Description of a Model Integrating Protein and Energy Metabolism in Preruminant Calves[1–4]

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ABSTRACT This paper describes the development of a mechanistic model integrating protein and energy metabolism in preruminant calves of 80–240 kg live weight. The objectives of the 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 nutrients from ingestion through intermediary metabolism to growth, consisting of accretions of protein, fat, ash and water. The model contains 10 state variables, comprising fatty acids, glucose, acetyl-CoA and amino acids as metabolite pools, and fat, ash and protein in muscle, hide, bone and viscera as body constituent pools. Turnover of protein and fat is represented. The model also includes a routine to check possible dietary amino acid imbalance and can be used to predict amino acid requirements on a theoretical basis. The model is based on two experiments, specifically designed for this purpose. Simulations of protein and fat accretion rates over a wide range of nutrient input suggest that the model is sound. It can be used as a research tool and for the development of feeding strategies for preruminant calves. J. Nutr. 127: 1229–1242, 1997.

KEY WORDS: • *veal calves* • *computer simulation* • *mathematical model* • *protein metabolism* • *energy metabolism*

During the last decade, the interest of meat producers has shifted from obtaining maximum growth of the animal to production of lean meat and an increase in the efficiency of nitrogen utilization. Simultaneously, interest in growth simulation models has increased because they provide a tool for better understanding of complex growth processes. Besides being of interest as a research tool, such models can be used in the development and evaluation of feeding strategies. For preruminant calves, no such models are available. Furthermore, the distribution of nutrients within the body of preruminant calves differs considerably from that of true monogastric animals (Gerrits et al. 1996) so that the same principles cannot be automatically applied. The objectives of the model described in this paper are to gain insight into the partitioning of nutrients in the body of preruminant calves and to provide a tool for the development of feeding strategies for calves between 80 and 240 kg live weight (Lw).[6]

This paper describes a dynamic, mechanistic model that simulates the partitioning of ingested nutrients through intermediary metabolism to growth, consisting of accretion of protein, fat, ash and water. The model is based on literature data and specifically designed experiments with Holstein-Friesian × Dutch-Friesian calves (Gerrits et al. 1996). Protein metabolism of muscle, visceral, hide and bone is explicitly considered, and a calculation routine for dietary amino acid imbalance is developed. The model is designed for evaluation of long-term feeding strategies for growing preruminant dairy calves and does not consider diurnal or postprandial metabolic events. In preruminants, feed completely bypasses the rumen. Therefore, the model may also provide a valuable tool for evaluating utilization of absorbed protein and, to a lesser extent, energy in ruminating calves.

EXPERIMENTAL DATA

To obtain data for the development of this model, two experiments were conducted with Holstein-Friesian × Dutch-Friesian male calves. These experiments started at 80 kg Lw so as to avoid large variation due to environmental factors

[1] Preliminary results of portions of this study were presented at the symposium: Veal, Perspectives to the Year 2000, September 12–13, Le Mans, France [Gerrits, W. J. J., Verstegen, M. W. A., France, J., Dijkstra, J., Tolman, G. H. & Schrama, J. W. (1996) Modelling growth of veal calves, pp. 243–253] and at the 26th meeting of the Agricultural Research Modellers' Group [Gerrits, W. J. J., Dijkstra, J. & France, J. (1995) Modelling growth of pre-ruminant calves. J. Agric. Sci. 125: 162].

[2] One of the authors (W.G.) was a visiting research worker at the Institute of Grassland and Environmental Research, Okehampton, U.K. He was in receipt of a bursary from the British Council, Amsterdam, which is gratefully acknowledged.

[3] This project was funded by the Dutch Commodity Board of Feedstuffs and by T.N.O. Nutrition and Food Research Institute, The Netherlands. These funds are gratefully acknowledged.

[4] The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 USC section 1734 solely to indicate this fact.

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[6] Abbreviations used: DAA, dispensable amino acids; EUN, endogenous urinary nitrogen; FDR, fractional degradation rate; FSR, fractional synthesis rate; Lw, live weight; Q_{Ew}, empty body weight; for other notation see Tables 1 and 2.

0022-3166/97 $3.00
Manuscript received 30 April 1996. Initial review completed 18 June 1996. Revision accepted 21 January 1997.

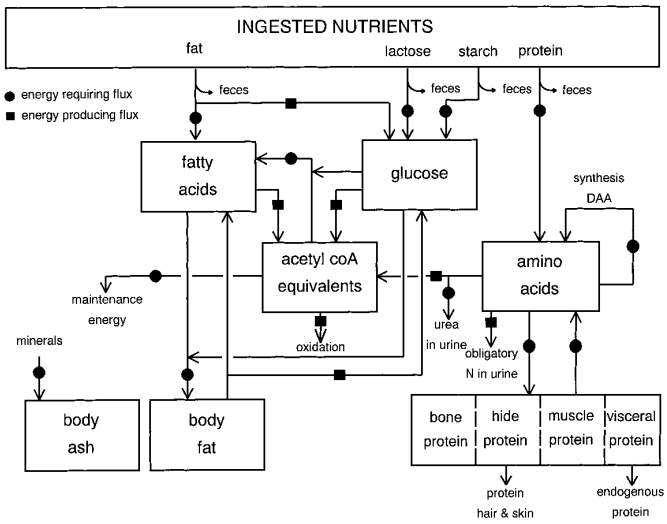

FIGURE 1 Diagrammatic representation of the model simulating metabolism of growing preruminant calves. Boxes enclosed in solid lines indicate state variables, arrows indicate nutrient fluxes. The composite box represents four body protein pools, each with a flux to and from the amino acid metabolite pool; urea in urine represents excretion of N after amino acid oxidation; DAA = dispensable amino acids.

dominating energy metabolism of very young calves (Schrama 1993). The experiments were similar in design and conducted in two weight ranges: 80–160 kg and 160–40 kg Lw (Gerrits et al. 1996). Briefly, in each experiment, 36 calves were assigned in a 6 × 2 factorial arrangement to one of six protein intake levels at one of two protein-free energy intake levels. Milk proteins were used as the only feed protein source. Calves were slaughtered at the beginning and end of each experiment and analyzed for nitrogen, fat, dry matter and ash content. At 120 and 200 kg Lw, total collections of feces and urine were obtained from all animals for 5 d. Feces were analyzed for dry matter, nitrogen, fat and ash content, and urine for nitrogen, energy and creatinine content. In designing the experiments, the attempt was made to establish relationships over a wide range of nutrient input. Depending on dietary treatment, average daily gain of the empty body varied between 640 and 1340 g/d and 420 and 1370 g/d for the growth ranges of 80–160 and 160–240 kg Lw, respectively.

MODEL DESCRIPTION

General

Choice of pools and representation of fluxes. A schematic representation of the model (principal pools and transactions) is shown in **Figure 1**. The model traces nutrients from ingestion through intermediary metabolism into body stores. The body storage pools comprise chemical fat, ash and four protein pools. Body protein is split up into protein pools from several anatomical tissues. The following types of protein were considered: muscle, viscera (including blood), bone and hide. Muscle protein is defined as non-bone, non-hide protein in carcass, head and tail and therefore includes small amounts of other tissues (brain, connective and adipose tissue). The metabolite pools, driving the transactions between pools, were selected to represent the major effects found in the experiments (Gerrits et al. 1996) and, additionally, to allow for distinguishing glucose from fat metabolism. The following metabolite pools are considered: amino acids, fatty acids, glucose and acetyl-CoA equivalents.

The model is driven by nutrient input, and for distribution of nutrients between pools, standard enzyme and chemical kinetic relationships were assumed, described by Gill et al. (1989a). This type of mathematical representation has been used before in aggregated models of metabolism (e.g., France et al. 1987, Pettigrew et al. 1992). Transactions between pools are characterized by substrate concentrations, a maximum velocity, affinity and inhibition constants and steepness parameters, as illustrated by Pettigrew et al. (1992). Two-letter symbols used in the model are listed in **Table 1**, the notation is summarized in **Table 2**, and parameter values are presented in **Table 3**. Simulations started at 80 kg Lw because that is the beginning weight in the experimental work.

Digestion. Milk replacers have been shown to be highly digestible in preruminants (ARC 1980). Simulation of the digestion process is kept simple, i.e., by multiplying ingested lactose, starch, fat and protein by a digestion coefficient. For lactose, the apparent fecal digestibility has been shown to be close to 99%, whereas the apparent ileal digestibility varies around 93% (Hof 1980, Toullec and Guilloteau 1989). To account for the energy yield from fermentation in the hindgut, lactose digestibility is set to an intermediate value of 95%. Starch digestibility has been shown to depend on age of the calves, source, pretreatment and level of inclusion in the diet (Toullec 1989, Van der Honing et al. 1974). For simulations in this paper, starting at 80 kg Lw, starch is assumed to be pregelatinized, it is included at a level of <5% in the diet and its digestibility is assumed to be 95%. Fecal digestibility of dietary fat varies between 94 and 97%, depending on, for example, dietary fat source (Hof 1980, Tolman and Demeersman 1991) and does not significantly differ from values ob-

TABLE 1

Two-letter abbreviations for entities used in the model simulating metabolism of growing preruminant calves

