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**A systems biology approach to bovine fertility
and metabolism:
Development of a glucose insulin model.³**

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Abstract

To counteract the antagonistic relationship between milk yield and fertility in dairy cow, a deeper understanding of the underlying biological mechanisms is required. For this purpose, we study physiological networks related to reproduction and metabolism in dairy cows. We interactively develop dynamic, mechanistic models by fitting the models to experimental data and mechanistic knowledge. We have already developed models for potassium balance and hormonal regulation of fertility in the dairy cow, which will briefly be reviewed here. The main focus of this article is a glucose-insulin model currently developed by us. This model links the bovine hormonal cycle and the potassium balance to glucose and thus to energy metabolism. The models can be applied in scientific research, education, experimental planning, drug development and production on farms.

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1 Introduction

In dairy cows, concurrent with increasing milk yield, decreasing fertility and increasing metabolic problems have been reported in the last decades [9]. To achieve a deeper understanding of the interactions between nutrition, fertility and the development of diseases we apply a holistic systems biology approach to hormonal regulation, energy and mineral metabolism in the dairy cow. In order to explore and to analyze the underlying biological interactions, we integrate experimental data into predictive, mechanistic, dynamic models.

In an interdisciplinary team with mathematicians and veterinarians, we develop these models on the basis of ordinary differential equations (ODEs), which describe the dynamic feedback mechanisms and alterations of the most relevant involved substances over time. Dynamic, mechanistic ODE models are developed iteratively by fitting parameters to field data and refining modeled processes with mechanistic knowledge according to the interest of application. By using SBML (Systems Biology Markup Language [21]) we make models accessible for a growing community and an increasing variety of software tools.

Currently, we are working with three mechanistic ODE models of physiological networks in dairy cows: a model for the bovine estrous cycle, named BovCycle [1], a model for potassium balance [6], and a recently developed model for the glucose metabolism. The latter will be introduced in this article.

With the BovCycle model the inter-individual variability in the number of follicular waves per estrous cycle has been analyzed, such that parameter sets for individuals under varying conditions could be found applying Fourier analysis [7] and an empirical approach [5]. In the potassium balance model we identified parameters for low potassium cows with an empirical approach [6], exploring the phenomenon that individuals show intracellular potassium concentrations of around either 20 mmol/l or 50 mmol/l [18].

In our work, parameter identification is performed with a Gauss-Newton algorithm called NLSCON [19], which is implemented in the freely available software package BioPARKIN [8] suitable for SBML models. The potassium model was partly developed and experiments were performed with the freely available software CellDesigner [20]. Prospectively, these models can be adapted for versatile applications in research, education, experimental planning, drug development and production on farms.

2 Models

2.1 BovCycle model

The BovCycle model has been built to describe the bovine estrous cycle with its key mechanisms and their regulation [1],[2]. The complete model description, parameters and initial values can be found in Stoetzel et al. 2012 [2]. With the BovCycle model, periodic estrous cycles lasting 21 days can be simulated, describing the growth and the decay of follicles and the corpus luteum (CL) and the key reproductive hormones regulating the system. The BovCycle model consists of 15 ODEs and 60 parameters and is available in SBML at the BioModels database¹. The mechanisms are depicted in Figure 1.

The hormone GnRH is synthesized in the hypothalamus and released into the pituitary. There it stimulates the release of the hormones FSH and LH into the blood. They are affecting the development of the follicles and the CL on the ovaries. The follicles produce estradiol (E2) and inhibin (Inh) and the CL produces progesterone (P4), which are released into the blood, and act locally at the ovaries. They influence GnRH, FSH and LH, and stimulate oxytocin and different enzymes that control the action of PGF2 α . This, together with several intra-ovarian factors, initiates the decay of the CL. These mechanisms are modeled in the BovCycle model as a closed system. No external stimuli are needed for the periodic behavior of the model, which results only

¹<https://www.ebi.ac.uk/biomodels-main/BIOMD000000481>

from the developed dynamics and the parametrization. In one application performed with this model, drug administration of prostaglandin F2 α (PGF2 α) has been successfully simulated [2].

