter  $a$  is close to zero, then the Michaelis-Menten term,  $\frac{ay}{y+L_0}$ , will disappear. In this case, the parameter  $b$  and  $L_0$  will be unrelated to the model. It reduces to the simplified model  $\dot{x} = \frac{rux}{u+L} (1 - \frac{x}{k_0})$ .

If the value of original parameter  $L_0$  is close to zero, the function of  $y$  will disappear. In this case, the parameter  $b$  is unrelated. The whole denominator becomes  $(k_0+a)$ , and it suggests that fetal development does not depend on the cumulative intake. The denominator  $(k_0+a)$  turns to be the ‘carrying capacity’ of  $k$ . It is still equivalent to the simplified model.

In conclusion, when fitting the full model with Rattray’s singletons data, it suggests that dropping some parameters is necessary to improve the fitting for the model. It is necessary to simplify the full model to achieve adequate fitting.

#### 4.1.1.2 Twins (function of $u(t)$ with quadratic fitting)

The quadratic fitting is used to model the function of daily nutrition intake for twins in this section. Five different sets of initial guesses are provided to evaluate final optimal value of each parameter. Results are shown in table 4.2.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final	(5) Initial	Final
$R$	-2.5	-2.5813	-2.4	-2.5813	-2.3	-2.5813	-2.4	-2.5813	-2.3	-2.5813
$Lt$	-2.0	-175.0004	-2.0	-151.0593	-1.8	-18.9212	-1.7	-75.5282	-1.3	-24.3360
$A$	0.5	-28.6264	-2.0	-26.3898	1.2	-15.0530	-1.5	-7.8879	-1.3	2.5741
$k_1$	2.0	2.5763	2.3	2.5763	2.1	2.5763	2.5	2.5763	2.3	-3.5250
$Lt_0$	0.2	-2.3285	-0.1	6.7920	-0.1	-0.5831	-0.5	28.2331	-0.3	-14.6114
$B$	0.2	-0.3797	-0.1	-0.4806	-0.1	-0.6986	-0.5	-24.6529	0.3	-138.4681
$X_0$	0.3	0.2470	0.3	0.2470	0.3	0.2470	0.25	0.2470	0.32	0.2470
SSE		12.0593		12.0593		12.0593		12.0593		12.0593

Table 4. 2: Parameter estimation for full model of twins (quadratic fitting of nutritional function)

From the five different sets of initial guesses provided, we notice that the same value of SSE (12.0593) has been achieved. This suggests that global minimum of SSE value is achieved with different initial guesses.

Examining the output carefully, we notice that the parameters  $R$  and  $x_0$  always achieve the same final optimal values, and hence those parameters are identifiable. In addition, the parameters  $Lt_0$  and  $A$  show large variation, however, they all show large negative values. In this case, the parameter  $L$  and  $a$  in original scale will be very close to zero.

When the parameter  $a$  is zero, the Michaelis-Menten term  $\frac{ay}{y+L_0}$  will disappear. It makes the parameter  $b$  and  $L_0$  unrelated to the model, and changes the model to one dimension. In addition, when the parameter  $L$  is zero, the nutrition intake function  $u(t)$ , will be unity. In this

way, the model becomes simple logistic model in original scale, i.e.  $\dot{x} = rx \left(1 - \frac{x}{k_0}\right)$ . This suggests that the fetal development is unrelated to the daily nutrition intake based on this model. This is not physiologically reasonable, so we are then forced to seek better models that show a relation to nutrition. The alternative models should have a simpler model structure than that of the full model.

#### 4.1.1.3 Twins (function of $u(t)$ with cubic fitting)

The cubic fitting is used to model the function of daily nutrition intake for twins in this section. Various sets of initial guesses are provided to evaluate final optimal value of each parameter. Results are shown in table 4.3.

Par	(1)		(2)		(3)		(4)		(5)		(6)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.3	-2.6773	-2.5	-2.6776	-2.4	-2.6785	-2.3	-2.6762	-2.3	-2.6752	-2.4	-2.6778
$Lt$	-1.8	-36.2340	-2.0	-11.1197	-2.1	-8.0677	-1.5	-6.9051	0.5	-4.2906	-1.7	-6.2956
$A$	1.2	2.8387	0.5	2.1317	0.6	2.2170	1.5	1.9898	0.5	2.5379	1.0	2.2817
$k_1$	2.1	-3.3153	2.0	2.2217	2.1	2.1261	2.2	2.3893	2.2	1.5061	2.2	2.0337
$Lt_0$	-0.1	-1.8305	0.2	-0.2035	0.15	0.2237	-0.1	0.0886	-0.1	0.5112	-0.2	-0.4615
$B$	-0.1	0.0012	0.2	-0.3511	0.15	-0.9816	-0.1	-0.0248	-0.1	-1.9886	-0.2	-0.4744
$X_0$	0.3	0.3036	0.3	0.3043	0.31	0.3049	0.3	0.3043	0.3	0.3105	0.28	0.3051
SSE		6.1383		6.1713		6.1758		6.1869		6.2171		6.1648

Table 4. 3: Parameter estimation for full model of twins (cubic fitting of nutritional function)

The output above shows that final values of SSE are all different based on those six different sets of initial guesses. It suggests that we cannot guarantee to get global minimum of SSE based on those initial guesses. The output indicates that final optimal value of each parameter is heavily dependent on the initial guesses provided. If we change the initial guess slightly for the parameter, it leads to the final optimal values being changed accordingly.

In this case, we cannot get a unique value of each parameter based on different sets of initial guesses. This suggests that our model is non-identifiable. We cannot actually estimate a reliable value of any parameter.

### 4.1.2 Cut-down model

The non-identifiability of full model for twins with cubic fitting of nutrition intake may be due to the complicated model structure. Raue et al. (2011) suggested simplifying the model structure to resolve the problem. We can assume that there is no effect of cumulative nutrition intake on the fetal development. We can ignore the equation (3.2), the ODE function of cumulative nutrition intake from the full model. In this way, the Michaelis-Menten term,  $\frac{ay}{y+L_0}$ , would be unrelated to the equation (3.1). Hence the full model has been reduced to a one-dimensional model. The ‘cut-down’ model shows in equation (4.1).

$$\dot{x} = \frac{rux}{u+L} \left(1 - \frac{x}{k_0}\right) \quad (4.1)$$

The parameter  $k_0$  in equation (4.1) represents the whole ‘carrying capacity’ in the cut-down model. The simplified model reduces the number of unknown parameters. It may help us to determine the value of each parameter uniquely.

There are only four unknown parameters in the cut-down model. These unknown parameters are  $r$ ,  $L$ ,  $k_0$  and initial fetal weight  $x_0$ . As discussed in previous chapter, we have to transform the parameters  $r$ ,  $L$  and  $k_0$  to address the positive constraints on those parameters for estimation. The cut-down model with appropriate transformation is shown in equation (4.2)

$$\dot{x} = \frac{e^R ux}{u+e^{Lt}} \left(1 - \frac{x}{e^{k_1}}\right) \quad (4.2)$$

The parameter estimation for the cut-down model is based on the equation (4.2). The following sections show the results of parameter estimation for singletons and twins based on the cut-down model.

#### 4.1.2.1 Singletons

The quadratic fitting is used to model the function of daily nutrition intake for singletons. Table 4.4 shows the final optimal value of each parameter based on four different sets of initial guesses provided.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.4	-2.5681	-2.8	-2.5681	-2.2	-2.5681	-1.5	-2.5681
$Lt$	-2.3	-0.8458	0.5	-0.8456	-1.5	-0.8456	-0.5	-0.8456
$k_1$	2.2	2.3498	1.8	2.3498	2.0	2.3498	1.5	2.3498
$X_0$	0.14	0.1907	0.18	0.1907	0.17	0.1907	0.23	0.1907
SSE		1.5860		1.5860		1.5860		1.5860

**Table 4. 4:** Parameter estimation for cut-down model of singletons

The table 4.4 shows that the final optimal values are identical based on those four different sets of initial guesses. In this case, we are quite confident to conclude that we get the best fitting for the model, and also estimate the unique value of each parameter. Thus, this model is fully identifiable.

The final optimal value of parameter  $R$ ,  $Lt$ ,  $k_1$  and  $x_0$  are -2.5681, -0.8458, 2.3498 and 0.1907 respectively. Those optimal values are consistent with the final value from the full model of singletons (section 4.1.1.1). This verifies that the cut-down model works well for singletons.

#### 4.1.2.2 Twins (function of $u(t)$ with quadratic fitting)

The quadratic fitting is used to model the function of daily nutrition intake for twins in this section. Three different sets of initial guesses are provided to evaluate final optimal value of each parameter. Results are shown in table 4.5.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final
$R$	-2.4	-2.5813	-2.2	-2.5813	-2.0	-2.5813
$Lt$	-2.3	-34.0443	-1.5	-34.7323	0.5	-34.8373
$k_1$	2.2	2.5763	2.0	2.5763	1.5	2.5763
$X_0$	0.14	0.2470	0.23	0.2470	0.3	0.2470
SSE		12.0594		12.0594		12.0594

**Table 4. 5:** Parameter estimation for cut-down model of twins (quadratic fitting of nutritional function)

The table 4.5 shows that the final SSE value is identical for those three different sets of initial guesses. The final values of the parameter  $Lt$  have some variation. However, they show really large negative values. If the final values of the parameter  $Lt$  are expressed in the original scale, they would all be effectively zero. Thus, the variation would be gone, in terms of expressing in the original scale. The rest of the parameters show that their final values are quite stable. This suggests that the model is identifiable.

In addition, since the parameter  $L$  in the original scale shows zero, this indicates that the function of daily nutrition intake would disappear. This suggests that there is no significant nutrition effect on fetal growth in this model.

#### 4.1.2.3 Twins (function of $u(t)$ with cubic fitting)

The cubic fitting is used to model the function of daily nutrition intake for twins in this section. Four different sets of initial guesses are provided to evaluate final optimal value of each parameter. Results are shown in table 4.6.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.4	-2.6403	-2.2	-2.6403	-2.0	-2.6403	-2.8	-2.6403
$Lt$	-2.3	-2.3540	-1.5	-2.3540	0.5	-2.3540	-0.2	-2.3540
$k_1$	2.2	2.7361	2.0	2.7361	1.5	2.7361	2.1	2.7361
$X_0$	0.14	0.3287	0.23	0.3287	0.3	0.3287	0.3	0.3287
SSE		6.9175		6.9175		6.9175		6.9175

Table 4. 6: Parameter estimation for cut-down model of twins (cubic fitting of nutritional function)

The table 4.6 shows that the final value of each parameter and the final value of SSE are identical based on different sets of initial guesses used. The final optimal value of parameter  $R$ ,  $Lt$ ,  $k_1$  and  $x_0$  are -2.6403, -2.3540, 2.7361 and 0.3287 respectively. The final value of each parameter is unique, which suggests that the model is identifiable.

In this model, the final value of SSE is 6.9175, which is much smaller than that of quadratic fitting with the function of daily nutrition intake (SSE = 12.0594) for twins. This suggests that the model with cubic fitting of  $u(t)$  function fits the data better. Recall that the cubic model interpolated the actual  $u(t)$  data means, whereas the quadratic model was a rougher approximation. So this illustrates that the more and better data we have on nutrition intake, the more likely we are to be able to find a relationship between nutrition intake and birth weight.

In addition, the parameter  $Lt$  is -2.3540, which would be 0.0950 in original scale of parameter  $L$ . The parameter  $L$  is positive, which indicates that fetal growth still depends on the nutrition intake in this model.

In other words, the cut-down model for twins with cubic fitting of nutrition intake function fits the data better than that of quadratic fitting. The model with cubic fitting indicates that the model parameters are identifiable, and there is the nutritional effect on fetal development.



The further analysis is required on this model to determine the optimal daily nutrition intake for sheep.

### 4.1.3 Hybrid model

In this section, we make some modifications from both full model and cut-down model which is named as the ‘hybrid model’. This is because it is somewhat of a compromise between the cut-down model and the full model proposed in Kiataramkul et al. (2011).

$$\dot{x} = \frac{rux}{u+L} \left(1 - \frac{x}{\frac{au}{u+L_0}}\right) \quad (4.3)$$

The equation (4.3) shows that hybrid model is simplified from the full model and is shown in one dimension. The cumulative intake function,  $y(t)$ , is no longer required in hybrid model. This is because the full model is too complicated and leads to either non-identifiability of model parameters, or forcing the parameters  $a$  or  $L_0$  to be zero. Dropping the cumulative intake function  $y(t)$  may improve the model identifiability.

In the cut-down model, the parameter  $k_0$  is simply representing the ‘carrying capacity’, which suggests that ‘carrying capacity’ does not dependent on other factors. However, in the hybrid model we assume that there is a nutritional effect on ‘carrying capacity’. We use a Michaelis-Menten term  $\frac{au}{u+L_0}$  to represent the ‘carrying capacity’ effect. In this way, the cut-down model becomes the special case of the hybrid model, when  $L_0$  equals zero.

The equation (4.4) shows the expression of the hybrid model with appropriate transformation of parameters. Various initial guesses of each parameter are provided to estimate final optimal value of each parameter. The detailed results on singletons and twins are shown below.

$$\dot{x} = \frac{e^R ux}{u+e^{Lt}} \left(1 - \frac{x}{\frac{e^A u}{u+e^{Lt_0}}}\right) \quad (4.4)$$

#### 4.1.3.1 Singletons

The quadratic fitting is used to model the function of daily nutrition intake for singletons. Table 4.7 shows the final optimal value of each parameter based on the seven different sets of initial guesses provided.

Par	(1)		(2)		(3)		(4)		(5)		(6)		(7)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.5	-2.7335	-2.6	-2.5681	-2.7	-2.6253	-2.4	-2.6690	-2.3	-2.5681	-2.3	-2.5742	-2.6	-1.5681
$Lt$	0.5	-2.2961	0.2	-0.8456	0.3	-1.2821	0.1	-1.6376	0.1	-0.8456	-0.1	-0.8817	-0.2	-0.8456
$A$	2.2	2.7515	2.5	2.3498	2.4	2.4763	2.5	2.5717	2.5	2.3498	2.5	2.3601	2.3	2.3498
$Lt_0$	0.2	-0.0724	0.1	-31.0332	0.3	-1.2024	0.1	-0.6691	0.1	-31.624	0.3	-3.8296	0.5	-30.747
$X_0$	0.23	0.1911	0.22	0.1907	0.21	0.1837	0.22	0.1843	0.22	0.1907	0.21	0.1902	0.2	0.1907
SSE		1.7849		1.5860		1.6349		1.6833		1.5860		1.5884		1.5860

Table 4.7: Parameter estimation for hybrid model of singletons

The table 4.7 shows that there are three sets of initial guesses, the sets (2), (5) and (7), which achieved the global minimum value of SSE at 1.5860 for the optimal estimation of final values. The final optimal values of parameter  $Lt_0$  showed slightly variation, those values are all around -31. Expressing the final value of parameter  $Lt_0$  in original scale, it would be very close to zero. The parameters of this model can be considered as identifiable, since all the parameters can be estimated uniquely in original scale.

Let us consider this model in original scale, which shows in equation (4.3). The parameter  $L_0$  has been determined as zero based on experimental data for singletons. In this way, the daily nutrition intake function of  $u(t)$  would be unity. This leads to parameter  $a$  representing the whole ‘carrying capacity’. Thus, this hybrid model is equivalent to the cut-down model. It also verifies that the cut-down model fits experimental data the best for singletons.

#### 4.1.3.2 Twins (function of $u(t)$ with quadratic fitting)

Again, the quadratic fitting is used to model the function of daily nutrition intake for twins in this section. The final optimal value of each parameter based on the four different sets of initial guesses provided.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.6	-2.5813	-2.5	-2.5813	-2.3	2.5813	-2.2	-2.5813
$Lt$	-0.2	-35.1555	-2.0	-33.7157	-1.1	-540.7616	-0.7	-36.0109
$A$	2.3	2.5763	2.2	2.5763	2.5	2.5763	1.8	2.5763
$Lt_0$	0.5	-45.3756	-0.2	-56.4319	0.1	-34.6158	-1.5	-192.4345
$X_0$	0.3	0.2470	0.28	0.2470	0.33	0.2470	0.27	0.2470
SSE		12.0594		12.0594		12.0594		12.0594

**Table 4. 8:** Parameter estimation for hybrid model of twins (quadratic fitting of nutritional function)

The final values of SSE are identical at 12.0594, which suggest that the model is suitable for parameter estimation. The parameters  $Lt$  and  $Lt_0$  are both shown large negative values, and also associated with some variation. It indicates that the parameters  $L$  and  $L_0$  would be both very close to zero in original scale. This results in the function  $u(t)$  will be unity, and suggests that there is no nutrition effects in this model.

#### 4.1.3.3 Twins (function of $u(t)$ with cubic fitting)

The cubic fitting is used to model the function of daily nutrition intake for twins. Table 4.9 shows the final optimal value of each parameter based on the four different sets of initial guesses provided.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.6	-2.6758	-2.5	-2.6758	-2.3	-2.6758	-2.2	-2.6758
$Lt$	-0.2	-32.3958	-2.0	-33.3321	-1.1	-59.9554	-0.7	-33.0983
$A$	2.3	2.8385	2.2	2.8385	2.5	2.8385	1.8	2.8385
$Lt_0$	0.5	-1.8342	-0.5	-1.8342	0.1	-1.8342	-1.5	-1.8342
$X_0$	0.3	0.3022	0.28	0.3022	0.33	0.3022	0.27	0.3022
SSE		6.1307		6.1307		6.1307		6.1307

**Table 4. 9:** Parameter estimation for hybrid model of twins (cubic fitting of nutritional function)

The table 4.9 shows the four different sets of initial guesses lead to the same final value of SSE at 6.1307. This suggests that the global minimum of SSE is reached, to provide the best fitting of the model with experimental data. The final values of parameter  $Lt$  in those four different sets of estimations are all large negative values. It indicates that parameter  $L$  in original scale is close to zero. Thus, all those parameters in the model are identifiable in the original scale.

As  $L=0$ , the function of daily nutrition intake associated with the growth rate  $r$  would be eliminated. Then the model would be simplified as equation (4.5) in the original scale.

$$\dot{x} = rx\left(1 - \frac{x}{\frac{au}{u+L_0}}\right) \quad (4.5)$$

The equation (4.5) shows that the ‘carrying capacity’ still has the nutritional effects. We can do further analysis on this model to determine the optimal daily nutrition intake in a later chapter.

Comparing quadratic fitting with cubic fitting of nutrition intake function in this hybrid model for twins, the value of SSE for cubic fitting is 6.1307 which is smaller than that of quadratic fitting (SSE = 12.0594). This indicates that the cubic fitting of the nutrition intake in this hybrid model shows the better fitting to experimental data.

#### 4.1.4 Summary

As discussed in previous sections, we can conclude that the best candidate model for singletons is the cut-down model. The results suggest that the parameter  $L_0$  can be forced to zero for the rest of the proposed models. This verifies that the cut-down model seems to be the most appropriate model for singletons. The biological interpretation is that, for singletons, the growth rate depends on the daily nutrition, but the final ‘mythical carrying capacity’ does not.