Symbol	Entity	Unit
Aa	Amino acids	mol
Ag	Additional energy costs for growth	—
As	Body ash	kg
At	ATP	mol
Ay	Acetyl-coenzyme A	mol
DMI	Dry matter intake	kg/d
En	Net endogenous protein losses	kg/d
Ew	Empty body weight	kg
Ex	Exogenous protein losses (skin and hair)	kg/d
Fa	Fatty acids	mol
Fb	Total body fat	kg
Fd	Dietary fat	kg
Gl	Glucose	mol
Lb	Lean body mass	kg
Ld	Dietary lactose	kg
Lv	Liver	kg
Lw	Live weight	kg
Ma	Maintenance energy	mol Ay/d
Md	Dietary minerals	kg
Ox	Oxidation of Ay	mol
Pb	Protein in bone	kg
Pd	Dietary protein	kg
Ph	Protein in hide	kg
Pm	Protein in muscle	kg
Pv	Protein in viscera	kg
Sd	Dietary starch	kg
Ue	Urinary excretion	—
Un	Obligatory urinary nitrogen losses	kg/kd
Ur	Urea	mol
Vi	Visceral mass	kg

TABLE 2

General notation used in the model simulating metabolism of growing preruminant calves[1]

Notation	Translation	Unit
A_i	Absorption costs for *i*	mol Ay/kg *i*
C_i	Concentration of *i*	(mol *i*)/kg Ew
D_i	Driving variable with respect to *i*	kg *i*/d
FDR_i	Fractional degradation rate of *i*	%/d
$J_{i,jk}$	Michaelis-Menten inhibition constant for $j - k$ transaction with respect to *i*	—
$M_{i,jk}$	Michaelis-Menten affinity constant for $j - k$ transaction with respect to *i*	—
$P_{i,jk}$	Rate of production of *i* by $j - k$ transaction	(mol or kg *i*)/d
PF_i	Crude protein factor for entity *i*	g crude protein/g N
PFT_i	True protein factor for entity *i*	g true protein/g N
Q_i	Quantity of *i*	mol or kg *i*
$R_{i,jk}$	Requirement for *i* in $j - k$ transaction	(mol *i*)/(mol or kg *j*)
$S_{i,jk}$	Steepness parameter associated with *i* for $j - k$ transaction	—
$U_{i,jk}$	Rate of utilization of *i* by $j - k$ transaction	(mol or kg *i*)/d
$V_{i,jk}$	Maximum velocity for $j - k$ transaction	mol/d
$Y_{i,jk}$	Yield of *i* in $j - k$ transaction	(mol or kg *i*)/(mol or kg *j*)

[1] *i*, *j*, *k* take values from Table 1.

tained at the terminal ileum (Hof 1980). A value of 95% is adopted. The true digestibility of milk proteins has been shown to be close to 100% (ARC 1980, Tolman and Beelen 1996). Consistent with this observation, apparent digestibility of milk proteins varies with protein intake, as observed in the experiments and discussed by Gerrits et al. (1996). In the model, the true digestibility of milk proteins is set to 100%, and, as described later, net endogenous protein losses are modeled as a drain from the visceral protein pool.

Absorption and transport of nutrients. In the context of this model, absorption is the transfer of nutrients from the intestinal lumen into the portal blood or lymph. Energy costs of nutrient absorption are represented in the model. Dietary fat (triglycerides) is assumed to be absorbed as mono-acylglycerol and two fatty acids (Brindley 1984). Laplaud et al. (1990) have shown that 80% of fat in intestinal lymph is present in the form of triglycerides. Therefore, energy costs are introduced for re-esterification of the fatty acids in the mucosal cell, set at 1.33 mol ATP per fatty acid equivalent (Stryer 1981). Re-esterification causes the absorption of fat to have no net effect on membrane potential. Therefore, no extra absorption costs were calculated.

Na^+-dependent transport is considered the major way of absorption of monosaccharides (Shirazi-Beechey et al. 1989). The energy costs involved are set to 0.33 mol ATP/mol monosaccharide, which is the amount of ATP needed to pump 1 mole Na^+ through a membrane (Mandel and Balaban 1981).

For amino acid absorption from the gut, several ways have been shown to exist: Na^+-dependent and Na^+-independent active transport and diffusion. Also, some of the protein may be absorbed as intact peptides (Webb 1990). Therefore, energy costs for absorption from the intestinal lumen are difficult to estimate. In analogy to absorption of monosaccharides, absorption costs are set to 0.33 mol ATP/mol amino acid, considering both lower costs for diffusion, Na^+-independent transport and peptide absorption, and higher costs if reabsorption of endogenous secreted protein is accounted for, as discussed by Gill et al. (1989b).

For transport of nutrients through a membrane other than membranes in the intestinal wall, a similar approach is used.

TABLE 3

Parameter values of the model simulating metabolism of growing preruminant calves[1]

Transaction, pool or dietary input	$M_{i,jk}$	$V^*_{i,jk}$	$S_{i,jk}$	$M_{Ay,jk}$	$J_{Ay,jk}$	$J_{Fa,jk}$	A_i	FDR_i
Aa, AaAy[2]	0.013	2.5	4					
Aa, AaPm	0.0017	0.26		0.00065				
Ay, AyFa	0.018	1.4				0.10		
Ay, AyAg	0.0035	0.04	3					
Fa, FaAy	0.002	0.053			0.004			
Fa, FaFb	0.002	0.27						
Gl, GlAy	0.0006	0.18						
Pb								0.061
Ph								0.040
Pm								0.020
Pv								0.245
Fb								0.010
Pd							0.247	
Fd							0.358	
Ld							0.153	
Sd							0.161	

[1] See Tables 1 and 2 for explanation of notation.
[2] The flux Aa, AaAy is calculated after checking dietary amino acid imbalance; see explanation in text and Table 8.

TABLE 4

Stoichiometry of principal transactions in the model simulating metabolism of growing preruminant calves[1,2]

Transaction	$R_{j,ik}$	$Y_{k,jk}$	$Y_{At,jk}$	Transaction	$R_{i,jk}$	$Y_{k,jk}$
Aa, PbAa		9.726		Ay, MdAs	2.398	
Aa, PdAa		8.987		Ay, PbAa	0.811	
Aa, PhAa		8.889		Ay, PhAa	0.824	
Aa, PmAa		9.691		Ay, PmAa	0.808	
Aa, PvAa		9.484		Ay, PvAa	0.790	
Aa, UnUe	53.204			Ay, UrUe	0.009	
At, AaAy			14.07	Fa, AyFa		0.102
At, AuUn			14.36	Fa, FbFa		3.394
At, FaAy			36.00	Fa, FdFa		3.224
At, FbFa			2.26	Fb, FaFb		0.295
At, FdFa			2.15	Gl, AyFa	0.157	
At, GlAy			14.00	Gl, FdFa		0.537
Ay, AaAa	0.250			Gl, LdGl		5.556
Ay, AaAy		0.780		Gl, SdGl		5.864
Ay, AaPb	0.361			Gl, FaFb	0.167	
Ay, AaPh	0.361			Gl, FbFa		0.566
Ay, AaPm	0.361			Pb, AaPb		0.1028
Ay, AaPv	0.361			Ph, AaPh		0.1011
Ay, FaAy		9.0		Pm, AaPm		0.1032
Ay, FaFb	0.282			Pv, AaPv		0.1054
Ay, GlAy		1.973		Ur, AaAy		0.671

[1] Expressed in moles, grams or kilograms per mole, gram or kilogram of principal substrate (*i*).
[2] See Tables 1 and 2 for abbreviations.

Transport costs are included in the stoichiometry of all reactions involving the use of glucose or amino acids as substrate. Other transport costs such as transport of blood are not specifically considered and are partly included in maintenance energy and partly in the flux additional energy costs for growth (AyAg); this is discussed later in this paper.

Stoichiometry. Stoichiometric yield and requirement factors are shown in **Table 4**. These factors include transport costs as mentioned above. Some of the major assumptions will be addressed. ATP requirement of incorporating amino acids into protein, excluding transport costs, is assumed to be 4 mol ATP/peptide bond (Gill et al. 1989b, Lobley 1990, McBride and Kelly 1990). According to reviews by Lobley (1990), McBride and Kelly (1990) and Simon (1989), ATP cost of proteolysis varies, depending on the mechanism involved (e.g., lysosomal vs. non-lysosomal). Energy costs are assumed to be 1 mol ATP/peptide bond cleaved, based on the energy yield of inhibiting intracellular proteolysis in reticulocytes described by Rapoport et al. (1985). Energy costs of urea synthesis are 4 mol ATP/mol urea synthesized and are included in the energy yield of amino acid oxidation.

Auxiliary variables. To relate pool sizes to empty body weight (Q_{Ew}), each protein pool is related to a certain amount of water. All relationships except the one relating bone protein to bone water were estimated from the experiments described above. For bone water, a relationship with bone protein was estimated from Nour and Thonney (1987) and Schulz et al. (1974). Describing the relationships between water and protein, allometric relationships are adopted because of the good fit of the data and sensible behavior of allometry close to zero and infinity. Empty body weight is approached by sum of the body fat, body ash and body protein pools, and the water attached to the protein pools. In the experiments, protein, ash, fat and water did not account for 100% of the fresh material analyzed in the body composition analysis. From the experiments, a multiplication factor of 1.03 was estimated for the conversion of the sum of the body pools and water into Q_{Ew} (Equation 11.1, see Table 7 for numbered equations). Live weight is assumed to be closely related to Q_{Ew}. From the experiments, a multiplication factor of 1.11 was estimated (Equation 11.2).

