MODELLING THE GROWTH OF PRERUMINANT CALVES

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Proefschrift

ter verkrijging van de graad van doctor
op gezag van de rector magnificus
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VOORWOORD

Met het risico dat ik iemand over het hoofd zie wil ik graag een aantal mensen bedanken dat een bijzondere bijdrage heeft geleverd aan dit boekje. Dit AIO onderzoek heeft na een wat moeizame start gelukkig weinig echte problemen gekend. Het kostte in het begin moeite het Produktschap voor Veevoeder te overtuigen van de noodzaak dure experimenten uit te voeren. Dat het Produktschap uiteindelijk toch over de brug kwam is voor een belangrijk deel te danken aan de flexibele opstelling van de leden van de werkgroep Voedingsonderzoek Vleeskalveren. De diskussies waren in het begin wel eens verwarrend (voor mij, in ieder geval), later ging dat steeds beter en ik heb de inbreng van deze werkgroep dan ook zeer gewaardeerd.

Zonder promotoren geen proefschrift. Martin, het kostte (nog steeds, trouwens) wel eens wat energie om diskussies to the point te houden. Een gesprek over aminozuurbehoefte bij kalveren eindigde niet zelden, via een diskussie over walvissen, zomaar ergens in Limburg. Toch heb ik altijd dankbaar gebruik gemaakt van je enthousiasme, je brede fysiologische kennis en je ongelooflijke netwerk. Seerp, bij jou kon ik behalve voor organisatorische zaken ook altijd terecht voor inhoudelijke diskussies, al heb ik van dat laatste naar mijn smaak te weinig gebruik gemaakt. Ik heb veel geleerd van jouw vermogen om bij- van hoofdzaken te onderscheiden en van je frisse kijk op overigens niet herkauwende herkauwers.

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Tja, en dan moeten de kalveren in monsterpotjes waar ze eigenlijk niet in passen. Wat een klus. Maar de vingers zitten er nog aan en ook jullie humeur heeft er niet onder geleden, Peter en Tamme. Ook jullie hulp heb ik gewaardeerd! Als het kalf ontbonden is dempt men de vrieskisten. Gelukkig had je de vleesjes, orgaantjes en niettevergeten de hakapétjes toen al in cijfertjes omgezet, Marian. Je denkt er,

STELLINGEN

1. Snel groeiende Holstein kalveren hebben geen hoge prioriteit voor het gebruik van voereiwit voor eiwitgroei: zelfs bij opname van hoogwaardige eiwitten wordt tussen de 50 en 60% van het verteerde eiwit als ureum via de urine uitgescheiden. *Dit proefschrift.*

2. Bij met melk(vervangers) gevoerde kalveren heeft een verhoging van de energie-opname, zelfs bij een lage eiwitopname, een betere benutting van het verteerde eiwit tot gevolg. *Dit proefschrift.*

3. De term "metaboliseerbare energie-opname" is niet de hoeveelheid energie die beschikbaar is voor het metabolisme van een dier, maar de hoeveelheid die beschikbaar is geweest. Dit maakt het gebruik van deze term in de veevoedingswereld op den duur onhoudbaar. Gebruik van de term "netto energie-opname" is hiervoor geen oplossing.

4. Het uitdrukken van nutriëntbehoeftes in procenten in het voer stamt uit het stenen tijdperk.

5. Simulatiemodellen zijn onmisbaar bij de invoering van op nutriënten gebaseerde voederwaarderingssystemen.

6. Het integreren van experimenteel onderzoek en computersimulatie heeft voordelen voor beide disciplines.

7. Een koe zonder pens is nog geen varken. *Dit proefschrift, tussen de regels.*

8. Extreme opvattingen zijn in het algemeen het gemakkelijkst te verdedigen.

9. Het wereldvoedselprobleem is vooral een machtsprobleem.

10. Het is maar goed dat de kreet "ik wil = ik kan" (*Emile Ratelband, 1996*) niet voor iedereen opgaat.

Walter J.J. Gerrits

Stellingen behorende bij het proefschrift:
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net als ik, vast met weemoed aan terug. Zonder jullie dus ook geen proefschrift.

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Mocht het niet uit het bovenstaande blijken, het internationale, sociale verkeer bij de vakgroep Veevoeding maakt het een aangename plaats om te werken. Daarom waardering voor René (die altijd koffie voor me zet), voor Josien en Maria (praatjes vullen geen gaatjes), maar ook voor alle anderen.

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Walter

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Gerrits, W.J.J. 1996. Modelling the growth of preruminant calves. The emphasis in meat production has shifted from maximizing production volume to the efficient production of lean meat. Body composition can to a large extent be manipulated by nutritional means. It does, however, require integrated knowledge of protein and energy metabolism. Two experiments were performed with male, preruminant calves, to quantify the relationship between nutrient intake (protein, energy) and the rate of gain of live weight, protein and fat in the live weight range 80 to 240 kg. These experiments showed that calves in this weight range respond to increased protein intake by increasing their protein and fat deposition rates. The utilization of digested protein decreased from 60% at low protein intake levels to 35% at high protein intake levels. Extra protein-free energy results mainly in extra fat deposition, but also increases protein deposition rate, even at low protein intakes. Subsequently, the results of these experiments were used for the construction of a dynamic, mechanistic growth simulation model. The model predicts rates of gain of body weight, body fat and body protein (in several tissues) from nutrient intake. It can also be used to predict amino acid requirements. Protein and fat accretion rates obtained in independent experiments could be simulated satisfactorily. Additional measurements, performed in the experiments showed that the rate of muscle protein degradation, measured by the urinary excretion of 3-methylhistidine, increased with increasing protein deposition rates. Furthermore, it was shown that plasma levels of triiodothyronine strongly respond to increased intake of protein-free energy but hardly to increased protein intakes. In contrast, plasma levels of thyroxine and insulin-like growth factor-1 were increased by increasing protein intake, but unaffected by increased protein-free energy intake levels.

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CONTENTS

GENERAL INTRODUCTION

VEAL PRODUCTION

Until the sixties, most male calves from dairy herds were slaughtered in the first week of life because of their poor performance in beef production. Some of them were allowed to suckle their mothers or were given whole milk by bucket for two to eight weeks. These calves produced a pale, tender, exclusive type of meat, called veal. Since the sixties, veal production has changed dramatically with the advent of milk substitutes. Today, almost all male dairy calves are used for veal production and raised exclusively on milk substitutes. In this way, calves are kept in a pre-ruminant stage until 2-5 months of age (100-250 kg live weight). Milk substitutes are powdered foods, which, after water reconstitution, can replace whole milk. The main ingredients are skim-milk powder, fat, and whey powder. In the last 10 years, increasing amounts of skim-milk powder are being replaced by soluble vegetable proteins, mainly originating from soy beans and wheat. Milk substitutes play an important role in the regulation of the dairy market, and allow the production of a large amount of meat from dairy calves which were previously slaughtered at birth (Toullec, 1992).

Veal production has always been greatly influenced by agricultural policies and public opinion. In the sixties, minimum prices for milk led to a surplus of milk on the E.C. market. The availability of skim-milk powder, resulting from that surplus has stimulated veal production since then. The introduction of the milk quota system in 1984 decreased the number of cows. Consequently, prices for newborn calves increased dramatically, and slaughter weights were increased in several countries so as to maintain the level of veal production. Recently, public opinion on human health, and on the health and welfare of calves, has put pressure on governments and on the E.C. to provide stricter regulations on animal housing, feeding and transportation.

The consumers' preference for lean meat and, to a lesser extent, the necessity to reduce environmental pollution, has forced meat producers to change their management strategy. Whereas maximizing the growth rates of animals was the main objective until the mid-eighties, during the last ten years, it has shifted towards the efficient conversion of feed into lean products. Research in animal nutrition has therefore focused on the development of feeding strategies which can help farmers to achieve this change in objective. This thesis is focused on the nutrition of calves as a means to manipulate body composition and to improve the conversion of nutrients into lean products.

MANIPULATION OF BODY COMPOSITION BY NUTRITIONAL MEANS

The dry matter of an animal diet generally consists of >90% proteins, fats and carbohydrates. The major elements are carbon (C), hydrogen (H), oxygen (O) and nitrogen (N). Protein is the major structural component of muscle (meat). Nitrogen, as a fixed proportion of protein, is a major undesirable component of animal excreta in countries like the Netherlands, which have a large animal production industry. The CHO part of proteins can be used in the body as a metabolic fuel, but can also be deposited as body fat. Nitrogen is consequently lost via urine, and rapidly volatilized as ammonia. The conversion of carbohydrates and fats (containing no N) into protein is impossible, however. A consequence of this biological fact is that if digested dietary proteins are not used for conversion into meat, unwanted products, i.e. fat and ammonia will result.

Amino acids are the basic components of proteins. Over 20 different amino acids have been shown to exist, some of which the body is unable to synthesize. The presence of each of the amino acids in body protein is fixed. If the amino acid profile of the dietary protein does not match the profile of body proteins, the animal may be limited in the amount of body proteins that can be built from these amino acids. Consequently, the amino acids supplied in excess are converted into body fat and urine-N. Some proteins, however, are needed for other body functions and are therefore also unavailable for growth.

Energy is needed to maintain vital life processes and for the deposition of body protein and fat. While excessive amounts of fat are unwanted in the bodies of farm animals, a certain amount is needed. Carbohydrates and fats are ideal sources of substrate for body fat synthesis and as a metabolic fuel for the body, but proteins can and are often also used for this purpose as well.

It has long been recognized that animals respond to increased feed intake by increasing their growth rate. However, the law of diminishing returns applies to this as much as it does to many other biological relationships.

For these reasons, nutrition provides a means to manipulate the body composition of animals. Providing an animal with the correct balance of nutrients, as well as understanding the metabolic fate of those nutrients is of vital importance for animal production.

EVOLUTION OF ANIMAL GROWTH MODELS

A model, like a map, is a simplification of reality. Models generally constitute a set of mathematical equations which describe the behaviour of a system (e.g. the animal) under changed (e.g. nutritional) inputs. By integrating existing knowledge, they can contribute to an increased understanding of the response of animals to nutrient intake.

Several types of models have been developed to model agricultural systems. For a comprehensive description of static versus dynamic, empirical versus mechanistic, and deterministic versus stochastic models, the reader is referred to France and Thornley (1984) and Thornley and Johnson (1990).

The earliest models of animal growth were regression equations which related live weight to age (Brody, 1945). In the late fifties and early sixties, the first animal growth models were developed which related growth performance to nutrient intake. These were static models, calculating requirements for protein and energy of animals at specific body weights (e.g. ARC, 1965; Blaxter, 1962). Such models are still often used for diet formulations. Parks (1970) developed equations that related growth output to both time and food input. In an attempt to gain wider applicability of models and an enhanced understanding of growth, dynamic simulation models were developed which represented underlying mechanisms of growth. The first models simulating growth in time and integrating protein and energy metabolism were developed in the mid-seventies, e.g. for milk-fed lambs (Black, 1974) and pigs (Whittemore and Fawcett, 1976) These models were largely based on empirical, but conceptual equations. The concept proposed by Whittemore and Fawcett (1976) has had a major impact on practical pig growth modelling and will be discussed separately. In the early seventies, Baldwin and Smith (1971) started the development of simulation models based on biochemical reactions. A strictly biochemical approach was applied by Schulz (1978) for simple-stomached animals. Baldwin and Black (1979), developed a general model for tissue growth of mammals, based on the representation of hyperplasia and hypertrophy of various organs. These principles were later simplified and applied in a model of beef cattle growth (Oltjen et al., 1986). These models, however, employed a very simplistic representation of nutrient entry, which was improved in mechanistic models for growing lambs (Gill et al., 1984; Sainz and Wolff, 1990) and steers (France et al., 1987). In this thesis, attention is focused on the preruminant calf, for which no such models have been developed.

REGULATING NUTRIENT PARTITIONING

There are several theories concerning the regulation of nutrient partitioning which can be a basis for animal growth models. Two important ones are discussed briefly below. The concept proposed by Whittemore and Fawcett (1976), is an example of a rather empirical approach. In their model, this concept was represented by a number of mathematical equations, which are called "conceptual equations". The metabolic approach, discussed subsequently, is based on the assumptions that the distribution of nutrients between body compartments is primarily controlled by substrate concentrations, and that these biological processes often follow the principles of saturable enzyme kinetics (Baldwin, 1995).

The concept of Whittemore and Fawcett (1976)

The theory proposed by Whittemore and Fawcett (1976) has had a large impact on practical pig growth modelling. Nutrient partitioning in pig growth models as developed by several research groups was originally based on this concept (e.g. Moughan et al., 1987; Pomar et al., 1991, TMV, 1991). The objectives of most of these models were similar to the model described in this thesis: they aimed to improve the understanding of nutrient partitioning in pigs and to obtain a practical tool for the development of feeding strategies. According to this concept, protein deposition increases linearly with increasing protein intake, up to the point where energy intake limits protein deposition. Increased protein intake above this point is not retained. It implies a preference for the utilization of ideal protein for protein deposition, provided that the fat:protein ratio in the gain exceeds some minimum, fixed value. This fixed value, together with the intrinsic maximum in protein deposition capacity would then determine the growth potential of pigs of a certain genotype. This definition of a genotype and the simplicity of the concept of partitioning of dietary protein and energy are important advantages of this concept.

The "conceptual equations" proposed, however, are not easily modifiable to include biological processes such as protein and fat turnover. Furthermore, throughout the years, improvements have been suggested (e.g. De Greef and Verstegen, 1993; Bikker et al., 1995), stressing that the concept of Whittemore may be an oversimplification.

A metabolic approach

A more metabolic approach has been described by France and Thornley (1984) and Gill et al. (1989), and has been applied in several whole-animal models of metabolism (e.g. France et al., 1987; Pettigrew et al., 1992; Sainz and Wolff, 1990). In these models, the whole animal is represented by metabolite pools and body storage pools. The metabolite pools (usually small and relatively constant in size) serve to partition absorbed nutrients into body storage pools, which increase in size with time and represent the growth of the animal. Nutrient partitioning in these models is assumed to follow standard enzyme and chemical kinetics (see Gill et al., 1989). Priorities of a specific metabolite (substrate) for various transactions have to be set according to experimental data, and if not available, by rule of thumb.

Hormones and nutrient partitioning

Several hormones have key-roles in the partitioning of nutrients over body components. However, hormone function is not included in the approach of Whittemore and Fawcett. Also, it is not often included in the metabolic approach either, probably due to the complexity of endocrinological mechanisms. The main hormones involved are growth hormone, insulin-like growth factor-1, androgens, oestrogens, insulin and thyroid hormones (for a review see Spencer, 1985). Insulin-like growth factor-1 and thyroid hormones are of particular interest in the long-term regulation of nutrient partitioning. They have an important role in nutrient partitioning and because plasma concentrations of these hormones are relatively constant over the day, they do not require frequent blood sampling, which makes them suitable for measurement in long-term experiments.

THIS THESIS

The main objective of the work described in this thesis, is to gain an insight into the partitioning of nutrients in the body of preruminant calves and to develop a simulation model, which can be used as a tool for the development of feeding strategies for these calves. Attention is focused on male calves from the dairy herd, representing the majority of the calves raised for veal production in the Netherlands.

Simulation models need to be based on good hypotheses and reliable

experimental results. In this thesis, the metabolic approach was chosen, because it allows representation of biological processes in as much detail as needed for the appropriate level of aggregation. A prototype model was designed, and two experiments were conducted to gather necessary information (**Chapter 1**). The design of these experiments was based on the concept of Whittemore and Fawcett (1976) and protein and protein-free energy intakes were varied over a wide range. **Chapter 2** describes the development of a mechanistic model which simulates nutrient partitioning in preruminant calves. The model is subsequently tested (**Chapter 3**) and its application for the development of feeding strategies is illustrated in **Chapter 4**.

The experiments, described in Chapter 1, were also used to measure the long-term effects of nutrient intake on muscle protein turnover and the nutritional regulation of circulating thyroid hormones and insulin-like growth factor 1. Unfortunately, results of these measurements, originally planned to be included in the model, only became available after completion of the model. The effects of the experimental treatments on muscle protein turnover are described in **Chapter 5** and the effects on circulating hormone concentrations are presented in **Chapter 6.** In the general discussion, attention is paid to the metabolic fate of amino acids in preruminant calves. Furthermore, the modelling approach is extensively discussed. Finally, attention is paid to the representation of factors, other than nutrition (e.g. climatic, genetic), in metabolic models. Finally, the main conclusions are summarized and practical implications are discussed.

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1 EFFECT OF PROTEIN AND PROTEIN-FREE ENERGY INTAKE ON PROTEIN AND FAT DEPOSITION RATES IN PRERUMINANT CALVES OF 80 TO 240 KG LIVE WEIGHT

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Effect of protein and protein-free energy intake on protein and fat deposition rates in preruminant calves of 80 to 240 kg live weight

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Abstract

Two experiments were conducted to quantify the effects of protein intake on protein and fat deposition rates at two protein-free, energy intake levels in 90 preruminant Holstein Friesian x Dutch Friesian calves. The two experiments were similar in design, but were performed in two different weight ranges: 80 to 160 kg BW and 160 to 240 kg BW in Exp. 1 and 2, respectively. In each experiment, calves were allocated to either an initial slaughter group or to one of 12 treatments (three calves per treatment), which consisted of six protein intake levels at each of two protein-free energy intake levels. Calves were slaughtered and analysed for body composition when they had reached the target weight. A balance study was conducted when calves reached 120 and 200 kg BW in Exp. 1 and 2, respectively. Protein digestibility increased with increasing protein intake in both experiments ($P < .001$). Average daily gain of the empty body varied between 640 and 1340 g/d and between 420 and 1,370 g/d in Exp. 1 and 2, respectively, and was affected by protein ($P < .001$) and protein-free energy intake ($P < .001$). The calves responded to increased protein intake by increasing their protein ($P < .001$) and fat ($P < .01$) deposition rates. Maximum protein deposition was reached in the second experiment at 244 g/d. Extra protein-free energy intake resulted mainly in extra fat deposition ($P < .001$), but also increased the protein deposition ($P < .01$), even at low protein intake levels. In both experiments, the response of protein deposition rate to increased protein intakes was low: about 30% of the extra ingested protein was deposited. These results clearly demonstrate a low priority for partitioning dietary protein into protein gain in these calves.

INTRODUCTION

The emphasis in meat production has shifted from maximizing production volume to the efficient production of lean meat. Research has therefore focused on ways to improve the efficiency of conversion of nutrients, especially protein, into lean products. Attempts vary from the use of growth promoters, repartitioning agents, and leaner genotypes to the adjustment of feeding strategies. The latter approach has received much attention in pork production and requires an integrated knowledge of protein and energy metabolism. Simulation models have proved to be a helpful tool in this respect and have been developed for many types of growing animals, e.g., pigs (Moughan et al., 1995), lambs (Sainz and Wolff, 1990), and beef steers (France et al., 1987). Such models, however, need to be based on sound experimental results, particularly concerning the response of protein and fat

deposition to nutrient intake. This information is lacking for preruminant calves.

Several species have been shown to utilize dietary protein preferentially for protein deposition, unless protein quality and/or energy availability are limiting (Miller and Payne, 1963; Gahl et al., 1991). The main objective of the experiments described in this paper was to quantify the response of protein and fat deposition rates to protein and protein-free energy intake in preruminant calves from 80 to 240 kg BW.

We hypothesized that protein deposition increases with increasing protein intake, showing a preferential utilization of dietary protein for protein gain up to the point where energy intake limits protein deposition. Increased protein intake above this point would be retained less efficiently. An increased energy intake would result in an increased fat deposition rate but should, at low protein intakes, not affect protein deposition rate.

EXPERIMENTAL PROCEDURES

Two experiments of similar design were performed with two weight ranges: 80 to 160 kg BW (Exp. 1) and 160 to 240 kg BW (Exp. 2).

Animals, housing and experimental treatments

Ninety male, Holstein Friesian x Dutch Friesian calves were used in the two experiments. In Exp. 1 and 2, 8 and 10 calves were used as a reference group, respectively. These calves were slaughtered at the beginning of the experiment (80 and 160 kg BW in Exp. 1 and 2, respectively) and analysed for body composition, as described below.

In each experiment, 36 calves were grouped in three blocks, based on BW, and within block, calves were randomly assigned to one of the 12 dietary treatments. The treatments were designed to quantify the response of protein and fat deposition over a wide range of protein intakes, at two energy levels.

The dietary treatments consisted of six protein intake levels at each of two protein-free energy levels (**Table 1**). Treatments were chosen around a presumed optimum of 14.25 and 12.25 g digestible protein intake per megajoule protein-free energy intake in Exp. 1 and 2, respectively (G. H. Tolman, personal communication; about 217 and 185 g digestible protein/kg feed).

Table 1

Experimental design

Item	Treatment number											
	1	2	3	4	5	6	7	8	9	10	11	12
Experiment 1: 80-160 kg BW												
Protein-free energy intake, kJ/MW·d[a,b]	663	663	663	663	663	663	851	851	851	851	851	851
Protein intake, g/MW·d[b,c]	5.6	7.2	8.9	10.3	11.7	13.3	7.2	8.9	11.1	13.3	15.1	17.0
Ratio protein/energy[d]	8.5	10.9	13.4	15.6	17.7	20.0	8.5	10.4	13.0	15.6	17.7	20.0
Experiment 2: 160-240 kg BW												
Protein-free energy intake, kJ/MW·d[a,b]	564	564	564	564	564	564	752	752	752	752	752	752
Protein intake, g/MW·d[b,c]	3.4	4.5	6.3	7.8	9.0	10.4	4.5	6.3	8.3	10.4	12.0	13.9
Ratio protein/energy[d]	6.0	8.0	11.2	13.9	16.0	18.5	6.0	8.4	11.0	13.9	16.0	18.5

[a]Digestible protein-free energy intake; digestibility assumed 95%
[b]MW (metabolic weight) = live weight$^{.75}$ (kg)
[c]Digestible crude protein intake; digestibility assumed 95%
[d]Expressed in g digestible crude protein/MJ protein-free energy

Proteins, carbohydrates, and fat differ in their ATP yield per MJ (McDonald et al., 1988). Therefore, the energy intake levels were kept constant on a protein-free basis. Moreover, the ratio of energy intake from carbohydrates to energy intake from fat was kept constant at around 1 in both experiments.

For each experiment, two basal milk replacers that varied in protein and protein-free energy content were used. All experimental treatments could be realized by mixing these milk replacers in different ratios and by varying the quantity fed. The nutrient and ingredient composition of the basal milk replacers is shown in **Table 2**.

The calves were weighed weekly, and the feed intake was adjusted twice weekly according to the measured BW and projected rates of weight gain. Milk replacers were reconstituted with water and supplied at a temperature of about 39°C. Calves were fed individually twice daily, at 8.00 h and 16.00 h. Calves were housed individually in wooden, slat-floored crates of 1.75 x .75 m and 1.85 x .85 m in Exp. 1 and 2, respectively. Both experiments were approved by the ethics committee of the TNO Nutrition and Food Research Institute, Zeist, the Netherlands.

Balance trial

In both experiments, a balance trial was performed using all calves, to measure apparent faecal nutrient digestibility and metabolizability of the ingested energy. When calves weighed 120 and 200 kg BW in Exp. 1 and 2, respectively, plastic bags were harnessed to the calves to allow quantitative collection of faeces. Urine was collected in buckets containing 50 ml of 9 *N* H_2SO_4 to prevent volatilization of ammonia. After a 5-d adaptation period, faeces and urine were collected daily for 5 d, pooled per calf over the collection period, and stored at -20°C pending analysis.

Slaughter procedures and sample preparation

The calves were slaughtered in the week in which their weight was closest to the target weight: 160 and 240 kg BW in Exp. 1 and 2, respectively. Calves were killed by stunning and exsanguination.

Body components were split into three fractions: 1) carcass; 2) hide, head, feet and tail (**HHFT**); and 3) organs. The organ fraction included blood, the emptied gastro-intestinal tract and all other organs. For analysis of the carcass fraction, the composition of the right carcass half (split longitudinally) was considered representative. All fractions were weighed and stored in plastic bags at -20°C. Prior

to mincing, the frozen body components were weighed again to account for moisture losses. The frozen carcass, organ and HHFT fractions were cut into small blocks and minced in a commercial butcher's mincer (45L, two speeds, Rohwer). The three fractions were sampled separately and stored in sealed plastic bags at

Table 2

Composition of basal milk replacer diets, as fed[a]

	Exp. 1		Exp. 2			Exp. 1		Exp. 2	
Ingredient, g/kg	Diet 1	Diet 2	Diet 1	Diet 2	Nutrient[e], g/kg	Diet 1	Diet 2	Diet 1	Diet 2
Skim milk	405.6	352.6	302.9	342.1	Dry matter	972.9	952.2	980.4	972.6
Ca-caseinate	-	260.4	-	100.0	Ash	56.2	55.5	55.9	54.3
Na-caseinate	-	206.4	-	70.0	Crude protein	159.8	556.3	113.9	275.9
Lactose	266.7	17.3	346.0	209.6	Crude fat	224.9	100.9	244.6	194.5
Gelatinized starch	40.0	39.8	40.0	40.0	GE (MJ/kg)	20.9	21.5	21.4	21.6
Fat[b]	230.1	106.2	241.0	187.7	DEpf[f,g] (MJ/kg)	17.6	8.3	18.1	14.3
Emulsifier[c]	24.0	5.0	25.2	19.9	Lysine[g]	12.9	45.2	9.5	23.2
Isoleucine	.5	.4	.4	.4	Methionine[g]	4.5	16.2	3.1	8.0
L-lysine-HCl	.3	-	.4	.5	Cystine[g]	1.2	2.7	.9	1.6
L-threonine	.2	.2	.2	.2	Tryptophane[g]	2.2	7.7	1.6	3.9
Premix[d]	5	5	5	5	Threonine[g]	7.3	25.3	5.3	12.8
MgO	.7	.5	.9	.7	Isoleucine[g]	9.2	31.9	6.6	16.1
KH_2PO_4	2.7	-	9.8	5.7					
$KHCO_3$	-	3.8	-	1.0					
NaH_2PO_4	6.2	-	4.2	-					
$NaHCO_3$	5.1	-	7.8	5.9					
$CaHPO_4.2H_2O$	5	-	5	5					
$CaCO_3$	5.3	-	8.7	3.8					
$CaCl_2.2H_2O$	2.5	2.5	2.5	2.5					

[a]Basal diets were mixed at different ratios and fed in different quantities to realize the experimental treatments, presented in Table 1.

