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# **A pharmacokinetic-pharmacodynamic model for single dose administration of Dexamethasone in dairy cows<sup>1</sup>**

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# A pharmacokinetic-pharmacodynamic model for single dose administration of Dexamethasone in dairy cows

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## Abstract

We present a mechanistic pharmacokinetic-pharmacodynamic model to simulate the effect of dexamethasone on the glucose metabolism in dairy cows. The coupling of the pharmacokinetic model to the pharmacodynamic model is based on mechanisms underlying homeostasis regulation by dexamethasone. In particular, the coupling takes into account the predominant role of dexamethasone in stimulating glucagon secretion, glycogenolysis and lipolysis and in impairing the sensitivity of cells to insulin. Simulating the effect of a single dose of dexamethasone on the physiological behaviour of the system shows that the adopted mechanisms are able to induce a temporary hyperglycemia and hyperinsulinemia, which captures the observed data in non-lactating cows. In lactating cows, the model simulations show that a single dose of dexamethasone reduces the lipolytic effect, owing to the reduction of glucose uptake by the mammary gland.


## Introduction

Glucose requirement in dairy cows early in lactation is dominated by the requirements of the mammary gland for milk synthesis [1]. Due to increased glucose requirements caused by milk production, high yielding dairy cows usually go through a period of negative energy balance that predisposes them at risk of developing hypoglycemia and ketosis. Hypoglycemia, which is often observed in early lactation, stimulates glycogenolysis in skeletal muscles and lipolysis in adipose tissues as an adaptation to manage the energy deficit. An increased rate of lipolysis often results in increased levels of non-esterified fatty acids in the blood, which causes an increase in its uptake by the liver. If the liver's capacity to oxidize mobilized non-esterified fatty acids is exceeded, fatty acids

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are accumulated in the liver, which may impair hepatic function. This gives rise to an increase in susceptibility to disease and several health problems such as ketosis, fertility disorders and impairment of immune function [2–5]. Ketosis in dairy cows is very common during the postpartum period. From a population study with large sample size (1717 cows), it was reported that the average incidence of ketosis was 43% and the peak incidence occurred at 5 days in milk [6].

Improved feeding management of cows during dry and early lactating periods has been shown to mitigate the degree and duration of negative energy balance and to reduce accumulation of fatty acids in the liver [7,8]. But if the increase of feed intake lags behind the demands for energy requirements, therapeutic intervention may be required to improve the energy balance of the cows. This treatment should regulate blood glucose and insulin concentrations in order to reduce fat mobilisation, thereby diminishing the risk of ketosis. Glucocorticoids such as dexamethasone are commonly known to treat and regulate mammalian glucose homeostasis. In other words, they play a protective role against glucose deprivation, especially under stress and starvation [9–12]. Glucocorticoids have been shown to be beneficial in the treatment of cows with clinical ketosis and fatty liver syndrome postpartum [13,14,14–18]. In particular, they are known to improve the energy balance of cows by decreasing the uptake of glucose in body tissues and by suppressing milk production [13,18–20]. Because of their ability to promote muscle and fat catabolism, glucocorticoids also stimulate gluconeogenesis during an energy deficit [13,21,22]. However, the use of glucocorticoids in these animals is still controversial because in addition to these beneficial effects, they may adversely result in even increased lipolysis and suppression of components of the immune system [23–25].

In the literature, there exists several pharmacokinetic-pharmacodynamic models developed for investigating the effect of glucocorticoids on rats [11,26–29]. For instance, the model in [11] quantitatively describes the induction of hyperglycemia as a result of methylprednisolone drug administration. In bovine, there are only a few studies focussing on the pharmacokinetics of glucocorticoids, e.g., dexamethasone [30,31]. So far, no combined mathematical model describing the coupling of pharmacokinetic and pharmacodynamic was introduced for dairy cows. Therefore, the aim of this paper was to develop a mechanistic pharmacokinetic-pharmacodynamic model for dairy cows. This model is used to simulate the effect of dexamethasone on the physiological behaviour of the system, especially glucose metabolism. In general, "In-silico simulations" play an important role in designing therapeutic strategies for ketosis and diseases related to the metabolic system. This can be done by varying drug and physiological parameters without performing animal experiments.

## 1 Pharmacokinetic-Pharmacodynamic Model

Dexamethasone is the main glucocorticoid drug which is used for the treatment of fatty liver syndrome or ketosis in postpartum dairy cows [2,17]. In the following, we introduce a pharmacokinetic model for dexamethasone in dairy cows. This model is based on the existing developed pharmacokinetic models. Then, we link the pharmacokinetic model to the metabolic-reproductive (MetRep) model

developed in [32] to investigate the pharmacodynamic characteristics of the drug.

## 1.1 Pharmacokinetic Model

The pharmacokinetics of dexamethasone in bovine have been reported in two published experimental studies [30,31]. In the experiment of Toutain et al. [30], four cows received dexamethasone alcohol and dexamethasone 21 isonicotinate as a solution by intravenous and intramuscular routes of 0.1 mg/kg (0.1 mg of the drug per kg body weight). Following intravenous administration, the disposition kinetics of both formulations were described by a two-compartment open model. The half times of elimination were almost similar, 335 and 291 min, for dexamethasone alcohol and dexamethasone 21 isonicotinate. No statistically significant difference was found for all other parameters. Following intramuscular administration, no significant difference in parameters was observed between the two formulations. Peak plasma concentrations were reached at 3 to 4 hours post injection. The bioavailability, denoted by  $F$ , which is the fraction of dose that reaches the systemic circulation, was estimated to be around  $F = 72\%$ . Following intramuscular administration, Toutain et al. [30] described the pharmacokinetics of dexamethasone again by a two-compartment model. In this model, one compartment described the distribution phase after intramuscular dosing, and the second compartment was considered to model the absorption phase into the central compartment.