Initial pool concentrations, reaction sites and maximum reaction velocities. Metabolite pool concentrations are expressed per kilogram Q_{Ew}, assuming that metabolites are distributed throughout the entire body, thus allowing the intracellular fluid to act as a substrate pool of metabolites. It is realized, however, that no data on concentrations in intracellular fluid are available. Hence, normal concentrations of metabolites in blood plasma are estimated from the literature to set initial metabolite pool sizes. The maximum velocity of a reaction (V_{max}) depends primarily on the availability of enzymes required for the transaction and is a function of the size of the reaction site and potential drive for the particular process. The size of the reaction site is considered to be the weight of the tissue in which the transaction is taking place. The metabolic activity of adipose tissue may decrease with increasing body fat pool size because of its vascular characteristics (low blood flow, compared with other tissues, Vernon and Clegg 1985). Therefore, for transactions that take place only in adipose tissue (FaFb, AyFa), the size of the reaction site is considered to be $Q_{Fb}^{0.67}$ (Equations 8.9 and 6.8, respectively).

Protein metabolism

General. For simplicity, all amino acids, available for synthesis or oxidation, are combined into one metabolite pool. Four body protein pools are represented, shown in the composite box in Figure 1. In the context of the present model, (crude) protein is defined as the sum of aminoacyl residues plus non-protein nitrogenous components. The amino acid composition of the protein pools and of the dietary protein, and the protein factors for the conversion of nitrogen into protein are discussed later in this paper. The fractional degradation rate (FDR) is assumed fixed for each protein pool. It was decided to make the development of the muscle protein pool dependent on substrate concentration and to relate the

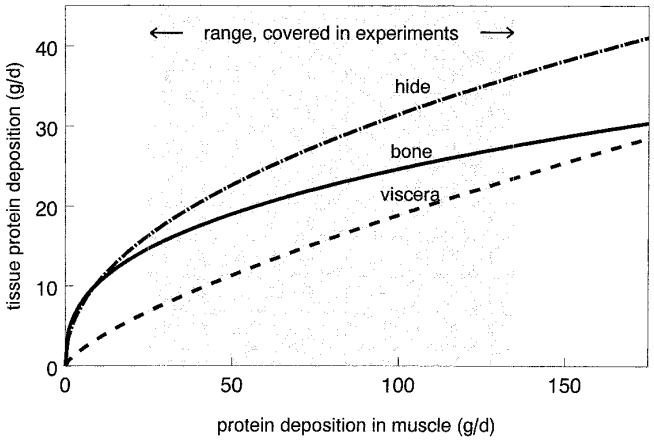

FIGURE 2 Protein accretion rate in bone, hide and viscera as a function of muscle protein accretion rate in preruminant calves between 80 and 240 kg live weight. Relationships estimated from experiments of Gerrits et al. (1996).

development of the other protein pools to muscle protein. For that purpose, relationships between accretion rates of protein in muscle and protein in bone, hide and viscera were estimated from the experiments (**Fig. 2**). In the model, the protein synthesis rate in bone, hide and viscera is calculated by summation of net accretion and degradation rates. Protein accretion in bone and hide appears to have some priority at low muscle protein accretion rates (Fig. 2). It may be argued that muscle growth follows rather than precedes skeletal development, and thus bone protein accretion should drive muscle protein accretion rather than the reverse. Relating muscle protein to bone protein, however, would mean a considerable enlargement of the errors made in the determination of the bone protein pool size. The approach used has the advantage of minimizing the error made because muscle protein is by far the largest of the protein pools.

Body protein pools. Estimation of synthesis, degradation and net accretion rates of the four body protein pools is discussed below.

Muscle protein pool, Pm. Williams et al. (1987) found an FDR of muscle protein of 1.9%/d in milk-fed calves weighing 120 kg, using urinary 3-methylhistidine excretion as a measure for myofibrillar protein degradation. Using the same method, Jones et al. (1990) found an FDR of 3.8%/d in freely fed steers of 320 kg Lw. The FDR is set at 2.0%/d, accounting for a slight overestimate of FDR due to the 3-methylhistidine method (Simon 1989).

Muscle protein synthesis is made dependent on the concentration of amino acids and acetyl-CoA, so that an increase in protein intake or an increase in protein-free energy intake will result in higher protein deposition rates (Gerrits et al. 1996). It is realized, however, that concentrations of amino acids and acetyl-CoA themselves are unlikely to drive muscle protein synthesis. It is, however, the simplest way of representing the effects of changed nutrient input and is considered appropriate for the level of aggregation applied in the present model (see also discussion). The V_{max} of muscle protein synthesis is expressed as a function of muscle protein mass (Equation 1.16). As discussed by Moughan (1994), maximum protein deposition capacity remains relatively constant over a large range of body weights for grower/finisher pigs and decreases to zero at maturity. However, for dairy calves, little is known about maximum (muscle) protein deposition capacity. Therefore, a simple function of muscle protein mass is used, which was calculated to result, in combination with the fixed FDR, in a slight increase in muscle protein deposition capacity with increasing muscle protein mass.

Visceral protein pool, Pv. Crudely estimated from the experiments, 30, 30, 15, 8, 4, 3 and 10% of visceral protein originates from the gastro-intestinal tract, blood, liver, lungs, heart, kidney and other organs, respectively. Reported fractional synthesis rates (FSR) of these organs vary widely, depending on species, age and the method used. From values reported by Early et al. (1990) for nonsomatotropin-treated steers of about 370 kg Lw, by Lobley et al. (1980) for two heifers of 250 kg Lw and pigs of about 75 kg Lw (reviewed by Simon 1989), a value of 25%/d is chosen for FSR. This FSR was used in combination with the visceral protein retention rates, measured in the experiments, to estimate a value for the FDR, which is used in the model. From these data, FDR was computed at 24.5%/d.

Net endogenous protein loss is modeled as a drain on the visceral protein pool (Fig. 1). The relationship describing net endogenous protein loss is based on the assumptions that true digestibility of milk proteins is close to 100% (ARC 1980, Tolman and Beelen 1996), and that the flow of endogenous protein depends on the dry matter intake. The equation used (Equation 5.2) is based on data obtained with 115 milk-protein–fed calves ranging in body weight from 60 to 270 kg and dry matter intakes varying from 1 to 3 kg/d (Gerrits et al. 1996; G. H. Tolman, T.N.O. Nutritional Food Research Institute, Wageningen, The Netherlands, unpublished). The value obtained is slightly higher than the value adopted by ARC (1980), i.e., 2.46 (R^2 = 0.59) vs. 1.90 g N/(kg dry matter intake · d). The relationship between visceral protein and muscle protein accretion was estimated from the experiments (Fig. 2). Protein synthesis (in g/d) is calculated as the sum of net accretion, degradation and endogenous losses (Equation 5.1).

Hide protein pool, Ph. FSR of hide protein is taken to be 4.7%/d (mean value of hide of two heifers of 250 kg Lw, Lobley et al. 1980). From this value and hide protein retention from the experiments, a corresponding FDR of 4.0%/d was calculated. Daily losses from this pool (hair and skin) are set at 0.11 $Lw^{0.75}$ (in g/d; ARC 1980). Protein degradation rate is calculated from pool size and FDR; synthesis rate is calculated as the sum of degraded protein, net protein accretion and hair and skin losses (Equation 3.1). The relationship between hide protein accretion and muscle protein accretion was estimated from the experiments (Fig. 2).

Bone protein pool, Pb. Bone protein has been shown to have a high protein turnover rate compared with muscle. Calculated by difference between muscle and minced carcass, FSR of bone protein of heifers of 250 kg Lw was 6.6%/d, about 3.5 times the FSR in muscle (Lobley et al. 1980). From this value and bone protein retention from our own experiments, a corresponding FDR of 6.1%/d was calculated, three times the chosen FDR for muscle protein. This value is chosen for the model. Bone protein synthesis is calculated as the sum of net accretion and degradation rate (Equation 2.1). Bone protein accretion rate is related to muscle protein accretion rate (Fig. 2).

Amino acid composition of the body protein pools. To calculate dietary amino acid imbalance (described below), an amino acid profile is assigned to each of the body protein pools and to the dietary protein (**Table 5**). The amino acid composition of bone protein is adopted from Mello et al. (young bovine, 1975), except for cystine and tryptophan, which is adopted from Wünsche et al. (pig bones, 1983). The amino acid profile of visceral protein is adopted from Williams (1978). The profiles for muscle and hide protein are estimated from the profiles of carcass and hide, head, feet and tail samples, analyzed by Williams (1978). This estimation involved

TABLE 5

Amino acid composition of bone, hide, muscle, viscera and endogenous losses in preruminant calves and of a typical milk replacer diet

	Bone[1]	Hide[2]	Muscle[3]	Viscera[4]	Endogenous[5]	Milk-replacer[6]
			g aminoacyl residue/kg aminoacyl residue			
Indispensable amino acids						
Threonine	25	36	45	48	81	46
Tryptophan	5	7	11	8	16	12
Valine	57	21	35	55	63	63
Methionine	16	9	22	17	13	24
Methionine + Cystine	22	37	33	33	40	33
Isoleucine	28	19	35	24	46	54
Leucine	70	50	73	99	65	92
Lysine	70	40	72	82	45	76
Histidine	31	8	27	38	21	34
Phenylalanine	50	24	33	56	62	48
Phenylalanine + Tyrosine	102	35	54	88	94	97
Arginine	77	81	75	58	39	34
Dispensable amino acids						
Aspartic acid/Asparagine	58	76	93	95	91	72
Serine	38	52	41	49	77	55
Glutamic acid/Glutamine	97	133	155	116	169	192
Glycine	122	155	81	65	44	16
Alanine	76	68	63	67	48	29
Proline	81	107	74	64	61	95
Hydroxyproline	41	75	33	11	0	0
Nitrogen content N (g/kg product)	31.84	39.37	28.96	20.16	2.80	34.41
True protein content (g/kg product)	169.81	209.17	153.23	106.34	11.77	198.57
Dummy amino acid (g/kg product)[7]	4.24	2.72	5.71	6.72	—	5.27
PFT (g true protein/g N)[8]	5.33	5.31	5.29	5.27	4.20	5.77
PF (g crude protein/g N)[8]	5.47	5.38	5.49	5.61	—	5.92
Average mol weight crude protein	101	98	106	107	—	114

