Evaluation of a Model Integrating Protein and Energy Metabolism in Preruminant Calves[1,2]

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ABSTRACT In a companion paper, a mechanistic model is described, integrating protein and energy metabolism in preruminant calves of 80–240 kg live weight. The model simulates the partitioning of nutrients from ingestion through intermediary metabolism to growth, consisting of accretions of protein, fat, ash and water. The model also includes a routine to check possible dietary amino acid imbalance and can be used to predict amino acid requirements. This paper describes a sensitivity and behavioral analysis of the model, as well as tests against independent data. Increasing the carbohydrate:fat ratio at equal gross energy intakes leads to higher simulated protein- and lower simulated fat-deposition rates. Simulation of two experiments, not used for the development of the model, showed that rates of gain of live weight, protein and fat were predicted satisfactorily. The representation of protein turnover enables the investigation of the quantitative importance of hide, bone and visceral protein in protein and energy metabolism. The model is highly sensitive to 25% changes in kinetic parameters describing muscle protein synthesis and amino acid oxidation. Comparing simulated with experimentally derived amino acid requirements shows agreement for most amino acids for calves of ~90 kg live weight. For calves of ~230 kg live weight, however, lower requirements for lysine and for methionine + cystine are suggested by the model. More attention has to be paid to the inevitable oxidative losses of amino acids. It is concluded that the model provides a useful tool for the development of feeding strategies for preruminant calves in this weight range. J. Nutr. 127: 1243–1252, 1997.

KEY WORDS: • *veal calves* • *computer simulation* • *mathematical model* • *amino acid requirements* • *energy metabolism*

In a companion paper (Gerrits et al., in press), a mechanistic growth simulation model is described, developed for preruminant calves between 80 and 240 kg live weight (Lw).[4] The objectives of this model are to gain insight into the partitioning of nutrients in the body of growing calves and to provide a tool for the development of feeding strategies for calves in this weight range. The model simulates the partitioning of ingested nutrients through intermediary metabolism to growth, consisting of accretions of protein, fat, ash and water. The model can also be used to predict amino acid requirements. It is based largely on data derived from two experiments with preruminant calves, especially designed for its construction (Gerrits et al. 1996). The objectives of the research reported in this paper are to evaluate model behavior, to test the sensitivity of model predictions to changes in model parameters, and to test the predictive quality of the model. To achieve this objective, several simulations were performed. First, driving variables (intake of nutrients) were varied. Second, model parameters were varied. Third, two published experiments, not used for the development of the model, were simulated and the results compared with the experimental observations. Finally, the behavior, sensitivity and predictive quality of the simulation of amino acid requirements were tested. Throughout this paper, results of the simulations performed are presented directly following the description of the simulation.

A reference simulation is chosen as the starting point for many of the simulations performed throughout this paper. It was decided to simulate a fast growing calf in the middle of the weight range for which the model was developed. The results of the reference simulation are obtained at 160 kg Lw, starting the simulation at 120 kg Lw. The nutrient intakes at 160 kg Lw are as follows: milk proteins (N × 5.92; Gerrits et al., in press), 556 g/d; fat, 428 g/d; lactose, 924 g/d; (pregelatinized) starch, 86 g/d.

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[4] Abbreviations used: DF, Dutch-Friesian; FDR, fractional degradation rate; HF, Holstein-Friesian; Lw, live weight; MSPE, mean square prediction error; V_{max}, maximum reaction velocity.

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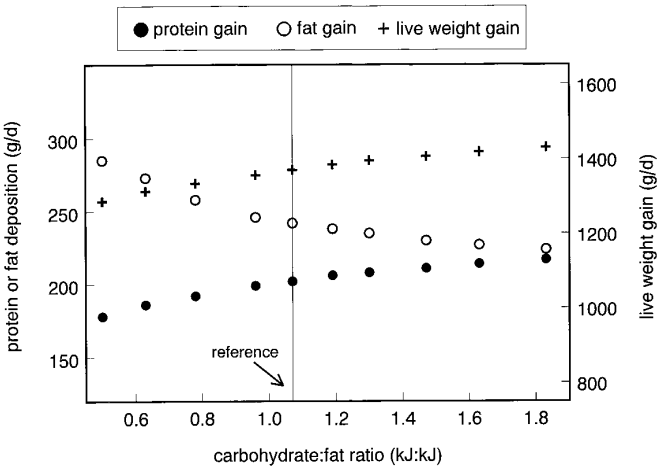

FIGURE 1 Sensitivity of model predictions of the rate of gain of protein, fat and live weight to changes in the ratio between energy intake from carbohydrates and fat at a constant energy intake. The reference simulation (see text) was used as a starting point.

BEHAVIORAL AND SENSITIVITY ANALYSIS

Changing driving variables

Protein and energy intake. In the companion paper, results of simulations of the experimental treatments of Gerrits et al. (in press) are presented and discussed. It is realized that these experimental data are not independent, because they are also used for parameterization of the model. Simulation of these experiments, however, illustrates the response of model predictions to intake of nutrients, varied independently over a wide range.

Dietary carbohydrate:fat ratio. Starting from the reference simulation, the ratio between energy intake from carbohydrates and fat was varied between 0.5:1 and 1.8:1. This range is comparable with the range tested experimentally by Donnelly (1983) using preruminant calves of 40–70 kg Lw. Gross energy intake was equal for all simulations. Daily fat intake decreased from 586 to 316 g/d with an increasing ratio. Daily carbohydrate intake (lactose + starch) increased from 606 to 1288 g/d with an increasing ratio. The apparent fecal digestibility of fat and carbohydrates in the model was set to 0.95, as discussed by Gerrits et al. (in press).