In this work, we also consider an intramuscular administration of dexamethasone 21 isonicotinate, which we call in the following dexamethasone only for the sake of simplicity. In this regard, we use the pharmacokinetic parameters reported by Toutain et al. [30] for the construction of our pharmacokinetic model. A graphical representation of this model is depicted in Fig 1. The figure illustrates the change of dexamethasone concentration, denoted by  $C$ , in the central compartment with the volume of distribution  $V_d$ . This change is controlled by two rate constants,  $k_a$  and  $CL$ . The first rate constant,  $k_a$ , describes the absorption phase, that is, the movement of the drug from its site of administration to the bloodstream. The second rate,  $CL$ , describes the volume of plasma from which the drug is totally removed per unit time. This rate can also be expressed in terms of the elimination rate  $ke$  and the volume of distribution  $V_d$ ,  $CL = ke \cdot V_d$ . The mathematical model describing the change in dexamethasone concentration is given by the following equation:

$$V_d \cdot \frac{d}{dt} C = dose \cdot F \cdot k_a \cdot e^{-k_a \cdot t} - CL \cdot C, \quad \text{with } C(t=0) = 0, \quad (1)$$

where  $dose$  is the total amount of dexamethasone given per kg body weight (mg/kg) following an intramuscular administration.

## 1.2 Integrating the Pharmacokinetic Model into MetRep Model

To couple the pharmacokinetic model to the MetRep model, a description of glucocorticoids' mechanisms of action on the system is presented in the following along with the coupling hypotheses.



Figure 1: **Illustration of the pharmacokinetic model.** This figure illustrates the absorption phase of dexamethasone described by first order rate  $k_a$  following intramuscular administration into the central compartment from which dexamethasone is cleared by an elimination rate of first order.

### Delayed Drug Effects

To observe the effect of a drug on the system, one has to wait the time it takes for the drug to induce an effect at the site of action. This can be ascribed to several mechanisms which explain delayed effects. For instance, after administrating a dose of drug, it takes time for the drug to reach the site of action (distribution) and then to bind to its receptor, which again takes time, thus contributing to a delay in response. Moreover, it may take time for the drug to change physiological intermediate substances before the drug response is observed. Commonly, these time lags can be seen as only one delay process. When the delay is short (minutes) then the mechanism is probably a distribution process, whereas if the delay is long (hours or longer) then the mechanism is more likely to be physiological [33]. When administering dexamethasone, it is not sufficient to directly model the effect as a function of systemic concentrations in the pharmacokinetic model, rather we assume that there exists a delayed effect. This is supported by the data in Fig 5, which show that glucose and insulin in the blood reach their maximum concentration about 24 hours after the drug administration. To account for this delay, an additional compartment called "the effect compartment" is considered linking the blood concentration in the central compartment to the systemic effect [34–36]. The equilibrium concentration in the effect compartment,  $C_e$ , is achieved by the flow of dexamethasone concentration,  $C$ , from the central compartment to the effect compartment with a rate constant  $k_{e1}$  and a dissipation from the effect compartment with a rate constant  $k_{eo}$ . Here we assume that  $k_{eo}$  is equal to  $k_{e1}$ . This parametrization of the model assumes that the average concentration in the effect compartment at steady state is the same as in the central compartment. If the value of  $k_{eo}$  is large, this means that the effect compartment is rapidly equilibrating and the concentration in this compartment closely follows the plasma concentration, whereas a small value of  $k_{eo}$  means that the effect compartment equilibrates slowly and hence effects are delayed relative to plasma concentrations. The temporal change of  $C_e$  is given by the following equation,

$$\frac{d}{dt} C_e = k_{e1} \cdot C - k_{eo} \cdot C_e = k_{eo} \cdot (C - C_e), \quad \text{with } C_e(t=0) = 0. \quad (2)$$

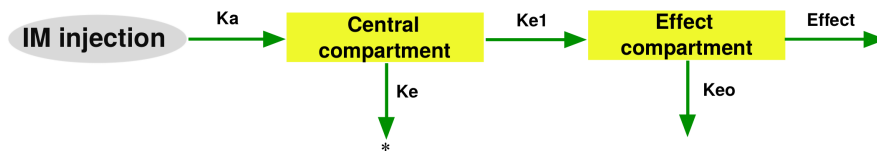


Figure 2: **Combined pharmacokinetic and effect compartment.** This figure shows the link of the pharmacokinetic model with the effect compartment to describe the indirect effect of drug at the site of action caused by the time lag. The central compartment is linked to the effect compartment with a rate constant  $k_{e1}$ . The elimination term from the effect compartment with a rate constant  $k_{eo}$  is assumed to be equal to  $k_{e1}$ .

### Glucocorticoid Effects

Several studies have analyzed the role of glucocorticoids in regulating glucose metabolism. In both human and animals, a common finding is that increased glucocorticoids induce peripheral insulin resistance by increasing hepatic glucose output and reducing the peripheral glucose uptake [11, 13, 37–39]. One mechanism by which glucocorticoids reduce glucose uptake is the inhibition of the insulin signalling cascade [13, 22, 38, 40, 41]. In contrast, reduced glucocorticoids signalling improves insulin-stimulated glucose uptake in peripheral tissues. At the level of alpha cells, this decrease would be regarded as glucose shortage in the system, which augments the responsiveness of alpha cells, thereby triggering glucagon secretion [42]. Studies in man and rats [21, 42–44] have reported that glucocorticoids increase glucagon secretion from alpha cells.