[1] Mello et al. (1975) and Wünsche et al. (1983), see text.
[2] Estimated from Williams (1978), see text.
[3] Estimated from analyses of carcass protein of veal calves by Williams (1978), see text.
[4] Williams (1978).
[5] Average of amino acid profiles of ileal digesta from Tolman and Beelen (1996) and Lallès et al. (1990), see text.
[6] Milk replacer based on milk proteins only, adapted from experiments (see text).
[7] Dummy amino acids, introduced to account for non-amino acid nitrogen compounds, see text.
[8] *PFT* = true protein factor; *PF* = crude protein factor.

separation of the bone parts of these fractions, based on the difference in ash content of these fractions. Considering the definition of muscle protein within the model, the amino acid profile as obtained via this estimation is preferred over the amino acid profile of veal, which does not include much collagen protein. The amino acid profile of muscle protein therefore contains fewer essential amino acids than reported for veal by Vervack et al. (1977). The amino acid composition of hair and skin losses is assumed equal to the amino acid composition of the hide. The amino acid profile of the net endogenous secreted protein is assumed to be identical to the amino acid profile of ileal digesta of milk-fed calves (averaged from Lallès et al. 1990 and Tolman and Beelen 1996). The dietary protein used for the simulations in this paper was based on milk proteins, as used in the experiments (Gerrits et al. 1996).

Protein factors for conversion of N into protein. Crude protein is defined as the sum of aminoacyl residues plus non-amino acid nitrogenous components. True protein is defined as the sum of aminoacyl residues. Derived from the literature described above, protein factors were computed for the conversion of total N into crude and true protein (Table 5). Two dummy amino acids were introduced to account for the non-amino acid nitrogen components: one for the body protein pools and a second one for the dietary protein. The molecular weight of both dummies was set to 100 and the N content was set to 20 and 36.5% for the body protein pools and the dietary milk protein, respectively, based on Rafecas et al. (1994) and Karman and van Boekel (1986). It is assumed that in all proteins, half of the aspartate and glutamate is in the amide form, which has been shown for casein by Walstra and Jenness (1984) and is often assumed for other proteins as well (e.g., Livesey 1984). The protein factors, presented in Table 5, are quite a bit lower than the factor 6.25, which is commonly applied. This factor, however, has been shown to be erroneous for many types of protein, mainly as a result of neglect of the amides glutamine and asparagine (Rafecas et al. 1994). The body protein pools and the amino acid pool are based on crude protein, as defined above. For the calculation of amino acid imbalance, the true protein factors are used.

Amino acid pool, Aa. Inputs to this pool are from absorption (Equation 1.2) and from degradation of body protein (Equations 1.4–1.7). Outputs are to body protein synthesis (Equation 1.9–1.12), to oxidation (Equation 1.8) and as endogenous urinary N losses (Equation 1.13). Stoichiometry of these transactions is based on equilibrium of nitrogen and expressed per mole average amino acid residue. This average amino acid residue was based on the weighted mean of the amino acid residues of the body protein pools. Synthesis of dispensable amino acids (DAA) is modeled as input and output of the amino acid pool (Equation 1.3). Therefore, energy consumption is the only effect of this flux. The flux is calculated in the imbalance routine described below and represents the

TABLE 6

A comparison between the inevitable amino acid oxidation rates from the model for preruminant calves (resulting from the application of different oxidation proportions), and minimum oxidation rates and amino acid requirements for maintenance in growing pigs

Indispensable amino acid	Oxidation proportion in model		Minimum oxidation rate, pigs[3]	Maintenance requirements, pigs[4]
	2% of flux[1,2]	5% of flux[1,2]		
			mg/(kg$^{0.75}$ · d)	
Threonine	34	85	14	53
Tryptophan	7	18		11
Valine	41	103		20
Methionine	14	35		12
Methionine + Cystine	24	60		56
Isoleucine	25	63		17
Leucine	68	170	28	27
Lysine	58	145		38
Histidine	25	63	3	
Phenylalanine	36	90	3	24
Phenylalanine + Tyrosine	62	155		47
Arginine	46	115		

[1] Simulated values at low protein intake level [7.2 g/(kg$^{0.75}$ · d)].
[2] Flux defined as the amount entering the amino acid pool, i.e., the sum of dietary and degraded body protein.
[3] Estimates of minimum rates of oxidation of indispensable amino acids by growing pigs, given diets devoid of that amino acid (adapted from review of Fuller 1994).
[4] Amino acid requirements for maintenance of growing pigs, estimated as the amount needed to maintain N equilibrium (Fuller et al. 1989).

difference between ingested and deposited DAA. Synthesis of DAA requires both a carbon and a nitrogen source. Although Kreb's cycle intermediates such as α-ketoglutarate, pyruvate and oxaloacetate usually provide the carbon, the nitrogen is provided by NH_3 from other amino acids. In the model, both carbon and nitrogen are assumed to be provided by nonlimiting amino acids. To correct for the difference in carbon source (Kreb's cycle intermediates vs. amino acids), energy costs were introduced. These energy costs were calculated as the difference between the energy costs for synthesis of DAA from glucose and NH_3, and the energy yield from oxidation of these amino acids. Stoichiometry of synthesis of DAA from glucose and NH_3, as well as energy yield from oxidation, was adopted from Schulz (1978), and average energy costs were estimated at 3 mol ATP/mol DAA synthesized. Transport and transamination costs are ignored.

There is no published information available for the use of amino acids for gluconeogenic purposes in preruminant calves. However, lactose intake in these calves usually accounts for 30–40% of the total energy intake. Hence, there should be no need for significant gluconeogenesis from amino acids. Therefore, this transaction is not included in the model.

Endogenous urinary nitrogen (EUN) loss is modeled as a drain on the amino acid pool and is assumed to be 180 mg N/(kg$^{0.75}$ · d). This is somewhat lower than reported for protein-free diets [200 mg N/(kg$^{0.75}$ · d); Roy 1980], because amino acid oxidation may well be increased in such situations. Endogenous urinary N excretion is assumed to be 52, 22, 13, 10 and 3% by origin from urea, creatinine, allantoin, amino acids and uric acid, respectively. Calculating this composition, literature values were adopted for excretion of allantoin and uric acid (Chen et al. 1990), amino acids (Lynch et al. 1978) and creatinine (Lindberg 1985 and our experiments). Urea was calculated to make EUN add up to 180 mg/(kg$^{0.75}$ · d). To correct for the difference in energy content per gram nitrogen between amino acids and EUN, an energy yield of 60 kJ/g N excreted is introduced, assumed to be released as ATP.

Amino acid oxidation is calculated after application of the imbalance routine, which is described below. In case of no limiting amino acid, oxidation depends on amino acid concentration (Equation 1.8; for references, see e.g., Liu et al. 1995). Amino acid oxidation is assumed to occur in the liver. The maximum velocity ($V_{Aa,AaAy}$), therefore, is expressed as a function of liver weight. Liver weight (in kg) was estimated from the experiments as a function of visceral protein mass (Q_{Pv}; Equation 11.3). The $\mathbf{V}_{max}$ was calculated from the N balance data of Robinson et al. (1996), who infused extremely large amounts of casein in the abomasum of Holstein steers (highest N input 231 g/d, steers of 200 kg Lw; 142 g N/d excreted with urine). Despite the extreme treatment of Robinson et al. (1996), the V_{max} derived from these data is most certainly an underestimate of the theoretical V_{max} because in in vivo experiments, conditions will never be optimal (Gill et al. 1989a). To approach a realistic V_{max}, the value obtained was increased by 33%. The effect of increasing the V_{max} on the rate of a transaction is discussed by Black and Reis (1979). Energy cost of urea excretion by the kidney is assumed to be 0.1 mol ATP/mol urea (Martin and Blaxter 1965).

Calculation of imbalance of dietary amino acids, synthesis of dispensable amino acids and requirements for indispensable amino acids. A calculation routine is introduced to account for possible imbalance of dietary amino acids. This routine is performed every iteration and, in the case of a limiting amino acid, results in increased oxidation. The calculation is based on the balance between amino acids, provided by either diet or degraded body protein, compared with the amino acids required for body protein synthesis. An amount of each amino acid inevitably oxidized is taken into account and discussed below. The routine also calculates the synthesis of DAA.