As shown in **Figure 1**, increasing the dietary carbohydrate:fat ratio at equal protein and gross energy intakes leads to an interesting shift in model predictions from fat to protein deposition. The simulated protein deposition rate increases from 178 to 217 g/d when the carbohydrate:fat ratio increases from 0.5 to 1.8. Fat deposition decreases from 285 to 224 g/d. Consequently, the rate of live weight gain increases from 1286 to 1430 g/d. In the model, the decreased availability of dietary fatty acids (244 g/d) with an increasing carbohydrate:fat ratio is only partly compensated for by an increased rate of de novo fatty acid synthesis (44 g/d) and a reduced fatty acid oxidation rate (144 g/d). The input into the acetyl-CoA pool from glycolysis increases with an increasing carbohydrate:fat ratio. This increase is larger than the increased net output to the fatty acid pool. Therefore, the acetyl-CoA concentration rises, causing the increased protein synthesis rates (see Gerrits et al., in press).

Unfortunately, the results of this modeling exercise could not be compared with experimental data in the live weight range 80–240 kg. The simulations, however, partly confirm the results of Donnelly (1983), who found similar effects with preruminant calves of 40–70 kg at a low dietary protein to energy ratio. At the high dietary protein to energy ratio, these effects were not observed, which was partly attributed to a narrower range in dietary carbohydrate:fat ratio tested with the high protein low energy diets. Model behavior, however, is similar when the carbohydrate:fat ratio is varied at different protein intakes (results not shown). It is known that a shift in energy intake from lipogenic to glycogenic sources increases the insulin production, which stimulates protein deposition (Reeds and Davis 1992). This is in line with the simulated shift in partitioning of nutrients, described above.

Changing major model assumptions

Maintenance energy and protein. To evaluate the quantitative importance of maintenance energy, the amount of energy spent, calculated as the sum of the requirements for the individual tissues, is varied by multiplication with a factor between 0.7 and 1.4. To relate this figure to practice, it is expressed in kJ/(kg$^{0.75}$ · d). The results are presented in **Figure 2**. As expected, rates of gain of fat, protein and live weight decreased with increasing maintenance energy requirements. The response of fat deposition rate to increased maintenance energy requirements is larger than that of protein deposition rate. The increased amount of energy spent on maintenance processes, however, is larger than the decrease in tissue deposition (expressed in energy units). A part (varying from 8 to 24%) of this increased energy expenditure is compensated by a decrease in the flux "additional costs of growth." This flux represents the increased costs of tissue deposition with increased tissue deposition rates (see Gerrits et al., in press).

The sensitivity of model predictions to variation in the daily expenditure on protein for maintenance is tested by separately varying the three maintenance components, represented by the model: **1**) endogenous urinary losses are varied from 50 to 350 mg N/(kg$^{0.75}$ · d) (default is 180); **2**) metabolic fecal losses are varied from zero to 7 g N/(kg dry matter intake) (default is 2.46); **3**) protein losses from skin and hair are varied from 3 to 47 mg N/(kg$^{0.75}$ · d) (default is 18). Increased N losses via all three pathways do not exert detrimental effects on rate of gain of protein, fat and live weight. The effects were all in a similar direction, which is illustrated in Figure 2 for increased metabolic fecal losses. In all three cases, the effect on protein deposition rate is less than expected on the basis of the increased N losses. Increased N losses lead to a decrease in the concentration of amino acids, which in turn decreases the rate of amino acid oxidation, provided that the increased losses do not cause an amino acid imbalance. By this mechanism, a large part of the increased N losses is compensated for. Seventy-nine percent of increased net fecal N losses, for example, is compensated for in this way. The small decrease in the fat deposition rate with increased N losses is caused by a lower energy yield from amino acid oxidation and increased energy expenditure on protein synthesis (in the second and third pathways). It is realized that the increased costs of protein synthesis are probably underestimated because only the net fecal endogenous losses, i.e., the difference between synthesis and reabsorption,.are modeled.

Protein turnover. The deposition rate of hide, visceral and bone protein is related to the muscle protein deposition rate (discussed by Gerrits et al., in press). Therefore, an increase in the fractional degradation rate (FDR) of hide protein, for example, is followed by an increased hide protein synthesis rate, because the model will try to keep the hide protein deposition rate proportional to the muscle protein deposition rate. Therefore, the turnover rate of protein in hide, bone and