A second major effect of glucocorticoids on glucose metabolism is that glucocorticoid excess can increase glucose production by stimulating proteolysis, lipolysis and hepatic gluconeogenesis. In particular, glucocorticoids increase the release of amino acids by decreasing protein synthesis and increasing the breakdown of proteins in several tissues. This leads to an increased uptake of hepatic amino acids, which serve as gluconeogenic precursors. In addition, glucocorticoids also increase the availability of gluconeogenic substrates by stimulating glycerol release from fat cells. The abundance of gluconeogenic precursors enhances hepatic gluconeogenesis, which results in an increase in glucose production particularly in case of an energy deficit [21, 22]. Glucocorticoid-stimulated proteolysis, lipolysis and hepatic gluconeogenesis can be mediated by glucagon. The major target for glucagon is the liver where glucagon stimulates gluconeogenesis, but it also stimulates proteolysis and lipolysis. Exton et al [45] reported that glucocorticoids stimulate gluconeogenesis by glucagon. The authors showed that subcutaneous injection of dexamethasone 30 min prior to perfusion restored glucagon activation of gluconeogenesis as revealed by increased glucose production and synthesis of glucose and glycogen from lactate. On the other hand, glucocorticoid deficiency showed to markedly impair the ability of glucagon to stimulate gluconeogenesis or glycogenolysis [21].

While the effect of glucocorticoids on alpha cells is to increase plasma glucagon lev-

els, their effects on beta cells, on the other hand, are conflicting [13,46]. Different results have been reported by in vitro studies in rats, mice and man treated with glucocorticoids, where insulin secretion was measured afterwards. These studies reported either increased [47–50], unchanged [46, 51–56], or decreased [46, 57–63] insulin secretion. These discrepancies cannot easily be explained and it seems that effects of glucocorticoids on beta cells function are highly dependent on the dosage and duration of administration, the experimental animal model and susceptibility of the population under investigation [46, 64]. However, some studies speculated that glucocorticoid treatment acutely (within less than one day) inhibits insulin secretion [22, 46, 57, 62, 63, 65], whereas, pancreatic islets isolated from healthy rats after more prolonged treatment (several days) with dexamethasone or hydrocortisone showed either unchanged or increased glucose-stimulated insulin secretion [22, 50, 63]. Chronic exposure likely results in beta-cell dysfunction in susceptible individuals [22, 66].

In dairy cows, results from some studies indicate that treatment with dexamethasone has the ability to enhance the metabolism of glucose and insulin. For instance, a single dexamethasone injection of 20 mg induced a sharp increase in blood glucose concentration and then a quick return to normal level [67]. Kusenda et al. [68] reported that the mean plasma glucose and insulin concentrations increased one day after treatment and were significantly higher on the second day in cows treated with a single dexamethasone injection of 0.04 mg/kg compared with control cows. In addition, the authors added that dexamethasone administration induced peripheral insulin resistance. Hammon et al. [69] reported an increase in both plasma glucose and insulin concentrations observed in calves treated with a single dexamethasone injection of 0.03 mg/kg. Jorritsma et al. [17, 70], observed in their studies a significant increase in the mean plasma glucose and insulin levels on the second day after treatment with a single dexamethasone injection of 0.02 mg/kg in cows fifteen days after calving versus the controls. This findings supported early work of Wierda et al. [18]. The same finding has been reported by van der Drift et al. [71] in a study where healthy Holstein-Friesian dairy cows in late pregnancy received a single dexamethasone injection of 0.02 mg/kg.

The effect of dexamethasone on reducing milk in dairy cows is well documented in the literature [18–20]. For example, in an experiment by Shamay et al. [20], a single intramuscular dose of 40 mg dexamethasone in multiparous cows at mid-late lactation caused a 45% reduction in milk yield after 24 hours. Milk yield then started to rise, but it took additional five days to return to the initial level. The decrease in milk yield was ascribed to the ability of dexamethasone to reduce glucose uptake in body tissues as well as the mammary gland, which leads to an insulin resistance state [19].

### Coupling Hypotheses

Based on the aforementioned mechanisms, we couple the pharmacokinetic model described above to the MetRep model, which incorporates glucose-insulin inter-regulations. From the above discussion, it is clear that glucocorticoids act on the alpha and beta cells, body tissues, mammary gland as well as on the liver. However, the results from different studies about the action of glucocorticoids



on insulin secretion in rodents and man are still contentious. As discussed previously, studies in dairy cows indicated an elevation in insulin level following a single dexamethasone injection. In addition, it is known that the effects of glucocorticoids counteract those of insulin, which enhances glucose uptake in peripheral tissues. Thus, to allow glucocorticoids to exert their responses, glucocorticoids need to antagonize insulin actions. Because of this, we rule out the possibility of glucocorticoids acting on insulin secretion in this work. Thus, we couple the two models according to the following hypothesis.

We assume that a rise in dexamethasone concentration stimulates glucagon secretion from pancreatic alpha cells, see Fig 3. The stimulation of glucagon secretion in turn will augment the availability of gluconeogenic precursors and enhance hepatic gluconeogenesis. On the other hand, glucocorticoids reduce insulin-stimulated glucose uptake in body tissues. In lactating cows, we assume that dexamethasone induces milk reduction by impairing glucose uptake in the mammary gland, see Fig 3. All these effects are mathematically expressed by altering the terms described in [32]. These terms are the secretion of glucagon represented by  $gluca_{sec}$ , glucose uptake in body tissues represented by  $glu_{lv-st}$  and  $glu_{lv-fat}$ , and the sink term of milk production  $glu_{milk-usage}$ :

$$gluca_{sec-dxm} = gluca_{sec} \cdot effect_{dxm-gluca}, \quad (3)$$

$$glu_{lv-st-dxm} = glu_{lv-st} \cdot effect_{dxm-bt}. \quad (4)$$

$$glu_{lv-fat-dxm} = glu_{lv-fat} \cdot effect_{dxm-bt}, \quad (5)$$

$$glu_{milk-usage} = c_{13} \cdot Milk \cdot effect_{dxm-bt}, \quad (6)$$

where

$$effect_{dxm-gluca} = \left( 1 + E_{max} \cdot \frac{C_e^{10}}{C_e^{10} + C_a^{10}} \right), \quad (7)$$

and

$$effect_{dxm-bt} = \left( 1 - \frac{C_e^7}{C_e^7 + C_b^7} \right). \quad (8)$$

Here  $E_{max}$  is the parameter representing the maximum possible stimulation of glucagon secretion  $gluca_{sec}$ . The constant  $C_a$  is the drug-specific parameter representing the concentration required for half-maximal stimulation of glucagon secretion. The constant  $C_b$  is the drug-specific parameter representing the concentration required for half-maximal inhibition of  $glu_{lv-st}$ ,  $glu_{lv-fat}$  and glucose uptake in the mammary gland. A full description of the combined pharmacokinetic and pharmacodynamic model of the system is depicted in Fig 4.