Inevitable oxidative losses. As stated by Heger and Frydrych (1989), the presence of degradive enzymes in tissues is presumably responsible for the inevitable loss of a fraction of amino acids, even at low levels of intake. Consequently, potential reutilization of degraded protein for protein synthesis is always

TABLE 7

Mathematical statement of the model simulating metabolism of preruminant calves[1]

	Protein metabolism	
Amino acid pool, Q_{Aa}, *mol*		
Concentration	$C_{Aa} = Q_{Aa}/Q_{Ew}$	(1.1)
Input	$P_{Aa,PdAa} = Y_{Aa,PdAa}D_{Pd}$	(1.2)
	$P_{Aa,AaAa} = U_{Aa,AaAa}$	(1.3)
	$P_{Aa,PbAa} = Y_{Aa,PbAa}U_{Pb,PbAa}$	(1.4)
	$P_{Aa,PhAa} = Y_{Aa,PhAa}U_{Ph,PhAa}$	(1.5)
	$P_{Aa,PmAa} = Y_{Aa,PmAa}U_{Pm,PmAa}$	(1.6)
	$P_{Aa,PvAa} = Y_{Aa,PvAa}U_{Pv,PvAa}$	(1.7)
Output	$U_{Aa,AaAy} = V_{Aa,AaAy}/[1. + (M_{Aa,AaAy}/C_{Aa})^{SAa,AaAy}]$	(1.8)
	$U_{Aa,AaPb} = Y_{Aa,PbAa}P_{Pb,AaPb}$	(1.9)
	$U_{Aa,AaPh} = Y_{Aa,PhAa}P_{Ph,AaPh}$	(1.10)
	$U_{Aa,AaPm} = V_{Aa,AaPm}/[1. + (M_{Aa,AaPm}/C_{Aa}) + (M_{Ay,AaPm}/C_{Ay})]$	(1.11)
	$U_{Aa,AaPv} = Y_{Aa,PvAa}P_{Pv,AaPv}$	(1.12)
	$U_{Aa,AaUn} = Y_{Aa,AaUn}(0.00018Q_{Lw}^{0.75})$	(1.13)
Differential equation	$dQ_{Aa}/dt = P_{Aa,PdAa} + P_{Aa,AaAa} + P_{Aa,PbAa} + P_{Aa,PhAa} + P_{Aa,PmAa} + P_{Aa,PvAa} - U_{Aa,AaAa} - U_{Aa,AaAy} - U_{Aa,AaPb} - U_{aa,AaPh} - U_{Aa,AaPm} - U_{Aa,AaPv} - U_{aa,AaUn}$	(1.14)
Auxiliary equations	$V_{Aa,AaAy} = V^{*}_{Aa,AaAy}Q_{Lv}$	(1.15)
	$V_{Aa,AaPm} = 1.25 + V^{*}_{Aa,AaPm}Q_{Pm}$	(1.16)
Remark	Amino acid oxidation ($U_{Aa,AaAy}$) and synthesis of dispensable amino acids ($U_{Aa,AaAa}$) are calculated after checking possible dietary amino acid imbalance in a separate calculation routine, see Table 8.	
Bone protein pool, Q_{Pb}, *kg*		
Input	$P_{Pb,AaPb} = FDR_{Pb}Q_{Pb} + PF_{Pb}0.02028[(dQ_{Pm}/dt)/PF_{Pm}]^{0.3753}$	(2.1)
Output	$U_{Pb,PbAa} = FDR_{Pb}Q_{Pb}$	(2.2)
Differential equation	$dQ_{Pb}/dt = P_{Pb,AaPb} - U_{Pb,PbAa}$	(2.3)
Hide protein pool, Q_{Ph}, *kg*		
Input	$P_{Ph,AaPh} = PF_{Ph}0.018Q_{Lw}^{0.75}/1000 + FDR_{Ph}Q_{Ph} + PF_{Ph}0.039316[(dQ_{Pm}/dt)/PF_{Pm}]^{0.4754}$	(3.1)
Output	$U_{Ph,PhEx} = PF_{Ph}0.018Q_{Lw}^{0.75}/1000$	(3.2)
	$U_{Ph,PhAa} = FDR_{Ph}Q_{Ph}$	(3.3)
Differential equation	$dQ_{Ph}/dt = P_{Ph,AaPh} - U_{Ph,PhEx} - U_{ph,PhAa}$	(3.4)
Muscle protein pool, Q_{Pm}, *kg*		
Input	$P_{Pm,AaPm} = Y_{Pm,AaPm}U_{Aa,AaPm}$	(4.1)
Output	$U_{Pm,PmAa} = FDR_{Pm}Q_{Pm}$	(4.2)
Differential equation	$dQ_{Pm}/dt = P_{Pm,AaPm} - U_{Pm,PmAa}$	(4.3)
Visceral protein pool, Q_{Pv}, *kg*		
Input	$P_{Pv,AaPv} = PF_{Pv}2.46DMI/1000 + FDR_{Pv}Q_{Pv} + PF_{Pv}0.06466[(dQ_{Pm}/dt)/PF_{Pm}]^{0.7376}$	(5.1)
Output	$U_{Pv,PvEn} = PF_{Pv}2.46DMI/1000$	(5.2)
	$U_{Pv,PvAa} = FDR_{Pv}Q_{Pv}$	(5.3)
Differential equation	$dQ_{Pv}/dt = P_{Pv,AaPv} - U_{Pv,PvEn} - U_{Pv,PvAa}$	(5.4)
	Energy metabolism	
Acetyl coenzyme A pool, Q_{Ay}, *mol*		
Concentration	$C_{Ay} = Q_{Ay}/Q_{Ew}$	(6.1)
Input	$P_{Ay,AaAy} = Y_{Ay,AaAy}U_{Aa,AaAy}$	(6.2)
	$P_{Ay,FaAy} = Y_{Ay,FaAy}U_{Fa,FaAy}$	(6.3)
	$P_{Ay,GlAy} = Y_{Ay,GlAy}U_{Gl,GlAy}$	(6.4)
Output	$U_{Ay,AyFa} = V_{Ay,AyFa}/[1 + (M_{Ay,AyFa}/C_{Ay}) + (C_{Fa}/J_{Fa,AyFa})]$	(6.5)
	$U_{Ay,AyMa} = (U^{\#}_{Ay,AyMa} - P_{At,FdGl} - P_{At,FbGl} - P_{At,AaAy} - P_{At,GlAy} - P_{At,FaAy} - P_{At,AaUn})/12$	(6.6)
	$U_{Ay,AyOx}$ = see auxiliary equations	
Differential equation	$dQ_{Ay}/dt = P_{Ay,AaAy} + P_{Ay,FaAy} + P_{Ay,GlAy} - U_{Ay,AyFa} - U_{Ay,AyMa} - U_{Ay,AyOx}$	(6.7)
Auxillary equations	$V_{Ay,AyFa} = V^{*}_{AyAyFa}Q_{Fb}^{0.67}$	(6.8)
	$U^{\#}_{Ay,AyMa} = 0.75(3.634Q_{Lb}^{0.75} + 1.6Q_{Fb}^{0.75} + 13.3Q_{Vi}^{0.75})$	(6.9)
	$P_{At,FdGl} = Y_{At,FdFa}D_{Fd}$	(6.10)
	$P_{At,FbGl} = Y_{At,FbFa}U_{Fb,FbFa}$	(6.11)
	$P_{At,AaAy} = Y_{At,AaAy}U_{Aa,AaAy}$	(6.12)
	$P_{At,GlAy} = Y_{At,GlAy}U_{Gl,GlAy}$	(6.13)
	$P_{At,FaAy} = Y_{At,FaAy}U_{Fa,FaAy}$	(6.14)
	$P_{At,AaUn} = Y_{At,AaUn}U_{Aa,AaUn}$	(6.15)
	$U_{Ay,AyOx} = R_{Ay,AaAa}U_{Aa,AaAa} + R_{ay,MdAs}0.1549[(dQ_{Pm}/dt)/PF_{Pm}]^{0.368} + R_{Ay,AaPb}U_{Aa,AaPb} + R_{Ay,AaPh}U_{Aa,AaPh} + R_{Ay,AaPm}U_{Aa,AaPm} + R_{Ay,AaPv}U_{Aa,AaPv} + R_{Ay,PbAa}U_{Pb,PbAa} + R_{Ay,PhAa}U_{Ph,PhAa} + R_{Ay,PmAa}U_{Pm,PmAa} + R_{Ay,PvAa}U_{Pv,PvAa} + A_{Pd}D_{Pd} + A_{Fd}D_{Fd} + A_{Ld}D_{Ld} + A_{Sd}D_{Sd} + R_{Ay,FaFb}U_{Fa,FaFb} + R_{Ay,UrUe}Y_{Ur,AaAy}U_{Aa,AaAy} + V_{Ay,AyAg}Q_{EW}/\{1 + [(M_{Ay,AyAg}/C_{Ay})^{SAy,AyAg}]\}$	(6.16)
Glucose pool, Q_{Gl}, *mol*		
Concentration	$C_{Gl} = Q_{Gl}/Q_{Ew}$	(7.1)
Input	$P_{Gl,LdGl} = Y_{Gl,LdGl}D_{Ld}$	(7.2)
	$P_{Gl,SdGl} = Y_{Gl,SdGl}D_{Sd}$	(7.3)
	$P_{Gl,FdFa} = Y_{Gl,FdFa}D_{Fd}$	(7.4)
	$P_{Gl,FbFa} = Y_{Gl,FbFa}U_{Fb,FbFa}$	(7.5)

(continued)

TABLE 7 (*continued*)

Mathematical statement of the model simulating metabolism of preruminant calves[1]