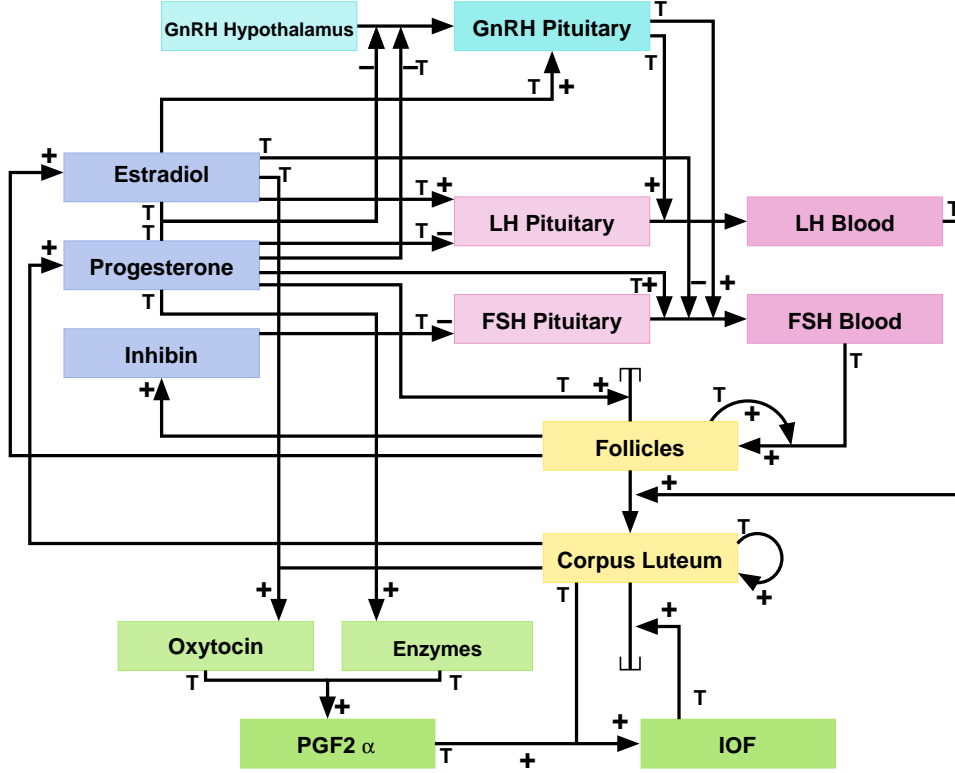


Figure 1: Graphical description of the estrous cycle model BovCycle in the dairy cow [2].

2.2 Potassium model

The model of potassium balance in dairy cows is the basis for computer simulations of potassium intake, distribution in the organism and excretion. The model has been developed and validated with experimental data from the Clinic for Ruminants at Freie Universität Berlin. The mechanisms are depicted in Figure 2. Through the component *DMI* (Dry Matter Intake) an amount of feed is taken in. K_{FEED} is the potassium fraction in the feed. The potassium passes to the digestive tract K_{GIT} and is then resorbed to the extracellular space of the blood K_{ECF} . Physiological extracellular potassium concentrations range between 3.5 and 5.8 mmol/l [22], if values fall outside this range, life-threatening conditions occur. In the model, potassium is distributed between extracellular space K_{ECF} , intracellular space K_{ICF} , and the tissues K_{TISS} . Potassium is excreted via urin K_{URIN} , saliva K_{SAL} , sudor, feces and milk K_{MILK} . Insulin, pH, the hormone Aldosterone, and the potassium amount in the food regulate the system.

2.3 Glucose insulin model

The motivation to build a glucose insulin model was to study the influence of this metabolic network on potassium balance and on hormonal regulation of fertility. Glucose metabolism and homeostasis are of special concern in every mammal, because glucose is a very important and, for some tissues, essential substrate [10]. For the potassium balance model the glucose-insulin metabolism is of special interest due to the interaction between insulin and the shift of potassium from extracellular to intracellular space. The interaction of the glucose-insulin system with the