[b]At least 95% of fat was sprayed on skim milk powder, the rest was added as stabilized fat. Fat composition: Coconut oil, 1/3; tallow, 2/3.

[c]Lecithin; composition: crude protein, 100 g/kg; sugars, 340 g/kg; crude fat, 505 g/kg.

[d]Contributed per kg of diet: vitamin A, 15,000 IU; vitamin D_3, 3,000 IU; vitamin E, 30 mg; vitamin K3, 4 mg; vitamin B1, 6 mg; vitamin B2, 10 mg; vitamin B6, 4 mg; vitamin B12 .025 mg; niacinamide, 40 mg; d-panthothenic acid, 25 mg; choline chloride 400 mg; vitamin C, 80 mg; ZnO, 100 mg; KI, .4 mg; $Na_2SeO_3.5H_2O$, .15 mg; $CuSO_4.5H_2O$, 30 mg; $MnSO_4.H_2O$, 30 mg; $CoSO_4.7H_2O$, 10 mg; MgO, 1 g; carrier: lactose. All diets contained avoparcin, 40 mg/kg and $FeSO_4.7H_2O$, 50 mg/kg;

[e]Analysed content, unless otherwise indicated;

[f]Protein free digestible energy;

[g]Calculated content.

-20°C. Because of the heterogeneity of the HHFT fraction, large samples (about 1.5 kg) were obtained from the mincer, autoclaved for 10 h at 124°C with a known amount of water and minced using a laboratory disperser prior to analysis. This procedure did not affect the components analysed, according to our unpublished observations.

Chemical analyses

All analyses were performed in fresh material. Crude protein was analysed in the milk replacer, faeces, urine, carcass, organ, and HHFT samples according to ISO 5983 (ISO, 1979). Crude fat was determined after acid hydrolysis in the milk replacer and faeces and in freeze-dried carcass, organ, and HHFT samples by extraction with petroleum ether (boiling range 40 to 60°C). After extraction, samples were dried in a vacuum oven at 80°C to a constant weight, according to ISO 6492 (ISO, 1985). Crude ash was determined in the milk replacer, faeces, carcass, organ and HHFT samples. All samples except milk replacer were dried for 16 hours at 120°C before the crude ash determination. Carcass, organ and HHFT samples were carefully carbonised using a gas burner, and incinerated in a muffle furnace at 550°C according to ISO 5984 (ISO, 1978). For the dry matter content, organ and HHFT samples were freeze-dried, faeces were dried in a forced air oven, and milk replacer samples were dried in a vacuum oven at 80°C at a vacuum below 13 kPa. All samples were dried to a constant weight according to ISO 6496 (ISO, 1983). Carcass samples were dried in a vacuum oven at 50°C at 10 kPa, using anhydrous calcium chloride as a drying agent. After 16 h, the vacuum was increased to 3 kPa for 8 h, and samples were subsequently weighed every 2 h until they reached a constant weight. Energy content was analysed in the milk replacer and freeze-dried faeces and urine samples using anisothermic bomb calorimetry (IKA-C700, Janke & Kunkel GmbH & CoKG, Staufen, Germany).

Calculations

In each experiment, protein deposition was calculated for each animal as the sum of the amount of protein in the three fractions minus the amount of protein present at the start of the experiment. The latter was estimated using initial BW and the body composition of the reference group. Fat deposition was calculated similarly. Corresponding deposition rates were calculated as deposition divided by the length of the experimental period. Empty body weight (**EBW**) was calculated as the sum of the three fractions. Metabolizable energy intake and digestible protein

intake during the slaughter trial were estimated by multiplying the GE and crude protein intakes during the slaughter trial by the ME/GE ratio and faecal protein digestibility, respectively, measured in the balance trial. Methane production has been shown to be negligible in preruminant calves (Meulenbroeks et al., 1986). Energy retention was calculated from protein and fat deposition rates, assuming an energy content of 23.8 and 39.6 kJ/g for protein and fat, respectively.

Statistical analysis

Three and five animals were excluded from the statistical analysis of the balance trial from Exp. 1 and 2, respectively, because of diarrhea or other illness. Because of illness, one and three animals had to be excluded from the statistical analysis of the slaughter trial in Exp. 1 and 2, respectively (see legend of Figure 1 for the number of animals used in each treatment).

The effects of protein and protein-free energy intake on digestibility, protein and fat deposition rates, and EBW gain were analysed using the following model:

$$y = \mu + E_i + \beta_1(X_j - \bar{X}) + \beta_{2i}(X_j - \bar{X}) + \epsilon_{ij} \quad [1]$$

in which y = dependent variable, μ = average intercept, E_i = fixed effect of protein-free energy intake level i, β_1 = effect of protein intake, β_{2i} = interaction between protein intake and protein-free energy intake level i, X_j = protein or digestible protein intake of calf j, $\bar{X}$ = the average experimental protein or digestible protein intake, ϵ_{ij} = error, i = 1, 2, and j = 1 .. 18.

For the analysis of treatment effects on nutrient digestibility, X and $\bar{X}$ were based on the protein intake during the N-balance period. For the analysis of treatment effects on protein and fat deposition rate and EBW gain, X and $\bar{X}$ were based on the digestible protein intake during the slaughter trial.

According to our hypothesis, protein deposition should increase with increasing protein intake (protein dependent phase) until the protein-free energy intake limits protein deposition (energy dependent phase). Ingested protein above this point should be retained less efficiently. To detect protein- and energy-dependent phases in protein deposition, the effects of digestible protein intake on protein and fat deposition rate and EBW gain were analysed per protein-free energy intake level using a two-phase linear model [2], based on Koops and Grossman (1993):

$$y = a + b_1 X - (b_1 - b_2) \ln(1 + e^{(X - c)}) \quad [2]$$

in which: y = dependent variable, X = digestible protein intake, a = intercept, b_1 = slope of the first linear phase, b_2 = slope of the second linear phase and c = point of transition for the independent variable.

In both experiments, the total energy intake increased with protein intake (Table 3). Therefore, protein deposition was expected to reach a plateau only when a maximum in the protein deposition capacity had been reached. This was tested by using a linear-plateau model, obtained by deleting the parameter b_2 from the model [2]. The variances explained by the two-phase linear and the linear-plateau models were compared with the linear model [1] for the corresponding protein-free energy intake level, by use of an F-test.

The energy requirement for maintenance can be estimated by both linear regression of energy retention on ME intake and linear regression of ME intake on energy retention. Usually, the independent variable is used as the regressor. In the present experiments, however, both ME intake and energy retention were dependent variables. Therefore, the energy requirement for maintenance was estimated by both methods.

Data were analysed by linear (GLM, SAS, 1989) and derivative-free nonlinear (NONLIN package, Dennis et al., 1981) regression procedures.

RESULTS

Nutrient intakes and live weight gain

Treatment means of nutrient intakes during the slaughter experiments and live weight gain are shown in **Table 3.**

Digestibility measurements and metabolizability

The effects of protein and protein-free energy intake on the digestibility of the nutrients are shown in **Table 4.** In both experiments, digestibility of dry matter, crude fat, and energy were ≥94%. Protein-free energy intake did not affect the digestibility of dry matter, crude fat, ash, or energy. The effects of protein intake were similar for both protein-free energy intake levels. Protein digestibility increased with increasing protein intake from 92 to 96% and from 86 to 95% for Exp. 1 and 2. This increase, however, diminished with increasing protein intake.

Table 3

Realized nutrient intake and live weight (LW) gain, averaged over the experimental period

	Treatment number											
Item	1	2	3	4	5	6	7	8	9	10	11	12
Experiment 1: 80 - 160 kg BW												
Total energy, MJ/d	28.6	30.4	31.7	33.2	34.2	35.7	37.0	38.4	40.2	42.4	44.4	44.7
Crude protein, g/d	215	279	337	395	449	512	281	345	428	513	587	650
Crude fat, g/d	305	308	306	307	305	304	396	396	392	394	395	383
Dry matter, g/d	1318	1399	1459	1526	1571	1633	1717	1787	1861	1964	2046	2056
LW gain, g/d	774	843	965	1062	1081	1174	1044	1179	1220	1401	1460	1482
Experiment 2: 160 - 240 kg BW												
Total energy, MJ/d	34.8	36.4	38.2	40.8	41.8	44.6	46.3	49.1	51.6	54.3	56.5	59.9
Crude protein, g/d	185	250	342	428	487	572	247	346	453	578	664	773
Crude fat, g/d	398	419	404	405	396	403	530	550	547	539	535	544
Dry matter, g/d	1599	1665	1760	1858	1905	2015	2125	2257	2356	2485	2586	2718
LW gain, g/d	528	680	827	929	1021	1131	827	1158	1343	1436	1522	1568

[a]For explanation of treatment numbers, see Table 1; For number of animals per treatment, see legend of Figure 1.

Addition of a quadratic component following deletion of the nonsignificant interaction term in model [1] led to a better description of this relationship in Exp. 1 ($P = .07$) and in Exp. 2 ($P < .01$). In Exp. 2, protein digestibility at the high protein-free energy intake level was lower, compared with the low protein-free energy intake level.

Table 4

Effect of protein and protein-free energy intake on the apparent faecal digestibility of nutrients and on the metabolizability of the digestible energy

Item	Effect protein-free energy intake[a]				Effect protein intake	Interaction ($.10^3$)[b]			
	LE %	P LE=HE[c,d]	HE %	sem[e]	P[f]	LE	P LE=HE[d,g]	HE	sem[e]
Experiment 1: 120 kg BW									
Crude fat	95.6	ns	93.8	.68	ns	7.5	ns	-1.2	5.15
Dry matter	96.5	ns	96.1	.27	ns	3.4	ns	-.1	2.01
Ash	86.2	ns	85.3	.92	ns	7.5	ns	-3.3	6.97
Crude protein	95.0	ns	94.3	.37	***	12.8	ns	7.0	2.83
Energy	96.6	ns	95.8	.35	ns	5.0	ns	.7	2.68
ME/DE[h]	96.1	***	96.9	.08	***	-3.0	*	-4.8	.60
Experiment 2: 200 kg BW									
Crude fat	94.8	ns	94.9	.93	ns	12.2	ns	4.2	5.51
Dry matter	95.6	ns	95.6	.35	*	4.6	ns	2.2	2.06
Ash	81.0	ns	82.5	.84	ns	2.0	ns	.2	4.94
Crude protein	93.8	**	91.7	.51	***	17.6	ns	14.4	3.02
Energy	96.0	ns	95.7	.50	*	7.8	ns	3.8	2.96
ME/DE[h]	95.3	ns	95.3	.14	*	-3.0	*	.3	.84

[a]LE and HE = low and high protein-free energy intake level, respectively; Values represent digestibility or ME/DE ratio (both in %) at the average crude protein intake (433 and 461 g/d in Exp. 1 and 2, respectively);

[b]Difference in regression coefficients, associated with protein intake, between LE and HE; Values are multiplied by 10^3

[c]Probability for test on effect of protein-free energy intake level;

[d]ns = $P > .05$; * = $P < .05$; ** = $P < .01$; *** = $P < .001$;

[e]Standard error of the mean;

[f]Probability for test if the regression coefficient, averaged over protein-free energy intake levels = 0;

[g]Probability for test if regression coefficients, associated with protein intake, are equal for LE and HE;

[h]Metabolizability of the digestible energy.

The metabolizability of the digestible energy (ME/DE ratio, Table 4) decreased with increasing protein intake. In Exp. 1, ME/DE was higher at the high protein-free energy intake level. In Exp. 2, this was not the case. The interaction between protein and protein-free energy intake was significant in both experiments. When urine samples were screened for glucose (semi-quantitatively; Glukotest®, Boehringer Mannheim, Almere, The Netherlands), calves receiving the low protein treatments (in Exp. 2 only at the high protein-free energy intake level), seemed to excrete considerable amounts of glucose with urine. This occurred in the calves receiving treatment 1, 2 and 7 in Exp. 1 (5 to 10 g/d) and calves of treatments 7,

Table 5

Effect of apparent faecal protein- and protein-free energy intake on live weight gain, empty body weight gain and protein- and fat deposition rates

	Effect protein-free energy intake[a]				Effect protein intake	Interaction[b]			
Item	LE g/d	P LE=HE[c,d]	HE g/d	sem[e]	P[f]	LE	P LE=HE[d,g]	HE	sem[e]
Experiment 1: 80-160 kg BW									
Live weight gain	1057	***	1241	15.4	***	1.38	ns	1.26	.127
EBW gain[h]	925	***	1108	13.1	***	1.38	ns	1.12	.108
Protein deposition	180	**	194	3.1	***	.28	ns	.25	.025
Fat deposition	129	***	218	5.3	**	.07	ns	.11	.044
Experiment 2: 160-240 kg BW									
Live weight gain	953	***	1239	26.7	***	1.56	ns	1.47	.173
EBW gain[h]	813	***	1078	23.5	***	1.40	ns	1.35	.153
Protein deposition	165	***	199	5.9	***	.29	ns	.25	.038
Fat deposition	127	***	268	11.3	***	.18	ns	.25	.074

[a]LE and HE = low and high protein-free energy intake level, respectively; Values represent gain of empty body, protein or fat (in g/d) at the average digestible crude protein intake (398 and 418 g/d in Exp. 1 and 2, respectively);

[b]Difference in regression coefficients, associated with protein intake, between LE and HE;

[c]Probability for test on effect of protein-free energy intake;

[d]ns = P>.05; * = P < .05; ** = P < .01; *** = P < .001;

[e]Standard error of the mean;

[f]Probability for test if the regression coefficient, averaged over protein-free energy intake levels = 0;

[g]Probability for test if regression coefficients, associated with protein intake, are equal for LE and HE;

[h]Empty body weight gain.

8 and 9 (70, 40 and 30 g/d, respectively) in Exp. 2.

Body weight gain and protein and fat deposition rates

The effects of protein and protein-free energy intake on live weight gain, EBW gain, and protein and fat deposition rates are shown in **Table 5**. All effects of protein intake were similar for the protein-free energy intake levels.

Protein-free energy intake markedly affected live weight gain, EBW gain and protein and fat deposition rates in both experiments. Empty body weight gains for calves fed the high protein-free energy level were, on average, 184 and 265 g/d higher than for calves fed the low protein-free energy level for Exp. 1 and 2. In both experiments, about 50% of this difference in EBW gain was accounted for as fat. Protein accounted for 7.6 and 12.8% of the difference in EBW gain in Exp. 1 and 2.

Empty body weight gain and protein and fat deposition rates increased with increasing digestible protein intake (Table 5). The existence of separate protein- and energy-dependent phases in this relationship could not be shown by the two-phase linear model. This model ([2]) did not provide a better fit of the experimental data than model [1].

At the high protein-free energy intake level in Exp. 2, EBW gain and protein deposition rate reached a maximum of 1,353 and 244 g/d, respectively. These maxima were reached at digestible protein intakes of 560 and 500 g/d, respectively. In these cases, the linear-plateau model provided a better fit of experimental data

Table 6

Parameter estimates for linear-plateau model[a], fitted for empty body weight gain and protein deposition rate at the high protein-free energy intake level of calves from 160 to 240 kg live weight

	a[b]	se[c]	b_1[d]	se[c]	c[e]	se[c]	P-value[f]
Liveweight gain	280	134.6	2.62	.411	463	30.3	.015
EBW gain[g]	403	84.5	1.69	.215	562	39.4	.058
Protein deposition	45	28.3	.40	.087	498	47.4	.012

[a]For description of linear-plateau model, see text; Value of plateau = a + b_1c (in g/d);
[b]Intercept at zero digestible crude protein intake;
[c]Standard error;
[d]Slope of linear phase;
[e]Point of transition from linear phase to plateau for the independent variable;
[f]Probability for test if linear-plateau model equals linear model, presented in Table 5;
[g]EBW = empty body weight.

(**Table 6**). The experimental observations and the results of the regression analysis are plotted in **Figure 1**. The increase in protein deposition rate per gram increase in digestible protein intake (marginal efficiency of protein utilization: slope of the lines in Figure 1), did not differ between protein-free energy intake levels and was between .25 and .3 in both experiments. The linear-plateau model, used for the high protein-free energy level in the second experiment, increased the estimation of this marginal efficiency as compared with the linear model: from .25 (over the whole range, linear model, Table 5) to .40 (linear phase, linear-plateau model, Table 6).

Maintenance energy requirements

For Exp. 1, the relationships between ME intake (in $MJ \cdot kg^{-.75} \cdot d^{-1}$) and energy retention (**ER**, in $MJ \cdot kg^{-.75} \cdot d^{-1}$) and between ER and ME intake are presented in the following equations:

$$ER = -.227\ (\pm .0311) + .58\ (\pm .033) \times ME\ intake \quad R^2 = .90 \qquad [3]$$

$$ME\ intake = -.447\ (\pm .0285) + 1.56\ (\pm .090) \times ER \quad R^2 = .90 \qquad [4]$$

The maintenance energy requirement and the efficiency of utilization of ME for growth, derived from these equations are .394 $MJ \cdot kg^{-.75} \cdot d^{-1}$ and .58 ([3]), and .447 $MJ \cdot kg^{-.75} \cdot d^{-1}$ and .64 ([4]), respectively.

Similarly, for Exp. 2, the relationships are presented in the following equations:

$$ER = -.302\ (\pm .0317) + .67\ (\pm .040) \times ME\ intake \quad R^2 = .90 \qquad [5]$$

$$ME\ intake = -.485\ (\pm .0191) + 1.35\ (\pm .081) \times ER \quad R^2 = .90 \qquad [6]$$

The maintenance energy requirement and the efficiency of utilization of ME for growth, derived from these equations are .452 $MJ \cdot kg^{-.75} \cdot d^{-1}$ and .67 ([5]) and .485 $MJ \cdot kg^{-.75} \cdot d^{-1}$ and .74 ([6]), respectively.

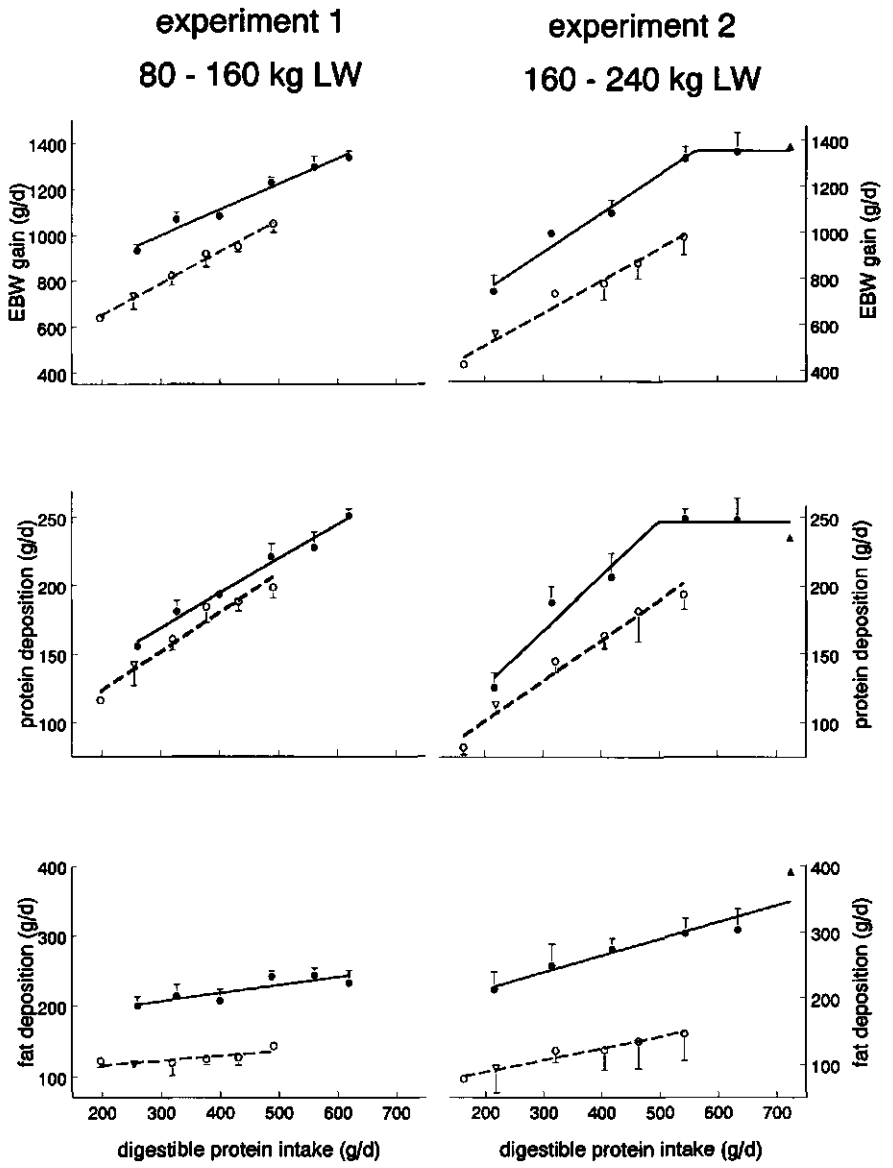

Figure 1. Response of gain of the empty body and protein and fat deposition rates to digestible protein intake at two protein-free energy intake levels in two weight ranges. High energy intake level (—, ●, ▲) and low energy intake level (- - -, ○, ▽). Values are means ± SEM, n = 3 (●, ○), n = 2 (▽), or n = 1 (▲). Regression coefficients are presented in Table 5 and 6.

DISCUSSION

Nutrient digestibility

The high apparent digestibility values for dry matter, crude fat and energy are in agreement with values reported by Ternouth et al. (1985), Tolman and Demeersman (1991), and Toullec (1989). Apparent ash digestibility was lower, compared with other nutrients, which is also in agreement with the work of Ternouth et al. (1985) and Tolman et al. (1993). Generally, the effects of treatment on apparent nutrient digestibility were more pronounced in Exp. 2, because of the slightly wider range in protein intakes and the larger contrast between protein-free energy intake levels.

The only important treatment effect observed was the effect of protein intake on apparent protein digestibility. Because the true digestibility of milk proteins is close to 100% (ARC, 1980; Tolman and Beelen, 1996), the increase in apparent protein digestibility with increasing protein intake may have been a result of the endogenous faecal excretion remaining constant and therefore decreasing as a proportion of the ingested protein. Similar effects were reported for veal calves of 60 kg BW by Donnelly and Hutton (1976), for rats by Donkoh and Moughan (1994), and for beef steers by Tritschler et al. (1984). The amount of endogenous secreted protein in animals fed milk-based diets, is affected by dry matter intake (ARC, 1980). This was most likely the cause of the higher protein digestibility at the low protein-free energy intake level. This effect was significant in Exp. 2 ($P < .05$) but not in Exp. 1. On average, the dry matter intakes during the N-balance at the high protein-free energy intake level were .4 and .6 kg/d higher than at the low protein-free energy intake level in Exp. 1 and 2. The increase in energy and dry matter digestibility with increasing protein intake in the second experiment was completely accounted for by the increase in the protein digestibility.

Metabolizability of the digestible energy

The metabolizability of the digestible energy at average protein intakes varied between 95 and 97% (Table 4). The decrease in the metabolizability with increasing protein intake, as well as the effect of protein-free energy intake in Exp. 1 corresponds with the treatment effects on the efficiency of utilization of digested protein, described later in this article. The glucose losses with urine, observed with the high energy, low protein treatments in Exp. 2, decreased the metabolizability in

these treatments, causing a significant interaction between protein and protein-free energy intake on the ME/DE ratio in Exp. 2. Substantial glucose losses with urine, affected by age and lactose intake, have already been reported in milk-fed calves and were attributed to lactose-induced insulin resistance (Palmquist et al, 1992; Hostettler-Allen et al., 1994). In addition, the results of present experiments suggest that the protein to energy ratio in the diet plays a role in the urinary glucose losses.

Maintenance energy requirements and efficiency of ME for growth

Estimates of ME requirements for maintenance and efficiency of utilization of ME for growth vary around the values, published by the ARC (1980) for preruminants: 428 kJ/kg $BW^{\cdot 75}$·d and .7, respectively. The lower estimates of the utilization of ME for growth in Exp. 1 as compared with Exp. 2 correspond with the slightly lower estimates for maintenance requirements in Exp. 1 as compared with Exp. 2. These slightly lower maintenance energy requirements for lighter calves may be surprising from the point of view that maintenance requirements decrease with increasing body weight (Blaxter, 1989). However, as stated by Blaxter (1989), the relationship between energy retention and ME intake is slightly curvilinear. The range of energy intakes covered in Exp. 2 was closer to the maintenance requirements than in Exp. 1 (see Table 3), which may explain the difference.