Glucose pool, (*continued*)	$U_{Gl,AyFa} = R_{Gl,AyFa}U_{Ay,AyFa}$	(7.6)
Output		
	$U_{Gl,FaFb} = R_{Gl,FaFb}U_{Fa,FaFb}$	(7.7)
	$U_{Gl,GlAy} = V_{Gl,GlAy}/[1 + (M_{Gl,GlAy}/C_{Gl})]$	(7.8)
Differential equation	$dQ_{Gl}/dt = P_{Gl,LdGl} + P_{Gl,SdGl} + P_{Gl,FbFa} + P_{Gl,FdFa} - U_{Gl,AyFa} - U_{Gl,FaFb} - U_{Gl,GlAy}$	(7.9)
Auxiliary equation	$V_{Gl,GlAy} = V^*_{Gl,GlAy}Q_{Ew}^{0.75}$	(7.10)
Fatty acid pool, Q_{Fa}, *mol*		
Concentration	$C_{Fa} = Q_{Fa}/Q_{Ew}$	(8.1)
Input	$P_{Fa,FdFa} = Y_{Fa,FdFa}D_{Fd}$	(8.2)
	$P_{Fa,AyFa} = Y_{Fa,AyFa}U_{Ay,AyFa}$	(8.3)
	$P_{Fa,FbFa} = Y_{Fa,FbFa}U_{Fb,FbFa}$	(8.4)
Output	$U_{Fa,FaAy} = V_{Fa,FaAy}/[1 + (C_{Ay}/J_{Ay,FaAy}) + (M_{Fa,FaAy}/C_{Fa})]$	(8.5)
	$U_{Fa,FaFb} = V_{Fa,FaFb}/[1 + (M_{Fa,FaFb}/C_{Fa})]$	(8.6)
Differential equation	$dQ_{Fa}/dt = P_{Fa,FdFa} + P_{Fa,AyFa} + P_{Fa,FbFa} - U_{Fa,FaAy} - U_{Fa,FaFb}$	(8.7)
Auxiliary equations	$V_{Fa,FaAy} = V^*_{Fa,FaAy}Q_{Lw}^{0.75}$	(8.8)
	$V_{Fa,FaFb} = V^*_{Fa,FaFb}Q_{Fb}^{0.67}$	(8.9)
Body fat pool, Q_{Fb}, *kg*		
Input	$P_{Fb,FaFb} = Y_{Fb,FaFb}U_{Fa,FaFb}$	(9.1)
Output	$U_{Fb,FbFa} = FDR_{Fb}Q_{Fb}$	(9.2)
Differential equation	$dQ_{Fb}/dt = P_{Fb,FaFb} - U_{Fb,FbFa}$	(9.3)
	Body ash	
Body ash pool, Q_{As}, *kg*		
Input	$P_{As,MdAs} = 0.3623(dQ_{Pv}/dt)/PF_{Pv} + 0.1933(dQ_{Ph}/dt)/PF_{Ph} + 0.3151(dQ_{Pm}/dt)/PF_{Pm} + 0.1549[(dQ_{Pm}/dt)/PF_{Pm}]^{0.368}$	(10.1)
Differential equation	$dQ_{As}/dt = P_{As,MdAs}$	(10.2)
	Summative equations	
Empty body weight, Q_{Ew}, *kg* Live weight, Q_{Lw}, *kg*; Liver weight, Q_{Lv}, *kg*	$Q_{Ew} = 1.03[Q_{As} + Q_{Fb} + Q_{Pb} + 11.605(Q_{Pb}/PF_{Pb})^{0.593} + Q_{Ph} + 13.879(Q_{Ph}/PF_{Ph})^{0.847} + Q_{Pm} + 24.294(Q_{Pm}/PF_{Pm})^{0.943} + Q_{Pv} + 30.536\ (Q_{Pv}/PF_{Pv})^{0.901}]$	(11.1)
	$Q_{Lw} = 1.11Q_{Ew}$	(11.2)
	$Q_{Lv} = 4.653(Q_{Pv}/PF_{Pv})^{0.81}$	(11.3)
Lean body mass, Q_{Lb}, *kg* Visceral mass, Q_{Vi}, *kg*	$Q_{Lb} = Q_{As} + Q_{Pb} + 11.605(Q_{Pb}/PF_{Pb})^{0.593} + Q_{Ph} + 13.879(Q_{Ph}/PF_{Ph})^{0.847} + Q_{Pm} + 24.294(Q_{Pm}/PF_{Pm})^{0.943}$	(11.4)
	$Q_{Vi} = Q_{Pv} + 30.536(Q_{Pv}/PF_{Pv})^{0.901}$	(11.5)

[1] See Tables 1 and 2 for explanation of notation.

<100%. Furthermore, potential reutilization decreases with protein intake and depends on the amino acid considered (Moughan 1994, Simon 1989). Unfortunately, information on the magnitude of the inevitable oxidative losses of specific amino acids in preruminants is scarce. It seems sensible, however, to make these losses for a specific amino acid dependent on the amount of that amino acid passing the site of oxidation. In the model, the proportion of each amino acid inevitably oxidized is set to 0.02 times the daily amount of that amino acid entering the amino acid pool. This represents a 2% chance of each amino acid being oxidized when passing the site of oxidation. This way, both increased protein turnover and increased protein intake lead to increased oxidative losses. To place this assumption in perspective, **Table 6** presents the effect of applying an inevitable oxidation proportion of either 2 or 5% on daily amounts of amino acids inevitably oxidized, compared with minimal rates of oxidation in pigs (reviewed by Fuller 1994) and with the maintenance requirements of growing pigs (Fuller et al. 1989). Maintenance requirements, estimated as the amount needed to maintain N equilibrium, would include these minimal oxidative losses (Fuller 1994). From Table 6, it appears that, for the amino acids reported by Fuller (1994), minimal oxidation is lower than the amount oxidized, caused by application of the 2%. Compared with the amino acid requirements for maintenance, which also includes other amino acid losses (e.g., endogenous fecal losses and scurf losses), application of 2% results in higher losses for most amino acids. The effect of changing this percentage on amino acid imbalance is discussed in a companion paper (Gerrits et al., in press).

The mathematical statement of the model simulating the metabolism of preruminant calves is presented in **Table 7**.

The calculation routine. The calculation routine, with numbered equations, is presented in **Table 8** and is briefly described below. First, all protein fluxes to and from the amino acid pool are converted into fluxes for individual amino acids by using the amino acid profiles presented in Table 5 (Calculations [1]–[3]). The inevitable oxidative losses for each amino acid are then calculated as the amount entering the amino acid pool, multiplied by its proportion inevitably oxidized (default 0.02) (Calculation [4]). Next, the supply of each amino acid, i.e., the amount entering the amino acid pool, is compared with the demand, i.e., the amount needed for protein synthesis increased by the amount needed to replace inevitable oxidative losses (Calculations [5]–[7]). If the supply of an indispensable amino acid is smaller than its demand, the rate of protein synthesis is calculated based on the supply of the limiting indispensable amino acid, and all amino acids supplied in excess are oxidized. If the supply of all indispensable amino acids exceeds the demand, amino acid oxidation is calculated according to (Equation 1.8, Table 7). Similarly, synthesis of DAA is calculated by difference between demand and supply (Calculations [8]–[9]). As described earlier, this flux is modeled as both input into and output from the amino acid pool. Therefore, energy consumption is the only effect of this flux

TABLE 8

Calculation of imbalance of dietary amino acids, synthesis of dispensable amino acids and requirements for indispensable amino acids in the model simulating metabolism of preruminant calves[1,2]

Protein fluxes are converted into fluxes for individual amino acids:

$$AaPx_i = [P_{Px,AaPx}(PFT_{Px}/PF_{Px})aa_{Pxi}]/MW_i \quad [1]$$

$$PxAa_i = [U_{Px,PxAa}(PFT_{Px}/PF_{Px})aa_{Pxi}]/MW_i \quad [2]$$

$$PdAa_i = [D_{Pd}(PFT_{Pd}/PF_{Pd})aa_{Pdi}]/MW_i \quad [3]$$

in which: $AaPx_i$ = flux of Aa_i from the Aa to body protein pool Px (in mol/d); Px stands for either Pb, Ph, Pv or Pm; aa_{Pxi} = the concentration of Aa_i in tissues Px [in g aminoacyl residue/kg aminoacyl residue; i = 1, . . ., 19; indispensables (i = 1, . . ., 12) and dispensables (i = 13, . . ., 19), see Table 5]; MW_i = molecular weight of aminoacyl residue i; $PxAa_i$ = flux of Aa_i from Px pool to the Aa pool (in mol/d); in the case of Pv, and Aa composition is calculated after subtraction of the amount of Aa_i disappearing to En; $PdAa_i$ = flux of Aa_i from Pd into the Aa Pool (mol/d)

Inevitable oxidative losses of Aa_i (i = 1, . . ., 19) are calculated as:

$$Aalo_i = (PbAa_i + PhAa_i + PvAa_i + PmAa_i + PdAa_i)aa_{loi}, \quad [4]$$

in which: $Aalo_i$ = amount of Aa_i inevitably oxidized; aa_{loi} = proportion of flux of Aa_i inevitably oxidized (default 0.02).

The supply of Aa_i (Aa_{si}) is defined as [5] and the demand of Aa_i (Aa_{di}) is defined as [6]. Subsequently, these are compared for i = 1, . . ., 19 by calculation of the ratio R_i [7].

$$Aa_{si} = PbAa_i + PhAa_i + PvAa_i + PmAa_i + PdAa_i \quad [5]$$

$$Aa_{di} = AaPb_i + AaPh_i + AaPv_i + AaPm_i + Aalo_i \quad [6]$$

$$R_i = Aa_{si}/Aa_{di} \quad [7]$$

If $R_i < 1$ for at least one indispensable Aa_i, R_{min} is defined as the minimum ratio R_i. Else, R_{min} is set to 1. $U_{Aa,AaAa}$, (mol/d) is calculated as [8]. If $R_{min} < 1$, $U_{Aa,AaAy}$, (mol/d) is calculated by summation of all Aa's, supplied in excess. $U_{Aa,AaAa}$ is added to this flux to prevent it from becoming negative in case of an extremely low supply of one or more dispensable Aa's [9]. If the ratio $R_i \geq 1$ for all indispensable Aa's, $U_{Aa,AaAy}$ is calculated according to (Equation 1.8, Table 7).

$$U_{Aa,AaAa} = \sum_{i=13}^{19}(Aa_{di}R_{min} - Aa_{si}), \text{ only for } R_i < 1 \quad [8]$$

$$U_{Aa,AaAy} = \sum_{i=1}^{19}(Aa_{si} - Aa_{di}R_{min}) + U_{Aa,AaAa} \quad [9]$$

The requirement for individual indispensable Aa_i (Rq, in mol aminoacyl residue/d) to support maximal protein gain [12] is calculated as the sum of the amount needed for tissue deposition ($Rqdep_i$) [10] and the amount needed to replace inevitable oxidative losses of Aa_i from protein degradation ($Rqox_i$) [11].

$$Rqdep_i = AaPb_i - PbAa_i + AaPh_i - PhAa_i + AaPv_i - PvAa_i + AaPm_i - PmAa_i \quad [10]$$

$$Rqox_i = (PbAa_i + PhAa_i + PvAa_i + PmAa_i)aa_{loi} \quad [11]$$

$$Rq_i = (Rqdep_i + Rqox_i)(1 + aa_{loi}) \quad [12]$$

[1] See Tables 1 and 2 for further explanation of notation.
[2] This calculation routine is included in the model (Table 7) and is performed every iteration.