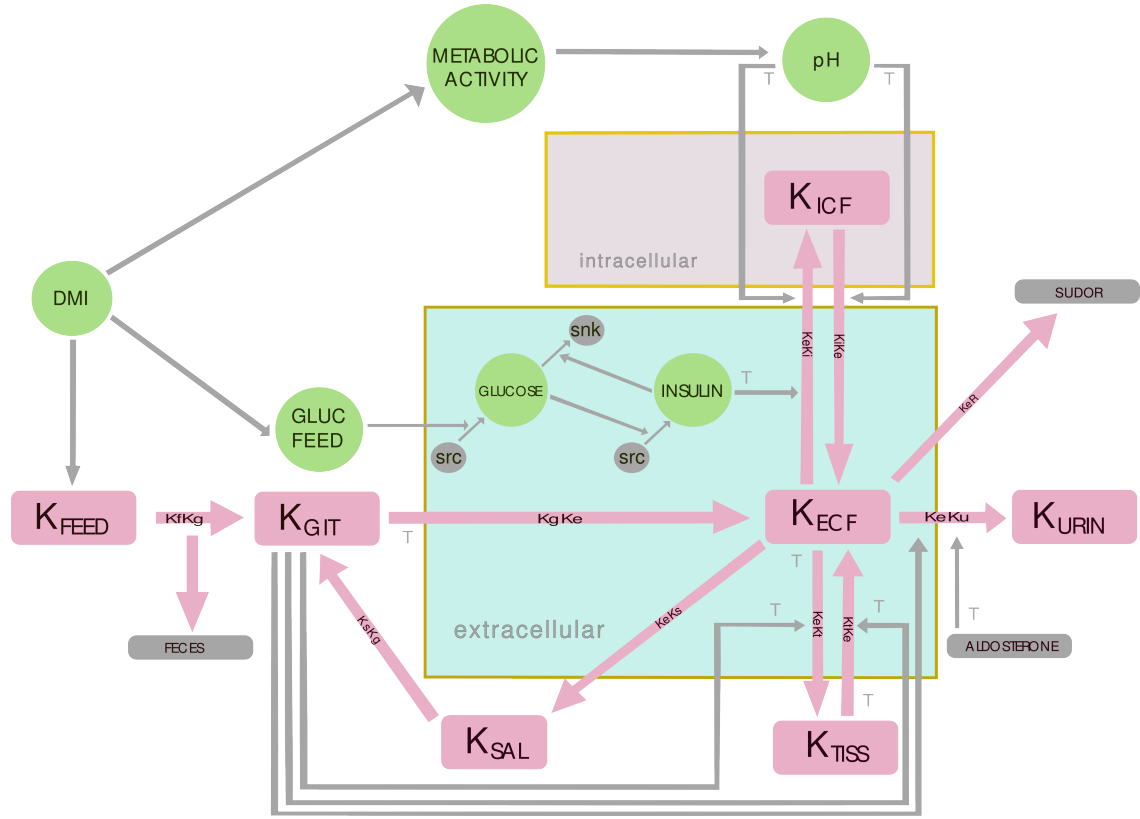


Figure 2: Graphical description of the model for potassium balance in the dairy cow.

hormonal regulation of fertility is characterized by long term effects, where long term food reduction may lead to reversible functional disturbances in follicular development up to anestrus [12]. There are common pharmaceutical interventions in the glucose metabolism, as e.g. application of glucocorticoids, whose effects on potassium balance and the hormonal reproductive system are rarely studied.

Due to the fermentative character of their digestion, glucose metabolism in ruminants is different compared to monogastrical species. Carbohydrates ingested with the *DMI* are processed by microbes into the intermediary product pyruvate and then processed into short chain fatty acids [11], which are resorbed and serve, amongst others, as precursor substances for endogen glucose production via gluconeogenesis. High amounts of starch in *DMI* cannot be metabolized in the rumen. So called bypass starch is split enzymatically in the small intestine and is then resorbed as glucose. The mechanisms of the glucose–insulin model are depicted in Figure 3. In the model, the fraction of substances in *DMI* that are glucose or can be transformed into glucose are pooled in the component $Gluc_{FEED}$. These glucoplastic substances are transported to the component $Gluc_{PROD}$ with a rate $GfGp$. In this component we pool the endogenous production of glucose. The dairy cow needs to cover up to 90% of her glucose demand by gluconeogenesis [13].

The remaining part of $Gluc_{FEED}$ is directly transported to blood glucose, $Gluc_B$. The corresponding rate $GfGb$ represents direct glucose resorption from the intestine, including bypass starch. Glucose production is also fed by the storage component $Gluc_{STOR}$ with the rate $GsGp$. Glucose produced in $Gluc_{PROD}$ is shifted to $Gluc_B$ with the rate $GpGb$. The excess is shifted back to $Gluc_{STOR}$ with the rate $GpGs$. Glucose is cleared from the blood at a rate $SnkGb$, representing the metabolic use of glucose. Storage of glucose is represented by the component $Gluc_{STOR}$. In vivo, glucose is stored as glycogen, with muscle, liver and kidney being the main tissues storing glucose. In the default condition, storage capacity for a cow with 600 kg body weight is calculated