The appropriate candidate models for twins are the cut-down model and hybrid model, and both of the models are associated with cubic fitting for the nutrition intake data. The results of parameter estimation show that only those two models are identifiable with our experimental data. The final values of SSE are 6.9175 and 6.1307 for cut-down model and hybrid model respectively. This suggests that the hybrid model for twins has a slightly better model fitting than that of the cut-down model. However, those two models have different model structures. The cut-down model has the same biological interpretation as for singletons, but with different parameters. The hybrid model, which fits better, has the biological interpretation that the growth rate  $r$  does not depend on daily nutrition but that for twins the ‘mythical carrying capacity’ is constrained. For example, the total weight of twins cannot grow as big as the total weight of two singletons could.

At the current stage, we are unsure about which model for twins will give us the optimal solution of daily nutrition intake for pregnant sheep. Hence further analysis will be conducted on both of those two candidate models.

## 4.2 Model diagnostics – ordinary least-squares

In this section, we will examine the fitting for those candidate models. The model diagnostics primarily focus on examining residuals. Checking the pattern of residuals carefully can tell us whether the model assumptions are reasonable and our choice of model is appropriate.

Residuals can be considered as elements of variation unexplained by the fitted model. Generally speaking, we expect the residuals to be normally and independently distributed, and also with a mean of zero and some constant variance. Violation of those assumptions usually indicates that the residuals contain some structure that is not accounted for in the fitted model. Then improvement of model fitting is required.

The graphic method is an excellent way to examine residuals. Generally, the common diagnostic tools include the plot of residuals versus fitted values and Quantile-Quantile plot (QQ plot).

Ideally, the residuals versus fitted values plot should show the residuals spread randomly around zero, regardless of the size of the fitted value. However, it is quite common to see that residual values increase as the size of the fitted value increases. The residual cloud looks like a ‘funnel’ shaped pattern with the larger end toward larger fitted values. This pattern suggests that the model has non-constant variance. The constant variance is one of the important assumptions of the ordinary least-squares method. If this assumption is violated, the results based on ordinary least-squares are not efficient.

A QQ plot is an excellent way to see whether the data deviates from normal distribution. It is similar to a probability plot. It shows the quantiles of the residuals against the quantiles of theoretical normal distribution. There is a complementary 45-degree reference line. The greater the departure from this reference line, the greater the evidence for the conclusion that the residuals are far from a normal distribution.

In the following sections, we are going to examine the diagnostic plots for each candidate model to assess the appropriateness of the model fitting.

### 4.2.1 Singletons – cut-down model

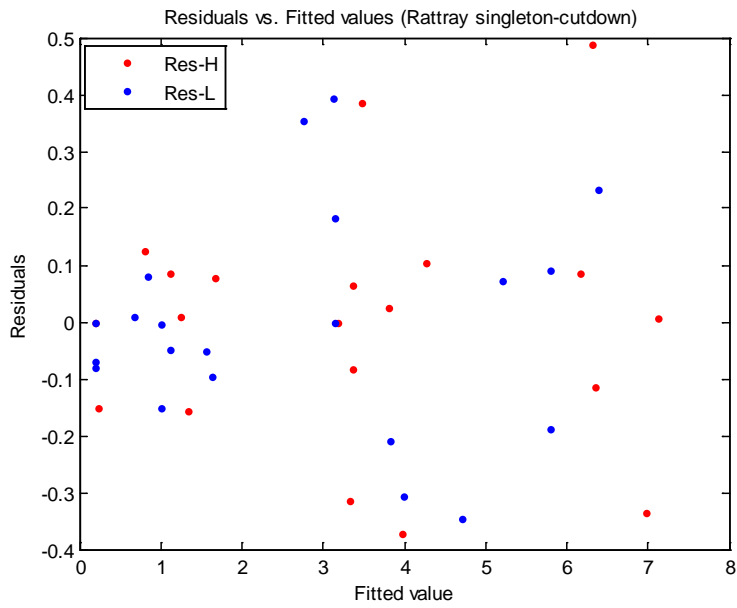


Figure 4. 6: Residuals vs. Fitted values (singletons – cut-down model)

The Figure 4.6 shows the residuals versus fitted values plot for the cut-down model of singletons. It seems that the residuals spread wider as the size of fitted value increases, especially for fitted values larger than 3. This indicates the variance is non-constant in this model. It also suggests that the ordinary least-squares fit seems to be inappropriate as the assumption of constant variance is violated.

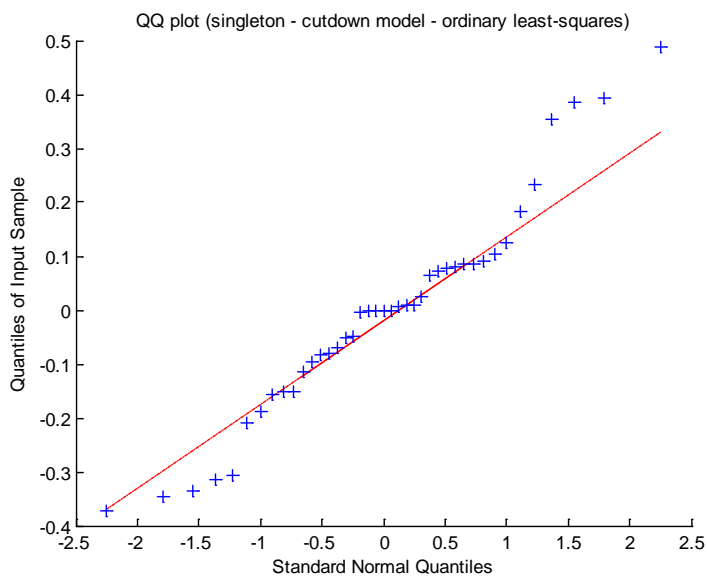


Figure 4. 7: QQ plot (singletons – cut-down model)

The figure 4.7 shows the QQ plot for the cut-down model of singletons. It shows that the middle points seem to be quite close to the reference line. However, some points near two ends deviate a lot from the reference line. This challenges the assumption of normality.

The diagnostic plots in this section suggest that the ordinary least-squares fit for the cut-down of singletons seems to be inappropriate. The assumptions of constant variance and normal distribution seem to be violated. The model fitting based on ordinary least-squares is inadequate. As a consequence, the estimated values of parameters are not able to be trusted.

#### 4.2.2 Twins – cut-down model

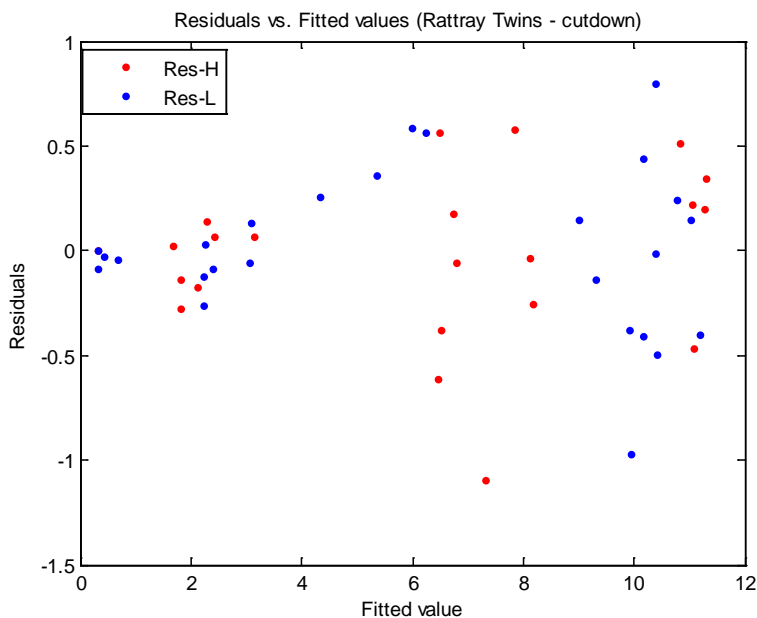
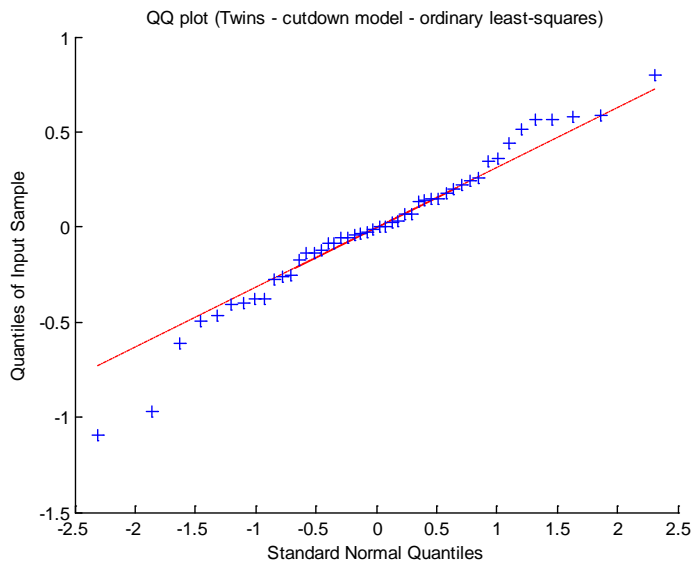


Figure 4. 8: Residuals vs. Fitted values (twins – cut-down model)

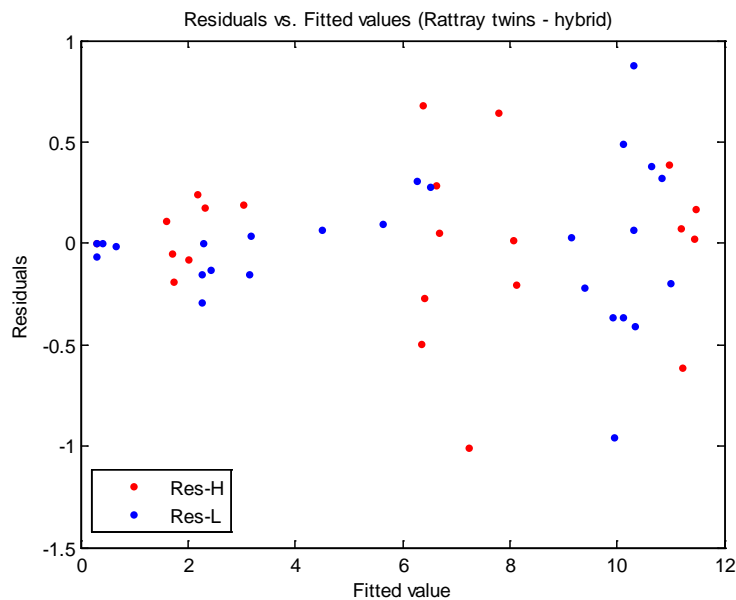
The figure 4.8 shows the plot of residuals versus fitted values for the cut-down model of twins. It shows obviously that the variance increases as the size of the fitted value increases. The residual cloud forms a ‘funnel’ shaped pattern with the larger end toward the larger fitted values. This pattern illustrates clearly there is non-constant variance in this model. This suggests that the ordinary least-squares is inadequate.



**Figure 4. 9: QQ plot (twins – cut-down model)**

The figure 4.9 shows the QQ plot for cut-down model of twins. It shows that the majority of the points lie close to the reference line. Just a few points at two ends show some deviation from the reference line. It indicates that the assumption of normality may still hold.

### 4.2.3 Twins – hybrid model

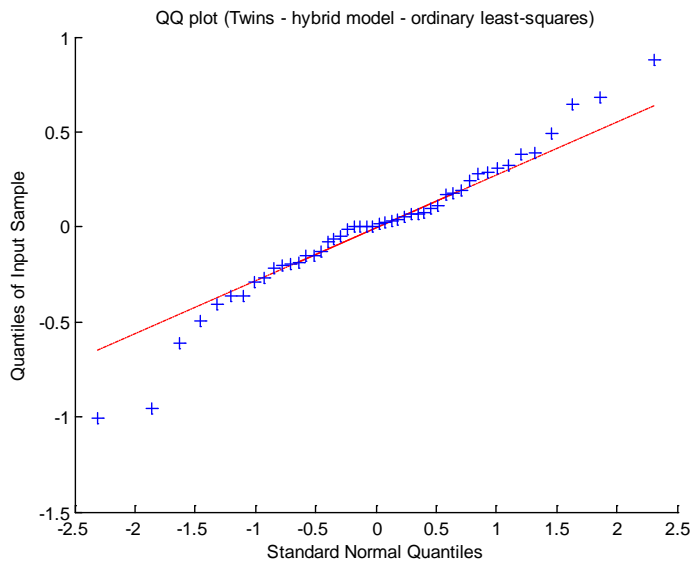


**Figure 4. 10: Residuals vs. Fitted values (twins – hybrid model)**

The figure 4.10 shows the plot of residuals versus fitted values for hybrid model of twins. It shows an obvious trend that variance increase as increasing size of the fitted value. This



indicates that the assumption of constant variance is violated. The estimation of parameter values based on ordinary least-squares fit is not reliable.



**Figure 4. 11: QQ plot (twins – hybrid model)**

The figure 4.11 shows the QQ plot for hybrid model of twins. It shows that the majority of the middle points seem to be quite close to the reference line. A few points around two ends are quite far from the reference line. This illustrates that the normality assumption seems to be violated.

#### **4.2.4 Summary**

The model diagnostic plots presented in this section are used to assess the adequacy of model fitting for candidate models. The plots above show that the assumption of constant variance is violated for all of the candidate models. The assumption of normality is challenged for most of those models. The estimation of parameters based on ordinary least-squares is not reliable in this case, as the assumption of constant variance is violated. Hence an improvement in the model fitting is required to produce accurate and reliable values of the parameters.

### 4.3 Model Improvement – Weighted Least-squares

The model diagnostics in previous sections show that the model fitting based on ordinary least-squares are not adequate. The major problem is the non-constant variance. This indicates that an improvement to the objective function is required. The weighted least-squares (WLS) criterion should be used to stabilise the variance.

Generally speaking, the objective function of weighted least-squares can be expressed in equation (4.6) below.

$$s = \sum_{i=1}^n w_i [x_i - \hat{x}_i]^2 . \quad (4.6)$$

where  $w_i$  is the weight, which help to stabilise the variance;

$x_i$  is the value of observation; and

$\hat{x}_i$  is the fitted value from the model; and

$s$  represents the objective function.

We use different regimes of weighted least-squares criteria for modelling the singletons and twins data to stabilise their variance. We will discuss the weights for singletons and twins separately in the following sections.

#### 4.3.1 Singletons

The weighted least-squares criterion is used for modelling singletons data. The objective function for singletons data is expressed in equation (4.7) below.

$$s = \sum_i^n \left( \frac{x_i - \hat{x}_i}{\sqrt{\hat{x}_i}} \right)^2 \quad (4.7)$$

We can arrange the expression of equation (4.7). It can be shown as  $\sum_{i=1}^n \left[ \frac{1}{\hat{x}_i} (x_i - \hat{x}_i)^2 \right]$ .

Comparing this expression with the general form in equation (4.6), we notice that the weight for this objective function is  $\frac{1}{\hat{x}_i}$ . Since the weight function is proportional to  $\frac{1}{\text{var}(\hat{x}_i)}$ . This assumption means that we are supposing the standard deviation of residuals increases proportionally to  $\sqrt{\hat{x}_i}$ , the square root of the fitted values. We use this weighted least-squares objective function to estimate the value of parameters for each model with singletons data.

#### 4.3.1.1 Full model

The quadratic fitting is used to model the function of daily nutrition intake for singletons. The result of final optimal value of each parameter based on ten different sets of initial guesses provided. The results are shown in table 4.10.

Par	(1)		(2)		(3)		(4)		(5)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.5	-2.5587	-2.6	-2.5600	-2.4	-2.5632	-2.3	-2.5587	-2.6	-2.5594
$Lt$	-2.0	-1.1388	-2.0	-1.1462	-2.4	-1.1727	-2.3	-1.1353	-1.4	-1.1434
$A$	0.5	1.4003	2.0	1.5326	1.4	2.3621	1.3	1.6985	1.9	1.6094
$K_1$	2.0	1.9551	0.8	1.8494	1.4	-1.4014	1.3	1.6797	2.3	1.7845
$Lt_0$	0.2	0.4351	-0.1	-0.1469	-0.3	-0.1475	-0.3	-0.3243	0.3	0.3886
$B$	0.2	-0.3060	-0.1	-0.0720	-0.2	-1.2244	-0.3	-0.2940	-0.6	-0.7437
$X_0$	0.2	0.1659	0.2	0.1659	0.22	0.1662	0.23	0.1661	0.17	0.1660
SSE		0.5753		0.5755		0.5759		0.5756		0.5755
Par	(6)		(7)		(8)		(9)		(10)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.3	-2.5595	-2.3	-2.5594	-2.3	-2.5601	-2.4	-2.5594	-2.4	-2.5600
$Lt$	-1.8	-1.1444	-1.7	-1.1454	-1.5	-1.1483	-1.9	-1.1409	-2.2	-1.1453
$A$	1.8	1.3800	1.7	1.3518	1.6	1.4658	1.9	1.7335	2.1	1.5990
$K_1$	2.4	1.9815	2.2	2.0197	2.1	1.9097	1.4	1.6465	1.2	1.7886
$Lt_0$	0.2	0.3941	0.3	0.6043	0.1	0.1119	-0.2	-0.1946	-0.2	-0.2818
$B$	-0.2	-0.1146	-0.3	-0.0881	-0.1	-0.1279	-0.3	-0.4440	-0.1	-0.0926
$X_0$	0.21	0.1659	0.22	0.1658	0.15	0.1659	0.19	0.1661	0.19	0.1660
SSE		0.5752		0.5751		0.5753		0.5756		0.5755

Table 4. 10: Parameter estimation for full model of singletons with WLS

The final values of SSE show that there is some variation. The smallest final value of SSE is 0.5751, which is from set (7). Based on the ten different sets of initial guesses, we cannot always reach the smallest final value of SSE. When we changed the value of initial guesses slightly, the final optimal value and final value of SSE being changed accordingly. This suggests that the full model is not stable for estimating the value of each parameter uniquely. In this case, we can conclude that the full model for singleton is non-identifiable. We are unable to estimate the reliable value of each parameter.

#### 4.3.1.2 Cut-down model

The quadratic fitting is used to model the function of daily nutrition intake for singletons. The result of final optimal value of each parameter based on four different sets of initial guesses provided. The results are shown in table 4.11.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.3	-2.5203	-2.4	-2.5203	-2.2	-2.5203	-1.5	-2.5203
$Lt$	-1.5	-0.8657	-2.3	-0.8657	-1.5	-0.8656	-0.5	-0.8657
$k_1$	1.5	2.2775	2.2	2.2775	2.0	2.2774	1.5	2.2775
$X_0$	0.11	0.1695	0.14	0.1695	0.17	0.1695	0.23	0.1695
SSE		0.5792		0.5792		0.5792		0.5792

Table 4. 11: Parameter estimation for cut-down model of singletons with WLS

The results in table 4.11 show that the final values of each parameter and SSE are almost identical. The slight difference related to parameter  $Lt$  and  $k_1$  in set (3), which may due to the rounding error. In this case, we are quite confident that we can estimate the parameter value uniquely. Therefore, we can conclude that the cut-down model for singletons is identifiable. We can obtain the precise estimated value for each parameter.