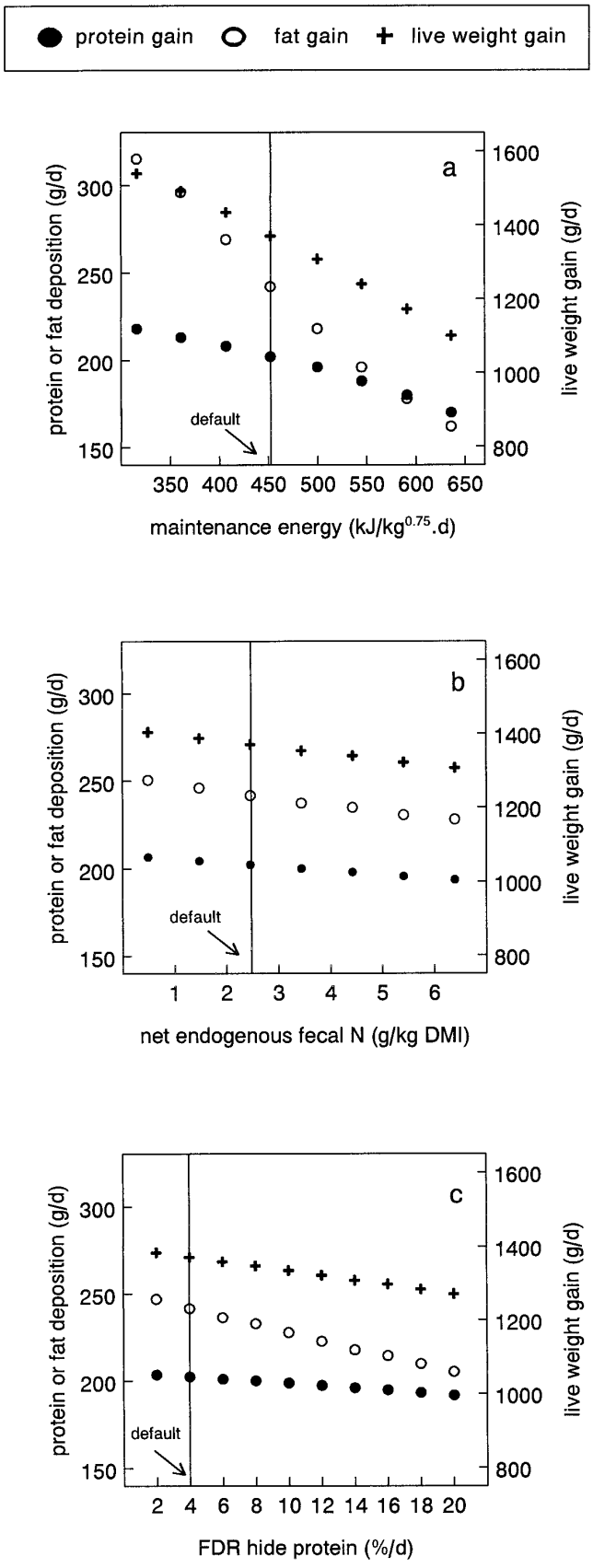

FIGURE 2 Sensitivity of model predictions of the rate of gain of protein, fat and live weight to changes in energy requirements for *a*) maintenance, *b*) net endogenous fecal N losses, and *c*) fractional degradation rates (FDR) of hide protein.

viscera can be varied by varying the respective FDR. The response of the model to changes in the FDR of muscle protein, however, is enlarged because of the dependency of hide, visceral and bone protein deposition on muscle protein deposition. An increase in the FDR of muscle protein leads to increased amino acid oxidation rates, because oxidation depends on the amino acid concentration. The muscle protein deposition rate is therefore decreased. Consequently, deposition of hide, visceral and bone protein is reduced as well. This, in turn, leads again to increased amino acid oxidation and thus enlarges the effect of the increase in FDR of muscle protein deposition rate. Increasing the FDR of muscle protein from 1.5 to 2.5%/d (default is 2%/d) doubles the amino acid oxidation, consequently decreasing the protein deposition rate from 283 to 121 g/d in the reference simulation.

As an example, the effect of increasing the FDR of hide protein from 2 to 20%/d on tissue deposition rates is shown in Figure 2. Increasing the FDR of hide protein slightly decreases the total protein deposition rate. The increased FDR leads to an increased amino acid concentration. This, in turn, leads to an increased amino acid oxidation rate, leaving less amino acid to be deposited. The decrease in protein deposition rate, however, is small, indicating that hide protein synthesis is increased to an almost similar extent as degradation. Increasing the FDR of hide protein from 2 to 20%/d increases the protein degradation rate with 662 g/d and increases the protein synthesis rate with 650 g/d, leaving a difference of 12 g/d. As expected, increasing the FDR of hide protein negatively affects the fat deposition rate. The response of fat deposition rate is larger than that of protein deposition rate and is caused by the increased energy expenditure on protein turnover. Furthermore, by increasing the amino acid fluxes, increased protein turnover will affect the amino acid requirements. This effect will be discussed later in this paper.

Energy requirements for tissue deposition. The effect of changing some of the main stoichiometric assumptions on the rate of gain of protein, fat and weight was tested, using the reference simulation as a starting point. The main assumptions tested were the amount of ATP required for **1**) protein synthesis and degradation, **2**) synthesis of dispensable amino acids, **3**) fat synthesis and **4**) the incorporation of Ca and P into bone ash, the latter being the only costs of ash deposition accounted for in the model. The results of these simulations are shown in **Table 1**. Additionally, Table 1 gives the amount of energy spent on these processes, expressed as a percentage of the total energy expenditure in the model. Increasing the amount of ATP needed for peptide bond synthesis considerably depresses the fat and to a lesser extent protein deposition rates. The effect of a similar increase of the amount of ATP needed for peptide bond degradation is smaller but still considerable. The effects of changing the amount of ATP needed for the synthesis of dispensable amino acids and incorporation of Ca and P into bone ash are quantitatively unimportant.

Sensitivity to changes in kinetic parameters

A sensitivity analysis was performed using the reference simulation as the starting point. The default values of all kinetic parameters used in the model, i.e., the maximum reaction velocities, affinity and inhibition constants and steepness parameters (see Table 3 in Gerrits et al., in press), were increased or decreased by 25%. The effects of changing these parameters on the rate of gain of protein, fat and live weight were analyzed. Also, the effect on the main transaction, which the parameter describes, is presented. The effects of changing the kinetic parameters of the fluxes involving protein are presented in

TABLE 1

The effect of changing stoichiometric assumptions in the model simulating metabolism of preruminant calves on the energy costs of protein turnover, synthesis of dispensable amino acids (DAA), fat synthesis and incorporation of Ca and P into bone ash, in the reference simulation[1]

Assumptions (default bold)	Protein deposition	Fat deposition	Live weight gain	% of total energy expenditure
		g/d		
mol ATP/peptide bond synthesized				Protein synthesis
3	207	262	1419	14.0
4	202	242	1370	17.7
5	197	222	1319	21.2
mol ATP/peptide bond degraded				Protein degradation
0	206	259	1411	0.0
1	202	242	1370	3.5
2	198	225	1327	6.8
mol ATP/mol DAA synthesized				Synthesis DAA
0	203	243	1374	0.0
3	202	242	1370	0.3
6	202	240	1366	0.6
mol ATP/mol fat synthesized				Fat synthesis
7	203	244	1375	0.9
10	202	242	1370	1.2
13	202	240	1365	1.6
mol ATP/mol Ca or P incorporated into bone ash				Ash deposition
0	203	243	1374	0.0
2	202	242	1370	0.3
4	202	240	1366	0.6

[1] For a description of the reference simulation, see text.