with 280 kg tissue being able to store glucose [14]. We assume that there is a maximum storage capacity that cannot even be exceeded with increased carbohydrate uptake. The dimension of this storage is calculated with 2% glycogen weight in the muscle tissue and 10% in liver [23]. Hence, in 9 kg liver an amount of 900 g glycogen can be stored, and in 265 kg muscle an amount of about 5300 g can be stored. Finally, a maximum storage capacity of 6200 g is obtained. Glucose is transported from the storage to the blood with a rate G_sGb , which increases for low levels of $Gluc_B$ and diminishes for high levels of $Gluc_B$.

Glucose and insulin influence each other. *Insulin* in the blood increases in response to rising $Gluc_B$, thus promoting the transport of glucose from $Gluc_B$ to $Gluc_{STOR}$ with the rate $GbGs$. If the storage $Gluc_{STOR}$ is already full or if the levels of $Gluc_{FEED}$ are low, this rate decreases.

In vivo, insulin is secreted from pancreatic beta cells in response to elevated blood levels of nutrients such as glucose or amino acids. It causes uptake of glucose as glycogen into cells of liver, muscle, and storage. Furthermore, insulin causes uptake of potassium in insulin sensitive cells by enhancing the activity of Na/K-ATPase [15]. In addition, the model is able to simulate glucose in milk by varying p_{55} , which represents the liter of milk produced per hour (see Appendix). In the simulations presented here, no milk production has been included.

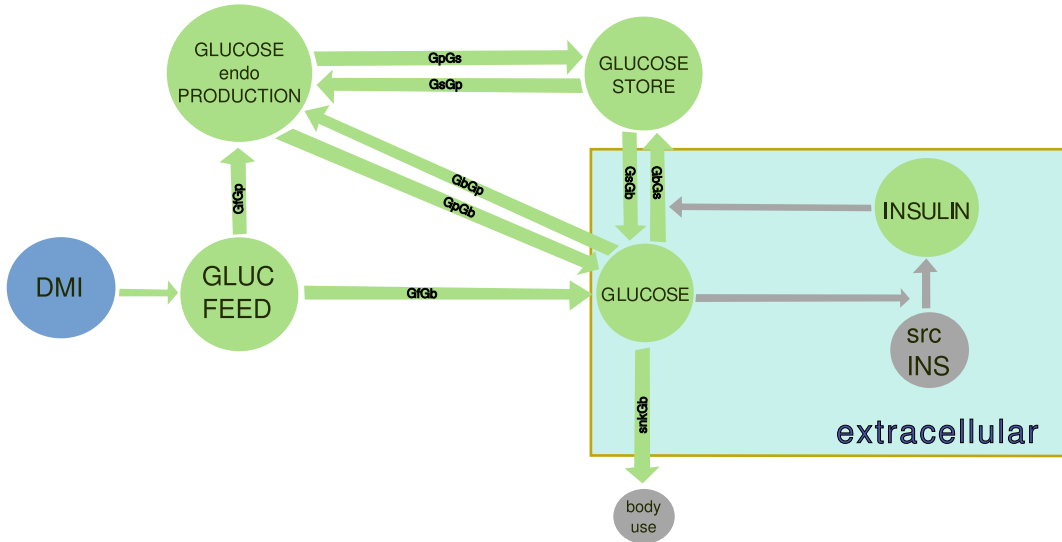


Figure 3: Graphical description of the model for glucose insulin metabolism in the dairy cow.

3 Simulation Results

The glucose-insulin model gives insight into dynamics of the glucose metabolism. In the following we present simulation outcomes with the glucose-insulin model for various amounts of food, modeled by varying the variable DMI . The first simulation shows results with 100% DMI , which is the default condition. In the second experiment we decreased the food to 10% of DMI , and in the third experiment we increased the food to 250% of DMI . The fraction of glucose and substances available for glucose production $Gluc_{FEED}$ was chosen as 8.54% of DMI and is the same in all presented experiments.