Nitrogen deposition measured by balance versus slaughter trial

It is known that, compared with slaughter experiments, balance trials overestimate, sometimes considerably, N deposition (e.g., Just et al., 1982; MacRae et al., 1993). Nitrogen deposition, measured by the balance technique (not presented) overestimated the N deposition measured in the slaughter trials by 17 and 10% in Exp. 1 and 2, respectively. This difference is considerably smaller than the 24% reported by MacRae et al. (1993). This may partly be due to the higher levels of N deposition of calves in the present experiments, compared with the lambs used by MacRae et al. (1993). Also, the greater accuracy of feed intake measurements in experiments with milk-fed animals, as compared with experiments with animals fed roughage probably plays a role.

Utilization of digested protein

In practice, the efficiency of utilization of digested milk proteins (deposition as a percentage of digestible intake) is usually around 65 to 70% at 50 to 70 kg

BW (Ternouth et al., 1985; van Weerden and Huisman, 1985) and decreases with increasing BW (or age) to about 50% at 100 to 120 kg BW (Ternouth et al., 1985; control groups of Williams et al., 1987) and to about 40% at 180 to 220 kg BW (Meulenbroeks et al., 1986). This efficiency depends on, amongst other factors, growth rate and feed intake. In both present experiments, the efficiency of utilization of digested protein (not presented) decreased from 60% at low protein intake levels to 35% at high protein intake levels and was, like protein deposition rate, mildly affected by the protein-free energy intake. When compared at similar growth rates, these efficiencies were in agreement with the work of Ternouth et al. (1985), the control groups of Williams et al. (1987), and the work of Meulenbroeks et al. (1986).

No protein and energy dependent phases in protein deposition

The existence of protein- and energy-dependent phases in protein deposition is a concept that is frequently applied in monogastric animals. Although over the years, modifications have been made, it is still the basis of many swine growth simulation models (Whittemore and Fawcett, 1976; Pomar et al., 1991; Moughan et al., 1995). This concept also formed the basis for our experiments. It implies a preference for the utilization of ideal protein for protein deposition, provided that the fat:protein ratio in the gain exceeds some minimal, fixed value. This fixed value, together with the intrinsic maximum in protein deposition capacity, would then determine the growth potential of a certain genotype.

The experimental results (summarized in Figure 1) however, do not support this theory. Firstly, no protein- and energy-dependent phases could be detected, using the two-phase linear model. Secondly, even at low protein intakes, protein deposition rate was indeed affected by protein-free energy intake. This effect was also reported by Donnelly and Hutton (1976) for preruminant Holstein calves of 40 to 70 kg BW. Third, the increase in fat deposition rate with increasing protein intake, observed in both experiments, does not support the theory that nutrients are used preferentially for protein deposition in the protein-dependent phase. A similar effect was reported by Donnelly and Hutton (1976), who found an increase in fat deposition rate with increasing protein intake at low protein intakes. However, as protein intake increased further, fat deposition rates dropped.

In Exp. 2, at the high protein-free energy level, protein deposition did not respond to increased digestible protein intake above approximately 500 g/d, indicating that a maximum protein deposition capacity had been reached. In Exp. 1,

this was not the case. The highest protein deposition rate obtained in Exp. 1 was equal to the maximum reached in the second experiment. The large standard error (47 g/d, Table 6) of the estimated "optimum" digestible protein intake in Exp. 2 (500 g/d) indicates that this optimum has to be interpreted carefully (see also discussion of Fuller and Garthwaite, 1993).

The marginal efficiency of protein utilization

Protein deposition increased with increasing digestible protein intake in both experiments. However, when expressed per gram of extra digestible protein intake (slope of the line or marginal efficiency of utilization of digestible protein), less than 30% was deposited in both experiments. This marginal efficiency is sensitive to the method of estimation: using the linear-plateau model for the high protein-free energy intake level in Exp. 2 increased the slope of the linear phase to about 40% (Table 6). However, even a marginal efficiency of 40% is low compared with results of experiments with younger calves. Donnelly and Hutton (1976) found a marginal efficiency of 45% in preruminant calves weighing 40 to 70 kg. Furthermore, this marginal efficiency is much lower than in other species. Batterham et al. (1990) and Bikker et al. (1993) found marginal efficiencies of 75 and 58%, respectively, in experiments with growing pigs weighing 20 to 45 kg. In their experiments, lysine was made the limiting amino acid. Therefore, the marginal efficiency of utilization of ideal protein would probably have been considerably higher. From data of Hegsted and Neff (1970) with growing rats, a marginal efficiency of protein (casein) utilization of 58% can be calculated. This would probably be somewhat lower when expressed as a percentage of digestible, rather than total protein intake.

It is obvious that these calves used a large part of the extra ingested protein for purposes other than for protein deposition. The reason for this, however, is not clear. Several possibilities to explain this include the following:

Imbalanced dietary amino acids. The marginal efficiency of protein utilization is affected by the amino acid pattern of the feed offered. Large deviations from ideal protein will therefore result in a low marginal efficiency (Black and de Lange, 1995). In the experiments reported here, however, the amino acid composition of the protein offered was matched with the amino acid pattern of casein. For lysine, methionine, cystine, tryptophan, threonine and isoleucine, the content (in grams per 100 g of protein) was in agreement with the recommendations of van Weerden and

Huisman (1985) for young veal calves. Therefore, it is not likely that this is the cause of the low marginal efficiency.

Genetic selection. It is possible that selection for milk production over many generations of the Holstein Friesian x Dutch Friesian calves used in these experiments, plays a role in the low marginal efficiency. Unfortunately, few data have been reported with which to test this hypothesis. Large differences in marginal efficiency should, however, also be reflected in differences in the (gross) efficiency of utilization of dietary protein at low protein intakes. An experiment with Large White vs Chinese Meishan pigs fed similar limiting amounts of protein showed that the efficiency of utilization of digestible protein has not been clearly improved by selection (Kyriazakis and Emmans, 1995). Vermorel et al. (1976) plotted protein gain against ME intake for preruminant double-muscled Charolais and for Friesian calves of about 100 kg BW. Their results, based on only four double-muscled Charolais calves, suggest a higher efficiency of utilization of extra ME for protein deposition for the double-muscled Charolais calves. Several experiments have been performed using rats or mice, selecting for many generations on lean growth, or even on lean efficiency (e.g., Wang et al., 1980). When tested with an adequate protein supply, the efficiency of protein utilization was increased in the selected rats (Wang et al., 1980). This does not, however, mean that the marginal efficiency was increased. If the theory of protein- and energy-dependent phases applies to rats, it could also mean that selection has reduced the minimal ratio fat:protein in the gain, thereby increasing the efficiency of protein utilization in the energy-dependent phase.

Need for gluconeogenesis from amino acids in ruminants. In general, functional ruminants absorb very little glucose. Under most conditions, however, ruminants have an obligate need to synthesize glucose. Although propionate is the predominant precursor, other sources such as glycerol, lactate, and also amino acids are important. Lobley (1992) suggested that a gluconeogenesis from amino acids is quantitatively important, even at higher feed intake levels. It can be speculated that gluconeogenesis from amino acids continues, even when glucose supply from the diet is abundant, as an inevitable consequence of the operation of mechanisms controlling the degradation of amino acids in (potential) ruminants.

In conclusion, in the present study, no protein- and energy-dependent phases

were detected in the relationship between protein intake and protein deposition rate. Protein and fat deposition rates increased with increasing protein intake, but the marginal efficiency of utilization of digestible protein was low: about 30% of the extra ingested protein was deposited. Maximum protein deposition capacity was reached in the second experiment at 244 g/d. Increased protein-free energy intake resulted in increased fat deposition rates, but also increased the protein deposition rate, even at low protein intake levels.

IMPLICATIONS

Preruminant calves weighing 80 to 240 kg respond to increased protein intake by increasing their protein and fat deposition rates. Extra protein-free energy intake will mainly result in extra fat deposition but will also increase the protein deposition rate. In general, ingested protein can be utilized more efficiently by decreasing the amount offered, but this has to be balanced against the reduced growth rate. The equations presented in this paper may be used to predict the response of protein and fat deposition to nutrient intake. These results were obtained as an average over two weight ranges and therefore do not necessarily reflect accurately the response of preruminant calves to nutrients for any given body weight.

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2 DESCRIPTION OF A MODEL INTEGRATING PROTEIN AND ENERGY METABOLISM IN PRERUMINANT CALVES

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Description of a model integrating protein and energy metabolism in preruminant calves

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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 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 the model is sound. It can be used as a research tool and for the development of feeding strategies for preruminant calves.

INTRODUCTION

During the last decade, interest of meat producers has shifted from obtaining maximum growth of the animal to production of lean meat and to increase 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 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., 1996c) so that the same principles cannot automatically be 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).

This paper describes a dynamic, mechanistic model which 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 x Dutch-

Friesian calves (Gerrits et al., 1996c). 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 of energy, in ruminating calves.

EXPERIMENTAL DATA

In order to obtain data for the development of this model, two experiments were conducted with Holstein-Friesian x Dutch-Friesian male calves. These experiments started at 80 kg Lw so as to avoid large variation due to environmental factors dominating energy metabolism of very young calves (Schrama, 1993). They were similar in design, and carried out in two weight ranges: 80-160 kg and 160-240 kg Lw, respectively (Gerrits et al., 1996c). Briefly, in each experiment, 36 calves were, in a 6 x 2 factorial arrangement, assigned to one of 6 protein intake levels at one of 2 protein-free energy intake levels. Milk proteins were used as the only feed protein source. Calves were slaughtered at the beginning and at the end of each experiment and analysed on nitrogen, fat, dry matter and ash content. Furthermore, at 120 and 200 kg Lw, total collections of faeces and urine were obtained from all animals for 5 days. Faeces were analysed 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. Average daily gain of the empty body varied, depending on dietary treatment between 640 to 1340 g/d and between 420 to 1370 g/d for the growth ranges of 80-160 and 160-240 kg Lw, respectively.

MODEL DESCRIPTION

General

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 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).

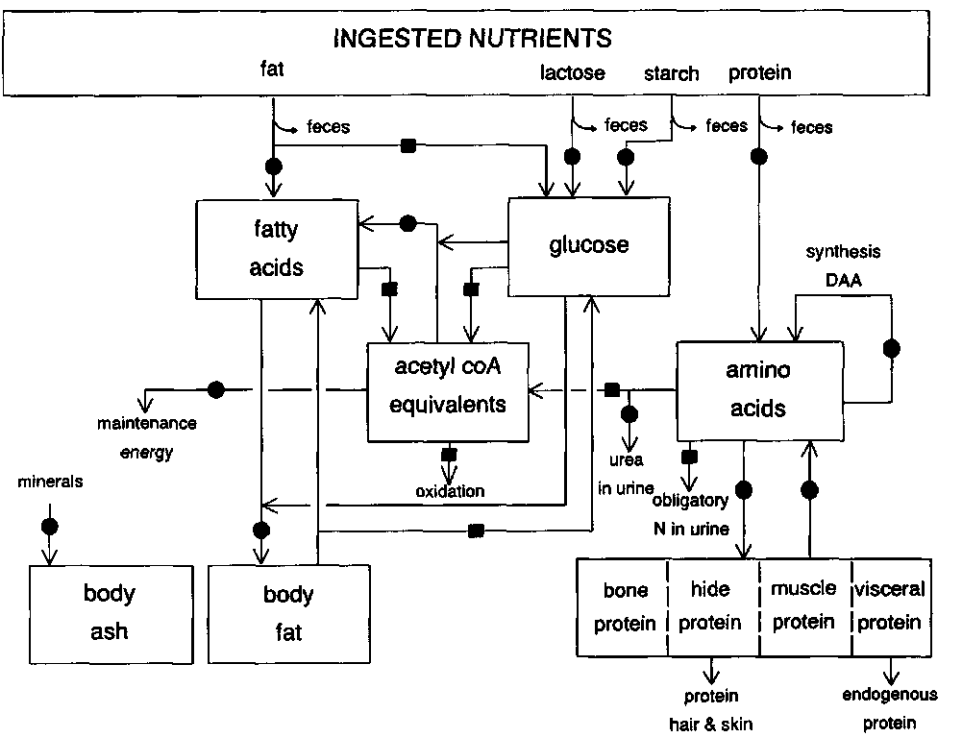

Figure 1. Diagrammatic representation of the model simulating metabolism of growing preruminant calves. Boxes enclosed in solid lines indicate state variables, arrows indicate nutrient fluxes. The composite box represents four body protein pools, each with a flux to and from the amino acid metabolite pool; Urea in urine represents excretion of N after amino acid oxidation; DAA = dispensable amino acids; Fluxes requiring energy are indicated by ●, those producing energy are indicated by ■.

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 as 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 faecal digestibility has been shown to be close to 99%, whereas 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, pre-treatment and level of inclusion in the diet (Toullec, 1989; Van der Honing et al., 1974). For

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 los (skin & 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/d
Ur	Urea	mol
Vi	Visceral mass	kg

simulations in this paper, starting at 80 kg Lw, starch is assumed pre-gelatinized, included at a level < 5% in the diet and its digestibility is assumed 95%. Faecal digestibility of dietary fat varies, depending on among others dietary fat source, between 94 and 97% (Hof, 1980; Tolman and Demeersman, 1991), and does not significantly differ from values obtained at the terminal ileum (Hof, 1980). A value of 95% is adopted. The true digestibility of milk proteins has been shown 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. (1996c). In the model, the true digestibility of milk proteins is set to 100%, and, as described later, net endogenous protein losses are modelled 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

Table 2

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

Notation	Translation	Units
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

(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 mol 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

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 Table 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 Appendix 2.

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 re-absorption 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. Transport costs are included in the stoichiometry of all reactions involving the use of glucose or amino acids as substrate. Other transport costs, like 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), which 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 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. In order 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 good fit of data and sensible behaviour 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 analysed 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 (eq.) 11.1, see **Appendix 1** 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 (eq. 11.2).

Initial pool concentrations, reaction sites and maximum reaction velocities. Metabolite pool concentrations are expressed per kg 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 literature to set initial metabolite pool sizes. The maximum velocity of a reaction (v_{max}) depends primarily on the

Table 4

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

Transaction	$R_{i,jk}$	$Y_{k,jk}$	$Y_{At,jk}$	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,AaUn			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.

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, due to its vascular characteristics (low blood flow, compared with other tissues, Vernon and Clegg, 1985), decrease with increasing body fat pool size. Therefore, for transactions that take place only in adipose tissue (FaFb, AyFa), the size of the reaction site is considered to be $Q_{Fb}^{0.67}$ (eq. 8.9 and 6.8, respectively).

Protein metabolism

General. For reason of simplicity, all amino acids, available for synthesis or oxidation, are combined in 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 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 (**Figure 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 (Figure 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 one 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 a 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 ad libitum 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., 1996c). 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. The v_{max} of muscle protein synthesis is expressed as a function of muscle protein mass (eq. 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 (FSRs) of these organs vary widely, depending on species, age and the method used. From values reported by Early

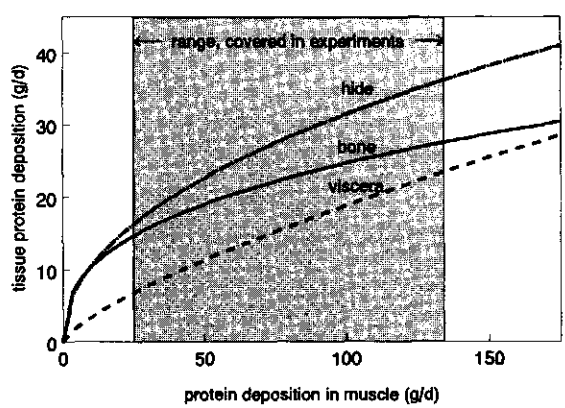

Figure 2. Protein accretion rate in bone, hide and viscera as a function of muscle protein accretion rate in preruminant calves between 80 and 240 kg live weight. Relationships estimated from experiments of Gerrits et al. (1996c).

et al. (1990) for non somatotropin treated steers of about 370 kg Lw, Lobley et al. (1980) for 2 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 modelled as a drain on the visceral protein pool (Figure 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 (eq. 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., 1996c; G.H. Tolman, unpublished). The value obtained is slightly higher than the value adopted by ARC (1980): 2.46 ($R^2 = 0.59$) vs 1.90 g N/kg dry matter intake per day. The relationship between visceral protein and muscle protein accretion was estimated from the experiments (Figure 2). Protein synthesis (in g/d) is calculated as the sum of net accretion, degradation and endogenous losses (eq. 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 (eq. 3.1). The relationship between hide protein accretion and muscle protein accretion was estimated from the experiments (Figure 2).

Bone protein pool, Pb. Bone protein has been shown to have a high protein turnover rate, compared with muscle. Calculated from 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, 3 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 (eq. 2.1). Bone protein accretion rate is related to muscle protein accretion rate (Figure 2).

Amino acid composition of the body protein pools. In order 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, analysed by Williams (1978). This estimation involved 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 above the amino acid profile of veal, which does not include much collagen protein. The amino acid profile of muscle protein therefore contains less 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., 1996c).

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 arbitrarily 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 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 due to neglection of the amides

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]
Indispensable amino acids	*g aminoacyl residue/kg aminoacyl residue*					
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 & 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.

glutamine and asparagine (Rafecas et al. 1994). The body protein pools and the amino acid pool are based on crude protein, as defined above. Only for the calculation of amino acid imbalance, the true protein factors are used.

Amino acid pool, Aa. Inputs to this pool are from absorption (eq. 1.2) and from degradation of body protein (eq. 1.4-1.7). Outputs are to body protein synthesis (eq. 1.9-1.12), to oxidation (eq. 1.8) and as endogenous urinary N-losses (eq. 1.13). Stoichiometry of these transactions is based on equilibrium of nitrogen and expressed per mol 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 modelled as input and output of the amino acid pool (eq. 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 difference between ingested and deposited DAA. Synthesis of DAA requires both a carbon and a nitrogen source. While Kreb's cycle intermediates like 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 non limiting amino acids. To correct for the difference in carbon source (Kreb's cycle intermediates versus 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 per 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 N-loss (EUN) is modelled 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 on 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 g N 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 (eq. 1.8; for references, see e.g. Liu et al., 1995, p.837). Amino acid oxidation is assumed to occur in the liver. The maximum velocity ($v_{Aa,AaAy}$), therefore, is expressed as function of liver weight. Liver weight (in kg) was estimated from the experiments as a function of visceral protein mass (Q_{Pv}; eq. 11.3). The 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). In order 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 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 results, in the case of a limiting amino acid, 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, needed 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 re-utilization of degraded protein for protein synthesis is

always less than 100%. Furthermore, potential re-utilization decreases with protein intake and depends on the amino acid considered (Simon, 1989; Moughan, 1994). 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 arbitrarily 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 leads to increased oxidative losses. In order to place this assumption in a 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 faecal 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., 1996b).

The calculation routine. The calculation routine, with numbered equations, is presented in **Appendix 2** 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 [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) [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 [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 (eq. 1.8, Appendix 1). Similarly, synthesis of DAA is calculated by difference between demand and supply [8-9]. As described earlier, this flux is modelled as both input into and output from the amino acid pool. Therefore, energy consumption is the only effect of this flux (eq. 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 [10-12].

Cystine, tyrosine and arginine. By considering the sum of methionine and cystine rather than methionine and cystine separately, and the sum of 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

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).

shown insufficient for maximum growth in growing pigs (see review of Fuller, 1994) and growing ruminants (Davenport et al., 1990). Therefore, it is assumed that a minimum of 40% of the arginine deposited needs 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 (eq. 9.1, 7.7). Outputs are to the fatty acid and glucose pool (eq. 9.2, 7.5). The body fat pool represents chemically determined fat, assumed to comprise only triacylglycerol. 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 rate of well fed heifers in vitro were shown to be 6 and 60 gram fatty acid/kg adipose tissue per day, respectively (Smith et al., 1992). Assuming 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 (eq. 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 underestimate 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 net lipid deposition rate with the rates measured in the experiments.

Fatty acid pool, Fa. Inputs to the fatty acid pool are from absorption (eq. 8.2), synthesis from acetyl-CoA (eq. 8.3) and from lipolysis (eq. 8.4). Outputs are to oxidation to acetyl-CoA (eq. 8.5) and to fat synthesis (eq. 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, arbitrarily, 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 re-utilization of fatty acids and a 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 (eq. 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. Maximum rate is set to be adequate to meet maintenance energy requirements, and is therefore represented as a function of $Q_{Lw}^{0.75}$ (eq. 8.5, 8.8)

Glucose pool, Gl. Inputs to the glucose pool are from dietary lactose (eq. 7.2), starch (eq. 7.3) and from glycerol, released during lipolysis (eq. 7.4) or fat absorption (eq. 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 energy source (catabolism to acetyl-CoA, eq. 7.8), as a source of glycerol in the esterification of fatty acids during fat synthesis (eq. 7.7), and as the major source of reduced NADPH in fatty acid synthesis (eq. 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 (eq. 7.7).

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

Acetyl-CoA is used as substrate in fatty acid synthesis (eq. 6.5), oxidized to satisfy maintenance energy needs (eq. 6.6) and to provide energy for various transactions (eq. 6.10-6.16). Additional to those represented in Figure 1, an extra 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). Also several substrate cycles, not represented in the model, may be part of this transaction (see a.o. Katz and Rognstad, 1976). The transaction is named "additional energy costs for growth" (Ag, see Table 1). Arbitrarily, the transaction is represented as a Michaelis-Menten function, dependent on acetyl-CoA concentration (eq. 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 eq. 11.4, 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 come to 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 Figure 1). The remaining part is met by oxidation of acetyl-CoA (eq. 6.6, 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 about 1% of maintenance energy expenditure. In total, 25% of the maintenance energy requirements is assumed double counted, and thus subtracted from the maintenance requirements (eq. 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 non-limiting. Ash accretion is considered to consist of two components: (i) ash in non-skeletal tissues: an amount depending on the pool size of visceral protein, hide protein and muscle protein and (ii) ash in skeletal tissue (eq. 10.1). The ratio between ash and protein in muscle (i) 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 analysed 12 German Friesian beef calves of either 150 or 270 kg Lw on body composition. The relationship between ash and protein in viscera is estimated from the experiments. The accretion rate of ash in skeletal tissue (ii) is made dependent on 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 per kg ash deposited in skeletal tissue, assuming, in analogy to France et al. (1987), a cost of 2 mol ATP per mol Ca or P incorporated in 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. (1996b).

Model calibration

The affinity and inhibition constants and steepness parameters were adjusted to obtain good fit of the experimental data, 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 Appendix 1. The calculation of amino acid imbalance, synthesis of dispensable amino acids and amino acid requirements is given in Appendix 2. 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

Results of simulations of the experimental treatments, described earlier in this paper are presented here. Model evaluation against independent data, sensitivity analysis and model application are presented in two companion papers (Gerrits et al., 1996a,b).

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 80-160 kg and between 160-240 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 are simulated satisfactorily. Also, the large contrasts in fat deposition rates between energy intake levels are represented quantitatively.

In order to demonstrate the effect of a stepwise reduction of the dietary intake of a specific amino acid on model behaviour, methionine intake was reduced from 9 to 2 g/d at 2 levels of protein intake. Results of the simulations are shown in **Figure 4**. While 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 Appendix 2, 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, needed 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 two companion papers (Gerrits et al., 1996a,b).