Table 2, those involving fatty acids or acetyl-CoA in **Table 3**. The effects of changing the kinetic parameters that involve glucose metabolism were negligible and are therefore not presented. As explained in Gerrits et al. (in press), these parameters were set to prevent accumulation of glucose in the glucose pool. In general, if a change in a model parameter increases the protein deposition rate, then the fat deposition rate is decreased, and vice versa (Tables 2 and 3). This is caused by two mechanisms: **1**) Increased amino acid oxidation rates will result in, or are a consequence of, lower protein synthesis (and therefore lower deposition) rates. These amino acids are converted into acetyl-CoA, which can be deposited as fat via increased fatty acid synthesis rates. **2**) When changing a model parameter does not affect amino acid oxidation but, for instance, increases the acetyl-CoA concentration, the concentration of fatty acids is increased too because of decreased fatty

TABLE 2

Sensitivity of rates of gain of protein, fat and live weight and of rate of principal transactions, predicted by the model simulating metabolism of preruminant calves, to 25% changes in all kinetic parameters involved in protein metabolism, compared with the reference simulation[1]

Model parameter[2]	Change %[3]	Protein deposition	Fat deposition	Live weight gain	Principal transaction	Effect on principal transaction
		Difference, compared with reference simulation, *g/d*				*mmol substrate/d*
$V^*_{Aa,AaAy}$	−25	6.5	−1.9	34	Amino acid oxidation	−48
	+25	−4.5	3.2	−22	Amino acid oxidation	59
$M_{Aa,AaAy}$	−25	−18.1	9.0	−91	Amino acid oxidation	180
	+25	11.9	−5.4	60	Amino acid oxidation	−115
$S_{Aa,AaAy}$	−25	−3.2	2.8	−15	Amino acid oxidation	48
	+25	2.2	−0.6	11	Amino acid oxidation	−14
$V^*_{Aa,AaPm}$	−25	−88.9	46.9	−440	Muscle protein synthesis	−781
	+25	82.5	−34.4	421	Muscle protein synthesis	715
$M_{Aa,AaPm}$	−25	15.2	−6.1	78	Muscle protein synthesis	73
	+25	−13.7	7.6	−68	Muscle protein synthesis	−69
$M_{Ay,AaPm}$	−25	8.7	−4.3	44	Muscle protein synthesis	72
	+25	−8.2	4.2	−41	Muscle protein synthesis	−69

[1] For description of reference simulation, see text.

[2] $V^*_{i,jk}$ = maximum velocity for $j - k$ transaction per kilogram tissue in which transaction occurs; $M_{i,jk}$ = Michaelis-Menten affinity constant for $j - k$ transaction; $S_{i,jk}$ = steepness parameter associated with i for $j - k$ transaction. Aa = amino acids; Ay = acetyl-CoA; Pm = muscle protein.

[3] Changes obtained by multiplying the default parameter values by 0.75 or 1.25; for default values see Table 3 in Gerrits et al. (in press).

TABLE 3

Sensitivity of rates of gain of protein, fat and live weight and of rate of principal transactions, predicted by the model simulating metabolism of preruminant calves, to 25% changes in all kinetic parameters involved in energy metabolism, compared with the reference simulation[1]

Model parameter[1]	Change %[1]	Protein deposition	Fat deposition	Live weight gain	Principal transaction	Effect of principal transaction
		Difference, compared with reference simulation, *g/d*				*mmol of substrate/d*
$V^*_{Fa,FaAy}$	−25	−9.6	21.0	−29	Fatty acid oxidation	−102
	+25	7.0	−11.7	26	Fatty acid oxidation	78
$J_{Ay,FaAy}$	−25	−5.4	12.6	−16	Fatty acid oxidation	−57
	+25	4.2	−7.1	15	Fatty acid oxidation	46
$M_{Fa,FaAy}$	−25	0.7	−1.2	3	Fatty acid oxidation	8
	+25	−0.7	1.3	−2	Fatty acid oxidation	−8
$V^*_{Fa,FaFb}$	−25	4.1	−47.2	−32	Fat synthesis	−76
	+25	−3.6	8.6	−10	Fat synthesis	41
$M_{Fa,FaFb}$	−25	−1.0	2.7	−2	Fat synthesis	13
	+25	0.6	−1.9	1	Fat synthesis	−10
$V^*_{Ay,AyFa}$	−25	3.4	−1.6	17	Fatty acid synthesis	−484
	+25	−3.1	1.9	−15	Fatty acid synthesis	441
$M_{Ay,AyFa}$	−25	−2.7	1.7	−13	Fatty acid synthesis	389
	+25	2.0	−1.0	10	Fatty acid synthesis	−280
$J_{Fa,AyFa}$	−25	0.1	0.0	1	Fatty acid synthesis	−13
	+25	−0.1	0.1	0	Fatty acid synthesis	8
$V^*_{Ay,AyAg}$	−25	4.8	21.7	52	Additional energy costs of growth	−1134
	+25	−4.9	−18.5	−48	Additional energy costs of growth	975
$M_{Ay,AyAg}$	−25	−1.6	−6.5	−16	Additional energy costs of growth	305
	+25	1.8	7.7	19	Additional energy costs of growth	360
$S_{Ay,AyAg}$	−25	1.4	6.2	15	Additional energy costs of growth	−284
	+25	−1.0	−4.2	−10	Additional energy costs of growth	196

[1] See footnotes Table 2; other abbreviations: $J_{i,jk}$ = Michaelis-Menten inhibition constant for $j - k$ transaction with respect to i; Ag = additional energy costs of growth; Fa = fatty acids; Fb = body fat.

acid oxidation and increased fatty acid synthesis rates. This, in turn, results in increased fat deposition rates. Protein synthesis (and consequently deposition) are decreased via the affinity constant of acetyl-CoA for muscle protein synthesis. The first mechanism causes large effects on protein deposition, but small effects on fat deposition rates. Effects caused by the second mechanism are the reverse. Live weight gain usually follows the response of protein deposition rate because the deposition of protein is accompanied by water. An exception to the two mechanisms described above is the oxidation of acetyl-CoA to meet the additional costs of growth. An increase in this flux decreases the amount of acetyl-CoA available for other purposes and thus depresses both protein and fat deposition rate.

As expected, the model is sensitive to changes in the parameters describing the amino acid oxidation transaction. Increasing the affinity constant by 25% decreases the amino acid oxidation rate by 115 mmol/d (about 12 g protein/d), which is consequently used for protein deposition. Changes in the steepness parameter for amino acid oxidation are hardly reflected in the amino acid oxidation rate. This may be specific, however, for the reference simulation. The rate of amino acid oxidation in the reference simulation is near 50% of its maximum velocity (V_{max}), a rate at which the transaction is not sensitive to changes in steepness parameters (Thornley and Johnson 1990). At both lower and higher amino acid concentrations, caused by lower and higher protein intakes, respectively, the model is more sensitive to changes in this steepness parameter. The model is highly sensitive to changes in all parameters in the muscle protein synthesis transaction, especially the V_{max}. This is a consequence of relating the deposition rate of the other protein tissues to muscle protein deposition rate, as discussed earlier in this paper.