(Equation 6.16). The requirement for each indispensable amino acid to support maximal protein gain in any specific situation can be directly derived from these calculations (Calculations [10]–[12]).

Cystine, tyrosine and arginine. By summing methionine and cystine rather than considering them separately, and summing phenylalanine and tyrosine (Table 5), synthesis of cystine from methionine and tyrosine from phenylalanine is accounted for. The maximum rate of arginine synthesis has been shown to be insufficient for maximum growth in growing pigs (see review of Fuller 1994) and ruminants (Davenport et al. 1990). Therefore, it is assumed that a minimum of 40% of the arginine deposited has to be supplied through the diet, as suggested by Fuller (1994). This has been included in the identification of the limiting indispensable amino acid.

Energy metabolism

Body fat pool, Fb. Inputs to the body fat pool are from the fatty acid and glucose pool (Equations 9.1 and 7.7). Outputs are to the fatty acid and glucose pool (Equations 9.2 and 7.5). The body fat pool represents chemically determined fat, assumed to comprise only triacylglycerol. The molecular weight is set at 884 g/mol. Data on body fat turnover in growing calves are scarce. Vernon and Clegg (1985) state that basal lipolytic rates measured in vitro are close to lipolysis in vivo. This may be true for well-fed animals, but is likely to underestimate lipolysis in underfed animals (Mersmann 1986). Basal and maximal lipolytic rates of well-fed heifers in vitro were shown to be 6 and 60 g fatty acid/(kg adipose tissue · d), respectively (Smith et al. 1992). Assuming that there is no need for preferential lipid degradation to provide energy, a fixed fractional degradation rate of 1%/d is adopted, close to the basal lipolytic rate reported by Smith et al. (1992).

Fatty acid esterification is dependent on fatty acid concentration (Equation 8.6). Maximum velocity, expressed as a function of $Q_{Fb}^{0.67}$, is set at the maximum observed lipid deposition rate in the experiments, assuming a fixed fractional degradation rate of 1%. The value obtained is most certainly an under-

estimate of the theoretical V_{max}, and is increased, as discussed previously, by 33% to approach a realistic V_{max}. The affinity constant was set to match the net lipid deposition rate with the rates measured in the experiments.

Fatty acid pool, Fa. Inputs to the fatty acid pool are from absorption (Equation 8.2), synthesis from acetyl-CoA (Equation. 8.3) and from lipolysis (Equation 8.4). Outputs are to oxidation to acetyl-CoA (Equation 8.5) and to fat synthesis (Equation 8.6). In transactions involving fatty acids, average stoichiometry of oleic acid is assumed. Molecular weight is set at 282 g/mol. Maximum rate of fatty acid synthesis could not be derived from in vitro data. Therefore, the maximum rate of de novo fatty acid synthesis was set to enable a fat deposition rate of 100 g/d for a calf of 100 kg Lw comprising 10 kg fat, assuming no reutilization of fatty acids and an FDR of 1%/d on a fat free diet. Fatty acid synthesis is inhibited by the end product, as observed by Wijayasinghe et al. (1986) and is stimulated by acetyl-CoA concentration, allowing excess energy to be deposited as fat (Equation 6.5).

Fatty acid oxidation is stimulated by substrate concentration and inhibited by a high acetyl-CoA concentration. The inhibition constant is set to allow fatty acid oxidation in case of energy shortage. The maximum rate is set to be adequate to meet maintenance energy requirements and is therefore represented as a function of $Q_{Lw}^{0.75}$ (Equations 8.5 and 8.8).

Glucose pool, Gl. Inputs to the glucose pool are from dietary lactose (Equation 7.2), starch (Equation 7.3) and from glycerol, released during lipolysis (Equation 7.4) or fat absorption (Equation 7.5). Degradation of lactose yields glucose and galactose. Galactose is assumed to be completely converted into glucose without energy costs because both molecules have the same net ATP yield during oxidation (Stryer 1981). Glucose is used as the energy source (catabolism to acetyl-CoA, Equation 7.8), as a source of glycerol in the esterification of fatty acids during fat synthesis (Equation 7.7) and as the major source of reduced NADPH in fatty acid synthesis (Equation 7.6, Wijayasinghe et al. 1986). The V_{max} of glucose oxidation is set to be sufficient to cope with a high input of glucose equivalents, based on the highest feeding level in the experiments. As discussed previously, the value obtained is increased by 33% to approach a realistic V_{max}. Glucose oxidation is dependent on the concentration of glucose. The affinity constant is set to prevent accumulation of glucose in the glucose pool (Equation 7.7).

Acetyl coenzyme A pool, Ay. In the interest of simplicity, acetyl-CoA is considered to be the energy supplier in the body (see also the discussion section). Stoichiometric factors for transactions yielding and requiring energy are calculated assuming 1 mol Ay equivalent to 12 mol ATP (Stryer 1981). Inputs are from oxidation of amino acids (Equation 6.2) and fatty acids (Equation 6.3) and from glycolysis (Equation 6.4).

Acetyl-CoA is used as substrate in fatty acid synthesis (Equation 6.5), oxidized to satisfy maintenance energy needs (Equation 6.6) and to provide energy for various transactions (Equations 6.10–6.16). Together with those represented in Figure 1, an additional energy-requiring transaction was introduced, representing increased energy costs per unit tissue deposition with increased tissue deposition rate. These costs represent increased energy costs of protein turnover (Lobley 1990, Millward 1989), ion pumping (Milligan and McBride 1985, Reeds 1991) and synthesis of endogenous protein (only net endogenous protein losses are represented in the model). In addition, several substrate cycles, not represented in the model, may be part of this transaction (see Katz and Rognstad 1976). The transaction is named "additional energy costs for growth" (Ag, see Table 1) and is represented as a Michaelis-Menten function, dependent on acetyl-CoA concentration (Equation 6.16). The V_{max}, affinity constant and steepness parameter are set to cover the discrepancy between the energy costs accounted for in the model and the energy balance measured in the experiments.

Maintenance energy. Baldwin et al. (1987) used empirical relationships to estimate basal energy expenditure of lean body mass, body fat and viscera, based on data for lactating cows and estimated originally by Smith (1970). Lean body mass, body fat and viscera are calculated from pool sizes (see Equations 11.4 and 11.5). For a calf of 162 kg Lw comprising 103 kg lean body mass, 21 kg viscera and 19 kg body fat, basal energy expenditure would be 0.456 MJ/$kg^{0.75}$. This is in good agreement with the estimates of metabolizable energy requirements for maintenance for preruminants by ARC (1980) and Van Es (1970) of 0.428 and 0.452 MJ/$kg^{0.75}$, respectively. This calculation of basal energy expenditure assumes a mean energy cost of ATP synthesis of 79 kJ/mol. Maintenance energy requirements are first met by all ATP-yielding transactions (see Fig. 1). The remaining part is met by oxidation of acetyl-CoA (Equations 6.6 and 6.9). During test simulations, it was verified that the sum of ATP-yielding transactions is indeed always lower than the maintenance energy requirements.

Several energy-consuming processes accounted for in the model are also part of maintenance energy. Protein synthesis may account for 15–25% of basal metabolic rate in several species (review, Summers et al. 1986). However, no data are available to quantify the contribution of protein degradation to basal metabolic rate. Considering the ATP costs for protein synthesis and protein degradation, and assuming no net protein deposition at maintenance, protein degradation may amount to 20% of the costs of protein synthesis. Furthermore, fat turnover and absorption costs of nutrients at the maintenance energy intake level were calculated to amount to ~1% of maintenance energy expenditure. In total, 25% of the maintenance energy requirements is assumed double counted and is thus subtracted from the maintenance requirements (Equation 6.9).

Body ash pool, As

The body ash pool represents chemically determined body ash. Supply of minerals and other nutrients required for body ash deposition is considered nonlimiting. Ash accretion is considered to consist of two components: *1*) ash in nonskeletal tissues: an amount depending on the pool sizes of protein in viscera, hide and muscle, and *2*) ash in skeletal tissue (Equation 10.1). The ratio between ash and protein in muscle is assumed constant and is adopted from Schulz et al. (1974). The relationship between ash and protein in hide is estimated from the same data. They analyzed 12 German Friesian beef calves of either 150 or 270 kg Lw for body composition. The relationship between ash and protein in viscera is estimated from the experiments. The accretion rate of ash in skeletal tissue is made dependent on the rate of muscle protein accretion, allowing for a higher priority in skeletal development in slower growing calves.

