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ANIMAL HUSBANDRY

Simulation Modeling of Substrate Homeostasis of Mammary Secretory Cells[1]

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Abstract—The results of simulation modeling, compared with the data of a physiological experiment, made it possible to reveal metabolic shifts in the systems transporting and utilizing milk precursors and to identify parameters of the substrate balance in secretory cells of the mammary gland.

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It is known that provision with substrates, blood supply level, and metabolic activity of cells of the mammary gland have a critical value for milk secretion; however, the mechanisms of the effect of these factors on milk productivity have been studied inadequately. The composition of milk is maintained relatively constant against the background of continuous variations of blood flow and level of substrates in the blood, which is related to the effect of mechanisms of homeostatic regulation at the organ, tissue, and secretory cell level. The problem is, having the results of in vivo investigations, that is, data of a blood analysis, measurements of blood flow, milk yield, and milk composition, how does one identify these mechanisms. If there are no direct measurements of the parameters, we can use indirect data, using methods of solving problems of parametric identification (inverse problems of kinetics). The existing experience of using this approach shows that dynamic functional models constructed on the basis of information about processes at the tissue and cell levels will enable a better interpretation of the experimental data and identification of indices of the substrate balance. This is important in connection with the fact that computer modeling showed substrate flows in mammary secretory cells are enveloped by a network of mutual influences, and bottlenecks limiting biosynthesis can migrate depending on the particular metabolic situation [1, 2].

The interrelations between the blood supply level, transport of substrates into mammary secretory cells, and rate of biosynthesis were studied and the parameters of these relations with shifts in the nutrition conditions of lactating cows and goats were assessed in the present work.

METHODS

The data of three physiological experiments, in which organ blood flow and milk yield were measured and the composition of arterial and venous blood and milk was analyzed, served as the material for analysis [3–5]. The activity of transport of water-soluble metabolites into secretory cells, expressed in clearance units, was determined by the method described earlier [6]. In the first experiment on four lactating cows, we studied the effect of eliminating for 24 h the concentrate part of the ration with subsequent renewal of feed in the full amount. The conditions of conducting the experiment are described in greater detail in [3]. The second experiment was conducted on three lactating goats, which after starving for 26 h were injected in the jugular vein for 6 h on different days with the following infusates: physiological solution (control), glucose (4 g/h), or a mixture of amino acids (2.5 g/h) in the ratio in which they are a component of milk protein [4]. The third experiment was conducted on lactating goats which at first were injected for 6 h in the jugular vein with physiological solution and then for the next two days with insulin (2 μg/kg per hour) in combination with glucose in the same prescription. The initial rate of injecting glucose was 0.15 g/kg per hour. Blood samples were taken periodically for determining the glucose content in them with subsequent correction of the rate of its injection for maintaining normoglycemia [5].

The conceptual principles, structure of the model, and complete scheme of the calculations used in numerical simulation of the processes occurring in the lactating mammary gland were described earlier [2, 7]. The values of internal metabolic parameters (constant coefficients) for the initial and experimental periods were determined by the best-fit test of the predicted and actual indices of milk formation by multiple running of the program with given concentrations of the substrates in the blood and values of the blood flow with variation of the values of the investigated parameters. The weight

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Table 1. Comparison of data of experiment and model prediction of milk formation indices of cows in initial state and during partial deprivation of feed

Index	Experiment		Prediction	
	initial	deprivation	initial	deprivation
Milk yield, kg/day	12.5	10.0	12.8	10.3
Butterfat, %	3.08	3.77	3.09	3.67
Butterfat production, g/day	385	377	395	378
Protein, %	3.01	3.08	3.00	3.01
Protein production, g/day	376	308	384	310

of the udder parenchyma was estimated indirectly by the daily milk yield (1/2 of the yield in kilograms). The Runge–Kutta method with a fixed 0.001 step was used for integrating the equations; the limits of integration were 0–0.06 (days).

It was shown in preceding investigations that, when analyzing various experimental situations, the model prediction agrees with the results obtained in experiments conducted on cows and goats [2, 3, 8–10].

RESULTS AND DISCUSSION

In the first experiment, in the initial phase of adaptation to partial feed deprivation the main role was played by factors having not a substrate but a signal function, since a change in the rate of blood flow was found already 3 h after excluding concentrates against the background of no shifts in the concentration of the main blood metabolites [3]. In the period between 8 and 23 h, an adaptive decrease in the activity of transport of amino acids, acetate, and β-hydroxybutyrate and an increase in the concentration and transport constant of nonesterified fatty acids (NEFA) into secretory cells were noted [3, 11]. During adaptation of the model to the period of deprivation, the values of the concentrations of the main substrates in arterial blood, transport rate constant of amino acids into the cells, entry of acetate from the interstitium into the cell (with consideration of the contribution of β-hydroxybutyrate, and magnitude of the blood flow measurements in the experiments were added to the composition of the input data. An increased value of the maximum rate of formation of butterfat from NEFA (with an excess of the substrate) was identified by the best-fit test of the predicted and actual data. With the use of new values of these parameters, the predicted values of milk yield and milk composition proved to be close to those observed in the experiment (Table 1).

Consequently, in this experimental situation, the critical role for the rate of milk formation and yield of milk protein was apparently played by organ blood flow and activity of transport of amino acids into secretory cells. A decrease of blood flow in itself should lead to a decline of glucose concentration in the intercellular fluid and inside the cell and, as a consequence of this, to a decrease in the rate of lactose synthesis—the main determinant of the volume of secretion. A decrease in the activity of transport of amino acids into the cell during deprivation of concentrates could be a consequence of either the direct hormonal effect on the system of membrane transporters or of an adaptive shift in this system of the down-regulation type owing to the excess of free essential amino acids that occurred as a result of their reduced need for synthesis of milk protein [12].