Model predictions are moderately sensitive to changes in the V_{max} for fatty acid oxidation, fat synthesis and the additional energy costs for growth (Table 3). Maximum velocities in this model are scaled with tissue size in which the transaction considered is expected to take place (Gerrits et al., in press). Therefore, in general, the model is more sensitive to changes in V_{max} than to similar changes in other model parameters.

COMPARISON WITH PUBLISHED EXPERIMENTS

Only two suitable experiments were found to evaluate model performance, i.e., an experiment of Van Es and Van Weerden (1970) in the weight range of 40–155 kg Lw and an experiment of Meulenbroeks et al. (1986) in the weight range of 180–230 kg Lw. As an indicator for the error of predicted values relative to experimental values, the mean square prediction error (MSPE) was computed in Equation (1):

$$\text{MSPE} = \sum_{i=1}^{N} (O_i - P_i)^2/n \qquad (1)$$

in which O_i and P_i are the observed and predicted values; i = 1,..., n; n = number of experimental observations (Bibby and Toutenburg 1977). The root MSPE is a measure in the same units as the output and is expressed as a percentage of the observed mean. The MSPE can be decomposed into *1*) the overall bias of prediction, *2*) deviation of the regression slope from 1, which is the line of perfect agreement, and *3*) the disturbance proportion (Bibby and Toutenburg, 1977). The first represents the proportion of MSPE that results from a consistent over- or underestimation of the experimental observations by model predictions. The second represents the proportion of MSPE that results from inadequate simulation of differences between experimental observations. The remaining proportion of MSPE represents the proportion that is unrelated to the errors of model prediction.

Van Es and Van Weerden (1970)

These authors conducted experiments in which they evaluated the effect of five feeding strategies for veal calves on rate of live weight gain and on N and energy balance in the weight range of ~40–155 kg Lw. Some of these data concerning live weight gain and N balances, published in an internal report (Van Weerden 1968), were available to test model performance. Van Es and Van Weerden used two Dutch Friesian male calves per treatment and fed milk replacers based on milk proteins only. In the five feeding strategies, both protein and energy intake were varied. Protein intake decreased with age at three different energy intake levels. Nutrient intakes, weight gain and N and energy balances were measured during four consecutive periods. The first period (d 0–30, weight range ~40–55 kg Lw) was considered too far outside the weight range for which the model was developed. Therefore, only the last three periods were simulated: period 2, d 31–58 (weight range ~55–85 kg Lw); period 3, d 59–87 (~85–110 kg Lw), and period 4, d 88–120 (~110–155 kg Lw). Nitrogen intake varied across treatments from 49 to 59, 38 to 54 and 57 to 97 g/d in period 2, 3 and 4, respectively. Fat intake varied across treatments from 125 to 388, 131 to 373 and 255 to 678 g/d in period 2, 3 and 4, respectively. Lactose intake varied across treatments from 598 to 783, 583 to 734 and 1058 to 1379 g/d in period 2, 3 and 4, respectively.

The results of the simulation of weight gain and N retention are presented in **Figure 3**. The root MSPE of live weight gain was 15.4% of the observed mean. Fifty-two per cent of MSPE was attributed to the overall bias and 48% to the disturbance proportion. The deviation of the regression slope from 1 did not contribute to the MSPE, indicating that the wide variation in growth rates caused by the feeding strategies was quantitatively predicted by the model. The consistent overestimation of growth rate was about 100 g/d. It is thought that the actual live weight gain was less than would have occurred in an optimal situation. In these experiments, the calves were transported each period to climate respiration chambers in which energy balances were measured during a 24-h period.

The predicted N retention is in general agreement with the observed values (Fig. 3). The root MSPE was 11.6% of the observed mean. Although the overall mean was well predicted (2% of the MSPE was attributed to overall bias), the observed variation in N retention, caused by the experimental treatments was less well predicted than the observed growth rates (deviation of regression slope from 1 contributed 35 and 0% to the MSPE, respectively). This may be due to the short period of collection in the N balance (6 d), whereas growth rates were determined over a longer period (28–33 d). A large part of the variation in the observed N retention was due to health problems during the collection period, rather than to the experimental treatments (Van Weerden 1968).