#### 4.3.1.3 Hybrid model

The quadratic fitting is used to model the function of daily nutrition intake for singletons. Table 4.12 shows the final optimal value of each parameter based on eight different sets of initial guesses provided.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.5	-2.6676	-2.3	-2.5585	-2.6	-2.5466	-2.4	-2.5585
$Lt$	0.5	-2.4126	0.1	-1.1288	-0.2	-1.0445	0.4	-1.1288
$A$	2.2	2.6622	2.5	2.3632	2.3	2.3383	2.4	2.3632
$Lt_0$	0.2	-0.0506	0.1	-1.6454	0.5	-1.9875	0.4	-1.6453
$X_0$	0.23	0.1608	0.22	0.1662	0.20	0.1670	0.14	0.1662
SSE		0.6007		0.5760		0.5763		0.5760
Par	(5) Initial	Final	(6) Initial	Final	(7) Initial	Final	(8) Initial	Final
$R$	-2.3	-2.5746	-2.4	-2.6465	-2.4	-2.5585	-2.5	-2.5585
$Lt$	0.3	-1.2559	0.3	-2.0365	0.2	-1.1288	0.1	-1.1288
$A$	2.3	2.4005	2.3	2.5940	2.1	2.3632	2.0	2.3632
$Lt_0$	-0.2	-1.2742	0.1	-0.2708	0.2	-1.6454	0.1	-1.6454
$X_0$	0.21	0.1651	0.21	0.1615	0.18	0.1662	0.17	0.1662
SSE		0.5765		0.5919		0.5760		0.5760

Table 4. 12: Parameter estimation for hybrid model of singletons with WLS

The results in table 4.12 show that the global minimum value of final SSE seems to be achieved at 0.5760 based on sets of initial guesses (2), (4), (7) and (8). For those sets of initial guesses which reach the global minimum of SSE, the final value of each parameter is almost identical. The final value of parameter  $Lt_0$  has slight variation in set (4), which may be due to the rounding error during the computation. In this case, the model parameters can be uniquely estimated. We can conclude this model is identifiable.

### 4.3.2 Twins

A different weighted least-squares criterion is used to estimate the model parameters for twins. This is because Figures 4.8 and 4.10 showed the standard deviation of residuals increasing much more than in Figure 4.6.

The weighted least-squares criterion is expressed in equation (4.8).

$$s = \sum_{i=1}^n \left( \frac{x_i}{\hat{x}_i} - 1 \right)^2 \quad (4.8)$$

We can arrange the equation (4.8) to form it in a generic format, as  $\sum_{i=1}^n \left[ \frac{1}{\hat{x}_i^2} (x_i - \hat{x}_i)^2 \right]$ . In this format, we can clearly see that the weight is  $\frac{1}{\hat{x}_i^2}$ . In other words we are modelling the standard deviation of residuals as increasing proportionally with  $\hat{x}_i$ . We used this weighted least-squares criterion as our objective function for optimising the parameters.

As discussed in the previous section, we notice that the cubic fitting for daily nutrition intake function performs better in parameter estimation. In the following sections, we will present the results of parameter estimation for twins associated with cubic fitting for modelling daily nutrition intake.

#### 4.3.2.1 Full model

The cubic fitting is used to model the function of daily nutrition intake for twins. Table 4.13 shows the final optimal value of each parameter based on ten different sets of initial guesses provided.

Par	(1)		(2)		(3)		(4)		(5)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.5	-2.6037	-2.3	-2.6040	-2.3	-2.6042	-2.4	-2.6040	-2.5	-2.6030
$Lt$	-2.0	-3.2437	-1.8	-3.2361	0.5	-3.2136	-1.7	-3.2264	-1.5	-3.2346
$A$	0.5	1.2706	1.2	1.3187	0.5	1.8967	1.0	1.4210	1.5	1.4093
$K_1$	2.0	2.5267	2.1	2.4771	2.2	2.1571	2.2	2.4297	2.5	2.5526
$Lt_0$	0.2	0.2938	-0.1	-0.0715	-0.1	-0.8697	-0.2	-0.2733	-0.5	1.4140
$B$	0.2	0.1068	-0.1	-0.1836	-0.1	-0.6658	-0.2	-0.3511	-0.5	-0.3375
$X_0$	0.3	0.2764	0.3	0.2764	0.3	0.2766	0.28	0.2765	0.25	0.2763
SSE		0.1720		0.1720		0.1718		0.1719		0.1721
Par	(6)		(7)		(8)		(9)		(10)	
	Initial	Final	Initial	Final	Initial	Final	Initial	Final	Initial	Final
$R$	-2.4	-2.6039	-2.3	-2.6038	-2.5	-2.6040	-2.4	-2.6039	-2.3	-2.6038
$Lt$	-1.3	-3.2391	-2.4	-3.2311	-2.3	-3.2255	-2.2	-3.2312	-1.2	-3.2375
$A$	2.1	1.2729	0.4	1.3082	1.4	1.4317	1.3	1.3349	2.3	1.2669
$K_1$	1.5	2.5031	2.5	2.4797	2.1	2.4246	2.0	2.4679	1.4	2.5072
$Lt_0$	-0.1	-0.0914	-0.4	-0.3314	-0.3	-0.3888	-0.2	-0.1868	-0.1	-0.0035
$B$	-0.1	0.1195	-0.5	0.0888	-0.3	-0.2692	-0.2	-0.1582	0.2	0.0820
$X_0$	0.26	0.2764	0.32	0.2764	0.31	0.2765	0.30	0.2764	0.27	0.2764
SSE		0.1720		0.1720		0.1719		0.1720		0.1720

**Table 4. 13: Parameter estimation for full model of twins with WLS**

The results above show the final optimal value of each parameter for full model of twins. The final value of SSE shows that it has some variation. The smallest value of final SSE is 0.1718 from set (3). We change the initial guesses slightly to assess how robust the final value of each parameter. The output shows that we fail to obtain the same SSE value of 0.1718 again in the other nine different sets of initial guesses. The final parameter values also vary. In this case, we are unable to conclude that the global minimum value of SSE is reached. If we take more trials, we may find an even lower value of SSE. This suggests that the full model for twins is unstable. In other words, the model is non-identifiable for parameter estimation. We cannot actually estimate the value of each parameter confidently.

#### **4.3.2.2 Cut-down model**

The cubic fitting is used to model the function of daily nutrition intake for twins. Table 4.14 shows the final optimal value of each parameter based on four different sets of initial guesses provided.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.4	-2.5746	-2.2	-2.5746	-2.0	-2.5746	-2.8	-2.5746
$Lt$	-2.3	-2.5645	-1.5	-2.5645	0.5	-2.5646	-0.2	-2.5645
$k_1$	2.2	2.6491	2.0	2.6491	1.5	2.6491	2.1	2.6491
$X_0$	0.14	0.2757	0.23	0.2757	0.3	0.2757	0.3	0.2757
SSE		0.1772		0.1772		0.1772		0.1772

**Table 4. 14: Parameter estimation for cut-down model of twins with WLS**

The results in table 4.14 show that the final value of SSE is stabilised at 0.1772. After examining the optimal value of parameters, we expect some rounding error related to parameter  $Lt$  in set (3), the other parameters are identical. In this case, we are really confident to conclude that this model is identifiable. We can estimate the reliable value of each parameter uniquely.

#### 4.3.2.3 Hybrid model

The cubic fitting is used to model the function of daily nutrition intake for twins. Table 4.15 shows the final optimal value of each parameter based on eight different sets of initial guesses provided.

Par	(1) Initial	Final	(2) Initial	Final	(3) Initial	Final	(4) Initial	Final
$R$	-2.6	-2.5746	-2.5	-2.6045	-2.3	-3.0201	-2.2	-2.5746
$Lt$	-0.2	-2.5645	-2	-3.2274	-1.1	-2.1388	-0.7	-2.5645
$A$	2.3	2.6491	2.2	2.7230	2.5	19.7952	1.8	2.6491
$Lt_0$	0.5	-28.8547	-0.5	-2.5932	0.1	-0.1945	-1.5	-115.2839
$X_0$	0.3	0.2757	0.28	0.2765	0.33	0.5427	0.27	0.2757
SSE		0.1772		0.1717		1.2934		0.1772
Par	(5) Initial	Final	(6) Initial	Final	(7) Initial	Final	(8) Initial	Final
$R$	-2.4	-2.6045	-2.7	-2.6045	-2.5	-2.6358	-2.6	-2.6045
$Lt$	-1.5	-3.2274	-0.8	-3.2274	-2.8	-126.3831	-1.0	-3.2274
$A$	1.5	2.7230	2.1	2.7230	2.7	2.7827	1.8	2.7230
$Lt_0$	-1.2	-2.5932	-2.2	-2.5932	0.4	-1.9476	-1.8	-2.5932
$X_0$	0.25	0.2765	0.29	0.2765	0.22	0.2773	0.28	0.2765
SSE		0.1717		0.1717		0.1768		0.1717

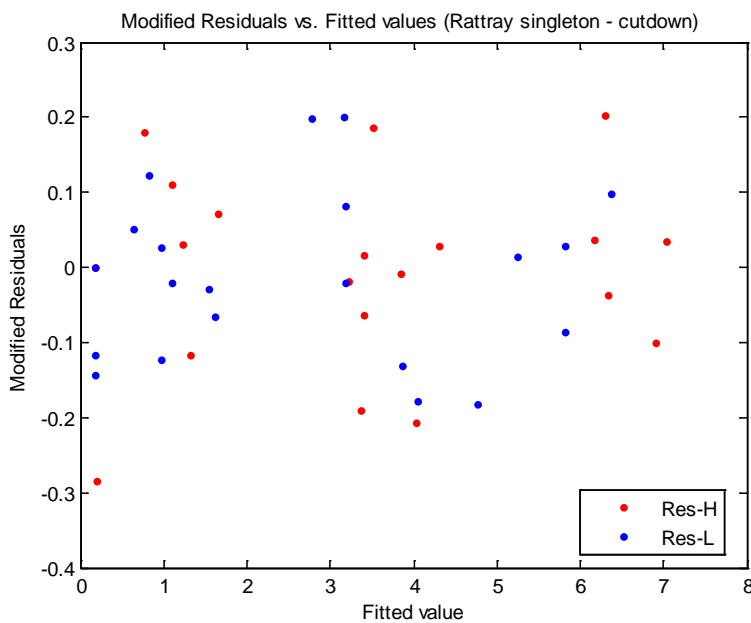
**Table 4. 15: Parameter estimation for hybrid model of twins with WLS**

The results in table 4.15 show that the smallest final value of SSE is at 0.1717. With these ten different sets of initial guesses, we obtained that the same smallest value of SSE four times. This suggests that the global minimum of SSE value has been reached, and the optimal value of each parameter has been obtained. It indicates the model is identifiable, and each parameter value is reliable.

## 4.4 Model Diagnostics – Weighted Least-squares

In this section, we will present the model diagnostic plots base on the objective function of weighted least-squares. For each model, we will show the plot of modified residuals versus fitted values to assess the assumption of constant variance. As well as the QQ normal plot to assess the normality of the residuals.

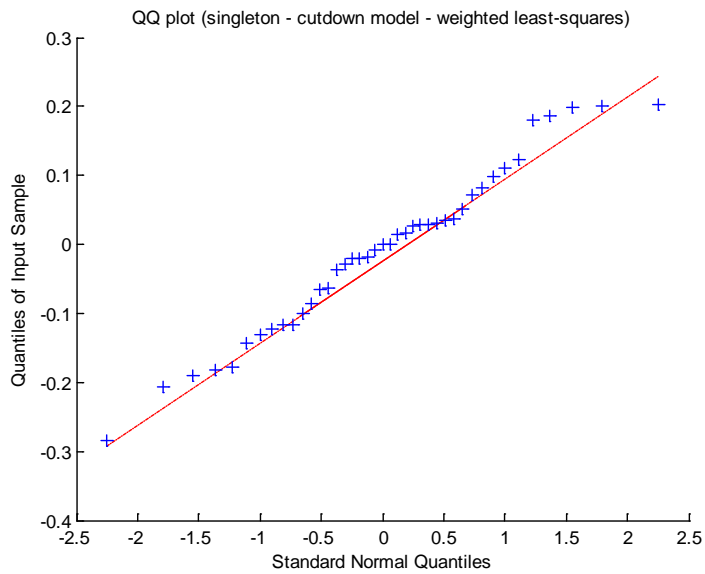
### 4.4.1 Singletons – cut-down model



**Figure 4. 12: Modified residuals vs. fitted values (singletons – cut-down model - WLS)**

The figure 4.12 shows that the plot of modified residuals versus fitted values. Modified residuals, also known as weighted residuals, are defined as  $\sqrt{w_i}(x_i - \hat{x}_i)$  where  $w_i$  is the weight. We notice that the modified residuals spread constantly around zero. There is no obvious pattern associated with those modified residuals. This indicates that the weighted least-squares fixed the problem of heteroscedasticity. It also suggests that the cut-down model fitting is adequate for singletons with weighted least-squares.

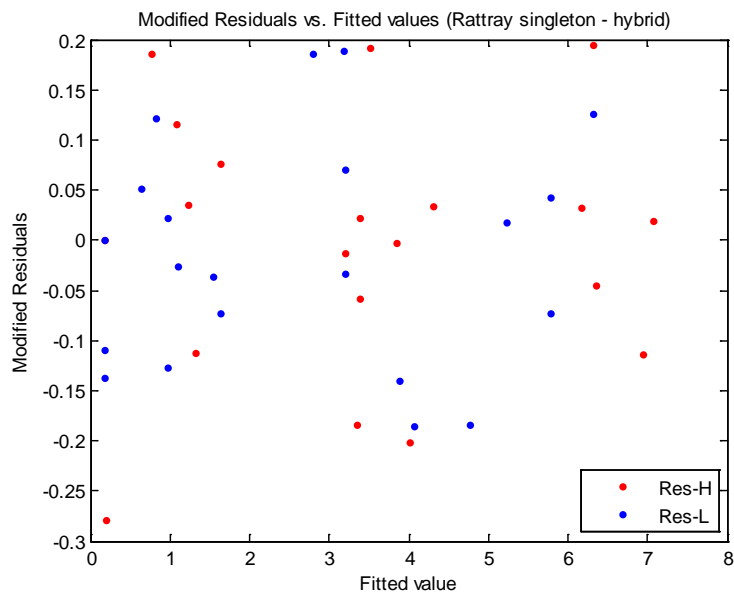




**Figure 4. 13: QQ plot (singletons – cutdown model - WLS)**

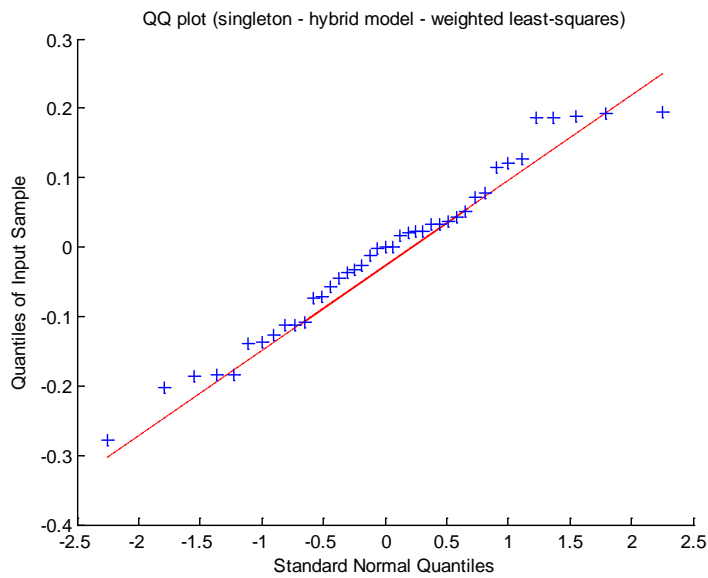
The figure 4.13 shows the QQ plot of the cut-down model for singletons. It shows that most of the residuals lie very close to the line. The modified residuals seem to be approximately normally distributed. The assumption of normality seems to be reasonable.

#### 4.4.2 Singletons – hybrid model



**Figure 4. 14: Modified residuals vs. fitted values (singletons – hybrid model - WLS)**

The figure 4.14 shows the modified residuals versus fitted values of the hybrid model for singletons with weighted least-squares fit. It does not show any obvious patterns. The variance spread constantly. It indicates that the hybrid model fitting is adequate for singletons with weighted least-squares fit.



**Figure 4. 15: QQ plot (singleton – hybrid model - WLS)**

The figure 4.15 shows the QQ plot of the hybrid model for singletons. It shows that the majority of the modified residuals are very close to the reference line. The assumption of normality seems to be appropriate.

### 4.4.3 Twins – cut-down model

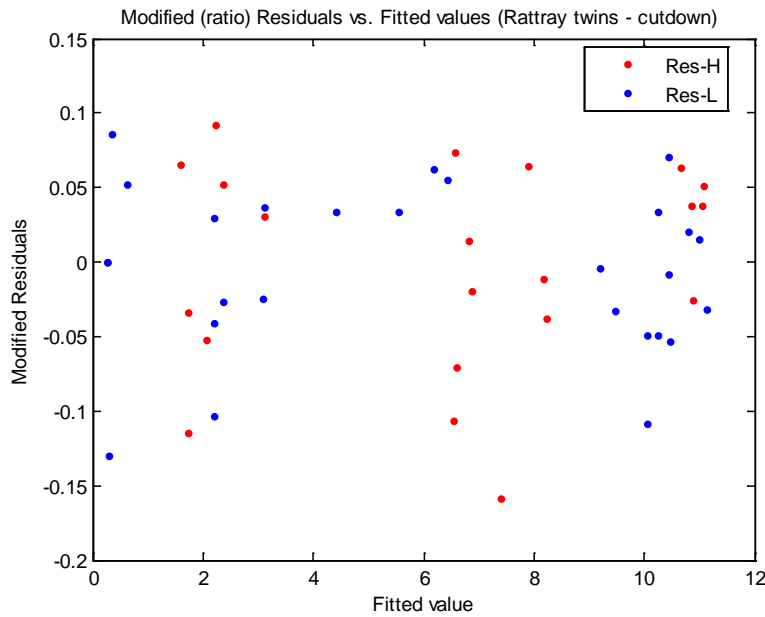


Figure 4. 16: Modified residuals vs. fitted values (twins – cut-down model - WLS)

The figure 4.16 shows the modified residuals versus fitted values plot of the cut-down model for twins. It shows that the modified residuals are spread constantly. It does not show any obvious patterns. The normality assumption seems to be reasonable.