In the second experiment, 26-h starvation led to a significant reduction of blood flow through the udder and it remained stable for 6 h after infusion of physiological solution. Intravenous injection of glucose led to an increase of blood flow, milk yield, and production of milk protein, and blood flow increased after injecting the mixture of amino acids, whereas milk yield and protein production did not change [4]. The experimental data were compared with the model prediction obtained with the use of two calculation variants: (1) with values of metabolic parameters (coefficients) changed for the period of infusions and (2) with the same values as for the period of starvation. In both variants the values of the arterial concentration of substrates and blood flow were introduced as the input variables (Table 2). The first variant proved to be more accurate, the second also gave a qualitatively correct milk yield prediction, but slightly underestimated the values of protein yield with

Table 2. Comparison of prediction according to model with correction of parameters by periods of the experiment* and average values of productivity indices of goats

Index	Initial period	Starvation	Glucose infusion	Amino acid infusion
Milk yield, l/6 h	0.4 (0.4)	0.20 (0.20)	0.28 (0.29)	0.20 (0.2)
Butterfat, %	4.7 (4.66)	5.47 (5.50)	3.97 (4.00)	4.33 (4.4)
Protein, %	2.85 (2.8)	3.36 (3.40)	3.02 (3.00)	3.35 (3.3)
Butterfat production, g/6 h	18.8 (19.0)	10.90 (11.0)	11.12 (11.60)	8.66 (8.8)
Protein production, g/6 h	11.4 (11.4)	6.72 (6.80)	8.46 (8.70)	6.70 (6.6)

* Calculation according to variant 1, see text.

Note: The average values of the quantities measured in the experiment are given in parentheses.

Table 3. Average values of indices of milk formation and organ blood flow in goats and data of model for initial state (infusion of physiological solution) and for period of infusion of insulin with glucose

Index	Infusion of insulin with glucose			
	experiment	model	experiment	model
Milk yield, l/day	1.54	1.52	1.97	2.09
Blood flow, l/day	598	601	922	929
Butterfat, %	5.2	5.2	4.0	4.0
Protein, %	3.2	3.2	3.0	3.0
Butterfat production, g	311	312	369	372
Protein production, g	191	192	277	279

the injection of glucose and overestimated the values of butterfat with the injection of amino acids. According to the data of the first calculation variant, with the injection of glucose the activity of glucose transport into the cell increased (from 1.2 to 1.5 l/day kg $\times 10^{-3}$) as did the maximum rate of milk protein synthesis (from 0.52 to 0.6 mol/day). With injection of amino acids, these parameters remained at the level noted during starvation, and the maximum rate of esterification of fatty acids, to judge for the calculation, decreased from 0.59 to 0.37 mol/day.

The absence of stimulation of milk yield after injecting starving animals with the amino acid mixture and the considerable effect after injecting glucose were reproduced qualitatively on the model even with consideration of possible adaptive changes in metabolic parameters. This gives grounds to assume that the specific relationships of intracellular concentrations and affinities (semisaturation points) in lactose and milk protein synthesis systems could be the cause of the noted qualitative difference in action of the substrates.

In the third experiment, intravenous injection of insulin with glucose did not have a noticeable effect on protein content in milk, substantially reduced the butterfat content in it, and stimulated organ blood flow, rate of milk formation, and protein production [5]. When analyzing the data of the experiment conducted, just as those of similar experiments described by other authors, it was important to obtain an answer to the question of whether shifts in milk yield and protein production are caused only by changes in blood flow. An increase in glucose and amino acid transport activity and positive correlation of amino acid transport activity with protein production were found in our experiment with injection of insulin with glucose, which indicated the presence of adaptive shifts in metabolic parameters [5]. The results of model calculations showed also that shifts in production indices were not caused by changes in blood composition and magnitude of blood flow.

During these calculations the model at first adapted to the initial state, when those blood indices which were observed during injection of insulin with glucose were introduced into the composition of the input variables; after this a value of the plasma flow close to that observed during experimental infusion was selected by varying the scale factor (basic plasma flow) during several runs of the program. It turned out here that such a variant of the model substantially underestimates the rate of milk formation and protein production compared with the experimental data. On the other hand, a good fit of the predicted and actual data was obtained with the use of increased, as compared with the initial period, indices of the capacity (maximum rate with excess substrate) of the amino acid–protein synthesis systems (V3: from 0.75 to 0.92 mol/day kg); acetate—milk fat (V4: from 0.3 to 0.39) and transport rate constant into cells of glucose (K1: from 0.35 to 0.45 l/day kg), amino acids (from 3.1 to 3.5), and acetate (K5: from 1.2 to 1.65) (Table 3). Consequently, a comparison of the data of the experiment and results of computer modeling indicates that the positive productive effect with respect to the rate of milk production and milk protein yield in the given experiment was due to stimulation of both the organ blood flow and metabolic activity of mammary secretory cells.

The recently revealed effect of stimulation of milk formation and protein production in cows and goats as a result of intravenous infusion of insulin with glucose [13, 14], judging by our data, can be related to activation of metabolism of amino acids in secretory cells, since shifts in substrate transport activity into the cell in the presence of active transport systems are usually due to variations in the number of molecules of transporters in the plasma membrane, and these variations are directly related to overall adaptation of cell metabolism to the altered status [15]. In itself the development of methods of quantitative assessment of amino acid transport under in vivo conditions is important in an applied aspect, since the search for amino acids limiting milk formation still remains among relevant unsolved problems despite the numerous investigations carried out. In a broader aspect, the search for substrate biosynthesis limiting points is met with on the path of gradual perfection of models of substrate homeostasis with subsequent uniting of theory and experiments for determining bottlenecks.

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