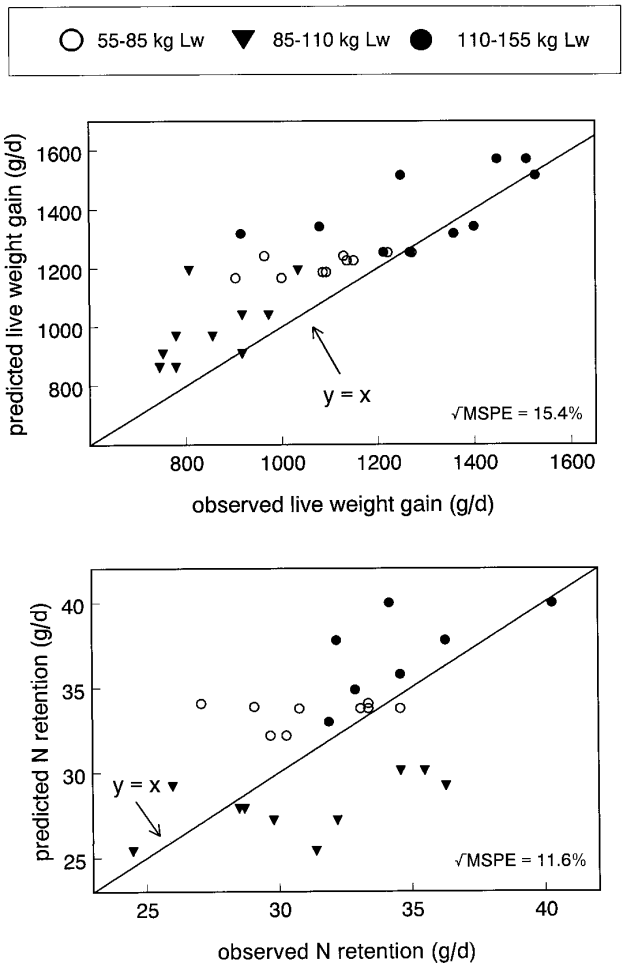

FIGURE 3 Comparison of experimental observations with model predictions of rate of live weight gain (*top*) and N retention (*bottom*) in the experiments of Van Es and van Weerden (1970). Values for live weight gain are averages over the live weight ranges 55–85, 85–110 and 110–155 kg. Values for N retention are determined in the middle of the respective weight range. √ MSPE = root mean square prediction error, expressed as a percentage of the observed mean, see Equation (1) in text.

Meulenbroeks et al. (1986)

These authors investigated the effect of genotype and feeding level on live weight gain, N and energy balance of preruminant calves in a 2 × 2 factorial arrangement. They used 20 male preruminant calves of either Dutch Friesian (DF) or Holstein Friesian (HF) × Dutch Friesian crossbreeds. The feeding levels used were 2.1 and 1.8 times metabolizable energy intake for maintenance. Energy balances were measured per group of five calves by indirect calorimetry, and N balances were measured by the total collection of urine and feces of each calf. Live weight gain, energy and N balances were measured weekly during the last 5 wk of the fattening period (180–230 kg Lw). The original data of 18 calves could be used for the simulations. These experiments revealed no effects of genotype. Therefore, the experimental results were averaged

TABLE 4

Comparison of experimental observations with model predictions of daily nitrogen retention, rate of fat deposition and live weight gain at two levels of intake in the live weight range 180–230 kg[1]

	High feeding level		Low feeding level			
	Observed	Predicted	Observed	Predicted	SEM[2]	√MSPE[3]
			g/d			
N retention	33.3	34.2	30.5	29.5	1.07	9.6
Fat deposition	273	325	149	206	6.5	27.2
Live weight gain	1265	1370	1071	1091	59.0	15.2

[1] Observations from Meulenbroeks et al. (1986).
[2] Pooled standard error of mean of experimental observations.
[3] Root mean square prediction error, expressed as % of mean of observed values; see Equation (1) in text.

over genotype, and the effect of feeding level, averaged over 5 wk, was simulated. Rates of fat gain are recalculated from the original data by Equation (2):

$$\text{fat gain (g/d)} = [\text{EB} - (\text{NB} \times 5.48 \times 23.9)]/39.8 \quad (2)$$

in which EB = measured energy balance (kJ/d); NB = measured nitrogen balance (g/d); 5.48 is the multiplication factor between nitrogen and protein in body protein of calves (Gerrits et al, in press); 23.9 and 39.8 are the energy contents of protein and fat, respectively (in kJ/g; Meulenbroeks et al. 1986).

The results are presented in **Table 4**. Generally, results of the simulations corresponded well with the observed rates of gain. Especially the predicted contrasts between the two feeding levels were predicted accurately. The deviation of the regression slope from 1 contributed only 6, 10 and 0% to the MSPE of live weight gain, N retention and fat deposition rates, respectively. Consistent with the observations, predicted rate of gain of weight, protein and fat decreased slightly with time. Simulated rates of weight gain were only slightly higher than the average observed rates. The simulated N retention agrees with the observed values. The predicted fat deposition rates are about 50 g/d higher than the observed values, whereas the observed contrast was predicted accurately. The overall bias proportion contributed 94% of the MSPE for fat deposition. The overestimation would be about twice the standard error of fat deposition, measured in slaughter experiments (Gerrits et al. 1996). The overestimation could be caused by inadequate representation of the fat metabolism in the model. Considering the close agreement between the predicted and observed contrasts in fat deposition rate between the two feeding levels, this is unlikely. Alternatively, the overestimation could be caused by a difference in the way the fat deposition is determined, i.e., calculated from the measured heat production and nitrogen balance vs. direct measurement in the slaughter experiments on which the model is based. According to Van Es and Boekholt (1987), however, this could explain only a small part of the overestimation. More likely, the difference between the observed and predicted values represents a real difference in fat deposition rates between our experiments and that of Meulenbroeks et al. (1986). Housing calves in metabolism crates in a respiration chamber could lead to increased maintenance energy requirements, which would be reflected in the fat deposition rate (Van Es and Boekholt 1987). An increase in maintenance requirement from 460 to 500 kJ/($kg^{0.75} \cdot d$), for example, could account for a difference of about 40 g/d in fat deposition in a calf of 200 kg Lw, assuming an energetic efficiency for fat deposition of 0.8. Additionally, the different genetic background of the calves used by Meulenbroeks (40% HF) and the calves used in our experiments (70% HF) could explain part of the difference (<20%) between observed and simulated fat deposition rates.

SIMULATION OF AMINO ACID REQUIREMENTS

As described in Gerrits et al. (in press), the model can be used to predict the requirement of indispensable amino acids. To study model behavior, amino acid requirements are simulated in different live weight ranges and at different protein deposition rates. Furthermore, the sensitivity of model predictions to changes in underlying assumptions is discussed. Finally, simulated amino acid requirements are compared with experimentally derived values.

Model behavior

Simulations were carried out to demonstrate the effects of body weight and protein deposition rate on the amino acid requirements. The effect of body weight was tested in two weight ranges, 80–100 and 220–240 kg Lw. The difference in protein deposition rate was created by using two levels of protein intake. The amount of each indispensable amino acid needed to support the maximum rate of protein deposition was simulated as described by Gerrits et al. (in press). For these simulations, daily nutrient intakes increased linearly with $Lw^{0.75}$. Daily intakes of fat, lactose and starch were 9.0, 18.5 and 1.6 g/($kg^{0.75}$), respectively, and daily protein intakes were 9 and 12 g/($kg^{0.75}$) for the low and high intake level, respectively. Simulations were started 20 kg below the start of the respective weight range, using an initial body composition estimated from the experiments described by Gerrits et al. (1996). Amino acid requirements, as well as rates of gain of live weight, protein and fat were calculated as an average over the simulated live weight range.