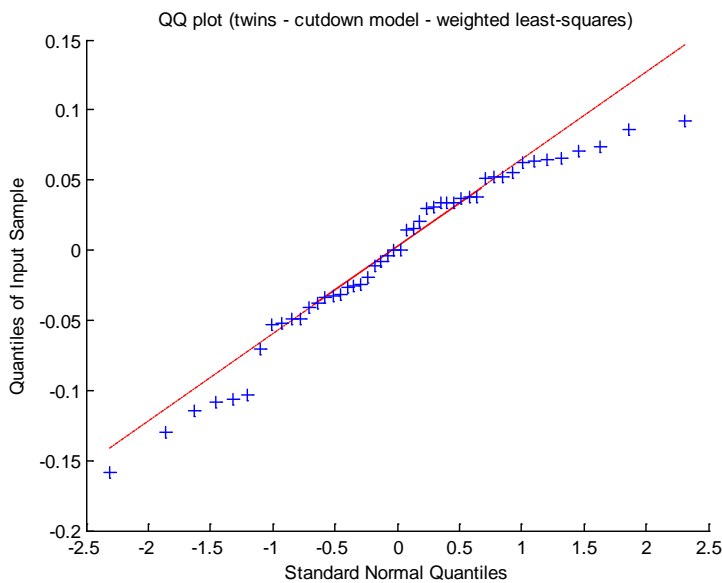
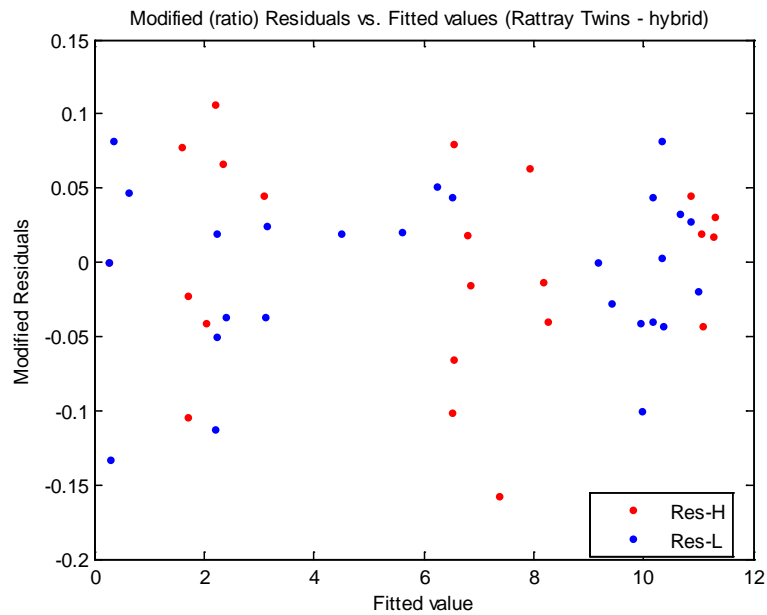


Figure 4. 17: QQ plot (twins – cutdown model - WLS)

The figure 4.17 shows the QQ plot of cut-down model of twins. There are few points at the upper quantile which have deviated a bit from the reference line. The majority of the points

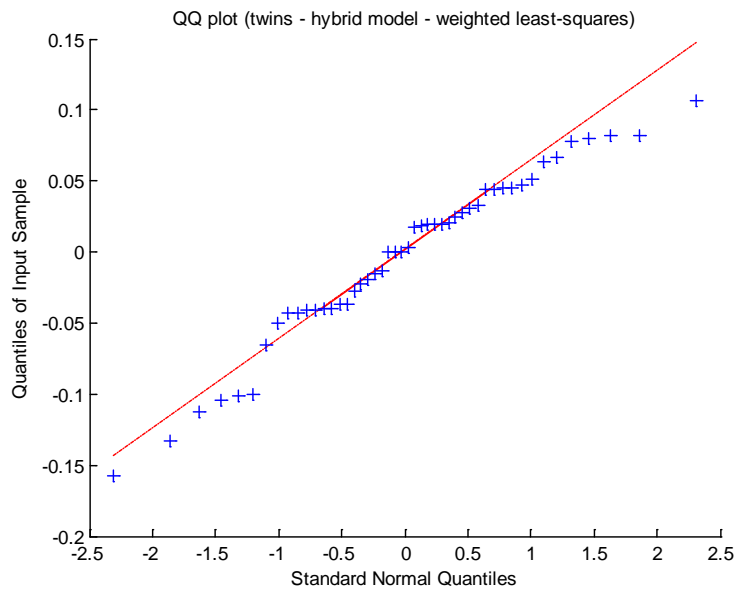
lie very close to the reference line. This suggests that the assumption of normality seems to be reasonable.

#### 4.4.4 Twins – hybrid model



**Figure 4. 18: Modified residuals vs. Fitted values (twins – hybrid model - WLS)**

The figure 4.18 shows the modified residuals against fitted values plot of the hybrid model for twins. It shows that the modified residuals spread quite constant around zero. The assumption of constant variance seems to be appropriate.



**Figure 4. 19: QQ plot (twins – hybrid model - WLS)**

The figure 4.19 shows the QQ plot of hybrid model for twins. The plot shows that the residuals are approximately normally distributed. The normality assumption seems to be appropriate.

## 4.5 Summary

In this chapter, we presented the results about the parameter estimation for our full model, and also some alternative models, such as the cut-down model and the hybrid model. We used appropriate model fitting to represent the daily nutrition intake function. The results show that the daily nutrition intake function with quadratic fitting is appropriate for singletons, and cubic fitting is appropriate for twins.

The weighted least-squares method is used as our objective function to optimise the parameters for each model. The weighted least-squares criterion is used to fix the problem of heteroscedasticity. It helps to achieve an adequate model fit.

The results suggest that the structure of the full model is too complicated. We cannot successfully estimate the unique parameters for the full model. In this case, we conclude that

the full model for both singletons and twins is non-identifiable. We failed to estimate reliable parameters based on this model for this reason.

We improved the models by simplifying the model structure. The alternative cut-down model can achieve the model identifiability with any initial guesses used. This suggests that the cut-down model is very stable. It always achieves the global minimum value of SSE to get the optimal value of parameters with the best model fitting.

Another alternative model is the hybrid model. This model seems to achieve the model identifiability as well. However, we have to choose the initial guesses very carefully for this model to achieve the model identifiability. Not all the initial guesses can help the model to reach the global minimum value of SSE. This suggests that the model is identifiable, but not as stable as the cut-down model. In addition, we noticed that the model fitting for the hybrid model is slightly better than that of the cut-down model.

In conclusion, both of the alternative cut-down model and hybrid model seem to be reasonable, and can achieve the model identifiability. We will do further analysis base on those two models in the following chapters, and then justify the suitability of each model.

# Chapter 5

## 5. Bootstrap Simulations

The bootstrap resampling is to resample the data with replacement from the original dataset. This method can test the reliability of the estimated parameters. In addition, this technique is very easy to implement. We program the bootstrap procedures, and the simulations will be done by the computer.

In this chapter, we will discuss the technique used for bootstrap simulations. The detailed procedures will be discussed in the methodology section. The results and relevant plots will be shown in the results section.

### 5.1 Methodology

In this study, our original datasets for singletons and twins are both from Rattray's experiments (1974a, 1974b). We estimated the unknown parameters based on those datasets. In this way, we can only get one estimated value for each parameter in the model. Due to the limited number of datasets available, we cannot test the reliability of the estimation. A key thing is that the estimation method inserts a system of differential equations between the data and the parameter estimates. The system does not have a solution in closed form. Therefore (except in the case of the cut-down model) it is not possible to get an analytical formula for the standard error based on the variation in the data. However, the bootstrap simulations provide us a way to estimate the accuracy of each estimated parameter. The detailed process will be described in the following sections.

Firstly, we create 5000 pseudo datasets based on the original one by using bootstrap resampling. The bootstrap resampling refers to sampling with replacement from the original dataset. The size of pseudo datasets should be the same as the original one. In addition, we adopt non-parametric resampling, which does not require assumptions associated with the distribution of the data. In our case, the fetal weight is always associated with a corresponding time point. Thus we have to make sure that we resample the paired data for fetal weight and corresponding time point.

Secondly, we estimate each parameter for the model based on every single simulated dataset. In this way, we can get 5000 estimated values for each parameter. Based on those simulations, we can construct a sampling distribution for each parameter.

Thirdly, we adopt the simple percentile confidence limits to construct the 95% confidence interval for each parameter. We sort those 5000 estimated values of each bootstrapped parameter in ascending order. Then the central 95% of the bootstrap distribution is taken as being a 95% confidence interval for each parameter. In our example, we take the 126<sup>th</sup> and 4875<sup>th</sup> of ordered estimated parameter values as the lower and upper confidence limits respectively.

Lastly, we assess the accuracy of the estimated parameters from the original dataset. If the original parameter estimate sits near the centre of the 95% bootstrapped confidence interval, this gives some assurance that the estimation method is unbiased and reliable.

## **5.2 Results**

In this section, we will show and discuss the results on two preferred models from the previous chapter. Those two preferred models are cut-down model and hybrid model. Both of those two models are associated with datasets of singletons and twins. The histogram of each parameter will be provided. The histograms are the main results for this section, as they show the bootstrapped distribution of each parameter. We label the upper and lower limit for each parameter, as well as the bootstrapped mean and bootstrapped standard deviation. In addition, we will discuss the results on each model.



## 5.2.1 Cut-down model for singletons

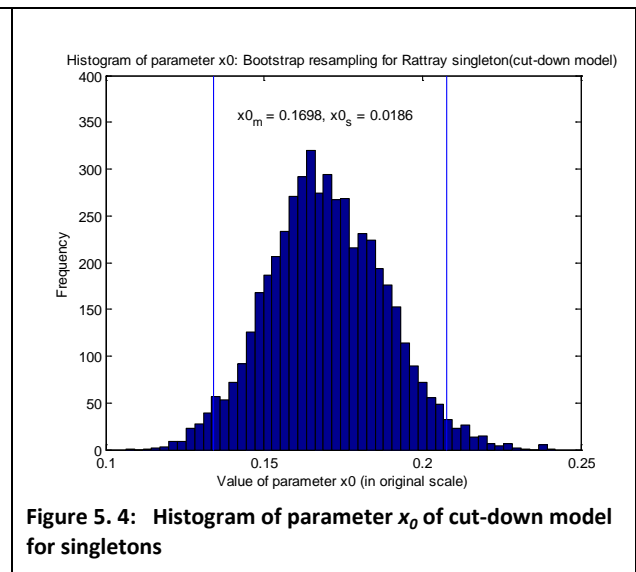
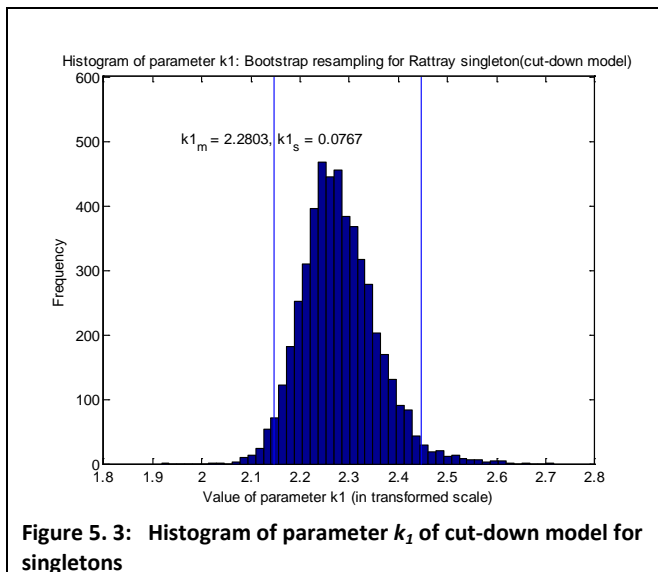
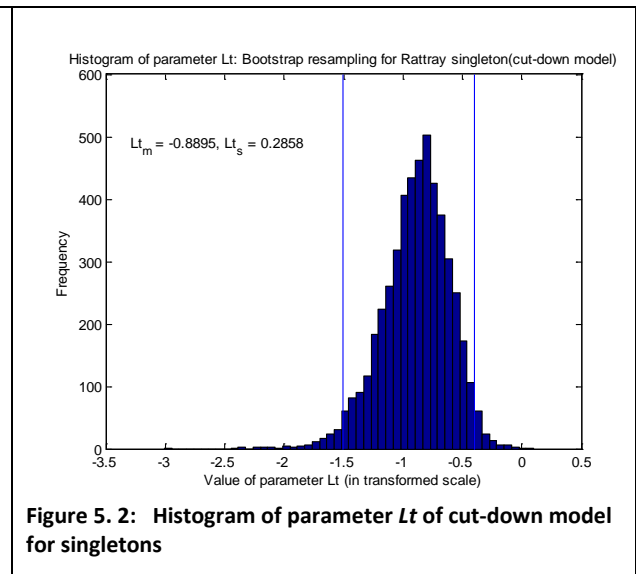
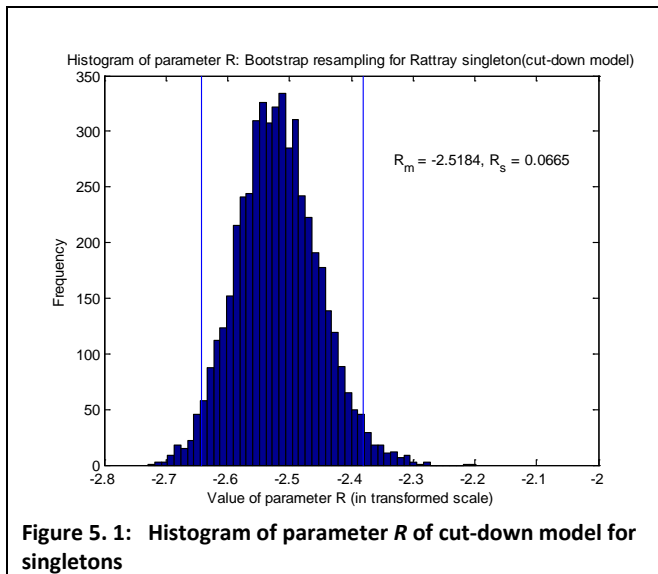
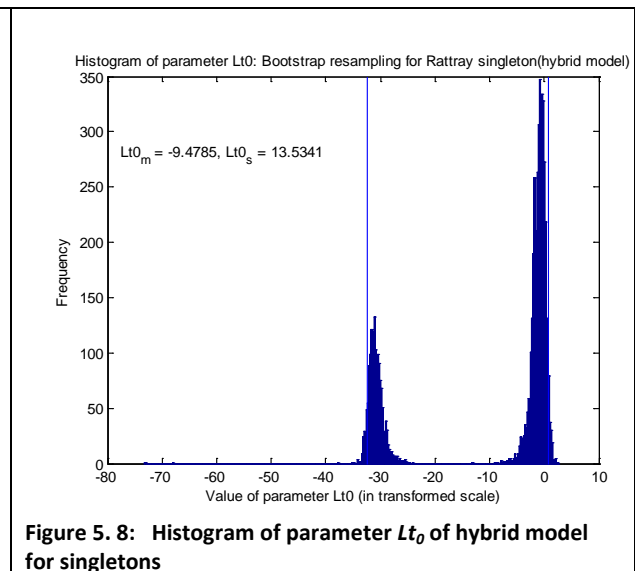
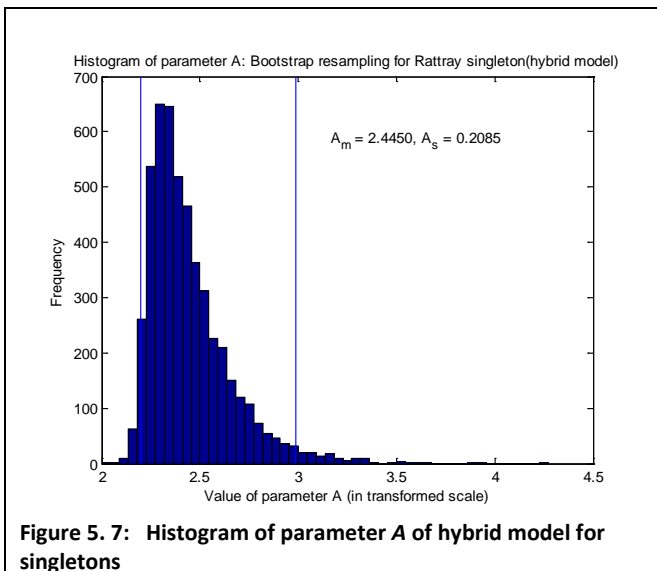
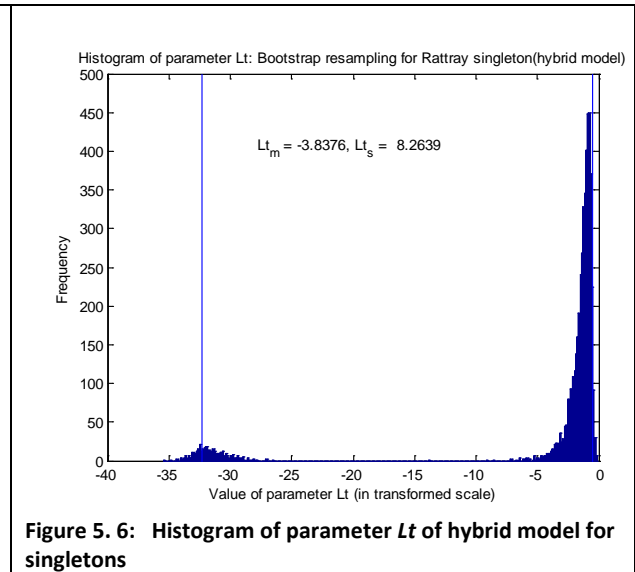
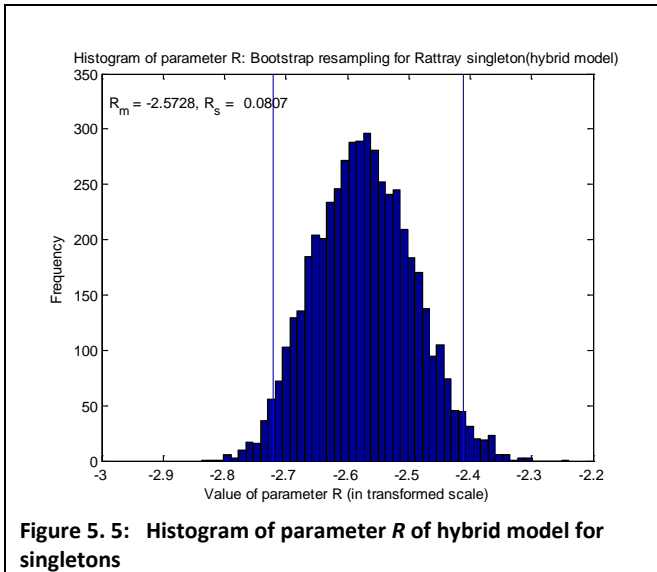
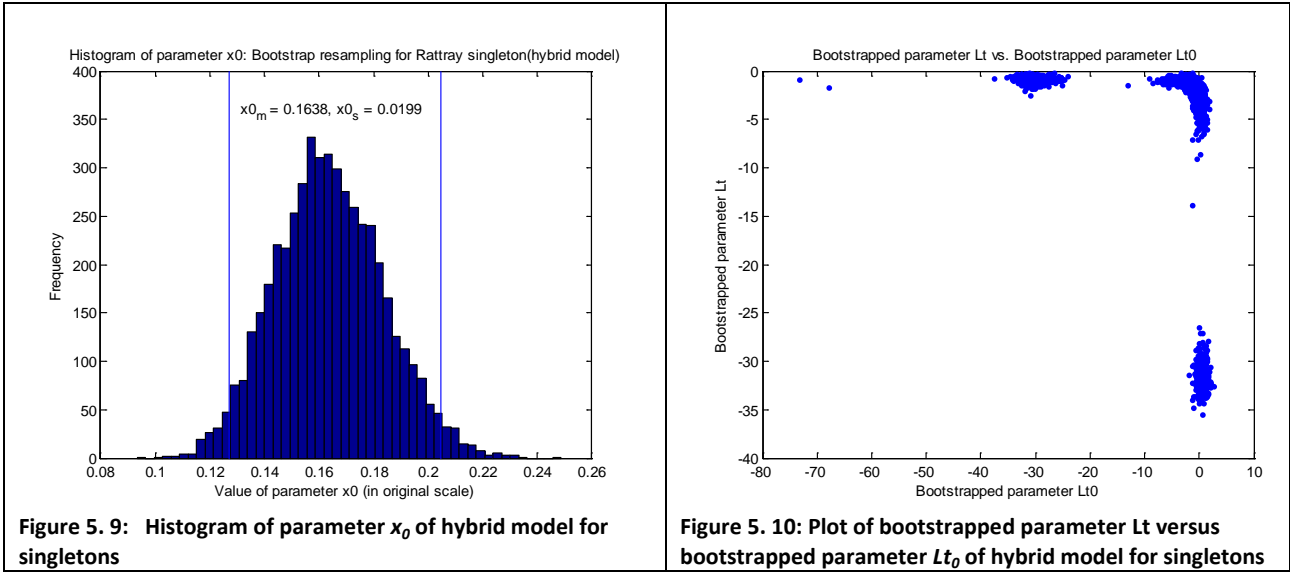


Figure 5.1 to figure 5.4 show the bootstrapped distributions of each parameter for the cut-down model applied to singletons. We notice that bootstrapped distribution of each parameter seems to be approximately a normal distribution. The estimated parameter values based on the original dataset for the cut-down model applied to singletons are  $-2.5203$ ,  $-0.8657$ ,  $2.2775$  and  $0.1695$  for the parameter  $R$ ,  $Lt$ ,  $k_1$  and  $x_0$  respectively. These estimated values all sit near the centre of the 95% confidence interval. In addition, the bootstrapped mean of each parameter is very close to the estimated value based on original dataset. In this way, we are

reassured that the parameter estimation method for the original dataset is very reliable for the cut-down model of singletons.