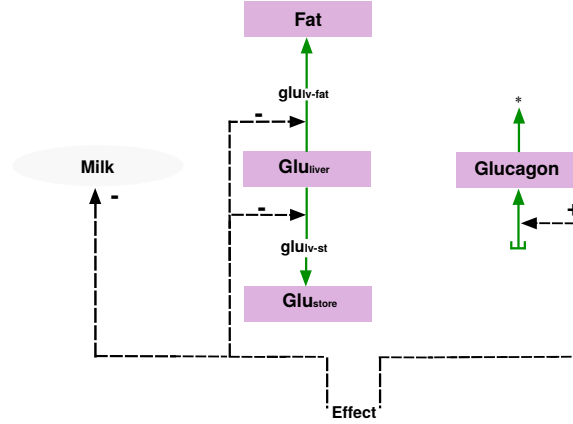


Figure 3: **Representation of possible drug effects on pancreatic cells, body tissues and mammary gland.** A rise in drug concentration stimulates glucagon secretion from pancreatic alpha cells and reduces glucose uptake into peripheral tissues, represented by  $glu_{lv-st}$  and  $glu_{lv-fat}$ , as well as uptake into the mammary gland. The green arrow with a star represents the decay term of glucagon.

## 2 Simulation and Discussion

In this section, we present the simulation results of the combined pharmacokinetic and pharmacodynamic model. We start by presenting the simulation results of the pharmacokinetic model for intramuscular administration of one single dose of dexamethasone of 0.02 mg/kg. Particularly, we explore the effect of administration on the dynamical behaviour of the model components in non-lactating cows. Subsequently, we examine the effect of administering one single dose of dexamethasone on the resumption of cyclicity of the estrous cycle during the postpartum period in lactating cows. Simulation results in non-lactating cows are compared with data of glucose and insulin concentration from 6 non-lactating cows that received 0.02 mg/kg dexamethasone 21 isonicotinate by the intramuscular routes, see Fig 5. It can be seen from Fig 5 that concentrations of glucose and insulin in the blood are not effected post drug administration until around 12 hours after treatment. Then an increase is observed and the peak is achieved at around 20 hours after treatment. However, we assume that the peak concentrations are already reached at around 18 hours since there are measurements missing for a time period of almost 5 hours. In addition, we assume that concentrations continue to decrease after 22 hours until return to the baseline.

### 2.1 Pharmacokinetics of Dexamethasone

As mentioned earlier, following intramuscular administration, the dexamethasone pharmacokinetics are described by a two-compartment model. The change in



**Figure 4: Combined pharmacokinetic and pharmacodynamic model.** This figure illustrates the proposed scenario linking the pharmacokinetic model with the pharmacodynamic model. The black dashed arrows describe the stimulatory and inhibitory effects on pancreatic alpha cells and body tissues.

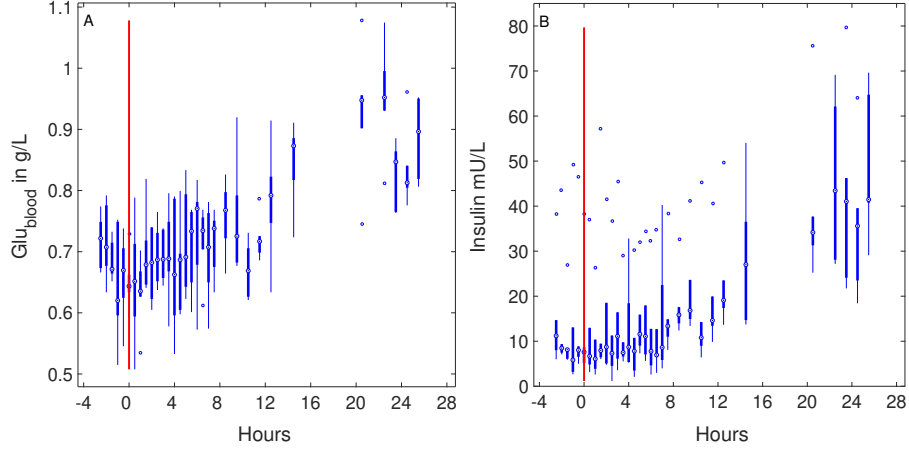


Figure 5: **Measured data of glucose and insulin concentrations.** This figure illustrates observed data of glucose and insulin concentrations collected from a clinical study in the Clinic for Ruminants of Freie Universität Berlin. These data were used for parameter estimation of the potassium balance model [72]. The data describe the change of glucose and insulin concentrations on an hourly basis of 6 non-lactating cows that received 0.02 mg/kg dexamethasone 21 isonicotinate by the intramuscular route. The time of administration is depicted by the red line. There are strong inter-individual variations in insulin measurements between the 6 study cows.

concentration  $C$  of dexamethasone in the central compartment as well as  $C_e$  in the effect compartment is expressed by eq.(1) and eq.(2), respectively. For a cow of weight 600 kg and a dose of 0.02 mg/kg, we consider an intramuscular administration of 12 mg dexamethasone. Only a fraction,  $F = 72\%$ , of the dose reaches the systemic circulation. The parameter values used to simulate eq.(1) and eq.(2) are shown in table 1. Simulation results are depicted in Fig 6. As one can see, the plasma concentration of dexamethasone in the central compartment,  $C$ , increases until around 3 hours post injection. At approximately 4 hours, the concentration reaches its maximal value of  $C_{max} \approx 8.7$  ng/ml and then subsequently decreases. The concentration of dexamethasone in the effect compartment,  $C_e$ , slowly increases until around 17 hours post injection. At around 18 hours, the concentration reaches steady-state with maximal concentration of  $C \approx 2.1$  ng/ml and then subsequently decreases.