In the model, amino acids are expressed as amino acyl residues. To allow comparison of the simulated requirements with literature values, the results, presented in **Table 5**, are already converted into grams amino acid per day. When compared at similar protein deposition rates (e.g., high protein intake in the range 80–100 kg Lw vs. low protein intake in the range 220–240 kg Lw, Table 5), the simulated requirement for all indispensable amino acids (in g/d) increases with increasing Lw. This is caused by *1*) increased endogenous amino acid losses, due to higher dry matter intakes at higher live weights; *2*) increased scurf losses from the hide protein pool at higher live weights; and *3*) higher inevitable oxidative losses

TABLE 5

The amount of each indispensable amino acid needed to simulate maximum protein (N × 5.48) deposition rate of preruminant calves in three live weight ranges at two protein (N × 5.92) intake levels

	80–100 kg		220–240 kg	
Protein intake, $g/kg^{0.75} \cdot d$	9	12	9	12
Average protein intake, *g/d*	263	352	531	709
Rates of gain in case of no limiting amino acids				
Protein, *g/d*	146	166	166	191
Nitrogen, *g/d*	26.7	30.2	30.3	34.8
Fat, *g/d*	100	110	216	242
Live weight, *g/d*	932	1056	1118	1283
	g/d			
Amino acid requirement				
Threonine	10.8	12.0	16.7	18.4
Tryptophan	2.1	2.3	3.1	3.5
Valine	10.4	11.5	16.4	17.8
Methionine	4.2	4.7	6.1	6.8
Methionine + Cystine	7.6	8.4	11.3	12.5
Isoleucine	7.1	8.0	10.5	11.6
Leucine	18.4	20.3	28.4	31.0
Lysine	16.3	18.0	24.7	27.1
Histidine	6.4	7.1	10.1	11.0
Phenylalanine	9.7	10.6	15.4	16.6
Phenylalanine + Tyrosine	15.9	17.4	24.8	26.9
Arginine	6.6	7.3	9.4	10.4

(in g/d). Inevitable oxidative losses of a specific amino acid depend on protein pool sizes and therefore also on live weight (Gerrits et al., in press). The relative contribution of these components to the increased requirements is not equal for individual amino acids. However, for all amino acids, the increased endogenous fecal losses are the most important factor, followed by the increased inevitable oxidative losses and the increased scurf losses.

Obviously, higher protein deposition rates lead to increased amino acid requirements because more substrate is needed for protein deposition (Table 5). However, when expressed per gram protein deposited, requirements decrease with an increasing deposition rate, caused by the same mechanisms as the increased amino acid requirements with increasing live weight, discussed above.

Changes in the requirements with changes in live weight or protein deposition rate are not always equal for all amino acids (Table 5). This is caused by changes in the composition of protein deposition. Muscle protein deposition, for example, becomes relatively more important with increasing protein deposition rates, causing a slight shift in the requirements for individual amino acids. Analogously, with higher dry matter intakes, the amino acid pattern of endogenous protein losses becomes more important.

Sensitivity of model predictions to the major assumptions

This sensitivity analysis is focused on evaluation of each of the following assumptions. First, the requirement for an amino acid depends on the amino acid composition of the protein tissue. As an example, the sensitivity of a 25% increase in the methionine + cystine content of muscle protein (from 33 to 41 g/kg) is tested. Second, the inevitable oxidative losses of a specific amino acid are made dependent on the amount (per day; flux) of that amino acid entering the amino acid pool (see Gerrits et al., in press). These inevitable oxidative losses were set to 2% of the flux (i.e., the daily amount of methionine + cystine entering the amino acid pool from dietary protein or degraded body protein) of that amino acid. The effect of increasing this proportion to 5 or 10% of the flux is tested. Third, the simulated requirements depend on the assumed protein turnover rates because increased protein turnover will lead to increased flux rates and consequently to higher inevitable oxidative losses. As an example, the effect of increasing the FDR of visceral protein from 24.5 (default) to 35%/d is tested. All simulations are performed using the reference simulation as a starting point and focused on the requirement for methionine + cystine, which are usually considered limiting amino acids for calves fed milk proteins (Williams 1994).

The simulated requirement for methionine + cystine in the reference simulation was 11.1 g/d. Increasing the methionine + cystine content of muscle protein by 25% increased the simulated requirement by 10%. Increasing the inevitable oxidative losses for methionine + cystine to 5 or 10% of the methionine + cystine flux increased the simulated requirement by 15 and 42%, respectively. Increasing visceral protein turnover by increasing its FDR to 35%/d increased the simulated requirement by 10%. The last is in agreement with the suggestion of Simon (1989) that visceral protein may be more important in defining amino acid requirements than can be expected on the grounds of its contribution to body protein. Increasing the methionine + cystine content of muscle protein (the largest protein pool) has an important effect on the requirement. It directly increases the need for deposition, but also increases the methionine + cystine flux, and thus the inevitable oxidative losses.

Comparison with experimentally derived amino acid requirements

Most experimentally derived amino acid requirements for preruminant calves are obtained between 40–80 kg Lw, as recently summarized by Williams (1994). Despite the lack of suitable experimental evidence in the weight range for which the model is constructed, the simulated requirements between 80 and 100 kg Lw at the low protein intake level (Table 5) are compared with the data of Van Weerden and Huisman (1985) and of Tolman (1996). They determined the requirements for methionine + cystine, lysine (Van Weerden and Huisman 1985) and threonine (Tolman 1996) for fast growing preruminant calves between 55 and 70 kg Lw as the maximum of a quadratic relationship between N retention and amino acid intake. When compared at similar N retention rates (27 g/d), the simulated methionine + cystine requirement is close to the value obtained by Van Weerden and Huisman (1985), 8 vs. 9 g/d. Similarly, the simulated threonine requirement is close to the value obtained by Tolman (1996), 11 vs. 12 g/d. The simulated lysine requirement, however, is lower than the value obtained by Van Weerden and Huisman (1985), 16 vs. 20 g/d. These authors also found upper limits for the requirements for arginine, tryptophan, valine, histidine and phenylalanine + tyrosine and found both upper and lower limits for the requirements for isoleucine and leucine. When compared at similar N retention rates (80–100 kg Lw, low protein intake; Table 5), simulated requirements for tryptophan, valine, phenylalanine + tyrosine and arginine (in g/d) are lower than the upper limits of Van Weerden and Huisman (1985), 2.1 vs. 2.5, 10 vs. 14, 16 vs. 20 and 7 vs. 8 g/d, respectively. If the semi-indispensability of arginine is not considered, the simulated arginine requirement would be about 17 g/d. Therefore, the upper limit found by Van Weerden and Huisman (1985) sup-