### 5.2.2 Hybrid model for singletons





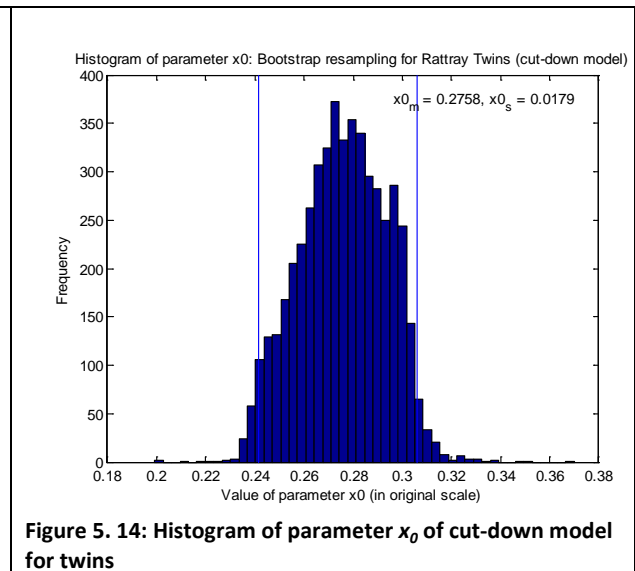
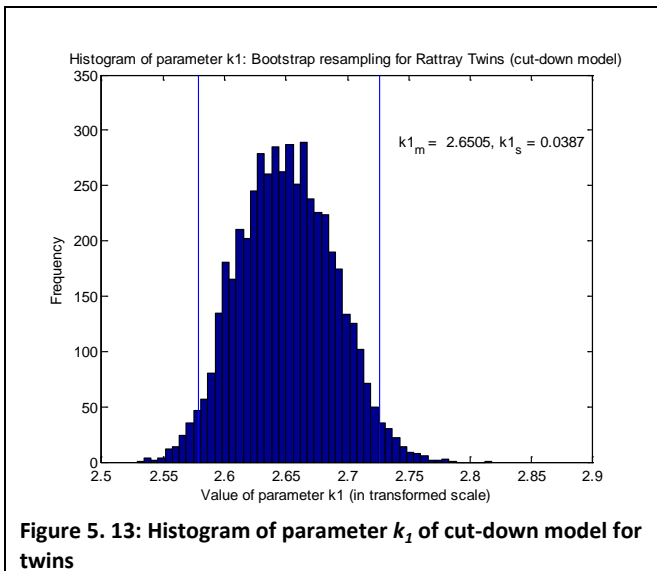
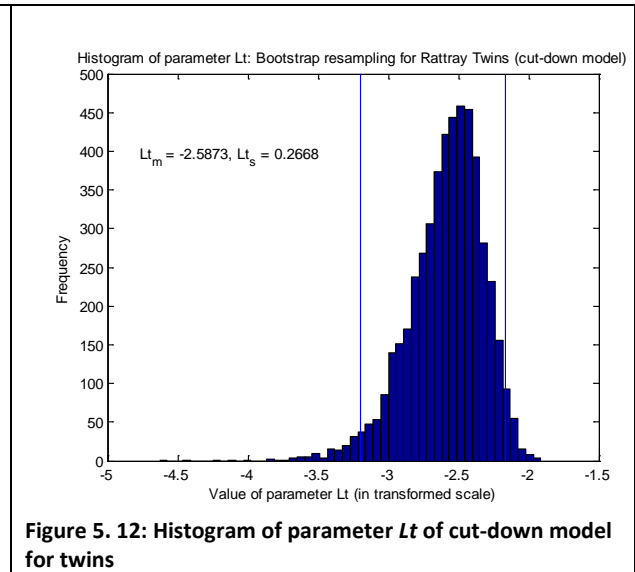
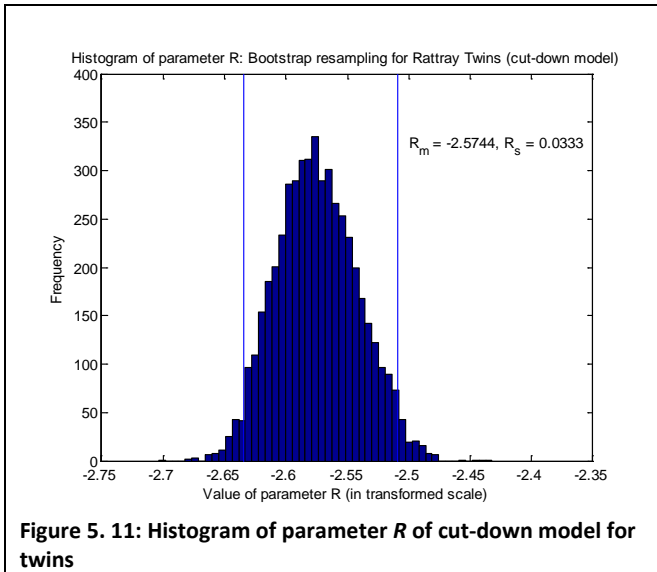
The figure 5.5 to figure 5.9 show the bootstrapped distributions of each parameter for the hybrid model applied to singletons. The bootstrapped distribution for parameter  $R$  and  $x_0$  seem to be normally distributed. It shows a highly right-skewed shape for bootstrapped parameter  $A$ .

However, the bootstrapped distribution of parameter  $Lt$  and  $Lt_0$  show a bimodal distribution. It shows the primary mode is around  $-1$  and secondary mode is around  $-33$  for parameter  $Lt$ . For parameter  $Lt_0$ , the primary and secondary modes are around  $0$  and  $-30$  respectively. The values of the secondary modes for both of these bootstrapped parameters are very close to zero in the original scale. The bootstrapped standard deviations for those two parameters are quite large, which indicate a large variation for the bootstrapped value for these two parameters with 5000 simulations. The estimated parameter values based on the original dataset for the hybrid model applied to singletons are  $-1.1288$  and  $-1.6454$  for the parameter  $Lt$  and  $Lt_0$  respectively. Although these estimated values sit well within the 95% confidence interval, this model still seems to be unstable based on the results of bootstrapped simulations.

The figure 5.10 shows the plot of bootstrapped parameter  $Lt$  versus bootstrapped parameter  $Lt_0$ . This plot is used to investigate the relationship between those two bootstrapped values. There are some points clustered at the top right corner that show reasonable values for both of the bootstrapped parameters. The rest of two clustered points show that one of the two bootstrapped parameter value is zero in original scale. This indicates that the hybrid model applied to singletons is unstable by bootstrapped simulations. In some cases, it forces one of

the parameters  $Lt$  or  $Lt_0$  to be zero in their original scales, in order to eliminate one of them from the model.

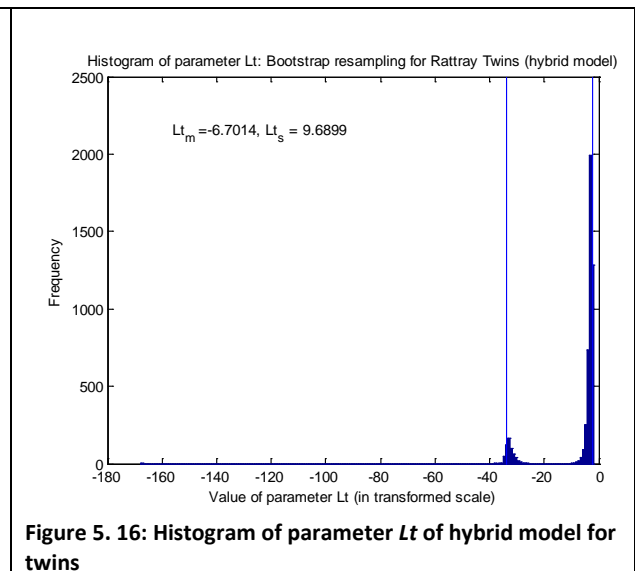
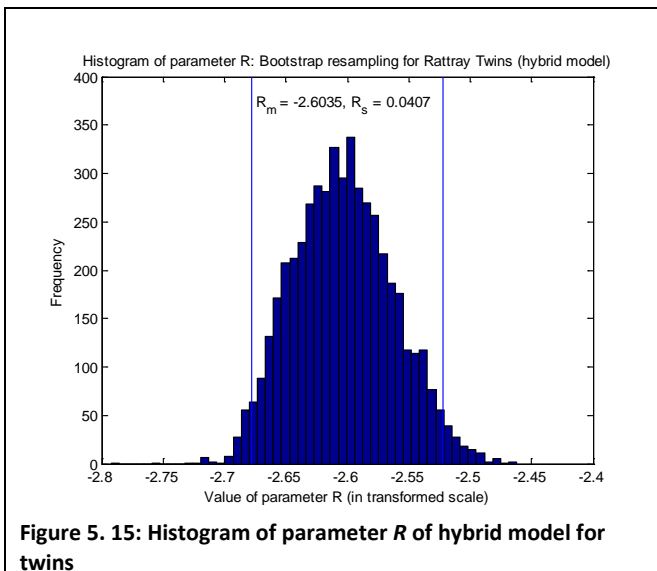
### 5.2.3 Cut-down model for twins

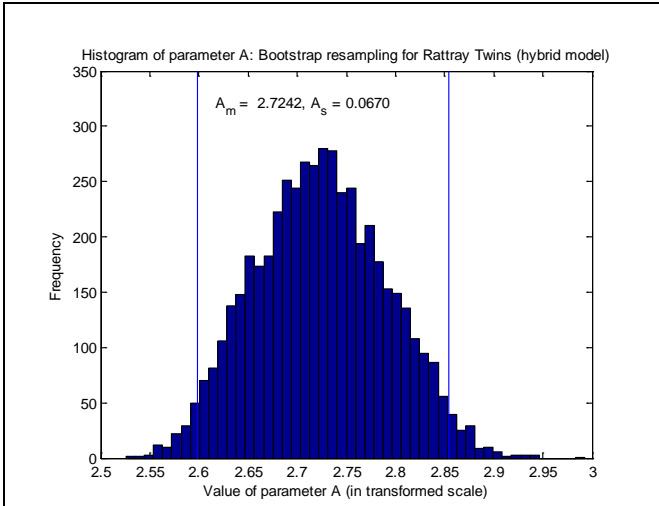


The figure 5.11 to figure 5.14 show the bootstrapped distributions of each parameter for the cut-down model applied to twins. The bootstrapped distribution of parameter  $Lt$  is slightly left-skewed. The rest of the bootstrapped parameters are approximately normally distributed.

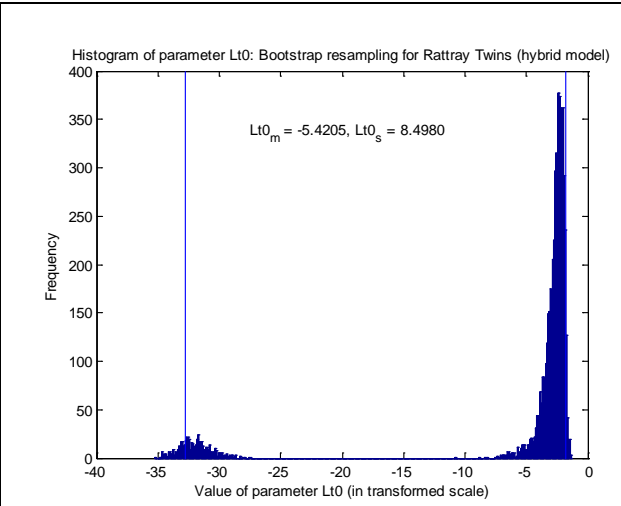
The estimated parameter values based on the original dataset for the cut-down model applied to twins are  $-2.5746$ ,  $-2.5645$ ,  $2.6491$  and  $0.2757$  for the parameter  $R$ ,  $Lt$ ,  $k_I$  and  $x_0$  respectively. These estimated values of each parameter are very close to each of the bootstrapped means. In addition, the each estimated parameter value sits near the centre of the 95% confidence interval. This indicates that the parameter estimation method for the original dataset is very reliable for the cut-down model of twins.

### 5.2.4 Hybrid model for twins

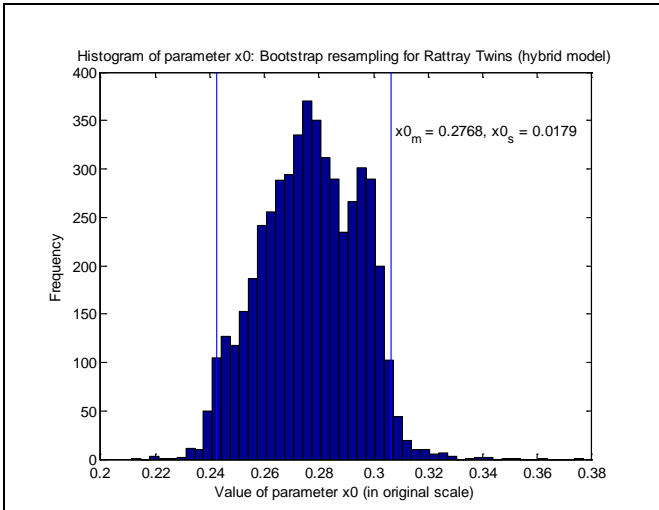




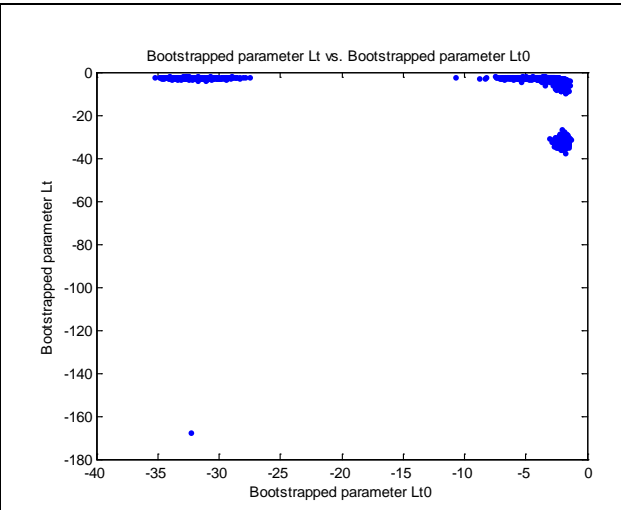
**Figure 5. 17: Histogram of parameter A of hybrid model for twins**



**Figure 5. 18: Histogram of parameter Lt0 of hybrid model for twins**



**Figure 5. 19: Histogram of parameter x0 of hybrid model for twins**



**Figure 5. 20: Plot of bootstrapped parameter Lt versus bootstrapped parameter Lt0 of hybrid model for twins**

The figure 5.15 - 5.19 show the bootstrapped distributions of each parameter for hybrid model applied to twins. The bootstrapped distribution of parameter  $R$ ,  $A$  and  $x_0$  are approximately symmetric and normally distributed. The estimated parameter values based on the original dataset for the hybrid model applied to twins are  $-2.6045$ ,  $2.7203$  and  $0.2765$  for the parameter  $R$ ,  $A$  and  $x_0$  respectively. We also noticed that the bootstrapped mean for these parameters are very close to the estimated value based on original dataset.

However, the bootstrapped distribution for the other two parameters  $Lt$  and  $Lt_0$  have showed a bimodal distribution. The figure 5.20 shows the detailed relationship between those two bootstrapped parameters. It shows that among those 5000 simulations, it has quite large probability for one of those two bootstrapped parameters to be the value around  $-30$ . It

indicates that the model forces one of those two parameters to be zero in original scale, in order to eliminate one parameter from the model in some cases.

### 5.3 Summary

In this chapter, we present the results of bootstrap simulations for the cut-down model and the hybrid model. The results are for both singletons and twins data. The histogram is used to show the bootstrapped distribution of each parameter. It gives us an overall view to justify how reliable is each estimated parameter, as well as how stable our models are.

The results suggest that the cut-down model is very stable for both singletons and twins data. The bootstrapped distribution of each parameter seems to be normally distributed. In addition, the bootstrapped mean is very close to our estimated value of each parameter. This suggests that the parameters from cut-down model are reliable.

However, the results suggest that the hybrid model is quite unstable for both singletons and twins data. The bootstrapped distribution for parameter  $Lt$  and  $Lt_0$  show the bimodal trend. The bootstrapped standard deviation is very large for those two parameters. This indicates the model parameter is not that reliable for the parameter  $Lt$  and  $Lt_0$ . With the bootstrapped datasets, in some cases, the hybrid model tried to eliminate one of the parameters  $Lt$  or  $Lt_0$ .

The results from the 5000 bootstrap simulations show that the cut-down model seems to be more stable than that of the hybrid model. This indicates that the cut-down model gives reliable estimates for its parameters.

# Chapter 6

## 6. Optimal solutions

In this chapter, we will present the optimal solutions of daily nutrition intake for the pregnant sheep during the second half of their pregnancy, with pre-determined desirable birth weight. The detailed methodology will be discussed.