3.1 Default Condition

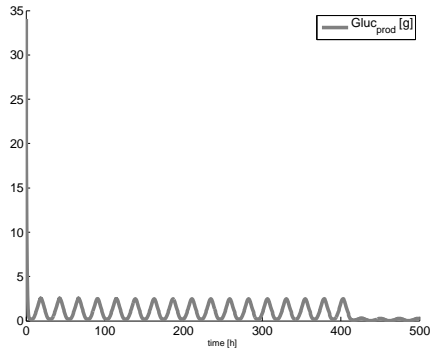
In the default condition the cow ingests 11700 g of feed. This amount was given from the study data we used to develop the potassium model (see Section 2.2). The simulation outcome for the default condition can be seen in Figure 6. The glucose production (a) is dependent on DMI and $Gluc_{FEED}$ (e). The amount of glucose produced by the cow per day is 927 g, and 8.54% of $Gluc_{FEED}$ are resorbed directly from the gastro-intestinal tract as hexose sugar and by-pass starch, here 80 g. Thus, the cow has 1007 g glucose available per day. The amount of glucose used in the model is 1000 g per day. This is in the expected range of the glucose production and demand per day [17] for a 600 kg non-lactating dairy cow. The storage (b) is filled up to about one half of its filling capacity and the oscillation is very low, as is to be expected when glucose demand is in the range of production. Glucose in blood (c) are in their physiological range of 0.39 - 0.59 g/l [16]. Insulin values in the blood (d) are also in the qualitative range given from the study data (f).

3.2 Decreasing Food

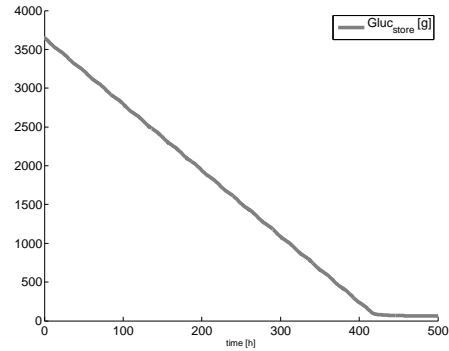
In the following experiment we decreased the DMI to 10% of the default condition. Within this experiment the cow ingests 1170 g of DMI per day, which is very little. In Figure 4 we observe that the glucose production (a) also decreases and now only 120 g of glucose are produced per day. Together with the glucose, which is directly resorbed from the digestive system, the cow has 129 g glucose available per day, while she is using 335 g per day. At the beginning of the experiment the glucose storage (b) is filled up to half of its maximum capacity. During the course of the experiment the levels are constantly depleting, since the cow is using up more glucose than it's intake provides. After 410 h (around 17 days) of simulation the glucose stores are nearly empty and no more glucose is available from there. The glucose levels in the blood (c) are in their physiological range of 0.39 - 0.59 g/l [16] as long as there is glucose available from the storage. It drops to very low and clinically critical values when the reserves are depleted. Insulin in the blood (d) follows the dynamic of glucose and also drops when storage is empty.

3.3 Increasing Food

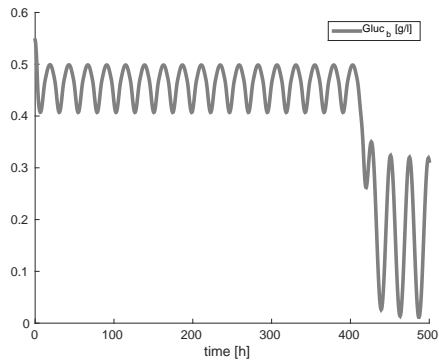
In the following experiment we increased the DMI to 250% of the default condition. The modelled cow now ingests 29250 g of DMI per day. In vivo, this amount of feed would be suitable for a high producing cow. Since our model cow is not producing milk, we suppose that she is doing much exercise, e.g. walking long distances, and/or is pregnant. In Figure 5 we can observe the simulation outcome for the components. The glucose production (a) is very high with 2303 g per day. Totally available glucose is 2503 g per day, and the daily use is 2244 g per day. The numbers for production and demand are in the expected range for a highly metabolic active cow [17]. The storage (b) is filling up over the simulated period but has not reached the maximum storage capacity yet. Glucose in blood (c) is in its physiological range of 0.39 - 0.59 g/l [16]. Insulin in the blood (d) follows the dynamics of glucose.