## 2.2 Effects of Administrating a Single Dose of Dexamethasone in Non-Lactating Cows

The effects of administration of a single dose on the dynamic of metabolic elements in non-lactating cows are shown in Fig 7. Simulation results show that the stimulatory effect of dexamethasone on alpha cells, eq.(3) and eq.(7), results in increased glucagon concentration from around 100 ng/ml to around 280 ng/ml,

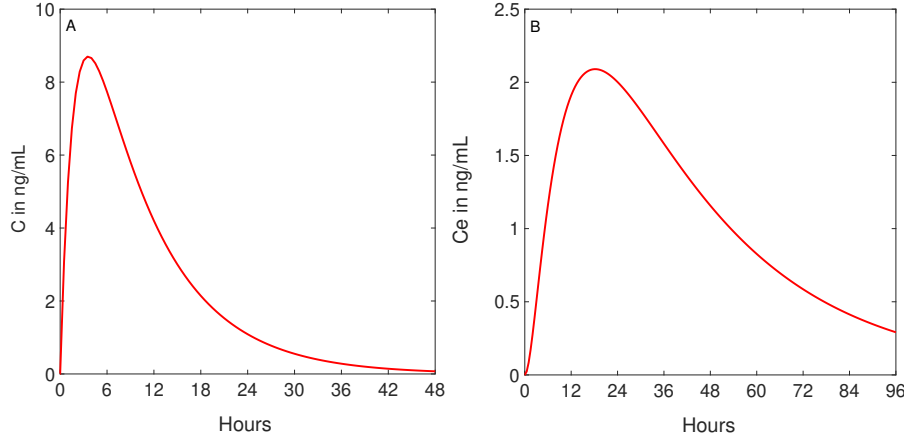


Figure 6: **Pharmacokinetics of dexamethasone upon administration of a single dose of 0.02 mg/kg intramuscular injection.** Sub-figure (A) depicts the plasma concentration  $C$  of dexamethasone in the central compartment. Sub-figure (B) depicts the concentration  $C_e$  of dexamethasone in the effect compartment.

Fig 7(E). Hammon et al. [69] reported an increase in glucagon concentration from around 125 ng/L to 175 ng/L in calves treated with dexamethasone administration of 0.03 mg/kg. In [44], the glucagon concentration increased from 150 ng/L to 350 ng/L following dexamethasone administration of 2 mg. As glucagon is a catabolic hormone that regulates glucose levels in the bloodstream, a rise in plasma glucagon concentration increases the supply of gluconeogenic precursors in the liver by stimulating the degradation and mobilization of glycogen and fat. This result is visible in Fig 7(D) and Fig 7(G), whereby the glucose production is enhanced by hepatic gluconeogenesis process, see Fig 7(H). As a result, the plasma glucose concentration temporarily increases from approximately 0.48 g/L to 0.72 g/L and quickly returns to the baseline on the second day, see Fig 7(C). The glucose concentration peaks at around 18 hours post drug administration. This time delay that dexamethasone takes until acting at the site of pancreatic alpha cells was modelled by the effect compartment, in which the time of the maximal concentration  $C_e$  is also around 18 hours post dexamethasone administration, see Fig 6(B). The glucose concentration fits to the measurement data as shown in Fig 7(A). Here, we scaled down the data of glucose concentration with a factor of 0.7 so that they match the baseline of the simulated glucose concentration. The discrepancy in the baseline levels of glucose in the blood between the modelled cow and the cows from the experimental study can be ascribed to several factors such as weight, age, diet, etc.

While insulin primarily stimulates glucose uptake into peripheral tissues, the antagonistic effect of dexamethasone onto this process, eq.(4), eq.(5) and eq.(6), leads to more available glucose in the systemic blood circulation as it can be seen in Fig 7(C). Biologically, this occurs through the mechanism by which dex-

amethasone directly interferes with the insulin signalling cascade in peripheral tissues [22], thereby inducing insulin resistance. The acute elevation in glucose concentration in the blood results in increased insulin concentration. This increase in insulin level is regarded as an adaptation of pancreatic beta cell function to insulin resistance by forcing beta cells to produce a compensatory increase in insulin secretion to maintain glucose homeostasis. In this case, the simulated insulin concentration follows the trend of increasing insulin data as shown in Fig 7(F). From this figure, we observe an increase in insulin concentration up to around 40 mU/L on the first day post dexamethasone administration, and a subsequent return to the baseline on the second day.

As it is evident from Fig 7(C) and (F), data of glucose and insulin concentrations resulting from a single dexamethasone administration of 0.02 mg/kg peak almost at the end of the first day post administration. Regardless of the day and the magnitude of this increase, the elevation in both concentrations following a single dexamethasone administration in dairy cows was also observed in early studies. For instance, Jorritsma et al. [17, 70] observed in their studies a significant increase in the mean plasma levels of glucose ( $3.55 \text{ mmol/L} = 0.639 \text{ g/L}$ ) and insulin ( $13.14 \text{ mU/L}$ ) on the second day after treatment with a single dexamethasone injection of 0.02 mg/kg 15 days after calving. In another study with healthy Holstein-Friesian dairy cows in late pregnancy treated with a single dexamethasone injection of 0.02 mg/kg, Van der Drift et al. [71] observed a significant increase in the plasma glucose level (median was  $4.2 \text{ mmol/L} = 0.756 \text{ g/L}$ ) on the second day and insulin level (median was  $15 \text{ mU/L}$ ) on the third day. These discrepancies in the magnitude of increase as well as the day of the peak seem to be dependent on the design of experiment and susceptibility as well as the metabolic condition of the animal under investigation.