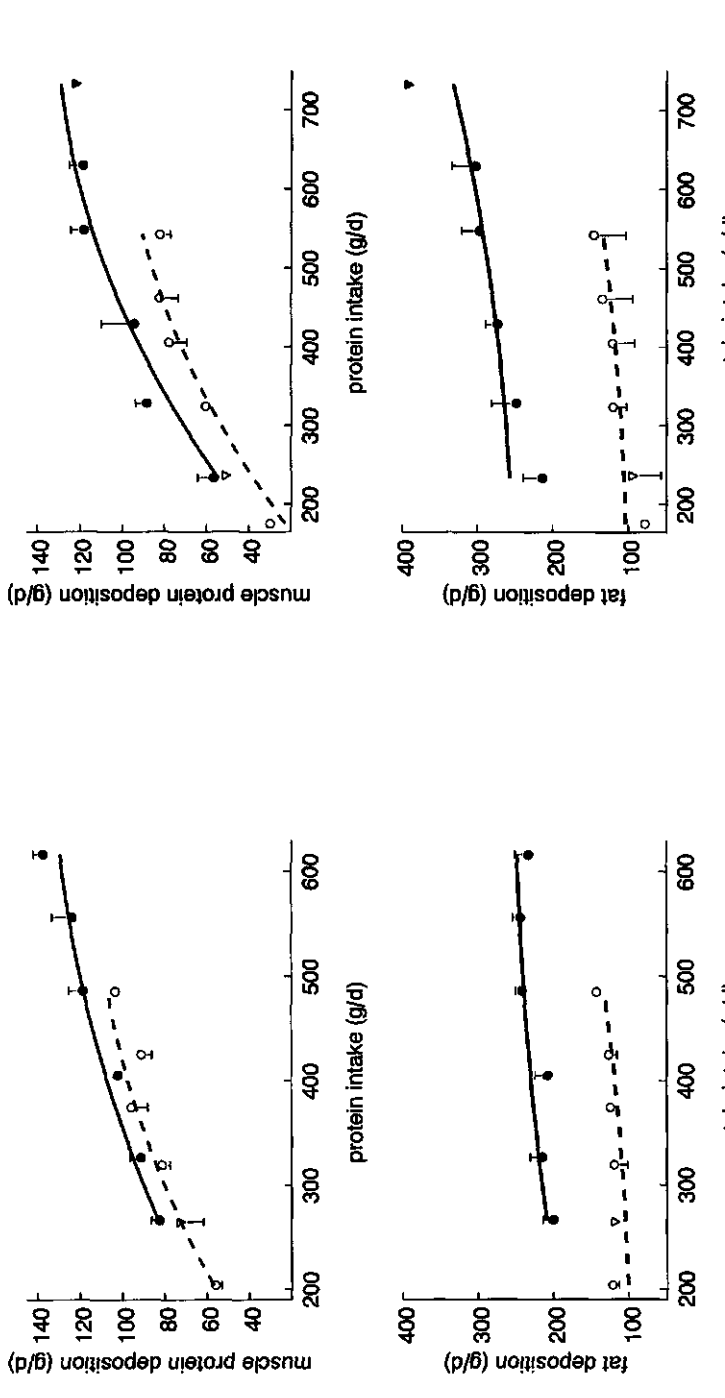

Figure 3. Simulated (lines) and observed (symbols) response of rate of muscle protein and body fat deposition in preruminant calves to protein intake at two protein-free energy intake levels in two live weight ranges. Low and high protein-free energy intake levels were 663, 851, 564 and 752 kJ/kg$^{0.75}$ · d) for the weight ranges 80-160 and 160-240 kg Lw, respectively. High energy intake level (— , ●, ▾) and low energy intake level (– – , ○, ▿). Values are means ± SEM, n = 3 (●, ○) or n = 2 (▾) or n = 1 (▿).

The relationships between the development of the protein pools, estimated from the experiments and shown in Figure 2, allow for a relative higher priority for bone and hide protein of slower growing calves. While the simplicity of this solution is attractive, it brings about a few problems. Firstly, the model becomes highly sensitive to changes in the parameters describing the development of the muscle protein pool (evaluated in Gerrits et al., 1996b). Secondly, the mathematical representation of the protein metabolism does not allow negative growth of the muscle protein pool (see Appendix 1). The model, therefore, is not valid for calves fed below maintenance.

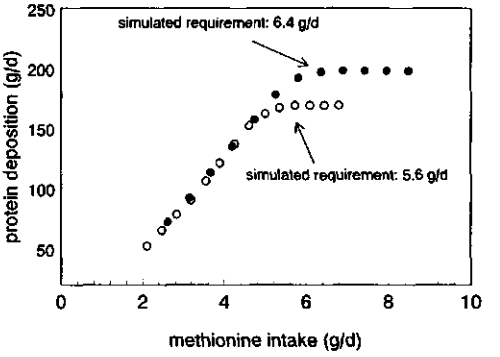

Figure 4. Simulation of the effect of a step-wise reduction of methionine intake on average protein gain of preruminant calves in the weight range of 80-160 kg Lw, at two protein intake levels: 13.1g/kg $Lw^{0.75}$.d (•) and 8.8 g/kg $Lw^{0.75}$.d (○). Protein-free energy intake was 820 kJ/($kg^{0.75}$ · d).

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, does probably not adequately represent the reduced muscle protein deposition capacity of calves, approaching maturity.

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 is 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., 1996c) and can therefore not be the basis for growth simulation in preruminants.

Extensive metabolic changes occur as calves develop from the non-ruminating to the ruminating state. The energy metabolism in preruminants is largely based 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 provides, 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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Appendix 1

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 Appendix 2.

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)

Continued

Appendix 1 *(continued)*

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

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)

Auxiliary equations: $v_{Ay,AyFa} = v^{*}_{Ay,AyFa}Q_{Fb}^{0.67}$ (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)

$U_{Ay,AyOx} = R_{Ay,AaAa}U_{Aa,AaAa} + R_{ay,MdAs}0.1549[(dQ_{Pm}/dt)/PF_{Pm}]^{0.368} + R_{Ay,AaPb}U_{Aa,AaPb} + R_{Ay,AaPh}U_{Aa,AaPh} + R_{Ay,AaPm}U_{Aa,AaPm} + R_{Ay,AaPv}U_{Aa,AaPv} + R_{Ay,PbAa}U_{Pb,PbAa} + R_{Ay,PhAa}U_{Ph,PhAa} + R_{Ay,PmAa}U_{Pm,PmAa} + R_{Ay,PvAa}U_{Pv,PvAa} + A_{Pd}D_{Pd} + A_{Fd}D_{Fd} + A_{Ld}D_{Ld} + A_{Sd}D_{Sd} + R_{ay,FaFb}U_{Fa,FaFb}$

Continued

Appendix 1 *(continued)*
Mathematical statement of the model simulating metabolism of preruminant calves[1]

$$+ R_{Ay,UrUe} Y_{Ur,AaAy} U_{Aa,AaAy} + v_{Ay,AyAg} Q_{Ew} / \{1 + [(M_{Ay,AyAg}/C_{Ay})^{SAy,AyAg}]\} \quad (6.16)$$

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)

Output: $U_{Gl,AyFa} = R_{Gl,AyFa} U_{Ay,AyFa}$ (7.6)

$U_{Gl,FaFb} = R_{Gl,FaFb} U_{Fa,FaFb}$ (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)

Continued

Appendix 1 *(continued)*

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

Summative equations

Empty body weight, Q_{Ew} (kg), Live weight, Q_{Lw} (kg); Liver weight, Q_{Lv} (kg)

$$Q_{Ew} = 1.03[Q_{As} + Q_{Fb} + Q_{Pb} + 11.605(Q_{Pb}/PF_{Pb})^{0.593} + Q_{Ph} + 13.879(Q_{Ph}/PF_{Ph})^{0.847} + Q_{Pm} + 24.294(Q_{Pm}/PF_{Pm})^{0.943} + Q_{Pv} + 30.536(Q_{Pv}/PF_{Pv})^{0.901}] \quad (11.1)$$

$$Q_{Lw} = 1.11Q_{Ew} \quad (11.2)$$

$$Q_{Lv} = 4.653(Q_{Pv}/PF_{Pv})^{0.81} \quad (11.3)$$

Lean body mass, Q_{Lb} (kg), Visceral mass, Q_{Vi} (kg)

$$Q_{Lb} = Q_{As} + Q_{Pb} + 11.605(Q_{Pb}/PF_{Pb})^{0.593} + Q_{Ph} + 13.879(Q_{Ph}/PF_{Ph})^{0.847} + Q_{Pm} + 24.294(Q_{Pm}/PF_{Pm})^{0.943} \quad (11.4)$$

$$Q_{Vi} = Q_{Pv} + 30.536(Q_{Pv}/PF_{Pv})^{0.901} \quad (11.5)$$

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

Appendix 2

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 tissue 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, the 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 for 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 (eq 1.8, Appendix 1).

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

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

Continued

Appendix 2 *(continued)*

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]

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].

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

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

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

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

[2] This calculation routine is included in the model (Appendix 1) and is performed every iteration.

3 EVALUATION OF A MODEL INTEGRATING PROTEIN AND ENERGY METABOLISM IN PRERUMINANT CALVES

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Evaluation of a model integrating protein and energy metabolism in preruminant calves

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Abstract

In a companion paper, the development of 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 protein, fat, ash and water. This paper describes a sensitivity and behavioural analysis of the model, as well as tests against independent data. The model is sensitive to changes in maintenance energy requirements, indicating their quantitative importance. It is, however, only marginally sensitive to changes in maintenance protein requirements. The representation of protein turnover, including its energy costs, enables the investigation of the quantitative importance of hide, bone and visceral protein. Protein turnover was identified as the major energy consuming process defined in the model. The model is highly sensitive to an increase or decrease of 25% in kinetic parameters describing muscle protein synthesis and amino acid oxidation. Simulation of the requirement for a specific amino acid at a given nutrient intake is sensitive to the inevitable oxidative losses, defined for that amino acid. Furthermore, it is sensitive to the amino acid content of the body protein and -to a lesser extent- to protein turnover rate. Simulation of two published experiments, not used for development of the model, showed that rates of gain of live weight, protein and fat were simulated satisfactorily in the live weight ranges 55-155 and 180-230 kg.

INTRODUCTION

In a companion paper (Gerrits et al., 1996a), a mechanistic growth simulation model is described, developed for preruminant calves between 80 and 240 kg live weight (Lw). 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 protein, fat, ash and water. The model can also be used to predict amino acid requirements. It is largely based on data derived from two experiments with preruminant calves, especially designed for its construction (Gerrits et al., 1996c). Using these data, it was shown that the model satisfactorily predicted growth and growth composition of preruminant calves in a wide range of nutrient intakes (Gerrits et al., 1996a). The objectives of the research, reported in this paper are to test (i) the sensitivity of model predictions to changes in model parameters and (ii) the predictive quality of the model.

Concerning (i), attention is paid to the sensitivity of model predictions to changes in some of the main assumptions concerning maintenance requirements (energy and protein), protein turnover, body fat degradation and the energy requirements for tissue deposition. Also, the sensitivity of model predictions to an increase or decrease of 25% in all kinetic parameters is analysed. Furthermore, the effect of changing the main assumptions on the simulation of amino acid requirements is tested. The predictive quality of the model (ii) is tested by simulating two published experiments, not used for the development of the model, and comparing the results with the experimental observations.

The reference simulation

A reference simulation is chosen as the starting point for the sensitivity analysis. For this purpose, 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 live weight (Lw), starting the simulation at 120 kg Lw. The nutrient intakes at 160 kg Lw are: milk proteins (N x 5.92; Gerrits et al., 1996a), 556 g/d; fat, 428 g/d; lactose, 924 g/d; (pre-gelatinized) starch, 86 g/d.

Sensitivity and behavioural analysis

Maintenance energy. In the model, energy requirements for maintenance are included as a function of lean body mass, body fat and viscera. Maintenance energy requirements are first met by all ATP yielding transactions in the model. The remaining part is met by oxidation of acetyl-CoA. In order 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). For the reference simulation, the default maintenance energy requirement, expressed in this way would be 453 kJ/($kg^{0.75}$·d).

As expected, rate of gain of fat, protein and live weight decreased with increasing maintenance energy requirements (**Figure 1**). 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 requirements, however, is smaller 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".

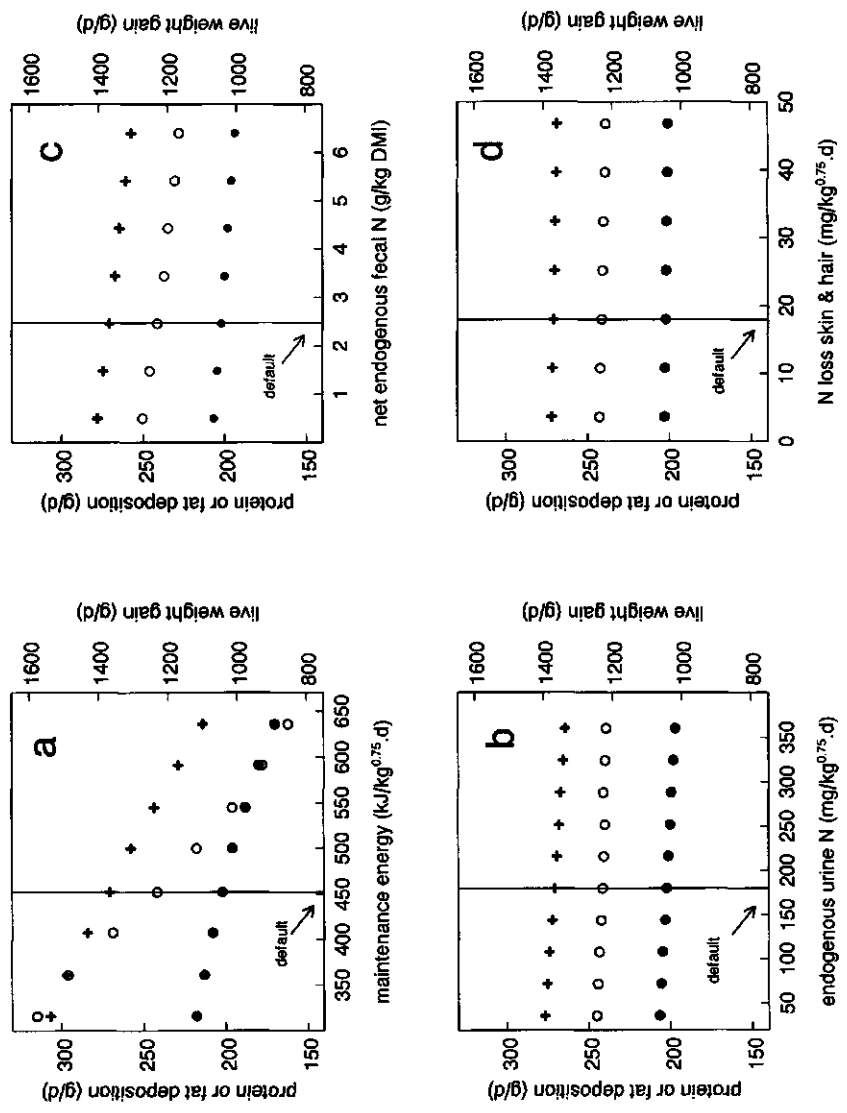

Figure 1. Sensitivity of model predictions on the rate of gain of protein (●), fat (○) and live weight (+) to changes in energy requirements for maintenance (a), endogenous urinary N losses (b), net endogenous faecal N losses (c) and N losses with skin and hair (d). DMI = dry matter intake.

This flux represents the increased costs of tissue deposition with increased tissue deposition rates (see Gerrits et al., 1996a). At maintenance levels, lower than 350 kJ/($kg^{0.75}$ · d), fatty acids are accumulating in the fatty acid pool, because the rate of fat synthesis is approaching its maximum.

Maintenance protein. According to classical protein metabolism concepts, nitrogen loss from the body at maintenance can be factored into three distinct fractions: (i) endogenous urinary losses (ii) metabolic faecal losses, (iii) losses of skin and hair (Owens, 1987). These three components are included in the model, expressed per kg $Lw^{0.75}$ (i and iii) or as a function of dry matter intake (ii). The sensitivity of the model to variation in the daily expenditure on protein for maintenance is tested by separately varying (i) from 50 to 350 mg N/($kg^{0.75}$ · d) (default is 180); (ii) from zero to 7 g N/(kg dry matter intake) (default is 2.46); (iii) from 3 to 47 mg N/($kg^{0.75}$ · d) (default is 18). The results are presented in Figure 1. Neither increased N losses via (i), (ii) nor (iii) exert detrimental effects on rate of gain of protein, fat and live weight. In all three cases, the effect on protein deposition rate is less than expected on grounds 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 endogenous faecal N losses, for example, is compensated for in this way. This compensation percentage depends on the protein intake level. Decreasing protein intake from 556 to 400 g/d in the reference simulation decreased the compensation from 79 to 73%. The small decrease in 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 case of ii and iii). It is realized that the increased costs of protein syntheses are probably underestimated, because only the net faecal endogenous losses are modelled, being the difference between synthesis and re-absorption.

Protein turnover. The deposition rate of hide, visceral and bone protein is related to the muscle protein deposition rate (discussed by Gerrits et al., 1996a). Therefore, an increase in the fractional degradation rate (FDR) of for example hide protein, is followed by an increased hide protein synthesis rate, because the model will try to keep hide protein deposition rate proportional to muscle protein deposition

rate. Therefore, the turnover of protein in hide, bone and viscera and bone can be varied by varying the respective FDRs. However, as discussed later, the deposition rate will be slightly negatively affected by increasing these FDRs. The response of the model to changes in the FDR of muscle protein, however, is exaggerated 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. 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 exaggerates 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.

The effect of changing the rate of turnover of visceral and hide protein is shown in **Figure 2**. Increasing the FDR of protein in viscera, hide and bone slightly decreases the protein deposition rate. The increased FDR of hide protein leads to an increased amino acid concentration. This, in turn, leads to an increased amino acid oxidation rate, leaving less amino acids to be deposited. The decrease in protein deposition rate, however, is small, indicating that protein synthesis is increased to an almost similar extent as degradation. Increasing for instance the FDR of hide protein from 2 to 20%/d increases the protein degradation rate with 662 g/d and an increases the protein synthesis rate with 650 g/d, leaving a difference of 12 g/d. As expected, increasing the FDR of these tissues negatively affects the fat deposition rate. The response of fat deposition rate is bigger than that of protein deposition rate, and is caused by the increased energy expenditure on protein turnover. The predicted effects of increasing the FDR, expressed per % increase, are biggest for hide protein, followed by bone and visceral protein, which is a direct effect of the difference in size of the pools. 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.

Fractional degradation rate of body fat. The fractional degradation rate of body fat is set to 1%/d (Gerrits et al., 1996a). Body fat synthesis is dependent on the concentration of fatty acids in the fatty acid pool. Taking the fixed FDR into

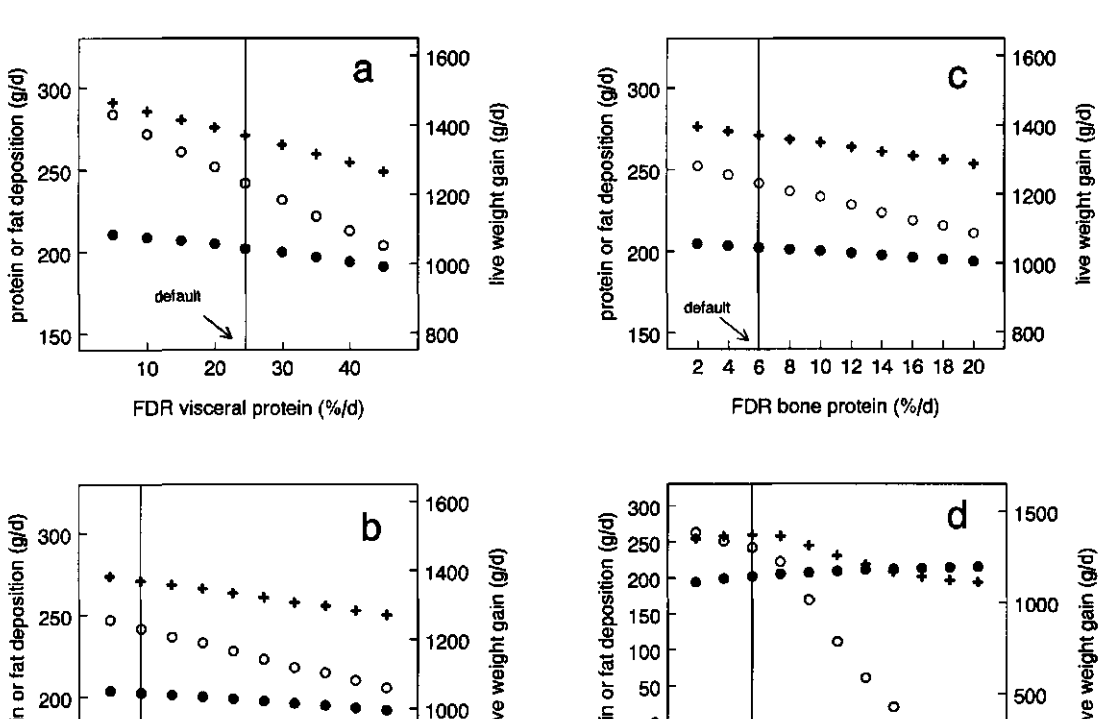

Figure 2. Sensitivity of model predictions on the rate of gain of protein (●), fat (○) and live weight (+) to changes in fractional degradation rates (FDR) of visceral (a), hide (b) and bone (c) protein and of body fat (d).

account, the parameters describing fat synthesis were set to match net fat deposition rates with the rates, measured in the experiments (Gerrits et al., 1996a). Changes in the FDR of body fat are not compensated for by an increase in the fat synthesis rate. Therefore, unlike the FDRs of protein in hide, bone and viscera, changes in the FDR of body fat do not represent changes in body fat turnover rate. As shown in Figure 2, the net fat deposition rate is clearly decreased by increased FDRs. At FDRs above 4%/d, no net fat deposition was predicted. Protein deposition rates are slightly increased. Increasing the FDR of body fat by 1%/d leads to a very large amount of fatty acids mobilized, causing the fatty acid concentration to rise. This is followed by an increase in fat synthesis and fatty acid oxidation rates, which both very soon reach their maxima. Mobilized fatty acids are then accumulated in the fatty acid pool.

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 (i) protein synthesis and degradation, (ii) synthesis of dispensable amino acids, (iii) fat synthesis and (iv) the incorporation of Ca and P in bone ash, which are 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 in 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., 1996a), were increased or decreased by 25%. The effects of changing these parameters on the rate of gain of protein, fat and live weight were analysed, as well as the effect on the transaction which the parameter is used to describe. The effects of changing the kinetic parameters of the fluxes involving protein are

presented in **Table 2**, those involving fatty acids or acetyl-CoA in **Table 3**. The effects of changing the kinetic parameters which involve glucose metabolism were negligible and are therefore not presented. As explained in Gerrits et al. (1996a), these parameters were set to prevent accumulation of glucose in the glucose pool.

In general, when changing a model parameter increases the protein deposition rate, the fat deposition rate is decreased, and vice versa (Table 2, 3). This is caused by two mechanisms: (i) 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

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 in 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 in 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.

increased fatty acid synthesis rates. (ii) 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 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 effects caused by mechanism (i) are large on protein deposition and small on fat deposition rates. Effects caused by mechanism (ii) are vice versa. 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.

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	"	59
$M_{Aa,AaAy}$	-25	-18.1	9.0	-91	"	180
	+25	11.9	-5.4	60	"	-115
$s_{Aa,AaAy}$	-25	-3.2	2.8	-15	"	48
	+25	2.2	-0.6	11	"	-14
$v^*_{Aa,AaPm}$	-25	-88.9	46.9	-440	muscle protein synthesis	-781
	+25	82.5	-34.4	421	"	715
$M_{Aa,AaPm}$	-25	15.2	-6.1	78	"	73
	+25	-13.7	7.6	-68	"	-69
$M_{Ay,AaPm}$	-25	8.7	-4.3	44	"	72
	+25	-8.2	4.2	-41	"	-69

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

[2] $v^*_{i,jk}$ = maximum velocity for *j-k* transaction per kg 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. (1996a).

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

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 on 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	"	78
$J_{Ay,FaAy}$	-25	-5.4	12.6	-16	"	-57
	+25	4.2	-7.1	15	"	46
$M_{Fa,FaAy}$	-25	0.7	-1.2	3	"	8
	+25	-0.7	1.3	-2	"	-8
$v^*_{Fa,FaFb}$	-25	4.1	-47.2	-32	fat synthesis	-76
	+25	-3.6	8.6	-10	"	41
$M_{Fa,FaFb}$	-25	-1.0	2.7	-2	"	13
	+25	0.6	-1.9	1	"	-10
$v^*_{Ay,AyFa}$	-25	3.4	-1.6	17	fatty acid synthesis	-484
	+25	-3.1	1.9	-15	"	441
$M_{Ay,AyFa}$	-25	-2.7	1.7	-13	"	389
	+25	2.0	-1.0	10	"	-280
$J_{Fa,AyFa}$	-25	0.1	0.0	1	"	-13
	+25	-0.1	0.1	0	"	8
$v^*_{Ay,AyAg}$	-25	4.8	21.7	52	additional energy costs of growth	-1134
	+25	-4.9	-18.5	-48	"	975
$M_{Ay,AyAg}$	-25	-1.6	-6.5	-16	"	305
	+25	1.8	7.7	19	"	360
$s_{Ay,AyAg}$	-25	1.4	6.2	15	"	-284
	+25	-1.0	-4.2	-10	"	196

[1]See footnotes Table 2; additionally used 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.

rate. This, however, may be specific 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., 1996a). Therefore, in general, the model is more sensitive to changes in v_{max} than to similar changes in other model parameters.

Sensitivity of simulation of amino acid requirements. As described in Gerrits et al. (1996a), the model can be used to predict the requirement of all indispensable amino acids. The requirement is defined as the intake below which protein deposition becomes depressed by the intake of a specific indispensable amino acid. This approach enables the estimation of amino acid requirements under various conditions, and is based on a number of assumptions. In this sensitivity analysis, the importance of each assumption is tested by simulating the requirements for methionine + cystine and threonine, usually considered limiting amino acids for calves fed milk protein diets (Williams, 1994). As stated in the companion paper (Gerrits et al., 1996a), amino acid requirements depend on the level of protein deposition, which depends on both protein and energy intake (Gerrits et al., 1996c). Also, simulated amino acid requirements are dependent on live weight (demonstrated by Gerrits et al., 1996b). This sensitivity analysis is focused on evaluation of the relative importance of each of the following assumptions to simulated requirements. The analysis is performed using the reference simulation as a starting point. 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 threonine and methionine + cystine content of muscle protein is tested.

Second, the inevitable oxidative losses of a specific amino acid is made dependent on the amount (per day; flux) of that amino acid entering the amino acid pool (see Gerrits et al., 1996a). Arbitrarily, these inevitable oxidative losses were set to two per cent of the flux of that amino acid. The effect of changing this assumption to one, five or ten per cent 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.