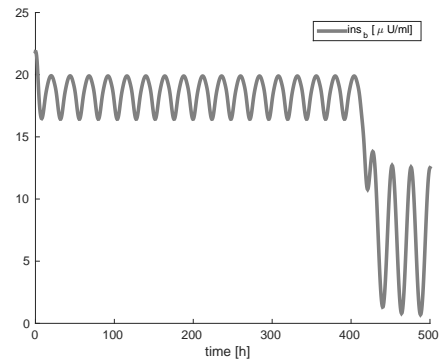
(a) Glucose production



(b) Glucose stored in tissues



(c) Glucose

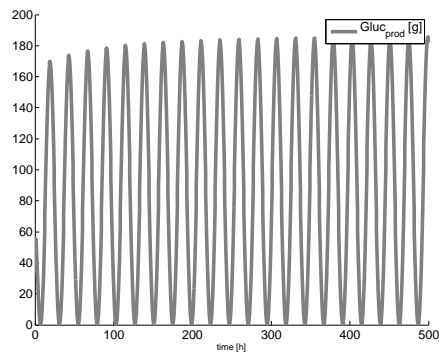


(d) Insulin

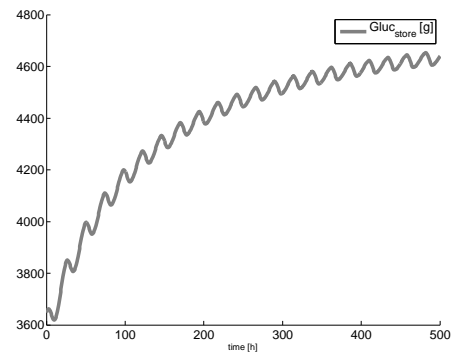
Figure 4: Simulation outcome for the components of the glucose-insulin model with 10 % *DMI*.

4 Conclusion

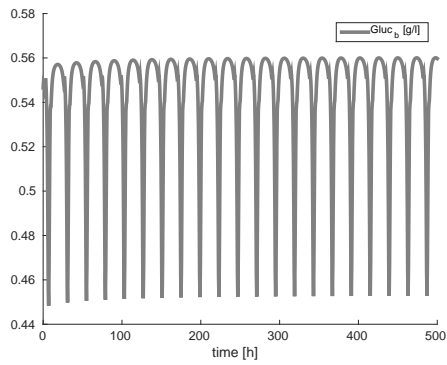
The herein presented glucose-insulin model is linking the previously developed physiological network models for the bovine estrous cycle and potassium balance to the glucose metabolism in the dairy cow. The model simulates the basic components on a level of organs and functional units. Simulation results qualitatively reproduce literature knowledge, particularly if environmental conditions are varied. Prospectively, we aim to couple the glucose-insulin model with the existing models to explore the interactions of these system. We are especially interested in conducting experiments with frequently performed interventions in veterinary practice, e.g. the application of glucocorticoides. Studying the system's behaviour will give new insights on short and long term effects and interactions without the need for new animal experiments.