### 2.3 Effects of Administrating a Single Dose of Dexamethasone in Lactating Cows

In this section, we explore the dynamical behaviour over time of the metabolic and the reproductive status in postpartum lactating cows after administrating a single dose of dexamethasone of 0.02 mg/kg.

#### Effects on the Metabolic Status

We assume that a cow of weight 600 kg gets an amount of DMI and produces an amount of milk as given in [32](Fig.9 therein). In addition, we assume that glucose content in DMI is  $c_0 = 20\%$ . Performed simulations with the MetRep model clearly show that for a low amount of glucose in DMI ( $c_0 = 20\%$ ), glucose and insulin concentrations in the blood drop towards their lower physiological limits around peak milk (Fig 10 (A),(D) in [32]). In order to compensate for the increase in milk production, the cow mobilizes her body reserves, represented in the model by glycogen and fat in the store (Fig 10 (B),(E) in [32]). The mobilisation of fat, however, is often associated with negative energy balance. The therapeutic treatment with dexamethasone is expected to up-regulate blood

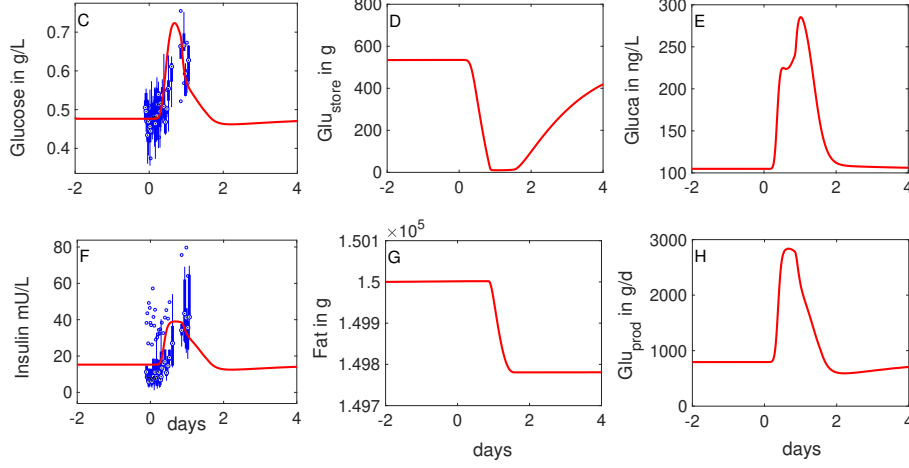


Figure 7: **Glucose and insulin changes in non-lactating cows following a single dexamethasone administration of 0.02 mg/kg.** Glucose (C) and insulin (F) concentrations peak almost at the end of the first day post administration. A stimulatory effect of dexamethasone on alpha cells increases glucagon concentration (E), which in turn increases the supply of gluconeogenic precursors in the liver by stimulating the degradation and mobilization of glycogen (D) and fat (G) from the depots, thereby increasing glucose production (H). An inhibitory effect of dexamethasone on body tissues impairs insulin-stimulated glucose uptake, thus allows more glucose in the systemic circulation.

glucose and insulin concentrations in order to decrease the rate of lipolysis. As it is shown in Fig 8(A) and (D), the administration of a single dose of dexamethasone of 12 mg on day 50 postpartum induces a temporary hyperglycemia with a significant increase in glucose concentration over 1 g/L. In their experiment, Shamay et al. [20] reported that a single intramuscular dose of 40 mg dexamethasone in multiparous cows at mid to late lactation caused a 45% reduction in milk yield after 24 hours. This resulted in elevated glucose concentrations in the treated cows to around 1 g/L one day after dexamethasone injection. Glucose was back in the normal range after the second day. Our model-simulated glucose is accompanied by a sharp spike in insulin concentration to around 40 mU/L. Hyperglycemia occurs due to increased glucose production (Fig 8(F)), which is stimulated by a rise in dexamethasone-stimulated glucagon secretion. Glucagon concentration sharply increases due to the stimulatory effect of dexamethasone, and subsequently decreases as a response to the significant increase in glucose level, see Fig 8(C). Interestingly, although dexamethasone stimulates the mobilization of glycogen and fat from the store, it is evident from Fig 8(E) that the rate of lipolysis is decreased. This originates from the fact that milk production is reduced through the mechanism by which dexamethasone impairs the glucose uptake into the mammary tissues. There is only a limited number of studies on the effect of a single dose of dexamethasone on the metabolic status during the postpartum period. All of them, however, report an improvement of the

Table 1: Values of rate and effect parameters in the pharmacokinetic-pharmacodynamic model.

Name	Description	Value	Unit	Source
$F$	The bioavailability which is the fraction of dose that reaches the systemic circulation	72%	-	[30]
$k_a$	Absorption rate constant from the central compartment	13.4352	1/d	[30]
$k_e$	Elimination rate constant from the central compartment	2.7086	1/d	[30]
$V_d$	Volume of distribution	1.105	L/kg	[30]
$k_{eo}$	Rate constant describing the rate of change of drug concentration in the effect compartment	0.7	1/d	Estimated
$C_a$	Concentration required for half-maximal stimulation of glucagon secretion	1.8	ng/mL	Estimated
$C_b$	Concentration required for half-maximal inhibition of glucose uptake	1.8	ng/mL	Estimated
$E_{max}$	Maximum stimulatory effect of drug on glucagon secretion	3	-	Estimated

metabolic status in early lactation period. However, all the effects were observed in short terms and there was no long time improvement of the negative energy balance [73].