The simulated requirements for threonine and methionine + cystine in the reference simulation were 16.2 and 11.1 g/d, respectively. The results of the sensitivity analysis are presented in **Table 4.** The changes in the threonine and methionine + cystine requirements in this sensitivity analysis are comparable to each other. Changing the inevitable oxidative losses has an important effect on the simulated threonine and methionine + cystine requirements. Increasing visceral protein turnover by increasing its FDR increases the simulated requirements. This illustrates the behaviour of the model and is in agreement with the suggestion of

Table 4

The effect of changing the inevitable oxidative losses, fractional degradation rate (FDR) of visceral protein and the threonine and methionine + cystine content in muscle protein on the threonine and methionine + cystine requirement, simulated by the model for preruminant calves in the reference simulation[1]

	Increase content in muscle with 25%[2]	Inevitable oxidative losses[3] 1%	Inevitable oxidative losses[3] 5%	Inevitable oxidative losses[3] 10%	FDR visceral protein 35%/d[4]
	Simulated amino acid requirement, in % of the reference simulation[4]				
Threonine	110	95	115	141	110
Methionine + Cystine	110	95	115	142	110

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

[2]The content of threonine and of methionine + cystine in muscle protein was increased from 45 to 56 and from 33 to 41 g aminoacyl residue/kg aminoacyl residue, respectively (for defaults see Table 5 in Gerrits et al., 1996a);

[3]Expressed as a percentage of the flux, i.e. the amount per day of that amino acid entering the amino acid pool from dietary or degraded protein;

[4]The simulated requirements are corrected for the decreased maximum obtainable protein deposition rate at this higher degradation rate. This effect is illustrated in Figure 1;

[5]Absolute values for the simulated requirements in the reference simulation are 16.2 and 11.1 g/d for threonine and methionine + cystine, respectively.

Simon (1989), that visceral protein may be more important in defining amino acid requirements than can be expected on grounds of its contribution to body protein. Increasing the threonine and methionine + cystine content of muscle protein (the largest protein pool) separately has an important effect on the respective requirements. Increasing the threonine content of muscle protein pool, for example, directly increases the need for deposition, but also the threonine flux, and thus the inevitable oxidative losses.

Comparison with published experiments

Only two suitable experiments were found to evaluate model performance: 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 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 (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 the overall bias of prediction (i), deviation of the regression slope from one, being the line of perfect agreement (ii), and the disturbance proportion (iii) (Bibby and Toutenburg, 1977). (i) Represents the proportion of MSPE, due to a consistent over- or underestimation of the experimental observations by model predictions. (ii) Represents the proportion of MSPE, due to inadequate simulation of differences between experimental observations. The remaining proportion of MSPE (iii) represents the proportion, unrelated to the errors of model prediction.

Van Es and Van Weerden (1970). These authors carried out 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 about 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.

They 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 balance were measured during four consecutive periods. The first period (day 0-30, weight range about 40-55 kg Lw) was considered too far outside the weight range for which the model is developed. Therefore, only the last three periods were simulated: period 2, day 31-58 (weight range about 55-85 kg Lw); period 3, day 59-87 (about 85-110 kg Lw), and period 4, day 88-120 (about 110-155 kg Lw). Nitrogen intake varied across treatments from 49 to 59, from 38 to 54 and from 57 to 97 g/d in period 2, 3 and 4, respectively. Fat intake varied across treatments from 125 to 388, from 131 to 373 and from 255 to 678 g/d in period 2, 3 and 4, respectively. Lactose intake varied across treatments from 598 to 783, from 583 to 734 and from 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 one 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 (Figure 3). The root MSPE was 11.6% of the observed mean. Two per cent of the MSPE was attributed to overall bias, 35% to deviation of the regression slope from one and 63% to the disturbance proportion. While the overall mean was well predicted, 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 one contributed 35 and 0% to the MSPE, respectively). This may be due to the short period of collection in the N-balance (6 days), while 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). The N-balance

technique, used by Van Es & Van Weerden (1970; not carried out in respiration chambers) usually leads to a higher estimation of N-retention than slaughter experiments, on which the model is based (Gerrits et al., 1996c; MacRae et al., 1987). It was therefore expected to lead to overestimation of the observed N-retention, which was not confirmed by the simulations. The underestimation for calves in the weight range 85-110 kg may indicate that calves in this weight range retain N with a somewhat higher efficiency than older calves. However, the model is based on the slaughter trials over a large weight range, and the short-term effects cannot be simulated adequately.

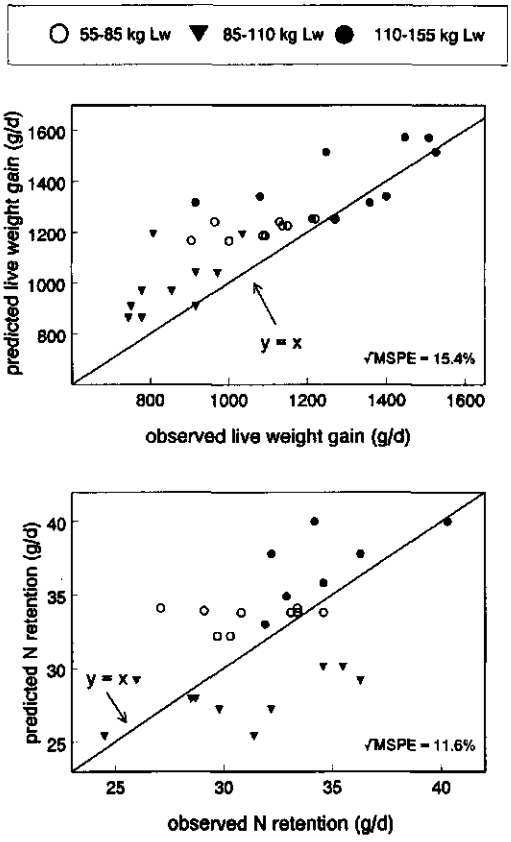

Figure 3. Comparison of experimental observations with model predictions of rate of live weight gain (top) and N-retention (bottom) in the experiments of Van Es & van Weerden (1970). Values for live weight gain are averages over the live weight ranges 55-85, 85-110 and 110-155 kg. Values for N-retention are determine in the middle of the respective weight range. √MSPE = root mean square prediction error, expressed as 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 2x2 factorial arrangement. They used 20 male preruminant calves of either Dutch Friesian (DF) or Holstein Friesian (HF) x Dutch Friesian crossbreeds. The

feeding levels used were 2.1 and 1.8 x 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 faeces of each calf. Live weight gain, energy and N balances were measured weekly during the last five weeks of the fattening period (180 - 230 kg Lw). The original data of eighteen calves could be used for the simulations. These experiments revealed no effects of genotype. Therefore, the experimental results were averaged over genotype, and the effect of feeding level, averaged over five weeks, was simulated. Rates of fat gain are re-calculated from the original data by (2):

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

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, 1996a); 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 5**. Generally, results of the simulations correspond well with the observed rates of gain. Especially the predicted contrasts between the two feeding levels are predicted accurately. The deviation of the regression slope from one 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

Table 5

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.

with time. Simulated rates of weight gain are only slightly higher than the average observed rates. The simulated N-retention agrees with the observed values. The general idea that the N-balance technique overestimates N-retention, compared with slaughter experiments, discussed earlier, applies to a lesser extent to these experimental data. Nitrogen losses via condensed water and air are accounted for in the open-circuit respiration chambers, in which these N-balances were measured. Calculated from the original data, N in condensed water and air accounted for on average 3% of the total N-intake, close to 8% of the N-retention in this experiment.The predicted fat deposition rates are about 50 g/d higher than the observed values, while 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., 1996c). The overestimation can be caused by (i) 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, (ii), the overestimation can be caused by a difference in the way the fat deposition is determined, i.e. calculated from the measured heat production and nitrogen balance (2) vs. direct measurement in the slaughter experiments, on which the model is based. According to Van Es & Boekholt (1987) however, this could only explain a small part of the overestimation. More likely (iii), the difference between the observed and predicted values represent real differences in fat deposition rates between the experiments of Meulenbroeks et al. (1986) and ours. 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 & Boekholt, 1987). An increased maintenance requirement from 460 to 500 kJ/($kg^{0.75}$ · d), for instance, 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 genetical 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.

DISCUSSION

The results of model tests against independent experimental data in the weight range 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, respectively). While model predictions, averaged over a relatively large weight range are reasonably 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. It can be questioned whether the low maintenance requirements, tested in the sensitivity analysis, are representative for practical situations. It does, however, illustrate model behaviour. The accumulation of fatty acids in the fatty acid pool could be an indication of metabolic disorders which occur under such circumstances. Increased maintenance energy requirements can have various reasons, e.g. illness, stress or increased activity of the animals (Baracos et al., 1987; Schrama et al., 1993; Verstegen et al., 1991). The observation from our modelling exercise, 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 seems therefore 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 in simulations compensated for roughly 70-80% by reducing the amino acid oxidation rate. While not likely to be so big, it is possible that such a kind of compensation mechanism exists. There are, for instance, some indications that the efficiency of utilization of absorbed protein for replacing endogenous urinary and endogenous faecal nitrogen losses in ruminants is higher than 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., 1996c). For endogenous urinary nitrogen excretion, this could be further supported by the fact that almost 40% of endogenous urinary nitrogen originates from creatinine, allantoin and uric acid (Gerrits et al., 1996a), which do not necessarily have amino acids as precursors. Compensation of increased losses of

skin and hair, however, is not likely to occur in vivo. 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 is appropriate for growing calves.

The design of the model allows evaluation of the effect of changes in turnover of hide, bone and visceral protein. These effects can be simulated by varying the respective FDRs. The direction of the predicted effects 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. Muscle protein and body fat turnover cannot be simulated by varying the respective FDRs.

Protein synthesis is quantitatively the most energy consuming process, defined in the model. Increasing the ATP requirement for protein synthesis from 3 to 5 moles ATP/mole 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. Energy expenditure on the synthesis of dispensable amino acids, fat synthesis and incorporation of Ca and P in bone ash is quantitatively less important than energy expenditure on protein turnover. In the reference simulation, these three processes together consumed less than 3% of the total energy expenditure.

The deposition rate of visceral, hide and bone protein is directly related to muscle protein deposition rate. This approach provides a simple solution to a complicated problem. It has the advantage that turnover of hide, bone and visceral protein can be varied by simply changing the FDR of these tissues. It has, however, the disadvantage that the model is highly sensitive to changes in the parameters determining muscle protein synthesis and also that the model will not respond satisfactorily to changes in the FDR of muscle protein.

The model provides a useful tool for estimating amino acid requirements.

Simulated amino acid requirements depend strongly on nutritional circumstances (Gerrits et al., 1996a) and respond to changes in the amino acid profiles of the tissues, body weight, protein turnover rate and inevitable oxidative losses. More attention has to be paid to the 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 the protein turnover rate. Application of the amino acid routine of the model is further discussed in a companion paper (Gerrits et al, 1996b).

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4 APPLICATION OF A MODEL INTEGRATING PROTEIN AND ENERGY METABOLISM IN PRERUMINANT CALVES

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Application of a model integrating protein and energy metabolism in preruminant calves

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Abstract

In two companion papers, a mechanistic growth simulation model, developed for preruminant calves in the live weight range 80-240 kg was described and evaluated. This model simulates the partitioning of ingested nutrients through intermediary metabolism to growth, consisting of protein, fat ash and water. It is developed for designing feeding strategies for preruminant calves. An optimal strategy results from the combination of the quality and quantity of the feed offered. In this paper, several simulations are performed, each dealing with an important issue in the development of a feeding strategy. Whenever possible, results of simulations are compared with experimental data. The model responds well to changes in protein and energy intake. Increasing the carbohydrate:fat ratio at equal gross energy intakes leads to higher simulated protein- and lower simulated fat- deposition rates. Amino acid requirements are simulated in three live weight ranges at two growth rates. Comparing simulated with experimentally derived amino acid requirements shows agreement for most amino acids for calves of around 90 kg live weight. For calves of around 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, especially leucine, isoleucine and histidine. Allocating the same amount of feed differently in the same time span hardly affected model predictions. Reducing daily feed intake markedly reduced performance, when compared at equal cumulative feed intakes. The efficiency of protein utilization, however, increased. It is concluded that the model provides a useful tool for the development of feeding strategies for preruminant calves in this weight range.

INTRODUCTION

In a companion paper (Gerrits et al., 1996a), a mechanistic growth simulation model is described, developed for preruminant calves between 80 and 240 kg live weight (Lw). 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 protein, fat, ash and water. In a second companion paper (Gerrits et al. 1996b) model performance was compared with independent data and the sensitivity of model predictions to changes in model parameters was tested. The main objective of the present paper is to study the effect of changes in the driving variables on model predictions or, in other words, to test its suitability for the development of a feeding strategy. An optimal feeding strategy depends on the

farmer's objective, but always results from the combination of the quality and quantity of a feed offered. How changes in the allocation of the feed are made over a period of time can also be important. Several simulations are performed, each demonstrating an important issue in the development of a feeding strategy. To simulate the effect of the quality and quantity of nutrients offered on animal performance, input of protein and protein-free energy and the composition of the protein-free energy (carbohydrates, fat) are varied independently. Also, the amount of individual amino acids, needed to support different growth rates at different body weights is simulated. Furthermore, simulations are performed to evaluate the response of the model to a certain amount of feed offered, allocated differently with time. Finally, the increase of live weight is monitored with time, using two feeding levels in two live weight ranges. Whenever possible, results of model simulations are compared with literature data.

METHODS

Varying the quantity and quality of feed offered

Protein and energy. To demonstrate the response of model predictions to changes in protein and protein-free energy intake between 80 and 240 kg Lw, results of simulations of the experimental treatments of Gerrits et al. (1996c) are presented. The protein intakes and deposition rates presented in the present paper are based on nitrogen, multiplied by 5.92 and 5.48, respectively, as discussed by Gerrits et al. (1996a). 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 the model to intake of nutrients, varied independently. Briefly, two slaughter experiments, similar in design, were carried out in two live weight ranges: 80-160 and 160-240 kg. Protein and protein-free energy intake varied, and calves were slaughtered at the beginning and at the end of each experiment, and analysed on chemical body composition. The wide range in nutrient intakes in these experiments, is shown in **Figure 1**. It was realized by mixing high protein-low energy with low protein-high energy diets in various ratios and by varying the quantity fed. Nutrient intakes increased linearly with $Lw^{0.75}$.

Dietary carbohydrate:fat ratio. The results of the experiments and simulations, presented in Figure 1 were obtained by using a fixed dietary carbohydrate:fat ratio. The design of the model allows evaluation of the effect of different dietary carbohydrate:fat ratios on tissue deposition rates. Starting from the reference simulation, described by Gerrits et al. (1996b), the ratio between energy intake from carbohydrates and fat was varied between 0.5:1 to 1.8:1.This range is comparable with the range, tested experimentally by Donnelly (1983) using preruminant calves of 40-70 kg Lw. The reference simulation was designed to simulate a fast growing calf of 160 kg Lw. Gross energy intake was equal for all simulations. Daily fat intake decreased with an increasing ratio from 586 to 316 g/d. Daily carbohydrate intake (lactose + starch) increased with an increasing ratio from 606 to 1288 g/d. The apparent faecal digestibility of fat and carbohydrates in the model was set to 0.95, as discussed by Gerrits et al. (1996a).

Amino acid requirements

The quality of protein intake is mainly determined by its amino acid composition. As described in the companion paper (Gerrits et al., 1996a), the model can be used to predict the requirements of individual, indispensable amino acids. The sensitivity of model predictions to changes in underlying assumptions is discussed by Gerrits et al. (1996b). Simulations were carried out to demonstrate the effect of body weight and protein deposition rate on the amino acid requirements. The effect of body weight was tested in three weight ranges: 80-100, 150-170 and 220-240 kg Lw, respectively. 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. (1996a). 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. (1996c). Amino acid requirements, as well as rates of gain of live weight, protein and fat were calculated as average over the simulated live weight range.

Varying the allocation of a fixed amount of feed with time

Three feeding strategies were designed to evaluate model responses to a different allocation of a fixed amount of feed with time. Simulations were started at about 80 kg Lw, and cumulative feed consumption was set to 300 kg. The dry matter, protein, fat, lactose and starch contents of the feed were: 960, 178, 210, 450 and 50 g/kg, respectively. Feeding strategy A started with a daily feed intake of 1.4 kg, with weekly increments of 100 g/d. Strategy B was designed to achieve an equal cumulative feed consumption in the same time span, but distributed differently. It started with a daily feed intake of 1.5 kg with weekly increments of 120 g/d for 7 weeks, and of 50 g/d thereafter. Daily feed consumption in strategy C was set at 90% of that of strategy A, i.e. starting at 1.26 kg with weekly increments of 90 g/d, until 300 kg of feed was consumed. Nutrient digestibility was set to the default values, described in Gerrits et al. (1996a).

Increase of live weight with time

The increase of live weight with time was evaluated using two feeding levels in two live weight ranges. The feeding levels and live weight ranges considered were taken from the experiments of Gerrits et al. (1996c), allowing comparison of experimental and simulated data. Low and high feed intakes in the weight range 80-160 kg were 43.2 and 55.5 g/(kg$^{0.75}$ · d), respectively. The dry matter, protein, fat, lactose and starch content of this feed were 968, 242, 195, 417 and 40 g/kg, respectively. Low and high feed intakes in the weight range 160-240 kg were 36.4 and 48.5 g/(kg$^{0.75}$ · d), respectively. The dry matter, protein, fat, lactose and starch contents of this feed were 975, 213, 210, 441 and 40 g/kg, respectively. Nutrient digestibility was set to the default values, described in Gerrits et al. (1996a).

RESULTS AND DISCUSSION

Varying the quantity and quality of feed offered

Protein and energy. The results of simulation of rates of gain of protein, fat and live weight are presented in **Figure 1**. The experimental results are extensively discussed by Gerrits et al. (1996c). Simulated, as well as observed protein deposition rates increase with increasing protein intake in both live weight ranges. Also, even at low protein intakes, increased protein-free energy intake leads to higher protein deposition rates in both weight ranges. The difference in the rate of

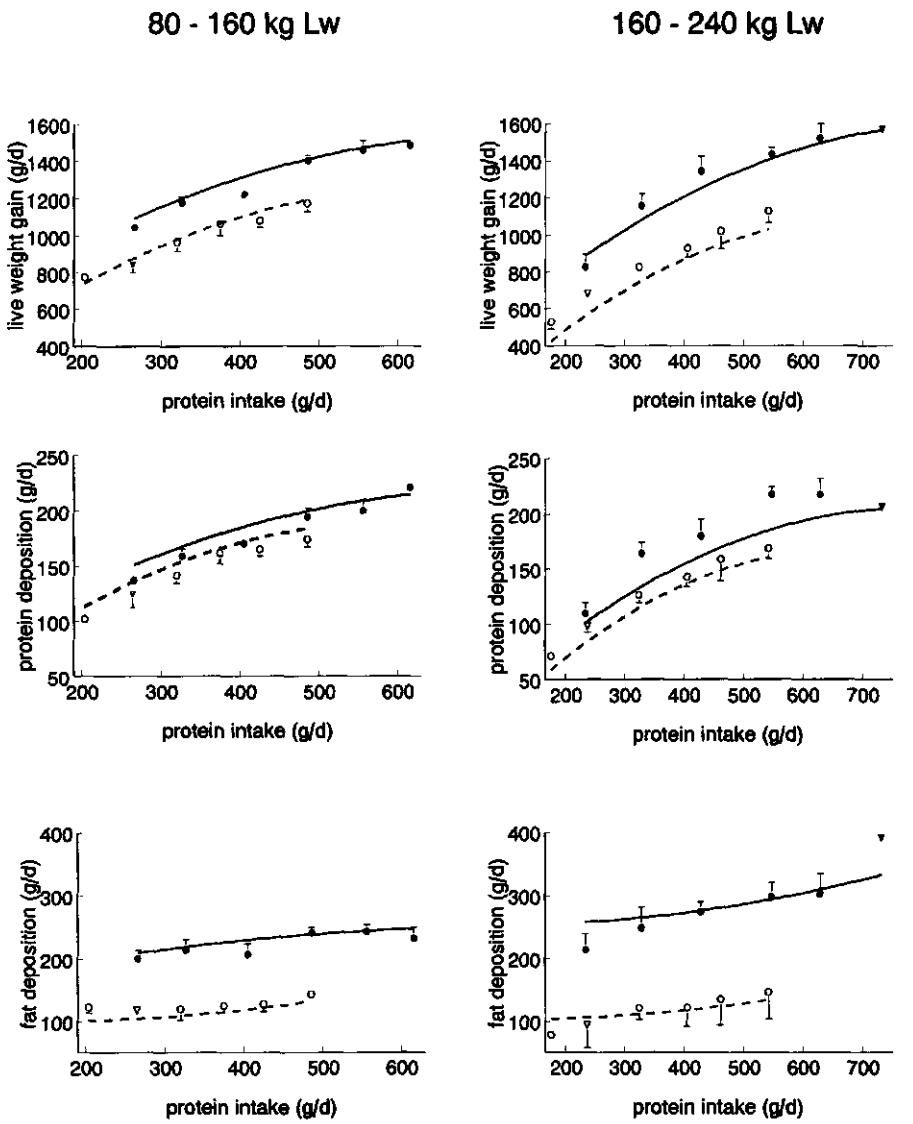

Figure 1 Simulated (lines) and observed (symbols) response of rates of gain of live weight (Lw), protein and fat in preruminant calves to protein intake at two protein-free energy intake levels in two live weight ranges. High (— , ●, ▼) and low protein-free energy intake levels (– – – , ○,▽) were 663, 851, 564 and 752 kJ/(kg$^{0.75}$·d), for calves between 80 and 160 and between 160 and 240 kg Lw, respectively, respectively. Values are means ± SEM, n=3 (●, ○), n=2 (▽) or n=1 (▼) . Data of Gerrits et al. (1996c).

live weight gain between the two protein-free energy intake levels can be explained for about 50% by the difference in fat deposition rates in both weight ranges.

Simulated values are in general agreement with the experimental data. The increase in rate of gain of live weight, protein and fat with increasing protein intake is simulated well, as is the effect of protein-free energy intake on rate of gain of live weight and protein. Also, the large contrasts in fat deposition rates in both experiments are represented quantitatively by the model. Rates of gain of protein and live weight are slightly overestimated between 80 and 160 kg Lw, and slightly underestimated between 160 and 240 kg Lw. This is probably due to a different ratio of hide and muscle protein deposition rates between the two experiments. This difference was in contrast to our expectations and may have been related to the different seasons in which the experiments were carried out (experiment 1 in the summer and 2 in the winter). Seasonal variation in hair development has been shown to exist, and may be related to day length (Hayman, 1965). Season, however, is not an input parameter in the model. Therefore, it was decided to neglect this effect. Model predictions are therefore based on the average relationship, computed using data of both experiments (Gerrits et al., 1996a). This resulted in the over- and under-estimations of the predicted rates of protein deposition and live weight gain.

Dietary carbohydrate:fat ratio. As shown in **Figure 2**, 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, rate of live weight gain increases slightly from 1286 to 1430 g/d. The changes in glycolysis, fatty acid oxidation and fatty acid synthesis are also presented in Figure 2, and illustrate how the responses occur. Increasing the carbohydrate:fat ratio decreases the amount of dietary fatty acids, entering the fatty acid pool. The amount of glucose, entering the glucose pool, however, is increased. The decrease in the availability of fatty acids from the diet (244 g/d) is only partly compensated for by an increased *de novo* fatty acid synthesis rate (44 g/d) and a reduced fatty acid oxidation rate (144 g/d). The concentration of fatty acids therefore decreases, leading to a decrease in the fat deposition rate (see Gerrits et al., 1996a). The input to the acetyl-CoA pool from glycolysis increases more than the net input from the fatty acid pool decreases (Figure 2). Consequently, the

concentration of acetyl-CoA increases, which causes the increased protein synthesis rates (see Gerrits et al., 1996a).

Unfortunately, the results of this modelling 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 they partly attributed to a narrower range in dietary carbohydrate:fat ratio tested with the high protein low energy diets. Model behaviour, 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.

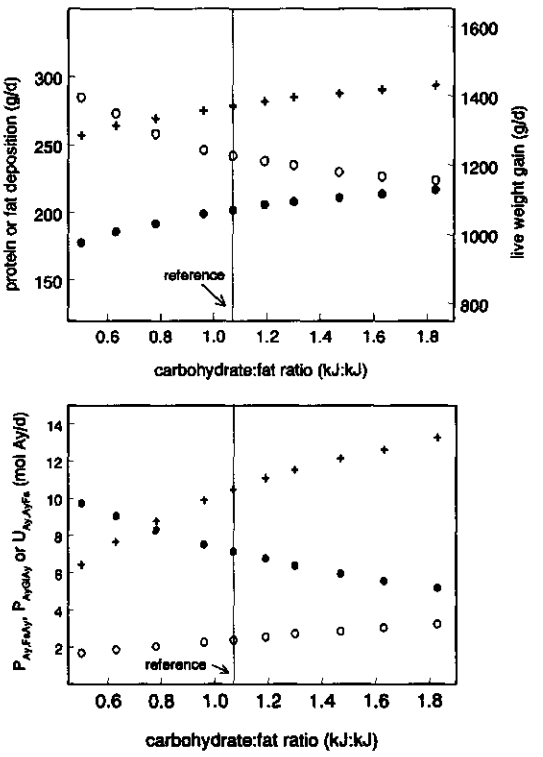

Figure 2. Sensitivity of model predictions to changes in the ratio between energy intake from carbohydrates and fat at a constant energy intake. Effects on the rate of gain of protein (●), fat (○) and live weight (+) (top graph) and on the production rate of acetyl-CoA (Ay) from fatty acids (Fa, ●) or from glucose (Gl, +) and on the utilization of Ay for fatty acid synthesis (○) (bottom graph). $P_{i,jk}$ = rate of production of i by j-k transaction; $U_{i,jk}$ = rate of utilization of i by j-k transaction. The reference simulation, described by Gerrits et al. (1996b) was used as a starting point.