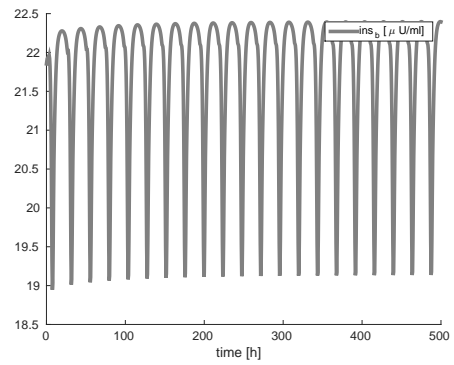
(a) Glucose production



(b) Glucose stored in tissues

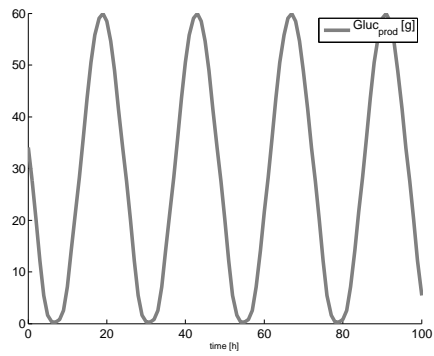


(c) Glucose

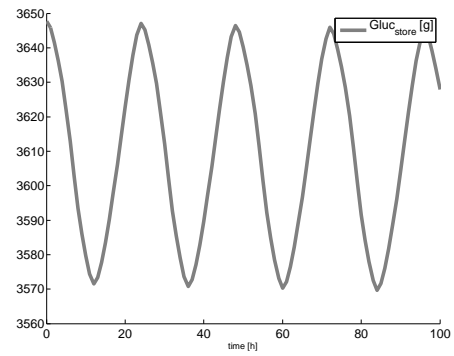


(d) Insulin

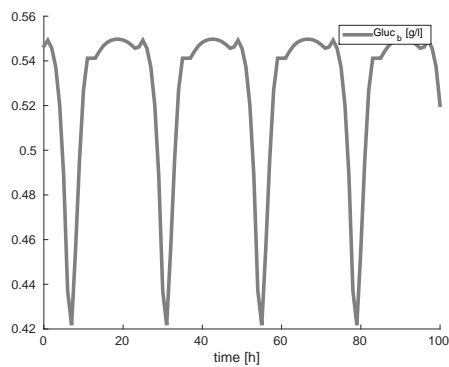
Figure 5: Simulation outcome for the components of the glucose-insulin model with 250 % *DMI*.



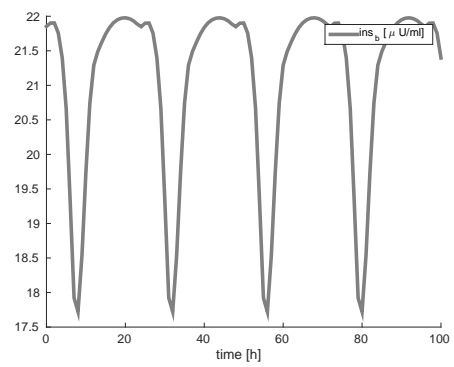
(a) Glucose production



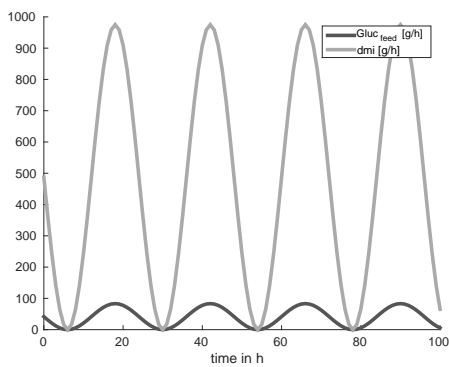
(b) Glucose stored in tissues



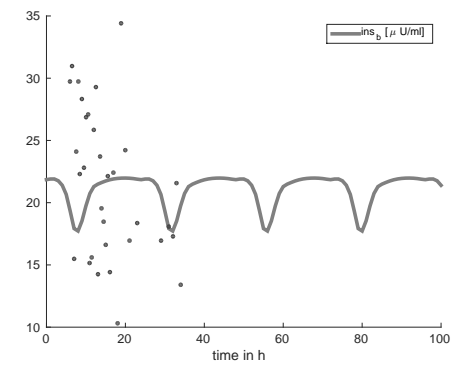
(c) Glucose



(d) Insulin



(e) DMI and $Gluc_{FEED}$



(f) Simulated insulin with data from one cow of the clinical study

Figure 6: Simulation outcome for the components of the glucose-insulin model with 100 % DMI .

A Equations

		Unit
<i>DMI</i>	$y_{DMI} = p_{54} \cdot 487.5 \cdot \left(1 - \sin\left(\frac{\pi \cdot t}{12}\right)\right)$	g/h
<i>GlucFEED</i>	$y_{GlucFEED} = p_{46} \cdot y_{dmi}$	g/h
<i>GlucPROD</i>	$\frac{d}{dt} y_{GlucPROD} = GfGp - GpGb - GpGs + GsGp$	g
<i>GlucB</i>	$\frac{d}{dt} (y_{GlucB} \cdot V_{extra}) = GfGb - GbGs + GsGb - SnkGb + GpGb$	g/l
<i>GlucSTOR</i>	$\frac{d}{dt} y_{GlucSTOR} = GbGs - GsGb + GpGs - GsGp$	g
<i>Insulin</i>	$\frac{d}{dt} (y_{Ins} \cdot V_{extra}) = p_{49} \cdot y_{GlucB} - p_{41} \cdot y_{Ins}$	$\mu\text{U/ml}$

B Initial values for ODEs

$$\begin{aligned}
 y_{GlucPROD}(0) &= 34.0337 \\
 y_{GlucB}(0) &= 0.5466 \\
 y_{GlucSTOR}(0) &= 3647.747 \\
 y_{Ins}(0) &= 21.853
 \end{aligned}$$