There are only few conducted studies on the effects of glucocorticoids in combination with or without other drugs on lipolysis in dairy cows, and the findings are inconsistent. In cows treated with dexamethasone on day seven and day eleven postpartum [74, 75], it was shown that in parallel with a significant increase in plasma glucose and insulin concentration, a significant decreases in the plasma concentrations of non-esterified fatty acids and beta-hydroxybutyrate occurs. This decrease can be used as a marker of excessive negative energy balance in dairy cows [76]. In other experiments, dexamethasone decreased blood ketone concentrations in ketotic and healthy fresh cows [18, 73]. On the other hand, the administration of a corticosteroid with or without insulin showed to have no therapeutic and preventive effects on ketosis one or two weeks after treatment in early lactation [77]. In other experiments, glucocorticoids did not lead to changes in non-esterified fatty acids concentrations in the blood [70, 71]. These aforementioned findings are discrepant, perhaps due to the difference in dosage, duration of treatment as well as the susceptibility of the cows under investigation. We think that increased lipolysis might occur only when the cow undergoes a consecutive multiple-day dosing of dexamethasone, where the accumulated concentration induces more fat degradation than glucose uptake into the mammary gland. This, however, should be supported by performing further in vivo and in silico experiments (simulating the model with multiple doses).



## Effects on Reproductive Performance

It was shown that an elevated concentration of beta-hydroxybutyric acid is associated with negative energy balance and poor fertility [78,79]. During the phase of negative energy balance, fat mobilization increases non-esterified fatty acids and beta-hydroxybutyrate concentrations. These elevations result in impaired *FSH* and *LH* secretion [78,80], resulting in a negative effect on follicle growth and ovulation. To assess the performance of our model, we administrate a single dose of dexamethasone at two different time points, days 50 and 52, and compare the two results. Simulation results before drug administration show that for a low glucose content ( $c_0 = 20\%$ ) in DMI the postpartum anestrous interval lasts until day 150, see Fig 9 (blue-solid line). After the administration of a single dose of dexamethasone on day 50, simulations show that dexamethasone induces estrous cyclicity with an elongated first cycle and normal cycles thereafter, see Fig 9 (red-dotted line). In contrast, administering a single dose of dexamethasone only two days later fails to reinduce the cyclicity of hormones, see Fig 9 (black-dotted line). This discrepancy can be ascribed to the time of administration. In other words, the dexamethasone dose results in a short term increase in glucose and insulin concentrations at days 50 and 52. Based on the linking mechanism of our model, a high insulin concentration stimulates *FSH*, *LH* and *IGF-1* hormones, see eq.(19) and eq.(20) in [32]. It turns out that the sharp increase in insulin temporarily stimulates *LH* and *IGF-1* around day 52, but in contrast to day 50, the *LH* magnitude does not surpass the threshold that allows follicles to ovulate.

To the best of our knowledge, there are only a few conducted studies about the effects of a single dose of dexamethasone or other glucocorticoids on reproductive performance in dairy cows in early lactation. For instance, Seifi et al. [77] reported that administration of a single dose of isoflupredone alone or in combination with insulin within the first 8 days after calving had no impact on reproductive performance. In other studies, it was reported that excess in glucocorticoid did not affect *LH* concentration of cows [81]. It was also shown in ewe that dexamethasone did not significantly modify reproductive function, in particular, it did not have any effect on *LH* [82]. Maciel et al. showed that dexamethasone did not affect *FSH* and *LH* concentrations, but the total number of follicles ( $\geq 5$  mm) and plasma estradiol concentrations were lower in the treatment group compared to control group [83]. However, in a recent study, Sami et al. [84] were the first to report an improving effect of a single injection of dexamethasone on pregnancy rate and reproductive performance. They concluded from their study that cows treated with an intramuscular injection of 20 mg at day three or ten of lactation had a shorter time to pregnancy than controls. In addition, dexamethasone-treated cows had a better pregnancy rate throughout the lactation period. In particular, the probability to develop pregnancy on day 120 postpartum was 2 times higher in cows that received dexamethasone in comparison to controls. This study was the first to suggest that a single dose of dexamethasone injection in early lactation might improve reproductive performance.

In general, the effects of glucocorticoids on fertility in dairy cows are not well understood. Future studies should explore mechanisms through which either dexamethasone concentration or its signalling pathways in the reproductive system

can be changed to improve the reproductive performance.