The whole dynamic system and pre-determined parameter values will be used in order to find the optimal solutions. The optimal control problem will convert to the boundary value problem. The boundary conditions for this problem are the initial fetal weight ( $x_0$ ) at the start of the second half of pregnancy ( $t_0$ ), and also the birth weight ( $x_b$ ) at the birth time ( $t_b$ ). The boundary conditions can be expressed in equation (6.1).

$$x(t_0) = x_0, \text{ and } x(t_b) = x_b. \quad (6.1)$$

Our aim is to solve the ordinary differential equation of daily nutrition intake with those boundary conditions to get the optimal solution, which is defined as nutrition intake at the second half of pregnancy ( $t_0 < t < t_b$ ). The derivative function for daily nutrition intake is denoted as  $\dot{u}$ . The original full model has been modified to form the cut-down model and the hybrid model to achieve identifiability of model parameters. The corresponding dynamic model system has been changed accordingly.

The optimal solutions can be estimated based on the following three steps. Firstly, we need to develop an expression for the ODE function of daily nutrition intake ( $\dot{u}$ ) based on each alternative model. Secondly, we set the desirable birth weight for each model as a boundary condition. Finally, using the estimated value of each parameter, we solve the model system with boundary conditions.

The Matlab built-in function 'bvp4c' is used to solve the dynamic systems with boundary conditions. The detailed results of optimal solutions will be shown in the following sections.



## 6.1 Cut-down model

Kiataramkul et al. (2011) proved a theorem that if  $\dot{x} = f(t, x, u) = f_1(x) \cdot f_2(u)$  is separable and  $g$  is not a function of  $x$ , where  $g = g(t, u)$  comes from  $J\{u\} = \int_{t_0}^{t_b} g[t, u(t)]dt$ , then  $\dot{u} = G(t, x, u) \equiv 0$  (where  $J\{u\}$  is the objective function that should be minimised). Thus the variable  $u$  is a constant.

The cut-down model is expressed as  $\dot{x} = \frac{rux}{u+L} \left(1 - \frac{x}{k_0}\right)$ . In this case, the function of  $u$  is separable, based on above theorem we can confirm that the variable  $u$  is a constant. The dynamic system for the cut-down model shows in equation (6.2) and (6.3).

$$\dot{x} = \frac{rux}{u+L} \left(1 - \frac{x}{k_0}\right) \quad (6.2)$$

$$\dot{u} = 0 \quad (6.3)$$

We set the desirable birth weight as one of the boundary condition, and the other boundary condition is predetermined by  $x_0$  based on the data. The numerical solutions are obtained by solving the boundary value problem with this dynamic system.

Alternatively, this dynamic system can be solved analytically. The ODE involving  $\dot{x}$  can be solved by treating variable  $u$  as a constant.

Since  $\frac{dx}{dt} = \frac{rux}{u+L} \left(1 - \frac{x}{k_0}\right) = \frac{rux}{u+L} \left(\frac{k_0-x}{k_0}\right)$ , then we can separate the variables  $x$  and  $t$ , and integral on both sides.

$$\int \frac{1}{\frac{rux}{u+L} \left(\frac{k_0-x}{k_0}\right)} dx = \int 1 dt, \text{ and solving both sides we can get}$$

$$\frac{1}{\frac{ru}{u+L}} \int \frac{1}{x} + \frac{1}{k_0-x} dx = t + c_1,$$

$$\frac{1}{\frac{ru}{u+L}} \cdot \ln \left( \frac{x}{k_0-x} \right) = t + c_1, \text{ for } 0 < x < k_0$$

$$\ln \left( \frac{x}{k_0-x} \right) = t \frac{ru}{u+L} + c_2, \text{ and then we obtain}$$

$$\frac{x}{k_0-x} = c_3 e^{\frac{rut}{u+L}} \quad (6.4)$$

where  $c_1, c_2$  and  $c_3$  are all arbitrary constants.

With initial condition  $x(t_0) = x_0$ , let us assume that  $t_0 = 0$ , and so we get  $x(0) = x_0$ .

Substituting the initial condition into equation (6.4), we get  $c_3 = \frac{x_0}{k_0 - x_0}$ .

Then the equation (6.4) becomes

$$\frac{x}{k_0 - x} = \frac{x_0}{k_0 - x_0} e^{\frac{rut}{u+L}},$$

After re-arranging the expression, finally we obtain  $x(t) = \frac{k_0 x_0}{x_0 + (k_0 - x_0) e^{-\frac{rut}{u+L}}}$ .

Let us set the birth weight of the lambs, which is denoted as  $x_b$  ( $x_b = x(t_b)$ ). After simplifying we get the analytical solution as

$$u = \frac{\ln\left(\frac{x_b(k_0 - x_0)}{x_0(k_0 - x_b)}\right) \cdot L}{rt + \ln\left(\frac{x_0(k_0 - x_b)}{x_b(k_0 - x_0)}\right)} \quad (6.5)$$

where  $t$  is the period of the second half of the pregnancy,  $t = t_b - t_0 = 147 - 70 = 77$  days.

### 6.1.1 Singletons

Pre-determined birth weight [ $x_b$ ] (kg)	Optimal daily nutrition intake [ $u$ ] (kg/day)
6.0	1.1219
6.5	1.3566
7.0	1.7063
7.5	2.3060
8.0	3.6496
8.5	10.1973

Table 6. 1: The optimal solution for the cut-down model of singletons

The table 6.1 shows the results of pre-determined birth weight for singleton lamb and with corresponding optimal nutrition intake. For the cut-down model, we have set various pre-determined birth weights for lambs to examine the corresponding optimal solutions. With increasing the pre-determined birth weight, the corresponding optimal daily nutrition intake increases accordingly.

In the last line, we set the desirable birth weight for the lambs extremely high, at 8.5 kg, which is quite impossible to achieve in reality. The corresponding daily nutrition intake is 10.2 kg/day. Of course, the optimal solution is unrealistic when there is an unreasonable pre-determined birth weight.

Based on our original dataset, we consider the reasonable birth weight for lambs is around 6.5 kg. The optimal daily nutrition intake associated with that birth weight stays constant at 1.36 kg/day. This optimal value seems to be reasonable. The experiments from Rattray (1974) suggested that the daily nutrition intake for the maintenance level of singletons is around 1.2 kg/day. The optimal value we estimated is a bit higher than this maintenance level. This suggests that the pregnant sheep carrying singletons should eat a bit more in the second half of gestation to give birth to a healthy offspring with desirable birth weight.

### 6.1.2 Twins

Pre-determined birth weight [ $x_b$ ] (kg)	Optimal daily nutrition intake [ $u$ ] (kg/day)
11.0	0.5725
11.5	0.8690
11.8	1.2872
11.9	1.5420
12.0	1.9309

12.1	2.5987
12.2	4.0174
12.3	9.0964
12.4	-30.7373
12.5	-5.5892
12.6	-3.0328
12.7	-2.0595
12.8	-1.5453

**Table 6. 2: The optimal solution for the cut-down model of twins**

The table 6.2 shows the results of pre-determined birth weight for lambs and with corresponding optimal nutrition intake for the cut-down model of twins. We notice that when the pre-determined birth weight is larger than 12.3 kg, the corresponding optimal nutrition intake is a negative value. The negative value of daily nutrition intake does not make sense for any solutions. For those negative values of daily nutrition intake, we fail to solve it with Matlab function 'bvp4c'. Those can only be solved analytically.

The reasonable total birth weight for twins based on our original dataset is supposed to be 12 kg. The corresponding optimal daily nutrition intake is 1.93 kg/day. Comparing this value with the solution of singletons, it suggests that in order to achieve the desirable birth weight, the pregnant sheep carrying twins must eat more than if they are carrying a singleton. This matches our expectation in real life. Thus we think the optimal solution of daily nutrition intake is 1.93 kg/day for twins, and 12 kg birth weight seems to be reasonable.

## 6.2 Hybrid model

Kiataramkul et al. (2011) developed a formula, which can be used to generate the algorithm for the function of optimal daily nutrition intake with a one dimensional dynamic system. The formula is shown in equation (6.6).

$$\dot{u} = \frac{g_x f_u^2 - g_{ux} f f_u - g_u f_x f_u + g_u f_{ux} f}{g_{uu} f_u - g_u f_{uu}} \quad (6.6)$$

where the partial derivatives are denoted as

$$g_x = \frac{\partial g}{\partial x}; \quad g_{ux} = \frac{\partial^2 g}{\partial u \partial x}; \quad g_u = \frac{\partial g}{\partial u}; \quad g_{uu} = \frac{\partial^2 g}{\partial^2 u}; \quad f_u = \frac{\partial f}{\partial u}; \quad f_x = \frac{\partial f}{\partial x}; \quad f_{ux} = \frac{\partial^2 f}{\partial u \partial x}; \quad f_{uu} = \frac{\partial^2 f}{\partial^2 u};$$

In our case,

$$g(t, x(t), u(t)) = u;$$

$$f(t, x(t), u(t)) = \frac{rux}{u+L} \left(1 - \frac{x}{a+u}\right).$$

We obtain the partial derivative for each component, as shown below:

$$g_x = g_{ux} = g_{uu} = 0; \quad g_u = 1;$$

$$f_x = -\frac{r(2L_0x - au + 2ux)}{a(L+u)}; \quad f_u = \frac{L_0rx^2 + Lrx(a-x)}{a(L+u)^2};$$

$$f_{ux} = \frac{2L_0rx + Lr(a-2x)}{a(L+u)^2}; \quad f_{uu} = -\frac{2L_0rx^2 + 2Lrx(a-x)}{a(L+u)^3}.$$

Finally, based on the formula in equation (6.6), we can get the ODE for the function of optimal nutrition intake in equation (6.7).

$$\dot{u} = \frac{L_0rx(L+u)}{2(La - Lx + L_0x)} \quad (6.7)$$

With appropriate boundary conditions, we can solve the dynamic system to get the optimal solution of daily nutrition intake for the hybrid model of both singleton and twins. The detailed results are shown in the following sections.

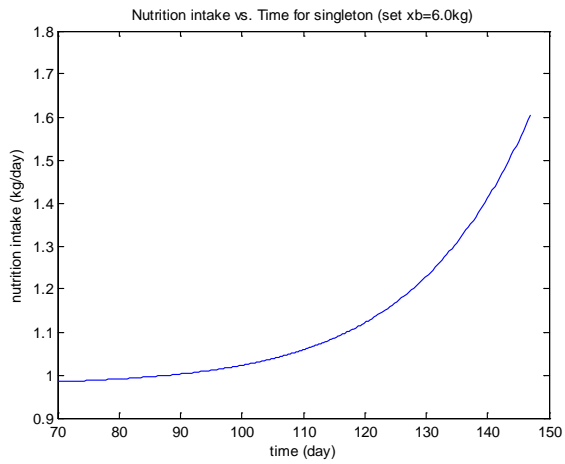
### 6.2.1 Singletons

Pre-determined birth weight [ $x_b$ ] (kg)	Optimal daily nutrition intake [ $u$ ] (kg/day)	
	$u(t_0)$	$u(t_b)$
6.0	0.9845	1.6047
6.5	1.1888	2.0043
7.0	1.4796	2.5873
7.5	1.9355	3.5221
8.0	2.7723	5.2721
8.5	4.8736	9.7391

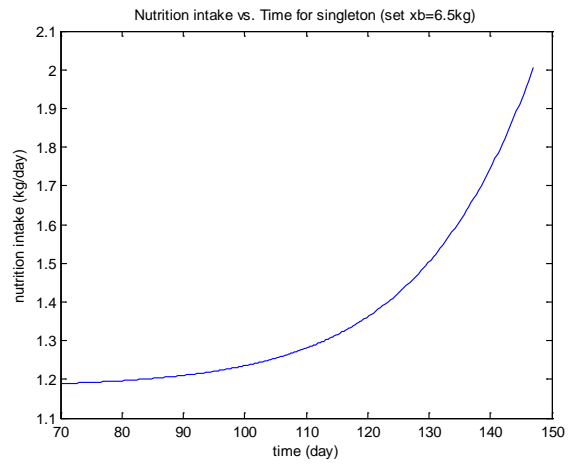
**Table 6. 3: The optimal solution for the hybrid model of singletons**

The table 6.3 shows the optimal solution of daily nutrition intake with various pre-determined birth weights for the hybrid model of singletons. The notation  $u(t_0)$  shows the optimal nutrition intake at the start of the second half of pregnancy, while the notation  $u(t_b)$  shows the optimal nutrition intake at the birth time.

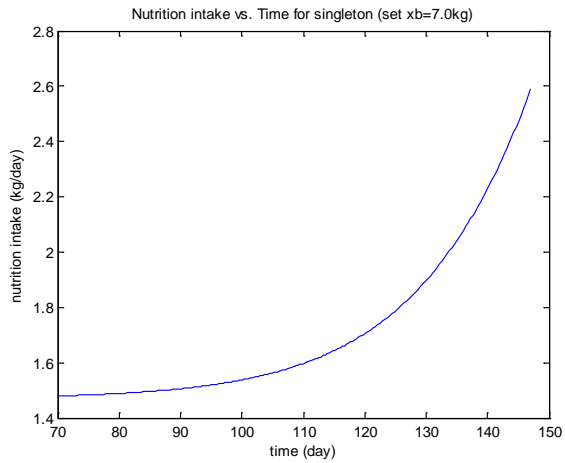
The figure 6.1 - 6.6 show the corresponding plots of optimal solutions. We notice that the curve of optimal nutrition intake is monotonically increasing for each of the pre-determined birth weight. With desirable birth weight of 6.5 kg, the corresponding optimal nutrition intake is from 1.2 kg/day to 2.0 kg/day during the second half of pregnancy. This seems to be reasonable for singletons. However, there is no evidence so far to support the monotonically increasing trend of nutrition intake during the second half of pregnancy. Hence the result of optimal nutrition intake for the hybrid model of singletons is not that convincing.



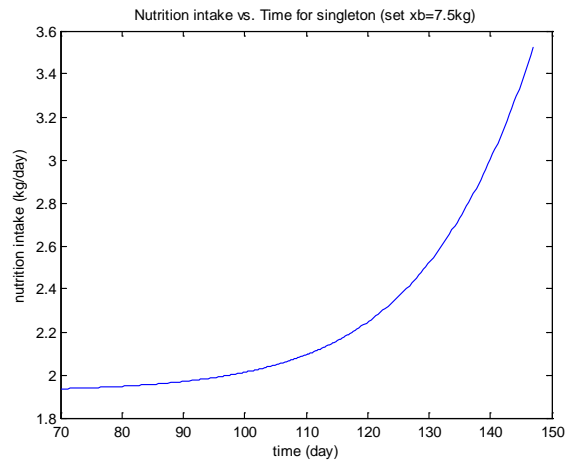
**Figure 6. 1: Optimal solution for the hybrid model of singletons ( $xb=6.0$  kg)**



**Figure 6. 2: Optimal solution for the hybrid model of singletons ( $xb=6.5$  kg)**



**Figure 6. 3: Optimal solution for the hybrid model of singletons ( $xb=7.0$  kg)**



**Figure 6. 4: Optimal solution for the hybrid model of singletons ( $xb=7.5$  kg)**

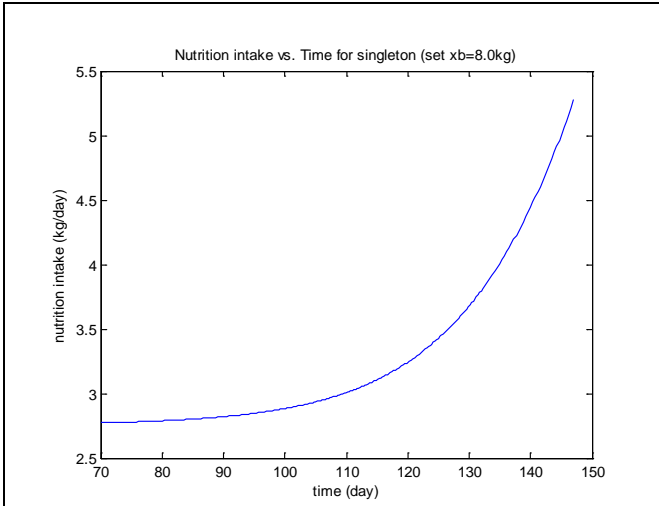


Figure 6. 5: Optimal solution for the hybrid model of singletons ( $x_b=8.0$  kg)

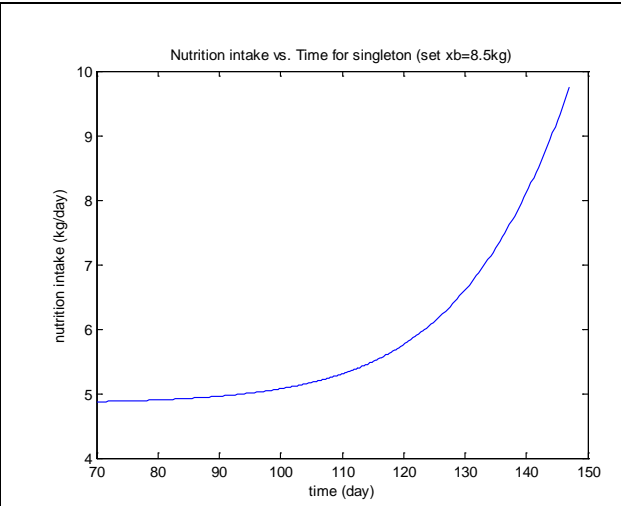


Figure 6. 6: Optimal solution for the hybrid model of singletons ( $x_b=8.5$  kg)