C Rates

$$\begin{aligned}
 GfGp &= (1 - p_{48}) \cdot y_{GlucFEED} \\
 GfGb &= p_{48} \cdot y_{GlucFEED} \\
 GsGp &= p_{60} \cdot H^-(y_{GlucFEED}, p_{61}, 5) \cdot H^+(y_{GlucSTOR}, p_{35}, 10) \\
 GpGb &= (p_{39} \cdot y_{GlucPROD}) \cdot H^-(y_{GlucB}, p_{50}, 10) \\
 GpGs &= p_{51} \cdot y_{GlucPROD} \cdot H^-(y_{GlucSTOR}, p_{57}, 10) \cdot H^-(p_{55}, p_{62}, 10) \\
 SnkGb &= H^+(y_{GlucB}, p_{58}, 10) \cdot (p_{28} \cdot y_{GlucB} + p_{59} \cdot y_{GlucPROD} \cdot e^{-p_{66} \cdot p_{55}} + p_{55} \cdot p_{47}) \\
 GsGb &= H^+(y_{GlucSTOR}, p_{35}, 10) \cdot (H^-(y_{GlucFEED}, p_1, 10) \cdot p_{17} \cdot (p_7 - y_{GlucB}) + H^+(p_{55}, p_{64}, 10) \cdot p_{63} \cdot H^-(y_{GlucB}, p_{65}, 10)) \\
 GbGs &= H^+(y_{GlucFEED}, p_1, 10) \cdot p_{12} \cdot y_{GlucPROD} \cdot H^-(y_{GlucSTOR}, p_{57}, 10) \cdot y_{GlucB} \cdot y_{Ins} \cdot H^-(p_{55}, p_{36}, 10)
 \end{aligned}$$

D List of Parameters

Parameters are denoted with p and are numbered according to Table 1.

Num	Value	Unit	Explanation
p_1	40.4	$\frac{g}{h}$	Threshold for $Gluc_{FEED}$
p_7	0.925	$\frac{g}{l}$	Glucose threshold
p_{12}	0.0105	$\frac{ml \cdot l}{\mu U \cdot h \cdot g}$	Factor for $Gluc_{PROD}$ influencing GsGb rate
p_{17}	16	$\frac{g}{h}$	$Gluc_B$ dependant glucose in GsGb rate
p_{28}	25	$\frac{l}{h}$	Glucose available for metabolic use from $Gluc_B$
p_{35}	100	g	Threshold for $Gluc_{STOR}$
p_{36}	1	$\frac{l}{h}$	Threshold for milk production
p_{39}	30	$\frac{l}{h}$	Fraction of $Gluc_{PROD}$ transported to $Gluc_B$
p_{41}	20	$\frac{l}{hl}$	Factor for insulin clearance
p_{46}	0.0854	-	Fraction of glucose and glucoplastic substances in DMI
p_{47}	72	$\frac{g}{l}$	Glucose in milk
p_{48}	0.08	-	Fraction directly resorbed from $Gluc_{FEED}$
p_{49}	800	$\frac{\mu U \cdot l}{mg \cdot h}$	Scaling factor for $Gluc_B$ influencing insulin production
p_{50}	0.4	$\frac{g}{h}$	Threshold for $Gluc_B$
p_{51}	0.1	$\frac{l}{h}$	Fraction of $Gluc_{PROD}$ transported to $Gluc_{STOR}$
p_{54}	1	$\frac{g}{h}$	Feeding amount as fraction of DMI
p_{55}	0	$\frac{l}{h}$	Milk produced
p_{57}	3800	g	Threshold for $Gluc_{STOR}$
p_{58}	0.01	$\frac{g}{l}$	Threshold for $Gluc_B$
p_{59}	1	$\frac{l}{h}$	Fraction of $Gluc_{PROD}$ for metabolic use
p_{60}	2	$\frac{g}{h}$	Basic glucose rate from $Gluc_{STOR}$ to $Gluc_{PROD}$
p_{61}	5	$\frac{g}{h}$	Threshold for $Gluc_{FEED}$
p_{62}	1	$\frac{l}{h}$	Threshold for milk production
p_{63}	800	$\frac{g}{h}$	Scaling factor for glucose in milk production
p_{64}	0.2	$\frac{l}{h}$	Threshold for milk production
p_{65}	0.3	$\frac{g}{l}$	Threshold for $Gluc_B$ in milk production
p_{66}	1.4	$\frac{l}{l}$	Exponent for milk production

Table 1: Parameter values.

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