Amino acid requirements

In the model, amino acids are expressed as amino acyl residues. Therefore, the requirements for aminoacyl residues rather than amino acids are simulated. In order to allow comparison of the simulated requirements with literature values, the results, presented in **Table 1** are already converted into g amino acid/d.

Effects of live weight and growth rate. 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 1), the simulated requirement for all indispensable amino acids (in g/d) increases with increasing Lw. This is caused by (i) increased endogenous amino acid losses, due to higher dry matter intakes at higher live weights; (ii) increased scurf losses from the hide protein pool at higher live weights; and (iii) higher inevitable oxidative losses (in g/d). Inevitable oxidative losses of a specific amino acid are calculated as a fixed proportion of the amount of that amino acid, entering the amino acid pool. The major part of this flux is from protein degradation. Fractional degradation rates of all protein pools are assumed fixed, as described in Gerrits et al. (1996a). Because pool sizes increase with live weight, these inevitable oxidative losses increase too. 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 faecal losses (i) are the most important factor, followed by the increased inevitable oxidative losses (iii) and the increased scurf losses (ii).

Obviously, higher protein deposition rates lead to increased amino acid requirements, because more substrate is needed for protein deposition (Table 1). However, when expressed per gram protein deposition, requirements decrease with 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 1). This is caused by changes in the composition of protein deposition. As shown in Figure 2 of Gerrits et al. (1996a), development of bone and hide protein has some priority above muscle protein deposition at low protein deposition rates. Therefore, with increasing protein deposition rates, muscle protein deposition becomes relatively more important, 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 become relatively more important. The amino acid patterns of the protein pools and

that of the endogenous protein losses, used in the model is presented in Table 5 of Gerrits et al. (1996a).

Comparison with literature data. Most experimentally derived amino acid requirements for preruminant calves are obtained between 40-80 kg Lw, 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 80-100 kg Lw at the low protein intake level (Table 1) 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

Table 1

The amount of each indispensable amino acid needed to simulate maximum protein (Nx5.48) deposition rate of preruminant calves in three live weight ranges at two protein (Nx5.92) intake levels

	80-100 kg		150-170 kg		220-240 kg	
Protein intake (g/kg$^{0.75}$ · d))	9	12	9	12	9	12
Average protein intake (g/d)	263	352	406	541	531	709
Rates of gain in case of no limiting amino acids						
protein (g/d)	146	166	159	180	166	191
nitrogen (g/d)	26.7	30.2	29.1	32.8	30.3	34.8
fat (g/d)	100	110	171	192	216	242
live weight (g/d)	932	1056	1052	1193	1118	1283
Amino acid requirement			*g/d*			
Threonine	10.8	12.0	13.9	15.2	16.7	18.4
Tryptophan	2.1	2.3	2.6	2.9	3.1	3.5
Valine	10.4	11.5	13.5	14.6	16.4	17.8
Methionine	4.2	4.7	5.2	5.7	6.1	6.8
Methionine + Cystine	7.6	8.4	9.5	10.4	11.3	12.5
Isoleucine	7.1	8.0	8.9	9.8	10.5	11.6
Leucine	18.4	20.3	23.5	25.6	28.4	31.0
Lysine	16.3	18.0	20.6	22.5	24.7	27.1
Histidine	6.4	7.1	8.3	9.0	10.1	11.0
Phenylalanine	9.7	10.6	12.6	13.6	15.4	16.6
Phenylalanine + Tyrosine	15.9	17.4	20.4	22.1	24.8	26.9
Arginine	6.6	7.3	8.0	8.8	9.4	10.4

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, respectively. 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, 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 1), 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 taken into account, the simulated arginine requirement would be about 17 g/d. Therefore, the upper limit, found by Van Weerden and Huisman (1985) supports 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 histidine requirement is, unlike the other amino acids, higher than the experimentally derived upper limit by Van Weerden and Huisman (6.4 vs. 5.7 g/d). This would indicate a lower inevitable oxidation proportion than the 2%, used in the model (Gerrits et al., 1996a). Reducing this proportion to 0% (utilization of absorbed histidine is then 100%) decreases the simulated histidine requirement from 6.4 to 5.8 g/d, still higher than the upper limit of van Weerden and Huisman (1985). There is some evidence in literature for the efficient utilization of histidine. Heger and Frydrych (1985) found a remarkably high utilization of histidine for growth (exceeding 100%) at low intake levels in growing rats. Similarly, histidine utilization was high in slaughter experiments of Bikker (1994), using pigs between 20 and 45 kg Lw. From these data, the efficiency of histidine utilization was calculated to be close to that of lysine, in diets which were designed to be 20% limiting in lysine. Wang and Fuller (1989) found no decreased N-retention in growing pigs, when histidine intake was reduced below 80% of their presumed optimal intake. It is likely that the minimal rate of histidine oxidation is lower than assumed in the model. Alternatively, it can be speculated that histidine may be conditionally indispensable. 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) only determined 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 1 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 hardly any 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 in response in these experiments.

There are several reasons why experimentally derived requirements can differ from the simulations. Obtaining requirements as the maximum of a quadratic relationship easily leads to overestimation, compared with other statistical methods, e.g. linear-plateau models (see Fuller, 1994; Williams et al., 1984). This may especially be the case for amino acids with a low response in N-retention per gram extra intake, e.g. lysine and leucine. On the other hand, by simply comparing simulated with experimentally derived amino acid requirements at similar N-retention rates, the way the N-retention data were obtained is neglected. As argued earlier (Gerrits et al., 1996b,c), N-balances tend to overestimate N-retention, when compared with slaughter experiments. The model is based on slaughter experiments. Comparing requirements at equal N-retention would therefore lead to higher estimations of amino acid requirements by the model, compared with requirements, derived from N-balance studies.

Varying the allocation of a fixed amount of feed with time

There are no important differences in performance criteria, body composition and the distribution of body protein between feeding strategy A and B (**Table 2**). Feeding strategy B resulted in a higher rate of live weight gain in the first ten weeks, compared with strategy A, and lower rates thereafter. Therefore, the

average live weight during the simulations was higher in strategy B, which explains the minor differences in protein and fat deposition rates and heat production.

Reducing the daily feed intake by 10% reduces the live weight gain on a similar cumulative feed consumption by 4 kg (Table 2, strategy C compared with A). Protein deposition is slightly increased, whereas fat deposition is decreased by almost 5 kg. The increased time span (132 vs. 142 d on strategy A and C, respectively) causes the total amount of energy, spent on maintenance to increase by 196 MJ. Total heat production is increased by 172 MJ. Similarly, the total amount of protein spent on maintenance is higher in strategy C. Despite this, the

Table 2

Effect of feeding strategies, equal in cumulative feed intake, on simulated performance criteria, composition of the empty body and distribution of protein over the protein pools in preruminant calves

	Feeding strategy[1]		
	A	B	C
Cumulative feed consumption (kg)	302.7	302.6	302.2
Time span (d)	132	132	142
Performance criteria			
live weight gain (kg)	173.2	172.3	169.1
protein deposition (kg)	23.85	23.73	24.17
fat deposition (kg)	37.38	37.23	32.68
heat production (MJ)	4025	4039	4197
Composition of the empty body (g/kg)			
protein (Nx5.48)	157.7	157.7	161.7
fat	182.4	182.4	164.7
ash	39.3	39.3	40.9
Distribution of body protein (% of total)			
muscle protein pool	58.0	58.0	57.4
hide protein pool	15.9	15.9	16.2
bone protein pool	14.3	14.4	14.6
visceral protein pool	11.8	11.8	11.8

[1] Feeding strategies: A: starting at 1.4 kg/d at 80 kg Lw, weekly increments of 100 g/d; B: starting at 1.5 kg/d at 80 kg Lw, weekly increments of 120 g/d until week 7 and 50 g/d thereafter; C: daily feed intake 90% of strategy A, equal cumulative feed consumption as strategies A and B.

overall efficiency of protein utilization (deposition as a percentage of intake) was slightly improved in strategy C: 47.9 vs 48.6% in strategies A and C, respectively. This illustrates the increased protein efficiency at lower levels of intake, as demonstrated by e.g. Meulenbroeks et al. (1986) and Ternouth et al. (1985). In practice, however, this advantage has to be balanced against the reduced growth rate when applying strategy C. Moreover, the slightly reduced proportion of total protein, deposited in muscle tissue in strategy C would probably be economically less favourable, compared with strategy A (Table 2).

Increase of live weight with time

Weekly body weights, recorded during the experiments (Gerrits et al., 1996c), averaged per treatment (3 calves per treatment) are plotted against time in **Figure 3**, as well as the simulated live weight. The simulated development of live weight with time matches the live weights, measured in the experiments. The contrasts between feeding levels in both weight ranges are well simulated. The experimental as well as the simulated rates of live weight gain at both feeding levels in both weight ranges (respective tangents in Figure 3) increase slightly with time. This is a consequence of the feed intake, which is based on $Lw^{0.75}$. This causes the amount of feed that can be used for growth to increase with time. However, at the low feeding levels, the simulated increase in the rate of live weight gain seems less than the observed increase in the experiments. In the simulations, the rate of live weight gain at the low feeding levels increases from 990 to 1110 g/d and from 850 to 870 g/d in the weight ranges 80-160 and 160-240 kg, respectively. The observed rates increased from 860 to 1230 and from 600 to 1090 g/d in the respective live weight ranges. The model is based on slaughter experiments, carried out over large live weight ranges (80 kg). Therefore, possible changes within such live weight range, such as adaptation of calves to for instance low feeding levels might not be represented adequately by the model.

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. More attention, however, has to be paid to the minimum oxidative losses of individual, indispensable amino acids. This was identified as the major unknown factor in estimating amino acid requirements in a companion paper (Gerrits et al., 1996b). Comparing simulated with experimentally derived amino acid requirements, the default values for the minimal oxidation proportion in the model may be too low for leucine and isoleucine and too high for histidine. Model predictions, averaged over a certain weight range are reliable, which was also shown using independent data by Gerrits et al. (1996b). Because the model is largely based on two slaughter experiments, carried out over a rather large live weight range, predictions at any given time or body weight are not necessarily accurate. Simulation of the increase of live weight with time, using different feeding regimes, however, is promising in this respect.

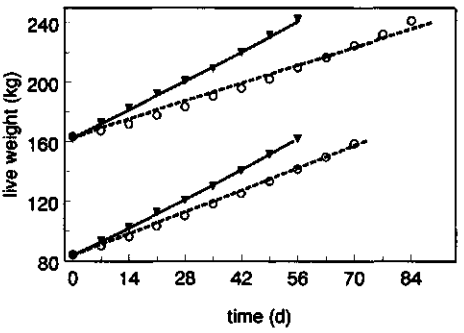

Figure 3. Simulated (lines) and observed (symbols) increase of live weight with time. Preruminant calves are fed either a high (— , ▼) or a low (– – – ,○) feeding level in the weight ranges 80-160 and 160-240 kg live weight. Symbols represent the mean live weight of three calves. Data from experiments of Gerrits et al., (1996c).

Apart from using metabolic models in research, there is, in our opinion, considerable scope for the use of these types of models to replace feeding tables, which are currently the basis for most feeding strategies.

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5 EFFECT OF PROTEIN AND PROTEIN-FREE ENERGY INTAKE ON MUSCLE PROTEIN TURNOVER IN PRERUMINANT CALVES OF 120 AND 200 KG LIVE WEIGHT

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Submitted

Effect of protein and protein-free energy intake on muscle protein turnover in preruminant calves of 120 and 200 kg live weight

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Abstract

In two experiments, the effect of protein and protein-free energy intake on the turnover of muscle protein in preruminant calves was studied. Both experiments were similar in design, but performed with two live weight ranges: 80 to 160 kg (Exp. 1) and 160 - 240 kg (Exp. 2). In each experiment, calves were allocated to either an initial slaughter group or to one of 12 treatments (3 calves per treatment), which consisted of six protein intake levels at each of two protein-free energy intake levels. Calves were slaughtered and analysed on body composition when they had reached the target weight. A balance trial was performed when calves had reached 120 and 200 kg LW in Exp. 1 and 2, respectively. Muscle protein degradation rate was measured by the urinary excretion of 3-methylhistidine. Correction of 3-methylhistidine excretion for muscle protein mass was performed based either on the urinary creatinine excretion rate or on estimated body composition during the balance trial. Additionally, fractional rates of muscle protein synthesis (FSR) and degradation (FDR) during the balance trial were calculated. In both experiments, FDRs and FSRs increased with increasing protein intake ($P<.01$). FSRs also increased with increasing protein-free energy intake ($P<.001$) in both experiments. FDRs increased with increasing protein-free energy intake in Exp. 1 ($P<.10$) and in Exp. 2 ($P<.05$). It was concluded that the increased muscle protein deposition rates, induced by nutrient intake are accompanied by increased muscle protein turnover rates. Furthermore, urinary creatinine excretion was considered unreliable as estimator of muscle protein mass, in experiments where a large range of nutrient intakes is applied.

INTRODUCTION

Growing animals generally respond to increased feed intake by increasing their rates of gain of weight, protein and fat. Increased rates of gain, however, are often associated with decreased efficiencies. In preruminant calves, the efficiency of utilization of digestible protein decreases with an increasing protein deposition rate (Gerrits et al., 1996). Also, the energy costs per gram protein deposited seem to increase with increasing protein deposition rate (Millward, 1989; Reeds, 1991). Protein turnover has been reported to increase with increasing protein deposition rates in for example rats (Jepson et al., 1988) and lambs (Liu et al., 1995) and has an impact on the decreased efficiencies mentioned above.

Little information is available about the changes in muscle protein turnover rate with changes in protein deposition rate in fast growing calves. The main objective of this paper is to study the effect of protein and protein-free energy intake on the rate of muscle protein turnover in preruminant calves weighing either 120 or 200 kg.

Urinary excretion of 3-methylhistidine (3-MH) is often used as an index for muscle protein degradation in cattle. Differences in urinary 3-MH excretion can be due to either differences in muscle protein mass or to differences in the turnover rate per unit muscle tissue. In order to separate these effects, urinary 3-MH excretion has to be corrected for differences in muscle protein mass. The urinary creatinine excretion rate is often used for this correction (e.g. McCarthy et al., 1983; Williams et al., 1987; Morgan et al., 1993; Funaba et al., 1996). Alternatively, fractional degradation rates are computed, in which the muscle (protein) mass is often assumed a fixed proportion of live weight (LW) (Gopinath and Kitts, 1984; Jones et al., 1990; Smith et al., 1992; Funaba et al., 1996). A secondary objective of this study is to compare the urinary creatinine excretion as a measure for muscle mass with an estimation, based on chemical carcass analysis. The chemical carcass analysis of the calves, used in these experiments has been presented elsewhere (Gerrits et al., 1996).

EXPERIMENTAL PROCEDURES

Two experiments of similar design were performed with two weight ranges: 80 to 160 kg LW (Exp. 1) and 160 to 240 kg LW (Exp. 2). The experimental design and slaughter procedures have been presented elsewhere (Gerrits et al., 1996) and are summarized below.

Animals and experimental treatments

Ninety male, Holstein Friesian x Dutch Friesian calves were used in the two experiments. In Exp. 1 and 2, 8 and 10 calves were used for a reference group, respectively. In each experiment, 36 calves were assigned to one of 12 dietary treatments. The dietary treatments consisted of six protein intake levels at each of two protein-free energy levels (see Gerrits et al., 1996). The digestible protein-free energy intakes were 663 and 851 $kJ.LW^{-.75}.d^{-1}$ in Exp. 1 and 564 and 752 $kJ.LW^{-.75}.d^{-1}$ in Exp. 2. Digestible protein intakes ranged between .90 and 2.72 g $N.LW^{-.75}.d^{-1}$ in Exp. 1 and between .54 and 2.22 g $N.LW^{-.75}.d^{-1}$ in Exp. 2. Digestible energy intakes were kept constant on a protein-free basis. The ratio of energy intake from carbohydrates to energy intake from fat was maintained at around 1 in both experiments. The nutrient and ingredient composition of the milk replacers, as well as the realized nutrient intakes, averaged over the experimental

period, are presented by Gerrits et al. (1996). Both experiments were approved by the ethics committee of the TNO Nutrition and Food Research Institute, Zeist, The Netherlands.

Balance trial

At an average weight of 120 and 200 kg LW in Exp. 1 and 2, respectively, plastic bags were harnessed to all calves to allow quantitative, separate collection of urine and faeces. Urine was collected in buckets containing 50 ml 9 *N* H_2SO_4. After a 5-d adaptation period, faeces and urine were collected daily for 5 d, pooled per calf over the collection period and stored at -20°C pending analysis. In order to avoid effects due to differences in body weight of the calves, the balance trials in Exp. 1 and 2 were carried out in 3 and 4 separate periods, respectively. Calves were selected for the balance trial in the period in which their expected LW was closest to the target weight.

Body composition analysis

The calves were slaughtered at the beginning (reference groups) and at the end of each experiment (160 and 240 kg LW in Exp. 1 and 2, respectively). Body components were split into three fractions (carcass, organs, and hide + head + feed + tail), which were analysed on nitrogen, fat, dry matter and ash content, as described by Gerrits et al. (1996).

Urine analysis

Urine samples were analysed on creatinine by colorimetry, based on Peters (1942). Extinctions were read at 500 nm, 20 min after the addition of 5 ml picric acid reagent (18 m*M* picric acid, .28 *M* NaOH) to 20 μl centrifuged urine (1160 *g*, 15 min).

Urinary 3-MH concentrations were determined by HPLC. Urine samples were prepared by centrifugation (15 min, 10,000 *g*). The supernatant was centrifuged (3 h, 5000 *g*) through a cellulose membrane (Ultrafree-CL 10,000 NMWL, Millipore Co., Bedford, MA). After centrifugation, 0.5 ml of filtrate was mixed with 0.5 ml *tetra*borate buffer (40 m*M* NaOH, 18 m*M* $Na_2B_4O_7$, 2% methanol) prior to filling HPLC loading vials. Separation and quantification of 3-MH was performed using an anion exchange column (250x4 mm, CarboPac PA1, Dionex Corporation, Sunnyvale, CA) with a similar guard column (50x4 mm). Column temperature was maintained at 22°C and the injection volume of each sample was 10 μl. A three-

step gradient was used to elute the amino acids as follows: 100% *tetra*borate buffer, 6 min; linear change to 100% sodium acetate buffer (.4 *M* NaOAc, 1 m*M* NaOH, 2% methanol) in 10 min; 100% sodium acetate buffer, 5 min. The column was regenerated between each sample by elution with borate buffer (.56*M* NaOH, .65*M* H_3BO_3) for 10 min, followed by equilibration with *tetra*borate buffer for 11 min. Flow rate of the eluent was 1 ml/min. Postcolumn derivatization was performed with *o*-phthalaldehyde followed by fluorescence detection (Waters M470, Milford, MA) with emission set at 338 nm and excitation set at 468 nm. 3-Methylhistidine eluted at 7.2 min and was quantified using external calibration. To ensure proper peak identification and recovery, a known amount of a 3-MH standard (Sigma Chemical Co., St Louis, MO) was added to three urine samples, and analysed as described above. The recovery of the 3-MH added was 98 ± 1.0%.

Calculations

Measurement of urinary 3-MH excretion is a technique to quantify myofibrillar protein degradation and has been validated for use in cattle (Harris and Milne, 1981; McCarthy et al., 1983). It is assumed that the average rate of breakdown of mixed muscle proteins is similar to those of actin and myosin, and the term "muscle protein" is used throughout this paper. The fractional degradation rate of muscle protein (FDR, in $\%.d^{-1}$) was calculated by using the 3-MH excretion rates and estimations of 3-MH pool size, the latter based on chemical carcass analysis:

$$\text{FDR, } \%.d^{-1} = (\text{3-MH excretion rate, } mg.d^{-1}/\text{3-MH pool size, mg}) * 100 \quad [1]$$

The size of the 3-MH pool in muscle was calculated by multiplication of the muscle protein mass by the 3-MH content in muscle protein (594 mg/kg mixed muscle protein; Nishizawa et al., 1979). As previously reported (Gerrits et al., 1986), the protein deposition rates were affected by the experimental treatments. Consequently, differences in muscle protein mass might be present during the balance period. Therefore, muscle protein mass was estimated from the fat-free dry matter (FFDM) of the carcass using the following two equations [2,3] :

$$\text{FFDM in muscle} + \text{FFDM in bone} = \text{FFDM in carcass} \quad [2]$$

$$\text{ash in FFDM muscle} + \text{ash in FFDM bone} = \text{ash in FFDM carcass} \quad [3]$$

The respective amounts of ash and FFDM in carcass were measured at the end of

each experiment (at 160 and 240 kg LW in Exp. 1 and 2, respectively; Gerrits et al. (1996). The ash content in the FFDM of bone and muscle tissue is assumed fixed: 548 g/kg (Nour and Thonney, 1987) and 48 g/kg (Schulz et al., 1974), respectively. From these two equations, the FFDM in muscle and FFDM in bone tissue can be calculated, and the amount of protein in muscle and protein in bone tissue can be calculated by difference between FFDM and ash. Muscle protein mass of each calf at the start of the experiment was estimated using initial body weight and the average ratio of muscle protein mass to body weight of the respective reference group. The muscle protein mass of the calves during the balance trial was estimated by assuming a linear increase in muscle protein mass between the start and end of the experiments.

The accretion rate of muscle protein was calculated as the difference between muscle protein mass at the end and beginning of the experiment, divided by the length of the experimental period. The fractional accretion rate (FAR) was then calculated by division of the accretion rate by the estimated muscle protein mass during the balance trial. The fractional synthesis rate (FSR) was calculated by summation of FDR and FAR.

Statistical analysis

Three and five animals were excluded from the statistical analysis of the balance trial from Exp. 1 and 2, respectively, due to illness or diarrhea. For the number of animals used in each treatment see the legend of Figure 1.

The effects of protein and protein-free energy intake on 3-MH and creatinine excretion rates, muscle protein mass, the 3-MH/creatinine ratio and the FDR and FSR of muscle protein, measured in the balance trials were analysed using the following model [4]. Due to the experimental design, the balance trials in both experiments were carried out in several periods. Nevertheless, some variation in body weight of the calves at the time of the balance trial existed. Therefore, the actual weight of the calves at the time of the balance trial was included as a covariate.

$$y = \mu + E_i + \beta_1(W_j - \bar{W}) + \beta_2(X_j - \bar{X}) + \beta_{3i}(X_j - \bar{X}) + \epsilon_{ij} \qquad [4]$$

in which: y = dependent variable, μ = average intercept, E_i = fixed effect of protein-free energy intake level i, β_1 = effect of live weight; β_2 = effect of protein intake, β_{3i} = interaction between protein intake and protein-free energy intake level

i, W_j = live weight of calf j during the balance trial, $\bar{W}$ = 120 and 200 kg live weight in Exp. 1 and 2, respectively, X_j = digestible protein intake (in g N/d) of calf j during the balance trial, $\bar{X}$ = the average experimental digestible protein intake during the balance trial (in g N/d), ϵ_{ij} = error, i = 1, 2, j = 1 .. 18. All regression procedures were performed using SAS (SAS, 1989).

RESULTS

The effects of protein and protein-free energy intake on the excretion rates of 3-MH and creatinine, muscle protein mass, the ratio between 3-MH and creatinine excretion and on the estimated FDR and FSR of muscle protein during the balance trial of Exp. 1 and 2 are presented in **Table 1**.

The excretion rate of 3-MH increased with increasing digestible protein intake in both experiments, and was higher at the high protein-free energy intake level in both experiments, although this effect was not significant in Exp. 1. Both measures of muscle protein mass (i.e. creatinine excretion and the muscle protein mass, estimated from chemical carcass composition) increased or tended to increase with protein intake in both experiments. However, the effects of protein-free energy intake on these measures of muscle protein mass were not similar. Muscle protein mass, estimated from chemical carcass composition tended to be higher ($P<.10$) at the low protein-free energy intake level in Exp. 1, but similar for both protein-free energy intake levels in Exp. 2. The creatinine excretion rates, however, were higher at the low protein-free energy intake in Exp. 1, while in Exp. 2, the opposite effect was found.

The 3-MH excretion rate, corrected for differences in muscle protein mass by division by the creatinine excretion rate (3-MH/creatinine ratio), did not vary with protein intake in either experiment. In Exp. 1, protein-free energy intake increased the 3-MH/creatinine ratio, whereas in Exp. 2, this ratio was unaffected by protein-free energy intake. Correcting the 3-MH excretion rate by dividing it by the 3-MH pool size, calculated from carcass analysis (FDR) resulted in a more consistent picture of treatment effects in the two experiments. The FDRs did not parallel the 3-MH/creatinine excretion ratios in either experiment. In both experiments, parallel increases in FDR and FSR occurred with increasing protein intake ($P<.01$), but also with increasing protein-free energy intake (see **Figure 1**; Exp. 1, $P<.10$; Exp. 2, $P<.05$).