**6.2.2 Twins**

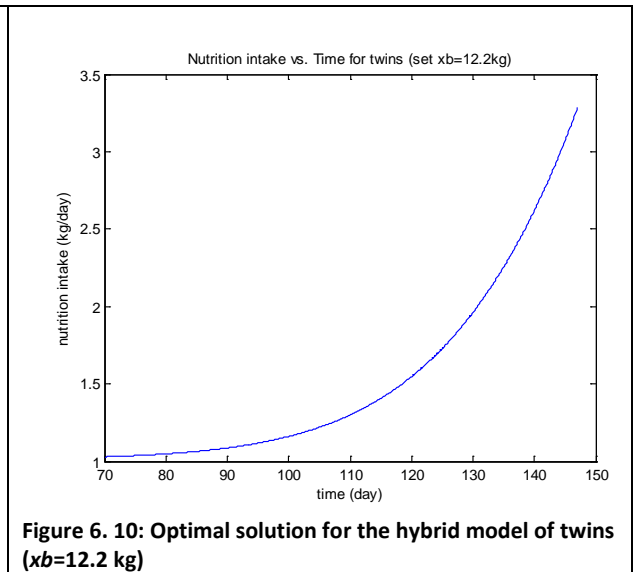
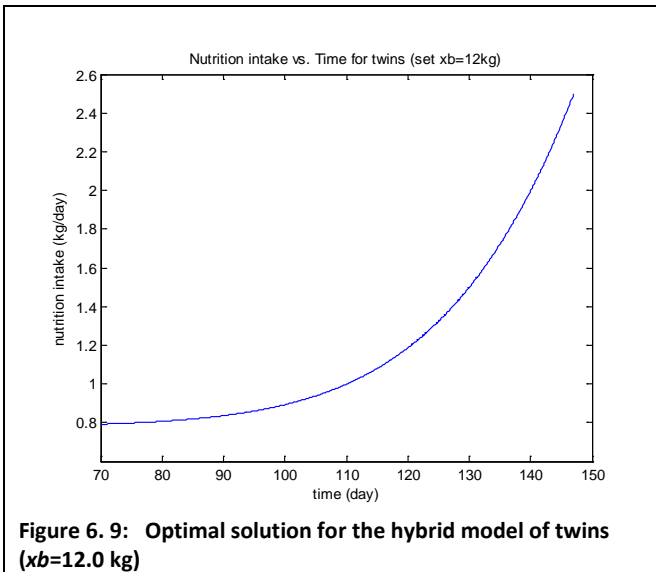
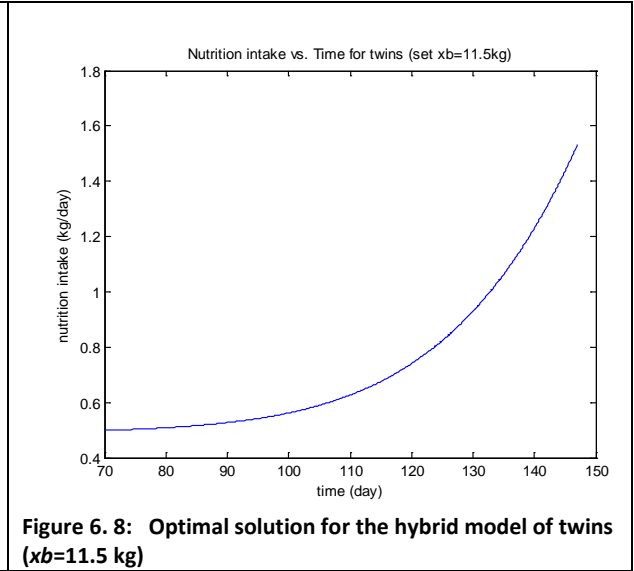
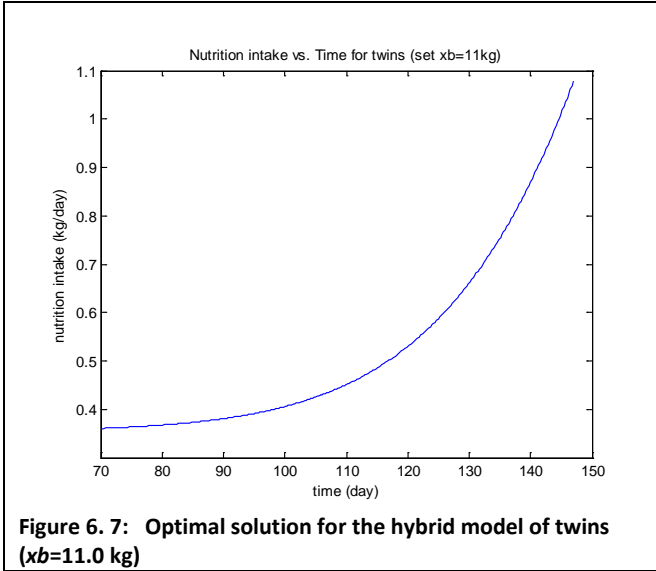
Pre-determined birth weight [ $x_b$ ] (kg)	Optimal daily nutrition intake [ $u$ ] (kg/day)	
	$u(t_0)$	$u(t_b)$
11.0	0.3605	1.0782
11.5	0.4990	1.5322
12.0	0.7918	2.4975
12.2	1.0289	3.2811
12.5	1.8512	6.0021

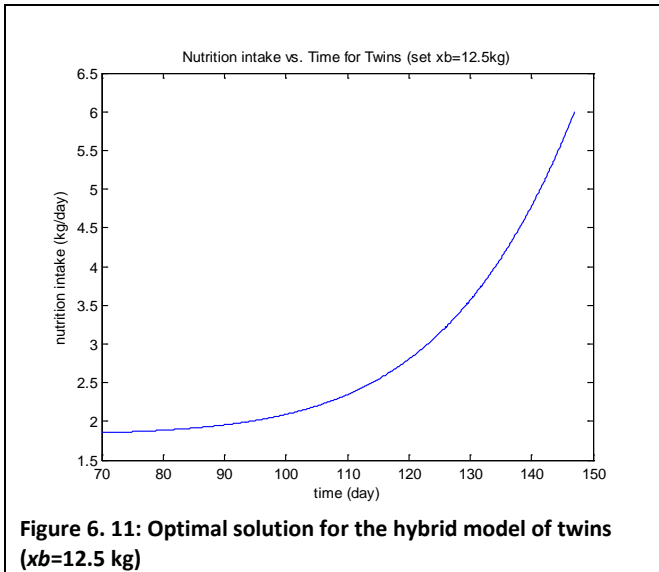
Table 6. 4: The optimal solution for the hybrid model of twins

The table 6.4 summarises the optimal nutrition intake for hybrid model of twins with various pre-determined birth weight. The corresponding plots are shown in figure 6.7 – 6.11. The plots suggest that the optimal nutrition intake for twins is monotonically increasing during the second half of pregnancy. The desirable total birth weight for twins is 12.0 kg, and the corresponding optimal solution is between 0.79 kg/day and 2.50 kg/day. The maintenance level of nutrition intake for twins is around 1.26 kg/day from Rattray’s experiments (1974). However, the optimal nutrition intake at the start of the second half of pregnancy seems to be



a bit low at 0.79 kg/day. The monotonically increasing trend of optimal nutrition intake during the second half of pregnancy also makes the optimal solution seem to be unconvincing.

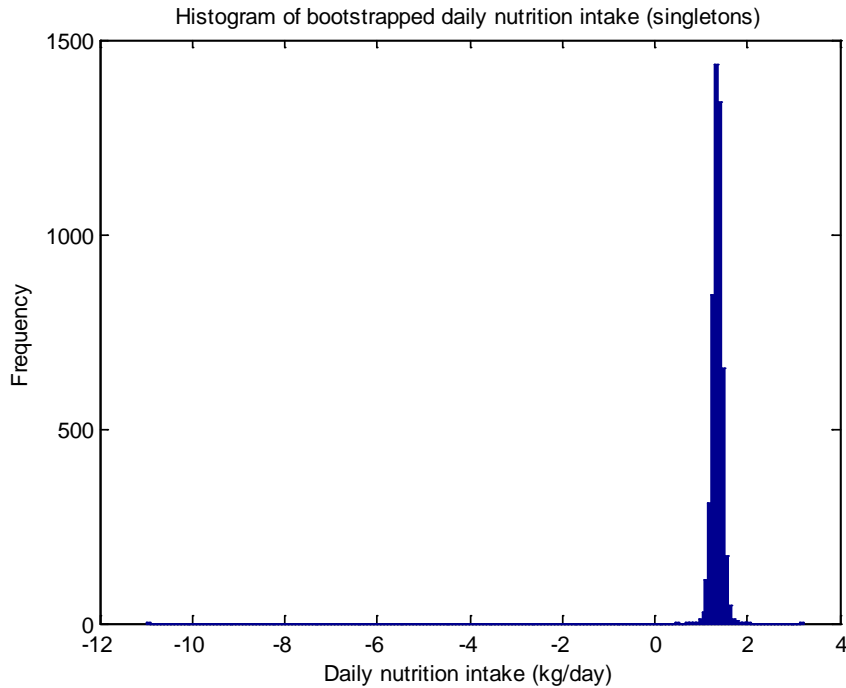




### 6.3 Simulations for optimal daily nutrition intakes

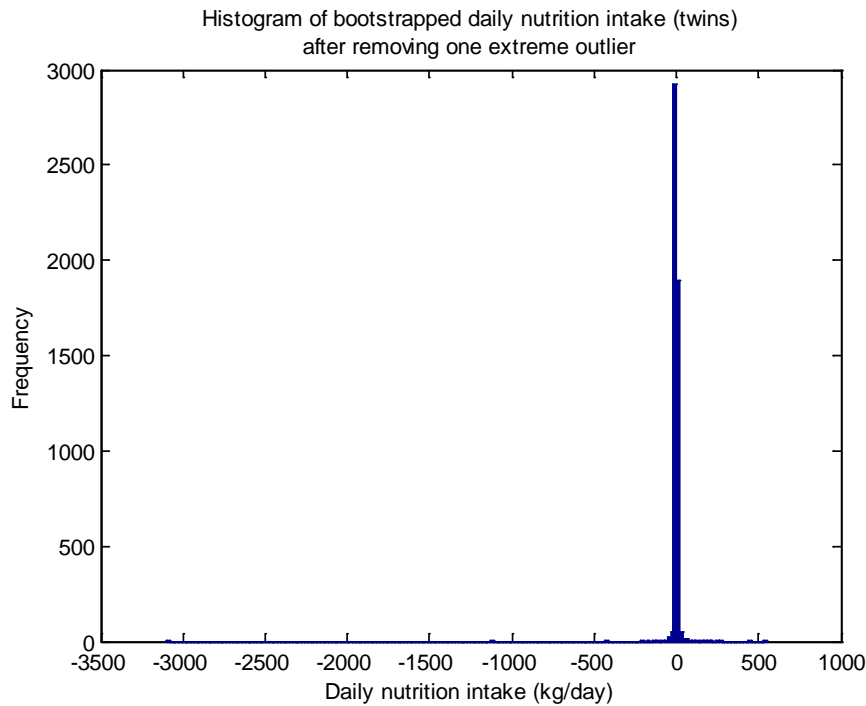
Previous sections suggest that the cut-down model provides reasonable optimal daily nutrition intakes for pregnant sheep carrying both singletons and twins. In this section, we will present the simulations for the optimal daily nutrition intakes based on the cut-down model.

The analytical formula for calculating the optimal daily nutrition intake based on the cut-down model was shown in equation (6.5). As we discussed previously in chapter 5, we did bootstrap simulations to test the reliability of each model parameter. We had 5000 bootstrapped values for each model parameter. Then we substituted these bootstrapped parameter values in the equation (6.5), with desirable birth weight of 6.5 kg for singletons, and 12 kg for twins, to get simulated optimal daily nutrition intakes. The distributions of simulated optimal daily nutrition intakes for singletons and twins are shown in figure 6.12 and 6.13.



**Figure 6. 12: Histogram of bootstrapped daily nutrition intake for singletons**

The figure 6.12 shows the distribution of bootstrapped daily nutrition intake for singletons. It shows two obviously outliers, one is at -10.97 and the other is at 3.21. As we discussed previously, the feasible value of the optimal nutrition intake for pregnant sheep should be strictly positive. Adopting the simple percentile method to obtain the 95% confidence interval, the upper and lower confidence limits are 1.55 and 1.14 kg/day respectively. The optimal nutrition intake based on the original dataset is 1.39 kg/day, which is very close to the centre of the confidence interval. This suggests that the optimal daily nutrition intake of 1.39 kg/day for singletons is reliable and unbiased.



**Figure 6. 13: Histogram of bootstrapped daily nutrition intake for twins (after removing one extreme outlier)**

The figure 6.13 shows the histogram of bootstrapped daily nutrition intake for twins after removing one extreme outlier (-88245). It shows a small proportion of values outside the feasible range of daily nutrition intake. The daily nutrition intake should be strictly positive. Based on some animal science literature, we believe that the optimal daily intake should be less than 3 kg/day for twins. In this case, the non-feasible daily nutrition intake may due to the sensitivity of the analytical formula. Based on this analytical formula, the optimal solution is heavily dependent on the parameter values, as well as the desirable birth weight. As we known, the sample size of the original dataset for twins is quite small, which is around 20 data points for low and high nutrition each. This may lead to the simulated daily nutrition intakes having a large variation based on the bootstrapped parameters. Thus, it does not give a sensible confidence interval.

The figure 6.14 shows the histogram of bootstrapped daily nutrition intake for twins with feasible range between 0 and 3 kg/day. We truncate the non-feasible simulated daily nutrition intakes at both ends. The histogram shows a right-skewed distribution. Seventy per cent of the 5000 simulated daily nutrition intakes are in the feasible range. This indicates that future work needs to be done on finding a better method for estimating confidence intervals for optimal daily nutrition intakes. At the moment about the best we can do is state that, based on quantiles of the bootstrapped estimates, a 50% confidence interval for the optimal daily intake is between 1.23 and 2.47 kg/day.

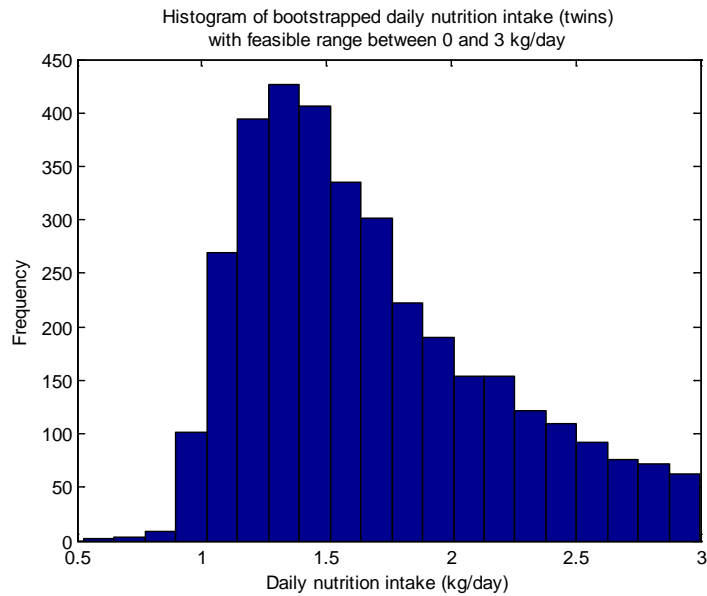


Figure 6. 14: Histogram of bootstrapped daily nutrition intake for twins with feasible range between 0 and 3 kg/day

## 6.4 Summary

In this chapter, we discussed the optimal daily nutrition intake function during the second half of pregnancy for sheep, according to the cut-down model and the hybrid model. We also illustrated the process for obtaining the optimal solutions step by step.

The results suggest that the cut-down model provides the appropriate solutions of constant optimal daily nutrition intake during the second half of pregnancy for sheep. The simulations indicate that the cut-down model is more applicable for the datasets of singletons than those for twins. The optimal solutions are summarised in table 6.5. With pre-determined desirable birth weight for singletons and twins, namely 6.5 kg and 12.0 kg respectively, the corresponding optimal daily nutrition intake is 1.36 kg/day and 1.93 kg/day for singleton and twins accordingly.

Those optimal solutions do not contradict the maintenance level of nutrition intake provided by literature. The maintenance level of nutrition intake from Rattray's experiments (1974) is 1.21 kg/day and 1.26 kg/day for singleton and twins respectively. Our optimal solutions suggest that the pregnant sheep have more nutrition intake in late gestation than in early gestation. In addition, it also indicates that the pregnant sheep carrying twins need a higher

level of nutrition intake than those carrying singletons. Those explanations seem to agree with common sense.

However, an important point worth noting is that the drop in sheep intake close to the time of birth is generally due to restriction of the rumen by the growing fetus, and twins cause more restriction than singletons. This means our model is a simplification of the biology, but with this proviso we believe the optimal results in table 6.5 provide us the feasible and plausible solutions.

Type	Pre-determined birth weight [ $x_b$ ] (kg)	Optimal daily nutrition intake [ $u$ ] (kg/day)
Singleton	6.5	1.36
Twins	12.0	1.93

**Table 6. 5: Summary of the best optimal solutions**

# Chapter 7

## 7. Conclusions and Future Study

This chapter focuses on conclusions for this study. A process has been developed for estimating the parameters for a dynamic optimal control model. The goal was to estimate the optimal daily nutrition intake for pregnant sheep during the second half of pregnancy. To achieve this goal, the software MATLAB was used to produce a comprehensive ‘black-box’ algorithm. The algorithm provides us the optimal solutions from the dynamic model applied to real data. In the following sections, we will summarise the main results, and also make some recommendations for the future research.

### 7.1 Conclusions

The ultimate goal of this study was to obtain the optimal daily nutrition intake for pregnant sheep during the second half of pregnancy, and with pre-determined desirable birth weight of offspring. The processes of achieving the primary objective of this study can be summarised into two steps. Firstly, estimation of model parameters; and secondly, obtaining optimal numerical solutions.

The results from parameter estimation suggest that weighted least-squares provides an adequate objective function for optimisation of model parameters. However, as in our example, different weights may be needed for modelling the datasets of singletons and twins to achieve the best model fitting for each.

We failed to estimate accurate values of the parameters from the original full model, since the dynamic model structure was so complicated, with too many unknown model parameters. The full model was non-identifiable for both singletons and twins data. The structure of the full model had been simplified to form alternative cut-down and hybrid models to achieve the model identifiability. The cut-down model could always achieve model identifiability with any initial guesses provided. However, the hybrid model could achieve the model identifiability only when the initial guesses are carefully chosen.

In addition, five-thousand bootstrap simulations were used to verify that the cut-down model was more stable than the hybrid model. Each estimated parameter value from the cut-down model sat well within the 95% bootstrapped confidence interval. Moreover, the bootstrapped mean was very close to the estimated value of each parameter. Hence we confidently conclude that the estimated parameters from the cut-down model are reliable, by contrast with the hybrid model.

The whole dynamic system of the cut-down model and estimated parameter values were used to obtain the optimal daily nutrition intake to achieve the target birth weight. The result suggested that the appropriate daily nutrition intake function for the cut-down model is a constant. With appropriate boundary conditions, we can estimate the optimal daily nutrition intake for pregnant sheep carrying singletons or twins during the second half of pregnancy. The results indicate that with pre-determined desirable birth weights 6.5 kg and 12.0 kg for singletons and twins respectively, the corresponding optimal daily nutrition intakes are 1.36 kg/day and 1.93 kg/day for singletons and twins accordingly.

Those optimal solutions do not contradict the maintenance level of nutrition intake suggested by the animal science literature. In addition, the optimal solutions suggest that the pregnant sheep carrying twins should have more nutrition intake than those carrying singletons, which meets our expectation with common sense. Thus we believe that those optimal solutions are convincing.

## **7.2 Future Research Direction**

As discussed previously in the thesis, the datasets used for this study are from the animal science literature. We have limited data points available for nutrition intake. In addition, we had to read off the data points associated with fetal weight, and corresponding time points, from a plot. To some extent, this may affect the accuracy of the results. Collecting more time series data points with corresponding fetal weight and especially nutrition intakes may provide more accurate and reliable results.

Parameter estimation for the fetal weight model was done by conditioning on the assumed polynomial model for nutrition intake. This ignores the errors in nutrition intake and the



polynomial assumption is strong one. Future work could consider simultaneously modelling the nutrition intake and the fetal growth. In addition, low-order natural splines, or even linear interpolation, might work better than using polynomial model for nutrition intake.

Nonlinear regression estimates can be severely biased, and attention needs to be given to this problem. The problem of bias can also be addressed by constructing a data set using the model of interest with known values for the model parameters, adding various degrees of noise and showing how well the parameters are recovered.

In this study, we used different weights for singleton and twin data to perform the parameter estimation based on the weighted least-squares. The weights for singletons and twins are  $\frac{1}{x_i}$  and  $\frac{1}{x_i^2}$  respectively. The weights were determined by the dataset used, as well as the model diagnostics. In future studies, the weight can be expressed in a generic form as  $\frac{1}{x_i^p}$ . For the different datasets used, we can always adopt that generic weight. The only thing we need to do is to determine the value of power  $p$ . The Breusch-Pagan test (Breusch & Pagan, 1979) may be required to assess the adequacy of the choice for value  $p$ , instead of visual checking on the plots of model diagnostics. Moreover, the general format of weight can be built into our ‘black-box’ algorithm to make it more comprehensive in the future.