There appears to be no published information on the energy cost of skeletal development. Therefore, it is set at 28 mol ATP/kg ash deposited in skeletal tissue, assuming, in analogy to France et al. (1987), a cost of 2 mol ATP/mol Ca or P incorporated into bone ash, calculating the Ca and P content of bone ash from Schulz et al. (1974). The sensitivity of the model to this assumption is described in Gerrits et al. (in press).

Model calibration and summary

The affinity and inhibition constants and steepness parameters were adjusted to obtain good fit of the experimental data,

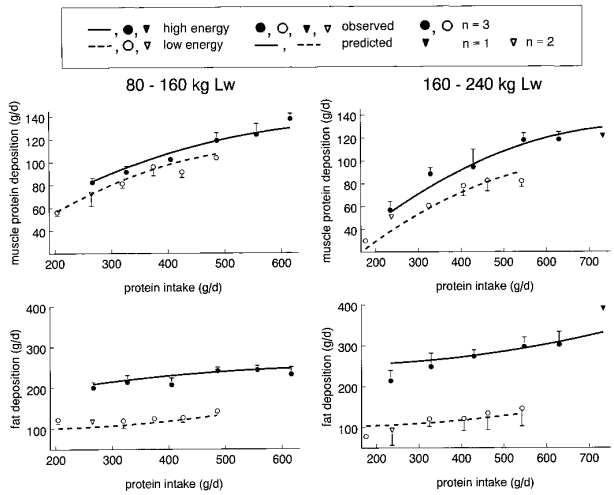

FIGURE 3 Simulated (lines) and observed (symbols) response of rate of muscle protein and body fat deposition in preruminant calves to protein intake at two protein-free energy intake levels in two live weight (Lw) ranges. Low and high protein-free energy intake levels were 663, 851, 564 and 752 kJ/(kg$^{0.75}$ · d) for the weight ranges 80–160 and 160–240 kg Lw, respectively. Values are means ± SEM.

i.e., the observed average muscle protein and fat deposition rates, for each weight range.

A complete listing of the equations that constitute the model is given in Table 7. The calculation of amino acid imbalance, synthesis of dispensable amino acids and amino acid requirements is given in Table 8. The model is programmed in ACSL (Mitchell and Gauthier 1981) and run on a VAX computer. The differential equations for the 10 state variables are solved numerically for a given set of initial conditions and parameter values. The integration interval used is 0.01 d, with a fourth-order, fixed-step–length Runge-Kutta method. The results presented are not sensitive to small changes in initial concentrations and smaller integration intervals.

RESULTS AND DISCUSSION

Model behavior and performance. Results of simulations of the experimental treatments, described earlier in this paper are presented here. An evaluation of model behavior, a comparison of model predictions with independent data and a sensitivity analysis are presented in a companion paper (Gerrits et al., in press).

Simulation of the experimental treatments revealed a slight amino acid imbalance (threonine shortage) only at the lowest protein intake level in the experiment with calves of 160–240 kg Lw. Results of experimental and simulated muscle protein and fat deposition rates for calves between 80 and 160 kg and between 160 and 240 kg Lw are shown in **Figure 3**. The increase in muscle protein deposition rate with increasing protein intake, as well as the effect of protein-free energy intake on muscle protein deposition rate, is simulated satisfactorily. Also, the large contrasts in fat deposition rates between energy intake levels are represented quantitatively.

To demonstrate the effect of a stepwise reduction of the dietary intake of a specific amino acid on model behavior, methionine intake was reduced from 9 to 2 g/d at two levels of protein intake. Results of the simulations are shown in **Figure 4**. Although the model simulates the response of protein deposition rate to intake of methionine residue (water-free) rather than methionine, the results in Figure 4 are already converted to methionine. The results clearly demonstrate the effect of methionine intake on average protein retention in this weight range. The methionine requirements, calculated as described in Table 8, are 6.4 and 5.6 g/d for the high and low protein intake level, respectively. At intakes below these requirements, protein deposition becomes depressed by methionine intake. Obviously, the simulated requirements (in g/d) depend on the nutritional circumstances in the specific simulation. In the example, the reduced methionine requirement at the low protein intake level is caused by a decrease in the amount of substrate required for protein deposition. The model provides the possibility of simulating the requirement for all essential amino acid in a wide range of nutritional input. More attention, however, has to be paid to the minimum oxidation proportion for individual amino acids. Also, more recent data on amino acid profiles of the tissues would improve the reliability of estimations of amino acid requirements. This approach to simulation of amino acid requirements is more extensively tested and studied in the companion paper (Gerrits et al., in press).

The relationships between the development of the protein pools, estimated from the experiments and shown in Figure 2, allow for a higher priority for bone and hide protein relative to muscle protein of slower growing calves. Although the simplicity of this solution is attractive, it brings about a few problems. First, the model becomes highly sensitive to changes in the parameters describing the development of the muscle protein pool (evaluated in Gerrits et al., in press). Second, the mathematical representation of the protein metabolism does not allow negative growth of the muscle protein pool (see Table 7). The model, therefore, is not valid for calves fed below maintenance.

The V_{max} of muscle protein synthesis was set to result, in combination with the fixed FDR, in a slight linear increase in muscle protein deposition capacity with increasing muscle protein mass. This approach resulted in an increase in growth rate with time on a feeding scheme based on Lw$^{0.75}$, consistent with observations in the experiments. This representation of the V_{max} of muscle protein synthesis, however, probably does not adequately represent the reduced muscle protein deposition capacity of calves approaching maturity.

Choice of pools. The choice of pools is important in the development of a growth simulation model. The choice of

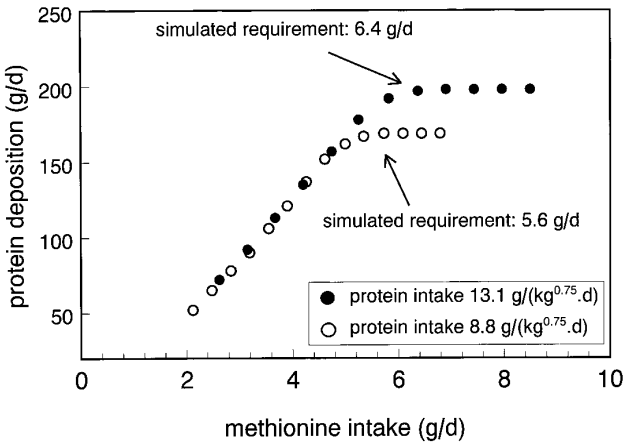

FIGURE 4 Simulation of the effect of a stepwise reduction of methionine intake on average protein gain of preruminant calves in the weight range of 80–160 kg live weight, at two protein intake levels. Protein free energy intake was 820 kJ/(kg$^{0.75}$ · d).

body storage pools is directly related to the model objective and available data, whereas the choice of metabolite pools is more delicate. In the present model, the simplest representation that would be sufficient to distinguish the effects of dietary protein, carbohydrates and fats on rates of gain of protein and fat was chosen. It has been assumed that substrate concentrations in the metabolite pools drive a particular process. The choice of the amino acid pool and especially the acetyl-CoA pool, however, compromises the biology. Acetyl-CoA in the body has an extremely high turnover rate. Because its metabolism is strictly intracellular (Stryer 1981), whole-body concentrations are unlikely to vary with nutrient input, at least not within a measurable range. Inclusion of an ATP pool would be an improvement in the respect that regulatory effects have been attributed to ATP (Gill et al. 1989a). It suffers the similar limitation, however, of having a high turnover rate. Moreover, it cannot replace acetyl-CoA because it is not a substrate for fatty acid synthesis. The choice of representation of regulatory effects of energy metabolites therefore remains a concern for future models of metabolism. Alternative structures such as transferring the regulatory effects to glucose and fatty acids in combination with a simple representation of the hormonal regulation of nutrient partitioning (see Baldwin et al. 1987) may be future options. To improve the link with experimental work and to stimulate meaningful research, attention should be focused on measurable entities.

Compared with pigs (see Moughan et al. 1995) and beef cattle (e.g., Di Marco et al. 1989, France et al. 1987, Oltjen et al. 1986), little attention has been paid to modeling growth responses to nutrient intake in preruminant calves. Clark et al. (1978) developed a simulation model for preruminant calves. This model, however, was developed to optimize the net returns to specialized veal resources. The response of protein and fat deposition rates to nutrient intake in this model was based on empirical growth equations, developed by Van Es (1970).

Nutrient partitioning in most pig growth models is based on protein- and energy-dependent phases in protein deposition (see Moughan et al. 1995). The experimental work, however, revealed that these principles do not apply to preruminant calves of 80–240 kg Lw (Gerrits et al. 1996) and therefore cannot be the basis for growth simulation in preruminants.

Extensive metabolic changes occur as calves develop from the nonruminating to the ruminating state. The energy metabolism in preruminants is based largely on glucose and long-chain fatty acids, whereas in ruminants, volatile fatty acids are the main energy source. These changes require a different representation of energy metabolism of preruminants compared with ruminants. There is, however, no reason why the metabolism of absorbed protein should be different as well. The approach to protein metabolism applied in this model, including the calculation of amino acid imbalance, may therefore be a valuable addition to existing models for beef cattle already mentioned.

In conclusion, this model is a useful step in the process of quantifying the connection between protein and fat retention and dietary input in preruminant calves. The representation of protein in bone, hide, muscle and viscera, in conjunction with turnover rates, provides a valuable tool for defining requirements of individual amino acids. The model can be improved further when more data are available, especially on rates of protein turnover, inevitable amino acid oxidation, and protein and fat retention at different fat and carbohydrate intake levels.

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