Table 1

Effect of apparent faecal digestible protein intake (in g N/d) and protein-free- energy intake on urinary excretion of 3-methylhistidine (3-MH) and creatinine, on estimated muscle protein mass and on the fractional degradation and synthesis rates (FDR, FSR) of muscle protein.

	Effect protein-free energy intake[a]				Effect protein intake	Interaction(.10^2)[b]			
Item[c]	LE	P LE=HE[d,e]	HE	sem[f]	P[g]	LE	P LE=HE[d,h]	HE	sem[f]
Experiment 1: 120 kg LW									
3-MH excretion (mg/d)	81	ns	85	3.8	***	39	ns	39	11.8
creatinine excretion (g/d)	4.56	*	4.24	.145	***	1.5	ns	1.1	.46
muscle protein (kg N)	1.78	ns[†]	1.74	.024	**	.20	ns	.18	.075
3-MH/creatinine (mg/g)	17.6	*	19.6	.99	ns	2.4	ns	4.1	3.1
FDR (%/d)[i]	1.22	ns[†]	1.31	.056	**	.4	ns	.4	.18
FSR (%/d)[i]	2.06	***	2.27	.056	***	1.1	ns	1.2	.18
Experiment 2: 200 kg LW									
3-MH excretion (mg/d)	115	*	124	3.4	***	67	*	30	12.9
creatinine excretion (g/d)	6.74	**	7.64	.261	***	2.5	ns	2.5	.87
muscle protein (kg N)	2.81	ns	2.80	.027	ns[†]	.16	ns	.09	.088
3-MH/creatinine (mg/g)	17.2	ns	16.5	.56	ns	4.6	*	-1.6	1.86
FDR (%/d)[i]	1.10	*	1.19	.035	***	.6	*	.2	.12
FSR (%/d)[i]	1.52	***	1.73	.044	***	1.0	ns[†]	.7	.14

[a]LE and HE = low and high protein-free energy intake level, respectively; Values represent intercept at the average digestible protein intake (69.3 and 73.8 g N/d in Exp. 1 and 2, respectively);

[b]Difference in regression coefficients, associated with protein intake (in g N/d) between LE and HE. Values multiplied by 10^2;

[c]Units in this column apply to column 2, 4 and 5 only;

[d]ns = P>.10; ns[†] = P<.10; * = P<.05; ** = P<.01; *** = P<.001;

[e]Probability for test on protein-free energy effect;

[f]Standard error of the mean;

[g]Probability for test if the regression coefficient, associated with digestible protein intake, averaged over LE and HE = 0;

[h]Probability for test if regression coefficients, associated with digestible protein intake, are equal for LE and HE;

[i]FDR calculated from 3-MH data according to equation [1] in text; FSR calculated as FDR + FAR (fractional accretion rate) the latter estimated based on chemical carcass analysis (see text).

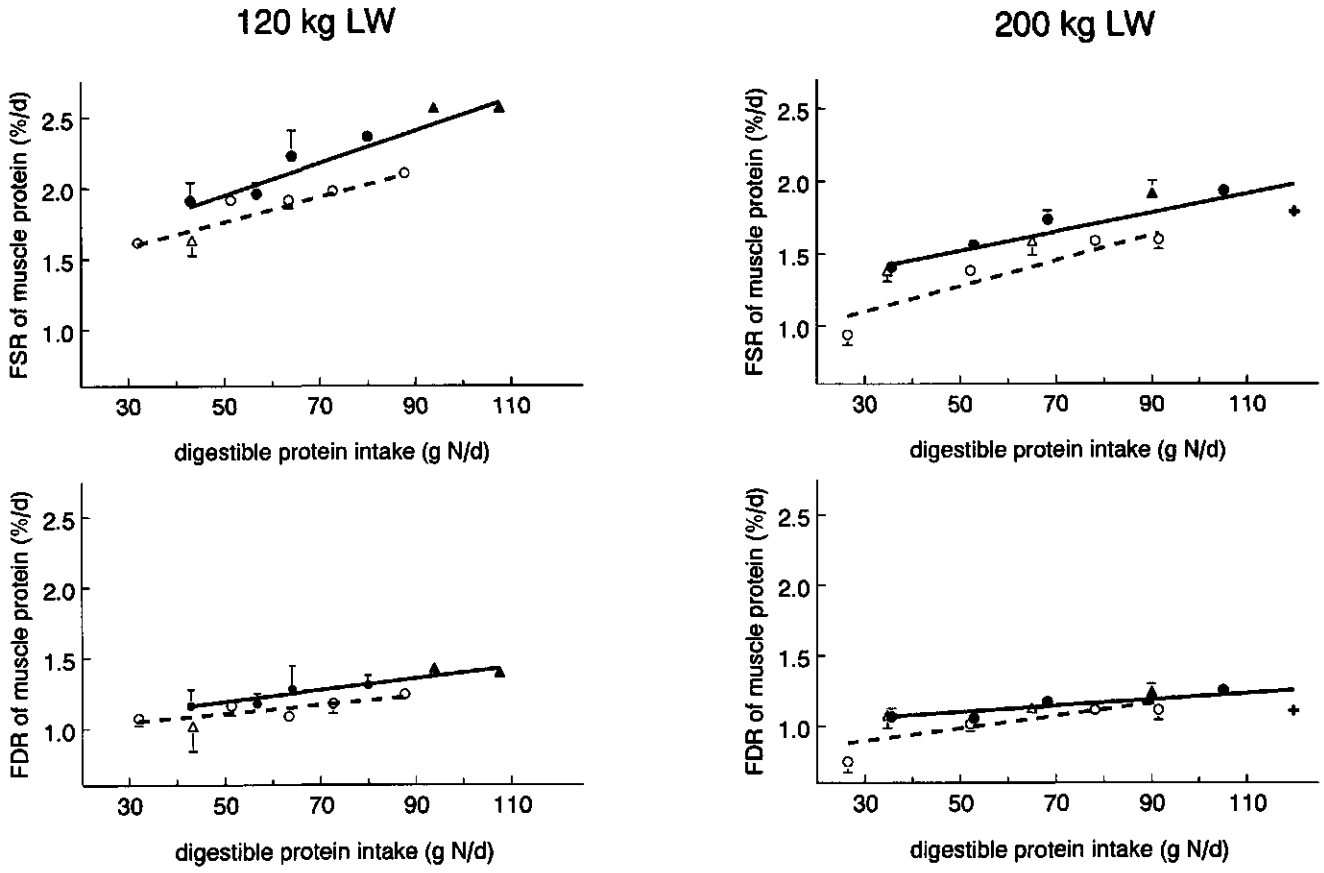

Figure 1. Response of fractional synthesis rates (FSR) and fractional degradation rates (FDR) of muscle protein to digestible protein intake at two protein-free energy intake levels at two different live weights (LW). High energy intake level (—, ●, ▲, +) and low energy intake level (- - -, ○, Δ). Values are means ± SEM, n = 3 (●, ○), n = 2 (▲, Δ) or n = 1 (+).

Calves at the lowest protein intake level at the low protein-free energy intake in Exp. 2 showed remarkably low 3-MH excretion rates (75 mg/d ±7.5; n=3). This also affected the 3-MH/creatinine ratio, the FSRs and the FDRs (see Figure 1), causing the interaction between protein and protein-free energy intake on these measures in Exp. 2. When this group was omitted from the statistical analysis, these interaction terms were not significant anymore ($P>.10$).

DISCUSSION

Urinary 3-Methylhistidine excretion

Urinary 3-MH is frequently used in cattle as an indicator for muscle protein degradation. The contributing sources other than skeletal muscle (e.g. the gastro-intestinal tract, skin) have not been properly quantified in bovine. Recently, Van den Hemel-Grooten (1996) showed that in pigs, the contribution of the gastro-intestinal tract to total 3-MH production was less than 6%. In cattle, contributing sources other than skeletal muscle are often assumed to be of minor quantitative importance, based on Nishizawa et al. (1979) and Harris (1981). The urinary 3-MH excretion rates, found in the present experiments (.4 to .8 $mg.LW^{-1}.d^{-1}$) are in a similar range as those found by others for a similar type of cattle (Harris and Milne, 1981; Williams et al., 1987; Funaba et al., 1996).

Methods of correction of urinary 3-MH for muscle protein mass

As stated previously, urinary 3-MH excretion rates have to be corrected for muscle protein mass in order to separate the effect of mass from the effect of the activity of muscle tissue. This correction was made by using either the urinary creatinine excretion rates (referred to as the "creatinine method") or by estimating muscle protein mass from chemical carcass analysis (referred to as the "carcass method"). The calculated FDRs (based on the carcass method) and urinary 3-MH to creatinine ratio (based on the creatinine method) were expected to respond similarly to the experimental treatments. However, protein intake increased the FDRs, while it did not affect the urinary 3-MH to creatinine excretion ratio (Table 1). This implies that the correction of the 3-MH excretion rate for muscle protein mass by the creatinine method is stronger than correction based on the carcass method. This difference can be due to errors in either method.

The analysis, commonly used for determination of creatinine, based on

Jaffé's reaction, is not entirely specific (see Spencer, 1986). Moreover, there are indications that the creatinine excretion partly reflects the nutritional status of the animal. According to Spencer (1986), creatinine excretion rate is dependent on protein intake. Also, Van den Hemel-Grooten (1996) found increased plasma creatinine levels in growing pigs, when fed a protein-free diet, compared with normally fed controls. They argued that the increased FDR of muscle protein at their protein-free treatment may have been partly responsible for the increased plasma creatinine levels.

Using the carcass analysis for the estimation of the size of the 3-MH-pool as described earlier assumes that (i) the 3-MH content of muscle protein is independent of nutritional input; (ii) carcass comprises bone and muscle tissue only, and (iii) muscle mass increases linearly between beginning and end of both experiments. Assumptions (i) and (iii) are probably valid, but assumption (ii) is doubtful. As discussed by Williams (1978), up to 30% of carcass protein is present in collagen tissue. Neglecting protein in connective tissues leads to an overestimation of the 3-MH pool and thus to underestimation of FDRs of muscle proteins. Considering the function of connective tissues, it is likely that the proportion of carcass protein, present in connective tissues is closely related to the bone proportion. In the separation of carcass protein into bone and muscle protein, a changed muscle to bone ratio with experimental treatments is taken into account. Therefore, the underestimation of the FDRs is likely to be independent of the experimental treatments. Assuming that muscle protein as estimated in this paper, consists, like carcass protein, for 30% of collagen protein, the average experimental FDRs would be increased from 1.27 to 1.81 $\%.d^{-1}$ and from 1.15 to 1.64 $\%.d^{-1}$ in Exp. 1 and 2, respectively.

In conclusion, the carcass method leads to estimations of muscle protein mass which may be too low, but are independent of the experimental treatments. Therefore, this method is preferred above the creatinine method.

Muscle protein turnover as affected by dietary treatments

There is little information on the separate effects of protein and protein-free energy intakes on the FDR and FSR of muscle protein in preruminants. In the present experiments, muscle protein turnover shows a similar response to nutrient intake as the protein deposition rates of these calves (Gerrits et al., 1996). Pearson correlation coefficients between the FDRs and the reported protein deposition rates are .54 ($P<.001$) and .65 ($P<.001$) in Exp. 1 and 2, respectively. In beef cattle,

several researchers found increased FDRs in response to increased feed intakes, measured by the 3-MH method, but often fail to reach a level of statistical significance. Smith et al. (1992) found increased FDRs of muscle protein when feed intake increased from 0.8 to 1.7 x ME intake for maintenance (MEm) in growing cattle. However, when feed intake was increased further to 2.1 x MEm, FDRs dropped. Jones et al. (1990) gave cattle of about 300 kg LW during 80d either restricted (LW gain .65 kg/d) or ad libitum access (LW gain 1.25 kg/d) to feed. They found the FDRs of the ad libitum group on average .7 %/d higher compared with the restricted group. This difference however, was statistically not significant, which is surprising, considering the large contrast between these dietary treatments. Possibly, the short urine collection periods (24h) in these experiments contributed to the large experimental error.

Increased rates of protein turnover with increasing growth rates have been reported in several species, e.g. rats (Jepson et al., 1988), lambs (Liu et al., 1995) and pigs (Reeds et al., 1980). In most cases, the increased growth rates were induced by increased protein rather than protein-free energy intakes, probably because by increasing substrate availability, protein deposition rates can be easily increased in most species, provided that energy is not limiting protein retention. In the present experiments, however, increased protein-free energy intakes also stimulated whole body protein deposition rates, and distinct protein and energy dependent phases in the deposition of protein could not be shown (Gerrits et al., 1996). In line with the whole body data, fractional muscle protein accretion rates were also increased at the high protein-free energy intake in both experiments (results not presented). It is therefore interesting to note that these increased deposition rates, obviously not caused by increased substrate availability, are accompanied by higher muscle protein turnover rates (Table 1). These findings are in contrast with the suggestion of Reeds et al. (1981) for growing pigs, that increased carbohydrate and fat intakes increase protein synthesis but not degradation rates.

In conclusion, these experiments show (i) that urinary creatinine excretion is unreliable as indicator for muscle protein mass in experiments where a wide range of nutrient intakes is applied. Furthermore, (ii) increased muscle protein deposition rates, caused by either increased protein or protein-free energy intakes are accompanied by increased fractional synthesis and degradation rates of muscle protein.

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6 EFFECT OF PROTEIN AND PROTEIN-FREE ENERGY INTAKE ON PLASMA CONCENTRATION OF INSULIN-LIKE GROWTH FACTOR-1 AND THYROID HORMONES IN PRERUMINANT CALVES OF 80 TO 240 KG LIVE WEIGHT

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Effect of Protein and Protein-Free Energy Intake on Plasma Concentration of Insulin-like Growth Factor-1 and Thyroid Hormones in Preruminant Calves of 80 to 240 kg Live Weight

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Abstract

Two experiments were conducted with preruminant calves weighing from 80-240 kg to study the long-term nutritional regulation of circulating insulin-like growth factor-1 (IGF-1), thyroxine (T_4) and triiodothyronine (T_3). The two experiments were similar in design, but performed with two live weight ranges: 80 to 160 kg (Exp. 1) and 160 to 240 kg (Exp. 2). In each experiment, 36 calves were allocated to one of twelve dietary treatments, which consisted of six protein intake levels at each of two protein-free energy intake levels. Blood samples were taken 5-6 h post feeding once every fortnight, until the calves reached their target weight. In Exp. 1, plasma IGF-1, and in both experiments, plasma T_4 levels increased with increasing protein intake ($P<.01$), but were unaffected by protein-free energy intake ($P>.10$). In both experiments, plasma T_3 levels were markedly higher at the high protein-free energy intake level ($P<.01$) and increased slightly with increasing protein intake in Exp. 1 ($P=.19$) and in Exp. 2 ($P<.01$). It was concluded that the effect of protein intake on plasma T_3 can be a direct effect of the increased amount of T_4, produced by the thyroid gland. The effect of protein-free energy intake on plasma T_3 is probably due to peripheral thyroid hormone metabolism, rather than to the actual thyroid gland function.

INTRODUCTION

Hormones of the somatotropic and thyroid axes have key-roles in the partitioning of nutrients over body components during growth (Millward, 1989). Circulating levels of these hormone, in turn, are to a large extent regulated by nutrition. Therefore, knowledge about the interaction between nutrient intake and hormone production is required to advance in the field of the hormonal regulation of growth. Insulin-like growth factor-1 (IGF-1) and thyroid hormones are of particular interest in the long-term nutritional regulation of nutrient partitioning. They have an important role in nutrient partitioning (Gluckman et al., 1987; Millward, 1989) and because plasma concentrations of these hormones are relatively constant over the day (Hammond et al., 1984; Gluckman et al., 1987), they do not require frequent blood sampling. In preruminant calves, essentially no information is available about the long-term nutritional regulation of circulating thyroid hormones and IGF-1. Furthermore, in functional ruminants, there is confusion about the role of specific macro nutrients in the regulation of hormone function (Gluckman et al., 1987). The objective of this study was to clarify the long-term nutritional regulation of plasma

concentrations of IGF-1, T_3 and T_4 in preruminant calves weighing from 80 to 240 kg. The rates of protein and fat deposition of these calves, measured in these experiments have been presented elsewhere (Gerrits et al., 1996).

EXPERIMENTAL PROCEDURES

Two experiments of similar design were performed with two live weight (LW) ranges: 80 to 160 kg (Exp. 1) and 160 to 240 kg (Exp. 2). The experimental design has been presented elsewhere (Gerrits et al., 1996) and is summarized below.

Animals, housing and experimental treatments

Seventy-two male, Holstein Friesian x Dutch Friesian calves were used in the two experiments. In each experiment, 36 calves were assigned to one of 12 dietary treatments. The dietary treatments consisted of six protein intake levels at each of two protein-free energy intake levels (see Gerrits et al., 1996). The digestible protein-free energy intakes were 663 and 851 $kJ.LW^{-.75}.d^{-1}$ in Exp. 1 and 564 and 752 $kJ.LW^{-.75}.d^{-1}$ in Exp. 2. Digestible protein intakes ranged between .90 and 2.72 g $N.LW^{-.75}.d^{-1}$ in Exp. 1 and between .54 and 2.22 g $N.LW^{-.75}.d^{-1}$ in Exp. 2. Digestible energy intakes were kept constant on a protein-free basis, and the ratio between energy intake from carbohydrates and fat was maintained at around 1 in both experiments. The nutrient and ingredient composition of the milk replacers, as well as the realized nutrient intakes, averaged over the experimental period, are presented by Gerrits et al. (1996). Blood samples were taken from all calves 5-6 hours post feeding from the jugular vein the day before the start of each experiment (representing day 0) and subsequently every 14 days. Blood samples of about 8 ml were collected in heparinized vacuum tubes, and stored on ice. Blood samples were centrifuged (15 min, 1160 *g*, 4 °C), plasma was removed and stored at -20°C.

Plasma hormone analyses

Plasma concentrations of triiodothyronine (T_3) were measured by radio immunoassay using a commercially available T_3 antiserum (Mallinckrodt) in combination with a specific tracer (1m321, Amersham). The plasma thyroxine (T_4) concentrations were assayed by using a tracer from Amersham and a laboratory raised rabbit T_4 antiserum. This T_4 antiserum had a 0.16% cross reactivity with T_3. Plasma IGF-1 levels were measured with a heterologous radio immunoassay

(Huybrechts et al., 1989).

Statistical analysis

One and three animals had to be excluded from the statistical analysis of Exp. 1 and 2, respectively, because of illness. For the number of animals used in each treatment see the legend of Figure 2. Plasma hormone concentrations were measured repeatedly during both experiments. The separation of time from treatment effects is illustrated in Figure 1. A straight line was fitted through plasma hormone concentrations against time, starting with the measurement on day 14. The existence of a quadratic component in this relationship was tested, but found significant in only a few animals (≤3 for all hormones tested in Exp. 1 or Exp. 2) and was therefore neglected. The slope of the straight line (α, see **Figure 1**) was considered to represent the effect of time (t) and the intercept (at t = $\bar{t}$) the average plasma hormone concentration during the respective experiment. Both slope and intercept were subsequently treated as dependent variables. The effects of protein and protein-free energy intake on slope and intercept were analysed, including the hormone concentration at day 0 as a covariate:

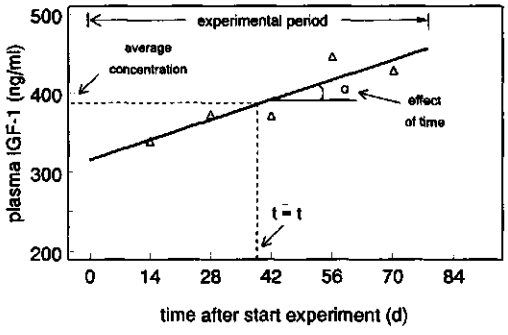

Figure 1. Example of separating time from treatment effects on plasma hormone concentrations. The individual measurements of IGF-1 in samples, taken once every fortnight, are presented for one animal (Δ). A straight line is fitted to these data. The slope of the line (α) represents the effect of time. The average concentration is read at the time, averaged between the start of the experiment and the time of slaughter of the animal (t = $\bar{t}$).

$$y = \mu + E_i + \beta_1(C_{0j} - \bar{C}_0) + \beta_2(X_j - \bar{X}) + \beta_{3i}(X_j - \bar{X}) + \epsilon_{ij} \quad [1]$$

in which: y = dependent variable, μ = average intercept, E_i = fixed effect of protein-free energy intake level i, β_1 = effect of initial hormone concentration, β_2 = effect of digestible protein intake, β_{3i} = interaction between protein intake and protein-free energy intake level i, C_{0j} = hormone concentration of calf j at the start of the experiment (day 0), $\bar{C}_0$ = average experimental hormone concentration at

the start of the experiment, X_j = digestible protein intake (in g N/d) of calf j during the experiment, $\bar{X}$ = the average experimental digestible protein intake (in g N/d), ϵ_{ij} = error, i = 1, 2, j = 1 .. 18. All regression procedures were performed using SAS (SAS, 1989).

While the experiments were designed to cover a fixed weight range, blood samples were taken at fixed time intervals (14 d). Consequently, the number of blood samples taken varied with the rate of weight gain of the animals. A preliminary analysis was performed for each experiment, using the plasma hormone concentrations, measured on day 14, 28 and 42 only. As this analysis led to similar conclusions as the analysis described above, the results are not presented.

RESULTS

Changes of plasma hormone concentrations with time

The changes of circulating plasma hormone concentrations with time are presented in **Table 1**. In Exp. 1, plasma IGF-1 increased with time. Unfortunately, samples of Exp. 2 could not be analysed on IGF-1. Furthermore, in both experiments, plasma T_3 and T_4 decreased with time. However, the decrease of T_4 in Exp. 2 was not significant. Although a direct statistical comparison cannot be made, comparison of the mean T_3 and T_4 concentrations between experiments (see Figure 2 and Table 2), stresses the decrease in these plasma hormones with time.

The reported changes with time were unaffected by protein and protein-free energy intake ($P>.05$), except in Exp. 1, where the reported increase in IGF-1 with time increased with protein intake ($P<.01$). No interactions between protein and protein-free energy intakes were found.

Response of plasma hormone concentrations to nutrient intake

The effects of protein and protein-free energy intake on the average plasma concentration of IGF-1, T_4 and T_3 (Exp. 1), and on T_4 and T_3 (Exp. 2) are presented in **Table 2** and graphically presented in **Figure 2**. In Exp. 1, the IGF-1 concentration increased with increasing protein intake, but was unaffected by protein-free energy intake ($P>.25$). In both experiments, plasma T_4 increased with increasing protein intake, but was unaffected by protein-free energy intake ($P>.10$). Plasma T_3 was markedly increased at the high protein-free energy intake in both experiments. In Exp. 2, T_3 increased ($P<.01$) with protein intake, whereas in Exp. 1, this effect was

not significant (P = .19).

DISCUSSION

Several studies have been done investigating the effect of nutrient intake on muscle protein metabolism and plasma hormone concentrations. Often, nutrient intakes are varied by fixing the feed intake and varying the diet composition, or the other way around. Alternatively, protein intake is varied isocalorically. These methods inevitably lead to changes in the composition of the energy intake. This was avoided in the design of this study, in which the composition of the protein-free energy intake was kept constant.

Changes of plasma hormone concentrations with time

The increase of plasma IGF-1 with time has been shown before in preruminant calves above 6 weeks of age (Breier et al., 1988; Hostettler-Allen et al., 1994) and is in agreement with Steele and Elsasser (1989) who stated that plasma IGF-1 typically increases during the first phase of growth. This increase, however, may have been partly due to the concomitant increase in protein intake with increasing live weight. In the present experiments, protein intakes increased linearly with $LW^{.75}$. The decrease in plasma T_3 with time in both experiments is in agreement with the results of Doppenberg and Palmquist (1991), obtained with

Table 1

Change of plasma concentrations of insulin-like growth factor-1 (IGF-1), triiodothyronine (T_3) and thyroxine (T_4) in experiment 1 and of plasma concentrations of T_3 and T_4 in experiment 2 with time ($ng.ml^{-1}.d^{-1}$)

Hormone		P[a,b]	sem[c]
Experiment 1			
IGF-1	1.55	***	.232
T_3	-.0038	**	.00123
T_4	-.162	*	.0737
Experiment 2			
T_3	-.0028	**	.00090
T_4	-.037	ns	.0596

[a]Probability for test if regression coefficient of hormone concentration, associated with time, averaged over the respective experiment = 0;
[b]ns = P>.05; * = P<.05; ** = P<.01; *** = P<.001;
[c]Standard error of the mean;

preruminant calves in a similar live weight range. The decrease of plasma T_4 with time, however, is in contrast with the reported increase by Doppenberg and Palmquist (1991). This difference may be related to differences in feeding schedule between the study of these authors and the present experiments. Doppenberg and Palmquist increased feed intake linearly with LW (dry matter fed at about 2% of LW), whereas in the present experiments, feed intakes increased linearly with $LW^{.75}$.