The bootstrapping was certainly useful for investigation the stability of the estimation, leading to some useful insights. The ‘semi-parametric’ bootstrap, in which the residuals from the fitted model are resampled as described in section 2.3, could be considered in the future work. This might be more appropriate as the data are from a planned experiment in which the time values were approximately chosen by design. It is well-known that for fitting nonlinear models the available values of the predictor variable can be very influential on successful parameter estimation. In particular a model postulating an upper asymptote (e.g. logistic growth) would require values reasonably close to the asymptote – Figure 1 of Rattray et al. (1974b) raises immediate concerns in this regard. It is not surprising then that some of the bootstrap datasets will omit the most useful points and seriously degrade the performance.

The comprehensive ‘black-box’ algorithm is especially developed for obtaining the optimal daily nutrition intake for pregnant sheep carrying singletons or twins. However, the algorithm is generic and can be easily applied to triplets or higher order multiple births, or with other species to achieve a better birth outcome. It is given a time series of weight and maternal

nutrition, to estimate the parameters as discussed in Chapter 4, then using these to determine the optimal maternal nutrition to obtain a pre-determined birth weight.

## Appendix

In this section, we will present the programming codes for the ‘black-box’ algorithm. The algorithm was written in Matlab. The logical process and relevant Matlab m.files are shown below.

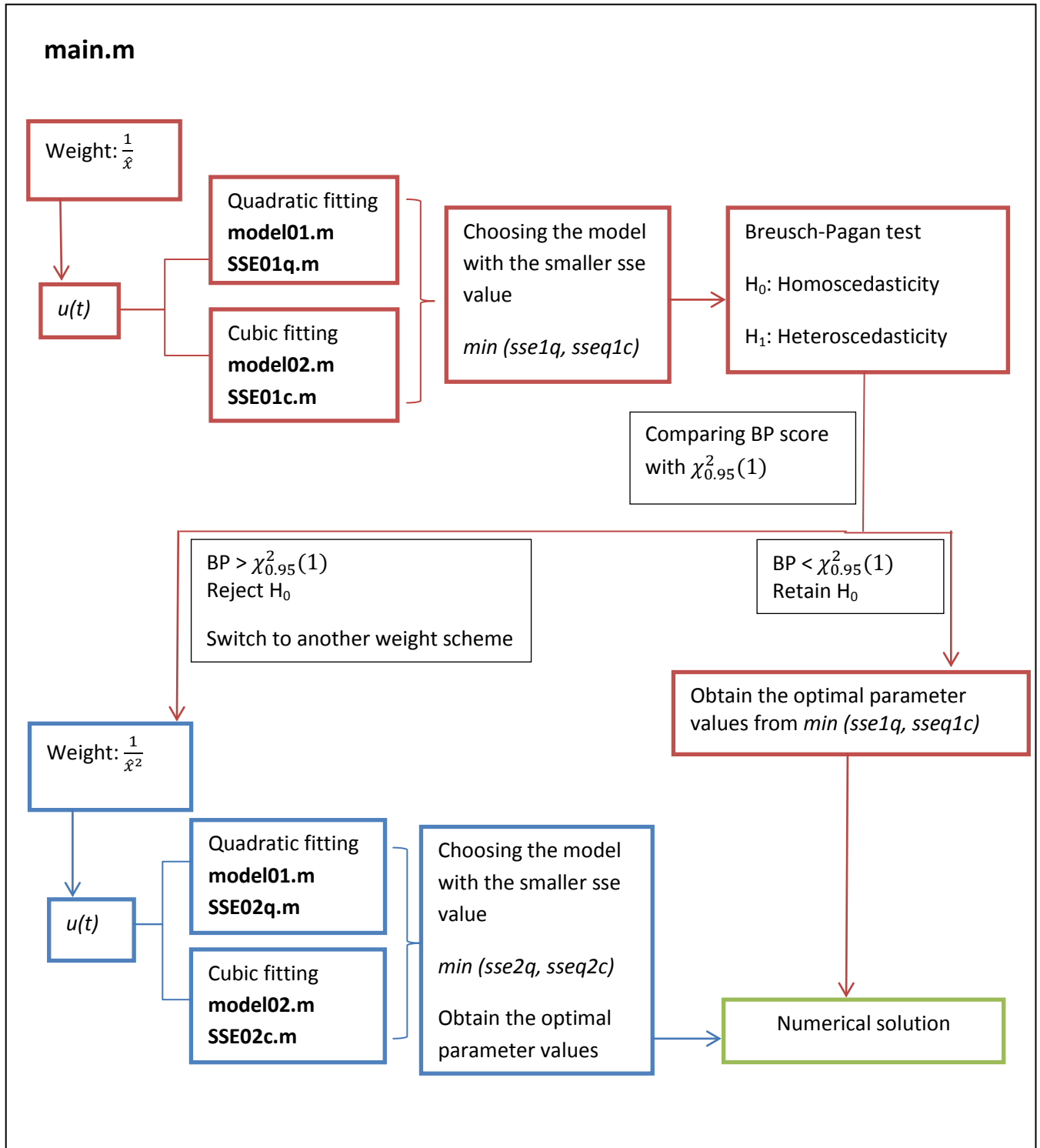


Figure A. 1: The logical process flow chart for Black-box algorithm

## main.m

```
%%% main coding %%%

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%% Part I
%%% PARAMETER ESTIMATION
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%clear everything
clear all

% *** value needed ***
%providing initial guesses for each unknown parameter
R=-2.6;Lt=-2.5;k1=2.5;x0=0.3;

%putting those initial guesses in vector 'c'
c=[R,Lt,k1,x0];

%changing the default settings
options = optimset('MaxFunEvals',10000,'MaxIter',10000);

%obtain the final value of each parameter and final SSE
[c01q sselq]=fminsearch('SSE01q',c, options);
[c01c sselc]=fminsearch('SSE01c',c, options);

%Comparing the model fit (quadric u(t) vs. cubic) with same weight
%adopt the model parameters with the smaller SSE value
if sselq < sselc
    c0=c01q;
else
    c0=c01c;
end

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Breusch-Pagan test
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% using pre-determined parameter values
R=c0(1); Lt=c0(2); k1=c0(3); x0=c0(4);

% *** load dataset ***
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% #Ratray Twins data
load rtwinH02.mat
load rtwinL02.mat
% *** value needed ***
% define the starting and ending time period.
% t0=70; tb=147; with 100 equal spaced points
T=linspace(70,147,100);

%adding an extra data point as the starting point for each level
%with corresponding datasets
fwh = rtwinH02(:,2);
fwh = [x0; fwh];
Th= rtwinH02(:,1);
Th=[70;Th];
fwl = rtwinL02(:,2);
fwl = [x0; fwl];
```

```

Tl= rtwinL02(:,1);
Tl=[70;Tl];

%define the initial conditions; i.e. x(t0)=x0
x_int(1) = x0; x_int(2) = x0;

%express model function in an Anonymous Function: modelfun
%choose the appropriate model
if sselq < sselc
    modelfun=@(t,x) model01(t,x,R,Lt,k1);
else
    modelfun=@(t,x) model02(t,x,R,Lt,k1);
end

%solve the model function
%store the solutions in variable 'sol'
sol=ode45(modelfun,T,x_int);

%obtaining the fitted values with pre-determined time points
Sxint_h=deval(sol, Th);
Sxint_l=deval(sol, Tl);

%Weighted Least-squares - weight: 1/xhat
%calculate modified residuals
LresidH = (fwh-Sxint_h(1,:))./sqrt(Sxint_h(1,:));
LresidL = (fwl-Sxint_l(2,:))./sqrt(Sxint_l(2,:));

%calculate SSE for each level
Lresid_sqH = LresidH.^2;
Lresid_sqL = LresidL.^2;
%total SSE
val = sum(Lresid_sqH)+sum(Lresid_sqL);

%each residual-squares
res_sq= [Lresid_sqH;Lresid_sqL];

%each xfit
xfit= [Sxint_h(1,:); Sxint_l(2,:)]';

%regress res_sq vs. xfit
p=polyfit(xfit,res_sq,1);
%calculate yhat
yhat = polyval(p,xfit);
%calculate ybar
ybar = mean(res_sq);

%calculate SSRegn
SSRegn= sum((yhat-ybar).^2);

%calculate sigma_sq
sigma_sq= val/length(res_sq);

%calculate B-P statistic
BP= SSRegn/(2*sigma_sq.^2);

%chi-sq statistic: p=0.95, df=1;
chi_sq= chi2inv(0.95,1);

```

```

% B-P test (compare BP with chi_sq)
% if BP<chi_sq, retain H0; it means constant variance
% if BP>chi_sq, reject H0; it means that the heteroscedasticity exists

```

```

if BP < chi_sq
    c0;
else
    [c02q sse2q]=fminsearch('SSE02q',c, options);
    [c02c sse2c]=fminsearch('SSE02c',c, options);
    if sse2q < sse2c
        c0=c02q;
    else
        c0=c02c;
    end
end

```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%% Part II
%%% NUMERICAL SOLUTIONS
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% solve it analytically
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

```

% using pre-determined parameter values
R=c0(1); Lt=c0(2); k1=c0(3); x0=c0(4);

```

```

% *** value needed ***
% time period: t=tb-t0;
t=77;
% *** value needed ***
% define the desirable birth weight
xb=12;

```

```

% analytical solution
s=x0*(exp(k1)-xb)/(xb*(exp(k1)-x0));
u=-log(s)*exp(Lt)/(exp(R)*t+log(s));

```

```

% display the optimal value of daily nutrition intake
disp(u);

```

```

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

```

## model01.m

```
function xdot=model01(t,x,R,Lt,k1)
%cut-down model for parameter estimation
%quadratic u(t) function
%xH=xdot(1); xL=xdot(2);

% *** value needed ***
% provide available data points for nutrition intake and corresponding time
% #Ratray Twins data
t0=[70; 100; 125; 140];
ul=[1.262; 1.425; 1.367; 1.648];
uh=[1.262; 1.869; 1.944; 1.919];

% quadratic fitting
pl = polyfit(t0,ul,2);
ph = polyfit(t0,uH,2);
% rounding the coefficients of nutrition intake function to 7dp
rpl = round(pl*10000000)/10000000;
rph = round(ph*10000000)/10000000;

% using the rounded coefficient of u(t)
xdot=[exp(R)*( rph(1)*t^2+ rph(2)*t + rph(3))*x(1)/ ...
      ((rph(1)*t^2+rph(2)*t+rph(3))+exp(Lt))*(1-x(1) ...
      /exp(k1));
      exp(R)*( rpl(1)*t^2+ rpl(2)*t + rpl(3))*x(2)/ ...
      (( rpl(1)*t^2+ rpl(2)*t+ rpl(3))+exp(Lt))*(1-x(2) ...
      /exp(k1))];
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

## model02.m

```
function xdot=model02(t,x,R,Lt,k1)
%cut-down model for parameter estimation
%cubic u(t) function
%xH=xdot(1); xL=xdot(2);

% *** value needed ***
% provide available data points for nutrition intake and corresponding time
% #Ratray Twins data
t0=[70; 100; 125; 140];
ul=[1.262; 1.425; 1.367; 1.648];
uh=[1.262; 1.869; 1.944; 1.919];

% cubic fitting
pl = polyfit(t0,ul,3);
ph = polyfit(t0,uH,3);
% rounding the coefficients of nutrition intake function to 7dp
rpl = round(pl*10000000)/10000000;
rph = round(ph*10000000)/10000000;
% using the rounded coefficient of u(t) (6dp)
xdot=[exp(R)*(rph(1)*t^3+ rph(2)*t^2+ rph(3)*t + rph(4))*x(1)/ ...
      ((rph(1)*t^3+ rph(2)*t^2+ rph(3)*t + rph(4))+exp(Lt))*(1-x(1) ...
      /exp(k1));
      exp(R)*( rpl(1)*t^3+ rpl(2)*t^2+ rpl(3)*t + rpl(4))*x(2)/ ...
      (( rpl(1)*t^3+ rpl(2)*t^2+ rpl(3)*t + rpl(4))+exp(Lt))*(1-x(2) ...
      /exp(k1))];
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

## SSE01q.m

```
function val= SSE01q(c)
%objective function for black-box algorithm
%SSE for cut-down model (weight: 1/xhat ; u(t): quadratic)
% minimising x(high)and x(low) simultaneously, then sum up their
% individual SSE.

% define the vector c of unknown parameters
R=c(1); Lt=c(2); k1=c(3); x0=c(4);

% *** dataset needed ***
% load available dataset for fetal weight and corresponding time
% #Rattray Twins data
load rtwinH02.mat
load rtwinL02.mat
% *** value needed ***
%define the starting and ending time period
T=linspace(70,147,100);
%adding an extra data point as the starting point for each level
%with corresponding datasets
fwh = rtwinH02(:,2);
fwh = [x0; fwh];
Th= rtwinH02(:,1);
Th=[70;Th];
fwl = rtwinL02(:,2);
fwl = [x0; fwl];
Tl= rtwinL02(:,1);
Tl=[70;Tl];

%define the initial conditions; i.e. x(t0)=x0
x_int(1) = x0; x_int(2) = x0;

%express model function in an Anonymous Function: modelfun
%model with quadratic fitting u(t)
modelfun=@(t,x) model01(t,x,R,Lt,k1);

%solve the model function
%store the solutions in variable 'sol'
sol=ode45(modelfun,T,x_int);

%obtaining the fitted values with pre-determined time points
Sxint_h=deval(sol, Th);
Sxint_l=deval(sol, Tl);

%weight: 1/xhat
%calculate modified residuals
LresidH = (fwh-Sxint_h(1,:))./sqrt(Sxint_h(1,:));
LresidL = (fwl-Sxint_l(2,:))./sqrt(Sxint_l(2,:));
%calculating SSE
Lresid_sqH = LresidH.^2;
Lresid_sqL = LresidL.^2;
%total SSE (objective function)
val = sum(Lresid_sqH)+sum(Lresid_sqL);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```



## SSE01c.m

```
function val= SSE01c(c)
%objective function for black-box algorithm
%SSE for cut-down model (weight: 1/xhat; u(t): cubic)
%minimising x(high)and x(low) simultaneously, then get total SSE.

% define the vector c of unknown parameters
R=c(1); Lt=c(2); k1=c(3); x0=c(4);

% *** dataset needed ***
% load available dataset for fetal weight and corresponding time
% #Rattray Twins data
load rtwinH02.mat
load rtwinL02.mat
% *** value needed ***
%define the starting and ending time period
T=linspace(70,147,100);
%adding an extra data point as the starting point for each level
%with corresponding datasets
fwh = rtwinH02(:,2);
fwh = [x0; fwh];
Th= rtwinH02(:,1);
Th=[70;Th];
fwl = rtwinL02(:,2);
fwl = [x0; fwl];
Tl= rtwinL02(:,1);
Tl=[70;Tl];

%define the initial conditions; i.e. x(t0)=x0
x_int(1) = x0; x_int(2) = x0;

%express model function in an Anonymous Function: modelfun
%model with cubic fitting u(t)
modelfun=@(t,x) model02(t,x,R,Lt,k1);

%solve the model function
%store the solutions in variable 'sol'
sol=ode45(modelfun,T,x_int);

%obtaining the fitted values with pre-determined time points
Sxint_h=deval(sol, Th);
Sxint_l=deval(sol, Tl);

%weight: 1/xhat
%calculate modified residuals
LresidH = (fwh-Sxint_h(1,:))./sqrt(Sxint_h(1,:));
LresidL = (fwl-Sxint_l(2,:))./sqrt(Sxint_l(2,:));
%calculating SSE
Lresid_sqH = LresidH.^2;
Lresid_sqL = LresidL.^2;
%total SSE (objective function)
val = sum(Lresid_sqH)+sum(Lresid_sqL);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

## SSE02q.m

```
function val= SSE02q(c)
%objective function for black-box algorithm
%SSE for cut-down model (weight: 1/(xhat)^2; u(t): quadratic)
%minimising x(high)and x(low) simultaneously, then obtain total SSE.

% define the vector c of unknown parameters
R=c(1); Lt=c(2); k1=c(3); x0=c(4);

% *** dataset needed ***
% load available dataset for fetal weight and corresponding time
% #Rattray Twins data
load rtwinH02.mat
load rtwinL02.mat

% *** value needed ***
%define the starting and ending time period
T=linspace(70,147,100);

%adding an extra data point at the starting point for each level
%with corresponding datasets
fwh = rtwinH02(:,2);
fwh = [x0; fwh];
Th= rtwinH02(:,1);
Th=[70;Th];
fwl = rtwinL02(:,2);
fwl = [x0; fwl];
Tl= rtwinL02(:,1);
Tl=[70;Tl];

%define the initial conditions; i.e. x(t0)=x0
x_int(1) = x0; x_int(2) = x0;

%express model function in an Anonymous Function: modelfun
modelfun=@(t,x) model01(t,x,R,Lt,k1);

%solve the model function
%store the solutions in variable 'sol'
sol=ode45(modelfun,T,x_int);

%obtaining the fitted values with pre-determined time points
Sxint_h=deval(sol, Th);
Sxint_l=deval(sol, Tl);

%weight: 1/xhat^2
%calculate modified residuals
LresidH = fwh./Sxint_h(1,:)-1;
LresidL = fwl./Sxint_l(2,:)-1;
%calculating SSE
Lresid_sqH = LresidH.^2;
Lresid_sqL = LresidL.^2;
%total SSE (objective function)
val = sum(Lresid_sqH)+sum(Lresid_sqL);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

## SSE02c.m

```
function val= SSE02c(c)
%objective function for black-box algorithm
%SSE for cut-down model (weight: 1/(xhat)^2; u(t): cubic)
%minimising x(high)and x(low) simultaneously, then obtain total SSE.

% define the vector c of unknown parameters
R=c(1); Lt=c(2); k1=c(3); x0=c(4);

% *** dataset needed ***
% load available dataset for fetal weight and corresponding time
%#Rattray Twins data
load rtwinH02.mat
load rtwinL02.mat

% *** value needed ***
%define the starting and ending time period
T=linspace(70,147,100);

%adding an extra data point at the starting point for each level
%with corresponding datasets
fwh = rtwinH02(:,2);
fwh = [x0; fwh];
Th= rtwinH02(:,1);
Th=[70;Th];
fwl = rtwinL02(:,2);
fwl = [x0; fwl];
Tl= rtwinL02(:,1);
Tl=[70;Tl];

%define the initial conditions; i.e. x(t0)=x0
x_int(1) = x0; x_int(2) = x0;

%express model function in an Anonymous Function: modelfun
modelfun=@(t,x) model02(t,x,R,Lt,k1);

%solve the model function
%store the solutions in variable 'sol'
sol=ode45(modelfun,T,x_int);

%obtaining the fitted values with pre-determined time points
Sxint_h=deval(sol, Th);
Sxint_l=deval(sol, Tl);

%Weighted Least-squares
%weight: 1/xhat^2
%calculate modified residuals
LresidH = fwh./Sxint_h(1,:)-1;
LresidL = fwl./Sxint_l(2,:)-1;
%calculating SSE
Lresid_sqH = LresidH.^2;
Lresid_sqL = LresidL.^2;
%total SSE (objective function)
val = sum(Lresid_sqH)+sum(Lresid_sqL);

%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
```

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