ports the assumption in the model that only 40% of the arginine needed for protein deposition has to be satisfied through dietary intake, based on Fuller (1994). The simulated requirements of leucine (18 g/d) and isoleucine (7 g/d) are lower than the experimentally derived lower limits (20 and 11 g/d, respectively; Van Weerden and Huisman 1985). The inevitable oxidation proportion of these amino acids is likely higher than assumed in the model. Possibly, this is due to the different location of oxidation of these branched-chain amino acids compared with other indispensables (muscle tissue, as opposed to liver; Benevenga et al. 1993). This would then also apply to valine, for which Van Weerden and Huisman (1985) determined only an upper limit.

The simulated methionine + cystine and the lysine requirement for calves between 220 and 240 kg at the high protein intake level in Table 5 can be compared with unpublished experiments of Tolman et al. (1991, internal report). They varied methionine + cystine and lysine intake from 13 to 21 and from 27 to 45 g/d, respectively, measuring N retention of 48 preruminant calves in the weight range 220–250 kg. They found little response of N retention to the increased amino acid intakes. The measured N retention varied around 36 g/d. The simulated requirements, 13 and 27 g/d for methionine + cystine and lysine, respectively, are just outside the measured range of Tolman et al. (1991). This may provide an explanation for the lack of response in these experiments.

DISCUSSION

The results of model tests against independent experimental data in the weight ranges of 55–155 kg and 180–230 kg Lw are promising. The model is based on experimental observations, which are averages over a large weight range (80–160 and 160–240 kg Lw). Although model predictions averaged over a rather large weight range are accurate, predictions at any given time or bodyweight do not necessarily reflect observed values accurately.

The model is quite sensitive to changes in maintenance energy requirements, indicating their quantitative importance. The simulated effects are generally in line with our expectations. The observation, however, that increased maintenance energy requirements can be partly compensated for by decreasing the additional energy costs for growth is not very likely. Reconsidering the representation of this flux therefore seems appropriate. For example, part of the additional energy costs for growth could be coupled to tissue deposition rate rather than to the concentration of acetyl-CoA. Before doing so, it would be important to decompose this flux into energy spent on defined physiological processes.

The model is marginally sensitive to changes in the protein requirements for maintenance. Increased maintenance protein losses are compensated for roughly 70–80% in simulations by reducing the amino acid oxidation rate. Although not likely to be so large, it is possible that a compensation mechanism of this kind exists. There are, for example, some indications that the efficiency of utilization of absorbed protein for replacing endogenous urinary and endogenous fecal nitrogen losses in ruminants is higher than that for growth. The estimated efficiencies vary between 0.5 and 1 (Owens 1987), whereas the gross efficiency of utilization of absorbed protein varies between 0.35 and 0.60 in fast growing, preruminant calves (Gerrits et al. 1996). The representation of protein requirements for maintenance in the model may be a crude approximation (Millward et al. 1990). Considering its quantitative importance, however, the simple approach used seems appropriate for growing calves.

The deposition rate of visceral, hide and bone protein is directly related to the muscle protein deposition rate. This approach provides a simple solution to a complex problem. It has the additional advantage that turnover of hide, bone and visceral protein can be varied by simply changing the FDR of these tissues. The direction of the predicted effects of changes in protein turnover is according to our expectations. However, quantitatively, the effects may be underestimated. About 20% of the increased expenditure on protein synthesis and degradation is compensated for by a decrease in the additional energy expenditure for growth. On the other hand, there are indications that the energy costs of growth do not increase proportionally with protein turnover rate. Summers et al. (1986) questioned whether the stoichiometry of peptide bond formation is fixed. Additionally, Lobley (1990) stated that increased protein turnover rates do not always lead to higher rates of oxygen consumption. A disadvantage of relating the deposition rate of the three protein pools to the rate of muscle protein deposition is that it makes the model highly sensitive to changes in the parameters determining muscle protein synthesis. Furthermore, variation in muscle protein turnover cannot be simulated by varying its FDR. This is also the case for body fat turnover (results not presented).

Protein synthesis is quantitatively the largest energy-consuming process defined in the model. Increasing the ATP requirement for protein synthesis from 3 to 5 mol ATP/mol peptide bond synthesis reduced the fat deposition rate by 40 g/d. In the reference simulation, 21% of the total energy expenditure is spent on protein turnover. Similar results were obtained by Gill et al. (1989), who simulated 19% for growing lambs. Also, the simulated increase in the ATP expenditure with increasing ATP requirement for protein synthesis and degradation corresponded well with their simulations.

The model provides a useful tool for estimating amino acid requirements. Simulated requirements depend strongly on nutritional circumstances and respond to changes in the amino acid profiles of the tissues, body weight, protein turnover rate and inevitable oxidative losses. More attention must be paid to these inevitable oxidative losses for individual amino acids. Also, recent data on the amino acid profiles of the tissues would improve the reliability of estimations of amino acid requirements. Obviously, the relative importance of an amino acid profile of a tissue depends on the contribution of that tissue to whole-body protein and on the protein turnover rate of that tissue.

It can be concluded that the model is a useful tool for the development of feeding strategies. The model responds well to changes in the quantity and the quality of the feed offered and provides a means for estimation of amino acid requirements. Apart from the use of the metabolic model in research, there is, in our opinion, considerable scope for using this type of model to replace feeding tables, currently the basis for most feeding strategies.

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