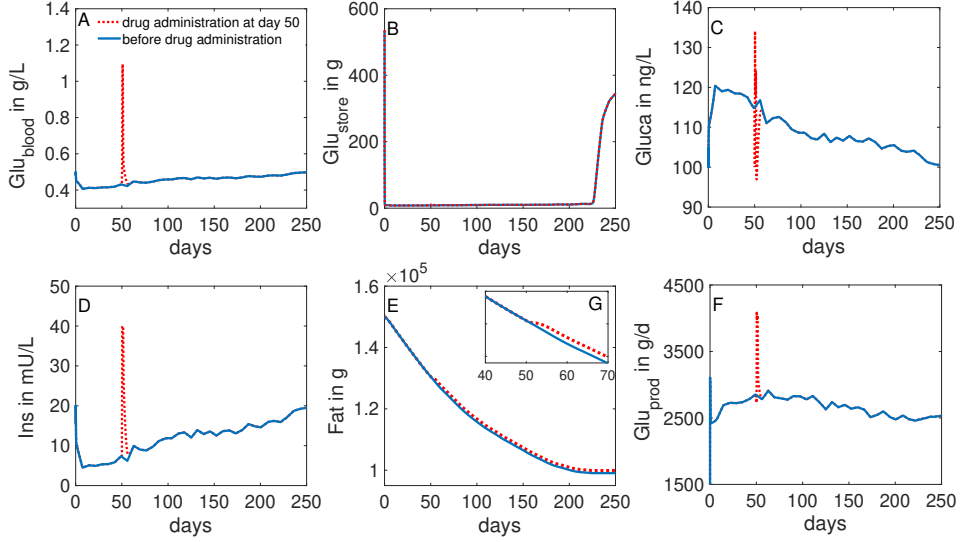


Figure 8: **Effects of administering a single dose of dexamethasone of 0.02 mg/kg on glucose-insulin dynamics in non-lactating cows.** This figure illustrates the changes in the metabolic components before (blue-solid line) and after (red-dashed line) dexamethasone administration. A sharp increase in glucose (A) and insulin (D) concentrations can be seen after administering a single dose of dexamethasone of 0.02 mg/kg on day 50. A stimulatory effect of dexamethasone on alpha cells increases glucagon concentration (C). Glucagon subsequently decreases as a response to the significant increase in the glucose level. The increase in glucagon concentration increases the supply of gluconeogenic precursors in the liver by stimulating the degradation and mobilization of glycogen (B) and fat (E) from the depots, thereby increasing glucose production (F). However, it can be seen that the magnitude of decrease in fat is reduced compared to the decrease observed before the administration of dexamethasone (E). This originates from the fact that milk production is reduced through the mechanism by which dexamethasone impairs the glucose uptake into the mammary tissues, thus leading to more glucose in the systemic circulation.

### 3 Conclusion

The mechanisms adopted for coupling the pharmacokinetic model to the pharmacodynamic model were based on the biological knowledge about the role of dexamethasone in stimulating glucagon secretion, glycogenolysis and lipolysis and in impairing the sensitivity of cells to insulin. Whether the stimulation of endogenous glucose production via gluconeogenesis is a primary dexamethasone effect or a secondary system change via dexamethasone-stimulated glucagon secretion remains unclear. An additional term which accounts for glucagon stimulation, was linked to  $glu_{prod}$  to avoid redundancy and overparameterization.

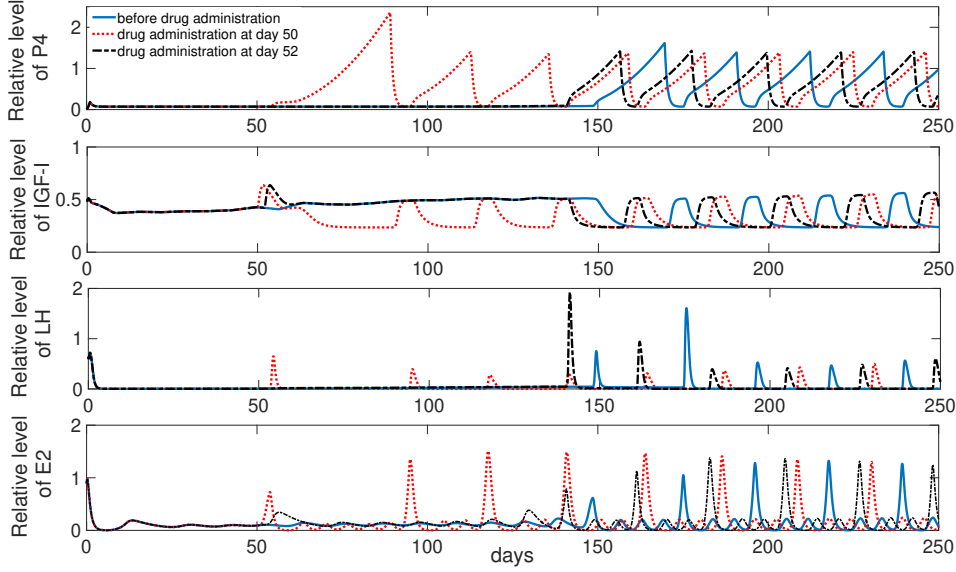


Figure 9: **Effects of a single dose of dexamethasone of 0.02 mg/kg on the estrous cycle in lactating cows.** This figure illustrates the dynamic behaviour of  $P_4$ ,  $IGF-1$ ,  $LH$  and  $E_2$  before and after dexamethasone administration. Simulation results before drug administration (blue-solid line) show that the postpartum anestrus interval last until day 150. Administering a single dose of dexamethasone on day 50 (red-dotted line) induces the cyclicity of  $E_2$ ,  $LH$  and  $IGF-1$ , thereby reinducing estrous cyclicity with an elongated first cycle and normal cycles thereafter. In contrast, administering a single dose of dexamethasone only two days later (black-dotted line) fails to reinduce cyclicity of hormones.

We have shown that the adopted mechanisms are able to induce a temporary hyperglycemia and hyperinsulinemia, which captures the observed data in non-lactating cows. In lactating cows, we have shown that a single dose of dexamethasone reduces the lipolytic effect, owing to the reduction of glucose uptake by mammary gland.

Although our model successfully simulates the acute effect of dexamethasone on the dynamics of metabolic hormones, the simulation of chronic dosing is infeasible due to the lack of mechanisms that describe the long-term effect of dexamethasone treatment on system homeostasis (such as insulin resistance caused via chronic hyperglycemia).

In summary, hyperglycemia and hyperinsulinemia can be simulated following treatment with a single dose of dexamethasone. However, while it is evident that glucocorticoids may acutely decrease or chronically increase insulin secretion in rodents and humans, available studies in dairy cows do not mention any acute decrease in insulin concentration. Since marked differences exist in digestion of

carbohydrates and regulation of glucose homeostasis between monogastric animals and ruminating cattle, acute effects of dexamethasone on insulin secretion as well as possible dysfunction of beta cells in chronic treatment regimes should be further investigated in dairy cows.

Mechanistic pharmacokinetic-pharmacodynamic modeling not only permits assessment of drug effects but also gives additional insights into the interacting components of the model. Improving our biological knowledge about the long term effect of glucocorticoids in dairy cows could entitle the proposed model to be further developed and used as a tool for exploring treatment strategies against ketosis and other diseases related to the metabolic system.

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