Response of plasma hormone concentrations to nutrient intake

Insulin-like growth factor-1. Plasma IGF-1 concentrations have been shown sensitive to feed intake in several species (Underwood et al., 1991), specifically so in ruminants (Elsasser et al., 1989; McGuire et al., 1992). A specific role of protein

Table 2

Effect of apparent faecal digestible protein intake (in g N/d) and protein-free energy intake on the plasma concentrations of insulin-like growth factor-1 (IGF-1), triiodothyronine (T_3) and thyroxine (T_4) in experiment 1 and on plasma concentrations of T_3 and T_4 in experiment 2.

	Effect protein-free energy intake[a]				Effect protein intake	Interaction[b]			
Hormone	LE ng/ml	P LE=HE[c,d]	HE ng/ml	sem[e]	P[f]	LE	P LE=HE[d,g]	HE	sem[e]
Experiment 1: 80-160 kg LW									
IGF-1	320	ns	305	9.4	***	2.56	ns	2.11	.486
T_3	2.01	***	2.52	.051	ns	.0028	ns	.0021	.0026
T_4	77	ns	82	2.6	**	.234	ns	.416	.140
Experiment 2: 160 - 240 kg LW									
T_3	1.45	**	1.70	.049	**	.0046	ns	.0037	.0020
T_4	45	ns	49	2.0	**	.182	ns	.175	.084

[a]LE and HE = low and high protein-free energy intake level, respectively; Values represent intercept at the average digestible protein intake (63.7 and 66.9 g N/d in experiment 1 and 2, respectively);

[b]Difference in regression coefficients, associated with digestible protein intake (in g N/d), between LE and HE;

[c]Probability for test on effect of protein-free energy level;

[d]ns = P>.05; * = P<.05; ** = P<.01; *** = P<.001;

[e]Standard error of the mean;

[f]Probability for test if the regression coefficient, associated with digestible protein intake, averaged over protein-free energy intake levels = 0;

[g]Probability for test if regression coefficients, associated with digestible protein intake, are equal for LE and HE.

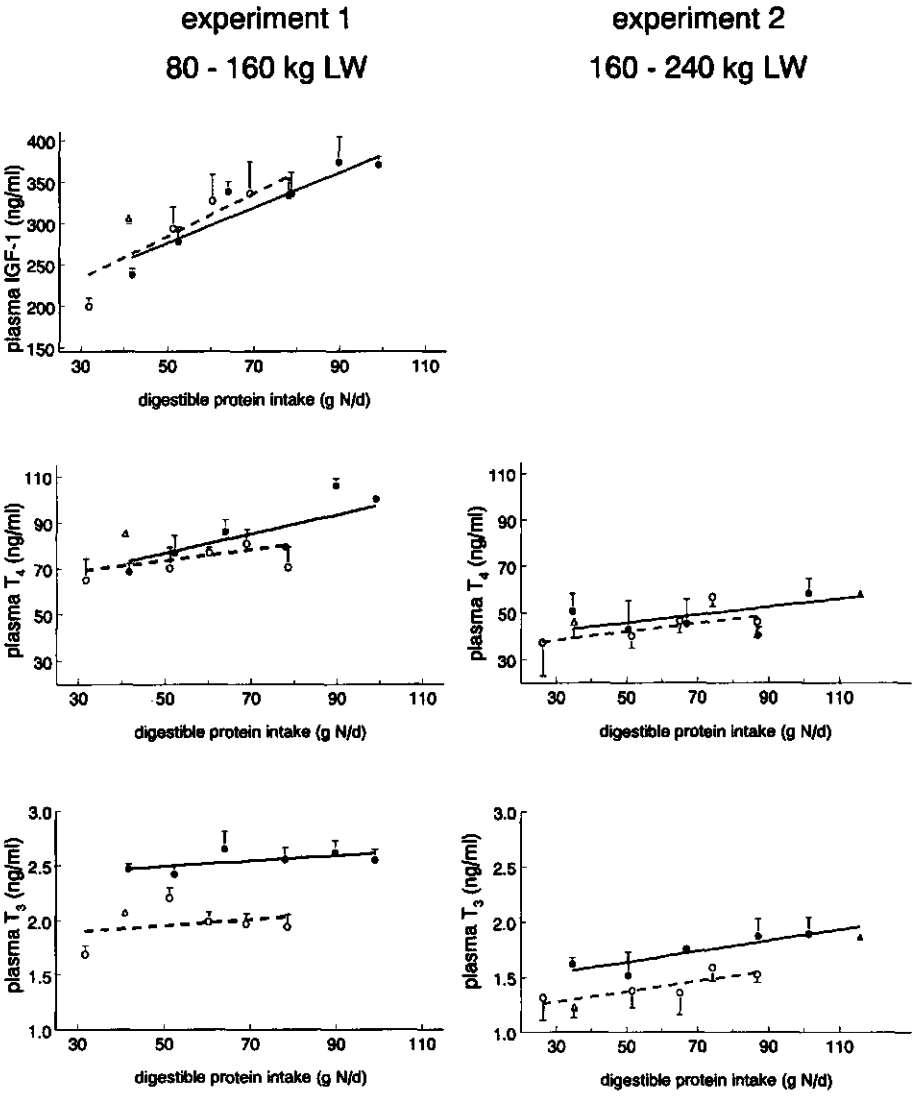

Figure 2. Response of plasma concentrations of insulin-like growth factor-I (IGF-1), triiodothyronine (T_3) and thyroxine (T_4) to digestible protein intake at two protein-free energy intake levels in two different live weight (LW) ranges. High energy intake level (—, ●, ▲) and low energy intake level (- - -, O, Δ). Values are means ± SEM, n = 3 (●, O), n = 2 (Δ) or n = 1 (▲).

intake in the regulation of circulating IGF-1 has been described in rats (Clemmons and Underwood, 1991; Dardevet et al., 1991) and in pigs (Campbell et al., 1990). However, there are reports that have failed to confirm this effect (Sève et al., 1993). Adequate energy intake has been suggested a prerequisite for the response of IGF-1 to dietary protein (Campbell et al., 1990, Clemmons and Underwood, 1991). In functional ruminants, there is confusion about the specific effects of protein and energy on circulating IGF-1 (McGuire et al., 1992; Elsasser et al., 1989). Lack of data on the long-term nutritional regulation of IGF-1 and thyroid hormones in preruminant calves negates the possibility of making a direct comparison between the present results and published information. Comparison of the present results with a study about the nutritional regulation of postprandial IGF-1 production (Coxam et al., 1989) suggests a clear difference between the short-term and the long-term regulation of plasma IGF-1. Coxam et al. (1989) studied the effect of amino acid infusion (physiological dose in the mesenteric vein for 3h) on either plasma IGF-1 levels or hepatic IGF-1 production during the infusion period or 3h thereafter. They did not find any change in either the hepatic IGF-1 production or the plasma IGF-1 level. Based on the results of infusion of chylomicrons, they suggested a role of dietary fat on the regulation of hepatic IGF-1 production. This contradicts the findings of the present study and may illustrate the difference between the short- and long-term nutritional regulation of IGF-1. However, it may be questioned whether the maximum response of IGF-1 to amino acid infusion in the study of Coxam et al. (1989) is reached within 6 hours post administration (Gluckman et al., 1987).

The results of the present experiments may illustrate the nutritional regulation of plasma IGF-1 in functional ruminants as well. The confusion about the role of protein and energy (Gluckman et al., 1987) may be partly cleared by the experimental design and the large contrasts in nutrient intakes in the present study. The latter is more difficult to achieve in functional ruminants. However, before extrapolating the results of this study to functional ruminants, it is important to consider the possible role of insulin, which differs considerably between preruminant and ruminant calves of similar age (Breier et al., 1988). Furthermore, it would be interesting to clarify the role of dietary fat and volatile fatty acids in the nutritional regulation of IGF-1 in functional ruminants.

Thyroid hormones. While T_4 is the major secretory product of the thyroid gland, T_3 is the biologically active form. Although never properly quantified in ruminants, the conversion of T_4 into T_3 by type I-deiodinase in liver and kidney is likely to be an important source of circulating T_3. It has been suggested that liver, heart and muscle tissue depend on this source of T_3 (Danforth and Burger, 1989).

In several species, plasma T_3 has been shown sensitive to dietary restriction (steers, Elsasser et al., 1989; rats, Jepson et al., 1988) especially when carbohydrate intake is reduced (see Danforth and Burger, 1989). Protein intake does not affect plasma T_3 in rats (Jepson et al., 1988) and growing steers (Hammond et al., 1984). Hammond et al. (1984) found plasma T_4 to increase with increasing protein intake in growing steers of 240 kg LW. However, these effects may have been confounded with energy intake. In preruminant calves, there appears to be no published information on the nutritional regulation of plasma T_3 and T_4 concentrations. From the present experiments, it is clear that plasma T_4 is affected by protein intake only. Furthermore, protein-free energy intake is responsible for the largest part of the variation in plasma T_3 concentrations. Increasing protein intake also increased plasma T_3 in Exp. 2, and not significantly in Exp. 1. It remains to be determined whether dietary carbohydrates or fats are responsible for the protein-free energy effect on T_3. Several authors attribute an important role to dietary carbohydrates (see Danforth and Burger, 1989). However, in rats, dietary fat restriction at equal protein and carbohydrate intakes was shown to depress both serum T_3 and T_4 concentration (Schalch and Cree, 1985). Doppenberg and Palmquist (1991) did not find differences in plasma concentrations of either T_3 or T_4 when dietary fat was exchanged isocalorically with lactose in preruminant calves of 160 kg LW. However, in their experiment, calves at the high lactose intake showed signs of severe insulin-resistance.

The effect of protein intake on plasma T_3 can be a direct effect of the increased amount of T_4, produced by the thyroid gland in Exp. 2 and, although the effect on T_3 was not significant, possibly also in Exp. 1. In contrast, the effect of protein-free energy intake is probably due to peripheral thyroid hormone metabolism rather than to the actual thyroid gland function. According to Rudman (1989) and Danforth and Burger (1989), the conversion of T_4 into T_3 by type I deiodinase in the liver is a carbohydrate-dependent process. Alternatively, is it is possible that the increase in T_3 is caused by an increase in circulating thyroid-binding proteins, because the synthesis and secretion of these proteins by the liver has been shown to depend on carbohydrate intake (Danforth and Burger, 1989). In order to study the

mechanisms involved, it would be interesting to study both total and plasma-free T_3 concentrations in combination with reverse-T_3, the latter being a sensitive indicator for type I deiodinase activity.

Relationship between plasma hormones and tissue gain

Plasma concentrations of IGF-1 and T_3 merely reflect the hepatic production of these hormones. Several tissues have been shown capable of producing IGF-1 (e.g. muscle and adipose tissue; Brameld et al., 1996), or convert T_4 into T_3 (for example kidney and brain tissue; Schröder-Van der Elst and Van der Heide, 1992). Furthermore, nutritional influences on hormone actions are in part mediated through changes in the numbers and/or affinity of receptors (Gluckman et al., 1987). Despite these limitations, it is assumed that changes in the plasma concentrations of these hormones represent a large proportion of the total IGF-1 and T_3 mediated action.

It is difficult to determine whether differences in plasma concentrations of IGF-1, T_3 and T_4 actually drive nutrient partitioning or reflect changes in metabolism, directly caused by nutrition or by other hormonal parameters. Nevertheless, it is important to compare the relationships between nutrient intake and plasma hormones with the relationships between nutrient intake and tissue deposition rates. Nutrient partitioning was measured in the present experiments, and protein and fat deposition rates have been presented by Gerrits et al. (1996). Briefly, the calves responded to increased protein intake by increasing their protein and fat deposition rates. Extra protein-free energy intake mainly resulted in extra fat deposition, but also increased the protein deposition rate, even at low protein intakes.

The effects of protein and protein-free energy intake on the rate of protein deposition are probably caused by different mechanisms. The observed increase in the rate of protein deposition with increasing protein intake corresponds with the effect of protein intake on plasma IGF-1, suggesting that these effects are related. The effect of protein-free energy intake on protein deposition, however, is not related to plasma IGF-1. This effect, which is clearly not caused by an increased substrate supply, may be related with thyroid hormone metabolism. It could, for instance, be related to the reported involvement of T_3 in the transcription phase in protein synthesis (Millward, 1989) or to improved transport of nutrients (glucose, amino acids) into cells (Danforth and Burger, 1989). The increased muscle protein

turnover rates with increased protein-free energy intake, measured in these experiments (Chapter 5) emphasizes the increased cellular energy demand.

It is realized, however, that the relationships suggested here may be confounded by interrelationships between IGF-1 and thyroid hormones. Triiodothyronine plays a permissive role in the release of IGF-1 (Kühn et al., 1990), but when administered on top of the normal T_3 production, it depresses the response of IGF-1 to growth hormone administration and consequently the rate of protein deposition (Elsasser et al., 1993).

In conclusion, these experiments show distinct effects of protein and protein-free energy intake on plasma hormone concentrations. Plasma IGF-1 increased with protein intake, while plasma T_3 mainly responded to protein-free energy intake. The small effect of protein intake on plasma T_3 can be a direct effect of the increased amount of T_4, produced by the thyroid gland. In contrast, the effect of protein-free energy intake is probably due to peripheral thyroid hormone metabolism, rather than to the actual thyroid gland function.

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GENERAL DISCUSSION

INTRODUCTION

The main objectives of the previous chapters were to gain an insight into the partitioning of nutrients in the body of preruminant calves, and to develop a tool for the development of feeding strategies for these calves. After designing a prototype model, two experiments were conducted to gather data necessary for model parameterization (Chapter 1). The model is described in Chapter 2 and bears the name SIMON (SImulation of Metabolism Of Nutrients in preruminants). Model performance is evaluated in Chapter 3 and application of the model for the development of feeding strategies is presented in Chapter 4. By integrating protein and energy metabolism, the model contributes to an increased understanding of nutrient partitioning in preruminant calves. The additional measurements performed in the experiments described in chapter 1 revealed that increased growth rates in calves are accompanied by increased muscle protein turnover rates (Chapter 5). Also, the nutritional regulation of circulating thyroid hormones and insulin-like growth factor 1 was studied (Chapter 6). This, however, is only the tip of the iceberg as far as the complex nutritional regulation of these hormones is concerned.

This discussion focuses not on the individual preceding chapters, but on gaps in knowledge in veal calf nutrition, on the modelling approach applied in SIMON and on possibilities to improve SIMON. It consists of three parts. First, the metabolic fate of amino acids is discussed, as, from a nutritional point of view, it appears to be one of the major limitations for efficient veal production. The second part of this discussion focuses on the model, with attention being paid to (i) general considerations on the approach, *(ii)* some of the basic choices made, and *(iii)* the representation of factors other than nutrition (ambient temperature, disease, sex, genotype) in metabolic models like SIMON. Finally, the main conclusions from each chapter are summarized and the implications for practice and research are discussed.

THE METABOLIC FATE OF AMINO ACIDS

From the experiments described in Chapter 1 it is clear that Holstein-Friesian x Dutch-Friesian calves, weighing between 80 and 240 kg, utilize absorbed dietary protein with a low efficiency (varying between 35 and 60%). This then led to the question of why such a large proportion of the absorbed amino acids were not

deposited. A brief overview of the metabolic fate of amino acids is presented below. Some issues have already been discussed in the Chapters 1 and 2.

Oxidation to provide energy or substrate for fat deposition

At low energy intakes, amino acids may be preferentially oxidized to provide energy, because maintaining energy flow within the body is of more importance to physiological viability than is the maintenance of protein deposition. Also, at extremely low protein-free energy intakes, amino acids may have to be oxidized to provide energy and substrate for a minimum amount of fat to be deposited. Although these calves are not striving to reach a minimal fat deposition per unit protein deposition, it does not seem unreasonable to assume that some minimum must exist. From the experiments in Chapter 1, the minimum ratios of fat to protein deposition found were about 0.7 for calves between 80 and 160 kg LW, and 0.8 for those between 160 and 240 kg LW.

Oxidative disposal due to a maximum in the animal's ability to deposit protein

It has been widely recognized that animals have a limited capacity to deposit body protein (Campbell, 1988). Dietary amino acids provided in excess are oxidized. This maximum, however, can only be determined under "optimal" conditions, which may well be beyond the animal's appetite. In these experiments, a maximum was found only for those calves weighing from 160 to 240 kg (Chapter 1) at protein intakes over 80 g N/d. It does, however, not explain the low efficiency of protein utilization at the lower protein intakes.

Gluconeogenesis from amino acids

Some tissues and processes specifically require glucose as an energy source, e.g. red blood cells, kidney medulla, fatty acid synthesis and to a large extent the central nervous system and brain (Freedland and Briggs, 1977; Wijayasinge et al., 1986). Furthermore, it is needed as a precursor for lactose in lactating animals. There may be, in the absence of dietary glucose and propionate, a specific need to use amino acids for gluconeogenesis, particularly in lactating animals (Black et al., 1990; Lobley, 1992). This is not included in the model because the dietary glucose supply is abundant in preruminant calves. There may be, however, at least two reasons for gluconeogenesis to be a major metabolic fate for amino acids in preruminants. Firstly, preruminant calves, being the progeny of a dairy herd, may use amino acids as an inevitable consequence of enzyme systems, operating in a

similar way to those in functional ruminants, which usually absorb very little glucose. Secondly, the clearance of glucose from the blood is relatively fast and the glucose storage capacity is limited. According to Palmquist et al. (1992), the half-life of plasma glucose in preruminant calves would be around 60 min. Therefore, gluconeogenesis from amino acids may be needed between meals. If this is the case, however, one should expect an improvement of nitrogen utilization with increased meal frequency. This effect, however, could not be shown in preruminant calves when the feeding frequency was increased from once to six times daily (Williams et al., 1986).

Use of amino acids in non-protein deposition pathways

Amino acids are used in non-protein deposition pathways, and may not be recycled back into protein metabolism when their products are broken down. Examples are shown in **Table 1**. So far, however, no attempts have been made to quantify these specific needs.

Oxidation because of imbalance

Amino acids may be oxidized when the composition of the absorbed protein is out of balance with the need for deposition. This mechanism is included in the model and is discussed extensively in chapter 2. In Chapter 1, it was hypothesized that a dietary amino acid imbalance was unlikely to be the main reason for the low efficiency of protein utilization. Based on the data presented in this thesis, however, it cannot be excluded. Therefore, feed and whole body samples of the calves used in both experiments, are currently being analysed for their amino acid composition. This hypothesis can be tested with these data, but it is beyond the scope of the current discussion.

Table 1

Non-protein deposition pathways of amino acid utilization

End product	Precursor amino acid
Serotonin	Tryptophan
Nicotinic acid	Tryptophan
Catecholamines	Tyrosine
Carnitine	Lysine (Methionine)
Taurine	Cysteine
Glutathione	Glutamate, cysteine, glycine
Nucleic acid bases	Glutamine, aspartate, glycine
Heme	Glycine
Creatine	Glycine, arginine, methionine
"Methyl group metabolism"	Methionine, glycine, serine
Bile acids	Glycine, taurine

from: Reeds and Mersmann (1991)

Oxidation due to diurnal protein cycling

According to Millward et al. (1990), the inefficiency of protein utilization is to some extent the consequence of the diurnal pattern of feed intake. Protein must be deposited in the fed state to match post-absorptive losses, which have been shown to vary with protein intake (Millward et al., 1990).

No firm conclusions can be drawn from the above about the cause of the low efficiency of utilization of digestible protein in preruminant calves. If an imbalance of dietary amino acids is not the cause of this low efficiency, results from literature may be used to quantify the use of specific amino acids in non-protein pathways. Valuable information could also be obtained by measuring the oxidation rates of individual amino acids at several low intake levels. This may shed light on whether the oxidation is related to a specific function of an amino acid, or alternatively, whether substrate dependence exists in the oxidation of that amino acid.

SIMON

General considerations of the modelling approach

The modelling approach, chosen for SIMON has not been discussed in the previous chapters. In this section, attention is paid to several aspects of the modelling approach, used for SIMON. The following topics are discussed: (i) Some of the basic choices made for SIMON; (ii) the choice of body constituent pools and the representation of fluxes in model construction; (iii) an interesting consequence of the substrate dependent approach; (iv) the approach to the simulation of the requirements for individual amino acids; (v) the possible role of hormones, regulating nutrient partitioning, in simulation models.

Basic choices

In this thesis, the metabolic approach (see General Introduction) was chosen, because it allows representation of biological processes in as much detail as needed for the appropriate level of aggregation. A prototype model was designed and experiments conducted, to gather the necessary information (Chapter 1). The design of the experiments was based on the concept of Whittemore and Fawcett (1976), because, independent of the outcome, it would provide the essential information required concerning the distribution of ingested protein and energy into body

protein, fat, ash and water over a wide range of protein and energy intakes. The experiments revealed no distinct protein and energy dependent phases in the deposition of protein. It was concluded that nutrient partitioning in preruminant calves does not follow the same principles as in true monogastrics such as pigs. The preruminant calf does not seem to strive to reach a minimal ratio of lipid to protein in the gain. Also, as discussed previously, the efficiency of protein utilization appeared remarkably low when compared with other species. Furthermore, even at low protein intakes, calves responded to increased protein-free energy intakes by increasing their protein deposition rates. For these reasons, it seems justifiable to conclude that preruminant calves may oxidize amino acids depending on the available energy substrates (amino acids, protein-free energy). The partitioning of ingested protein, carbohydrate and fat was therefore modelled using the principles of standard enzyme and chemical kinetics, and the model was parameterized to match the experimental observations (Chapter 2).

Choice of pools

One of the most important phases in the construction of a metabolic model is the choice of body constituent and metabolite pools, which will be appropriate for the level of aggregation chosen (Gill et al., 1989). The choice made is often a compromise between the true and a simple representation of biology.

The choice of body constituent pools in SIMON was related to its objective to predict body composition and to make progress in the definition of amino acid requirements. For the metabolite pools, the simplest representation was chosen which would be sufficient to distinguish the effects of dietary protein, carbohydrates and fats. It has been assumed that substrate concentrations in the metabolite pools drive a particular process. In the animal, however, substrate concentrations themselves are sometimes unlikely to do so. In SIMON, for example, muscle protein synthesis is driven by the concentrations of amino acids and acetyl-CoA. The concentration of amino acids was chosen to represent the effect of changed protein intake on protein synthesis (and therefore on deposition), and acetyl-CoA to represent the effect of protein-free energy on protein deposition. Acetyl-CoA in the body, however, has an extremely high turnover rate, and as metabolism of acetyl-CoA is strictly intracellular (Stryer, 1981), whole body concentrations of acetyl-CoA are unlikely to vary with nutrient input, at least not within a measurable range. Similarly, a higher concentration of amino acids available for protein synthesis does not always reflect an increase in substrate availability. In

fasted calves, for example, increased (muscle) protein breakdown may lead to an increased, rather than a decreased concentration of most amino acids in whole blood (McCormick and Webb, 1982). The amino acids mobilized are then preferentially partitioned between the synthesis of vital proteins and oxidation to provide energy. The use of substrate concentrations to direct nutrient partitioning is, however, by far the simplest way to represent the effects of changed nutrient input on the metabolism of preruminant calves, and although it compromises the biology to some extent, is therefore the method of choice. An alternative way of representing nutrient partitioning would be to include of hormone function, which will be discussed later.

Representation of fluxes

After choosing compartments ("pools"), choices about the representation of fluxes have to be made. While some fluxes depend on pool sizes (or on live weight, being the sum of pool sizes), and can therefore be represented by mass-action equations or allometry for example, others depend on substrate concentrations. To ensure the robustness of the model, at least two of the fluxes connecting a metabolite pool to other pools (leaving or entering) have to be made dependent on the metabolite concentration in that pool. In that way, the metabolite concentration can vary within narrow limits for its regulatory function, and at the same time, accumulation of substrate in that metabolite pool can be avoided. For the representation of fluxes, depending on substrate concentration, Michaelis-Menten type equations are often used (also in SIMON). The general form of the Michaelis-Menten equation, commonly used for the representation of standard enzyme and chemical kinetics is shown below. Transactions between pools are characterized by substrate concentrations (S_i), a maximum velocity (v_{max}), affinity (K_i) and inhibition (I_i) constants and steepness parameters (θ_i).

$$flux = \frac{v_{max}}{\left(1 + \left(\frac{K_1}{[S_1]}\right)^{\theta_1} + \left(\frac{K_2}{[S_2]}\right)^{\theta_2} + \ldots + \left(\frac{[S_3]}{I_3}\right)^{\theta_3} + \ldots\right)}$$

As argued by Gill et al. (1989), such expressions have several advantages for the representation of nutrients fluxes in mechanistic models:

"... (1) all the parameters are in units of velocity or concentration and can thus be ascribed biological meaning;

(2) the affinity constants (K_i) reflect priorities between fluxes for a

particular substrate, e.g. if more than one flux utilizes the same substrate then the flux which has the highest priority for that substrate will have the smallest K value in its reaction velocity equation; and

(3) hormonal influences can be mediated through changes in K and V_{max}, with rising levels of anabolic hormone concentration decreasing K, and rising levels of catabolic hormone concentration increasing V_{max}.

..." (Gill et al. ,1989).

Furthermore, many metabolic systems are saturable, and when numerical methods are used to solve the equations, these models are very robust (Baldwin, 1995). In the practice of model construction, the V_{max} is often the only parameter in the equation that can be ascribed a true biological meaning. Experimental evidence is rarely available at the same level of aggregation as the transactions that are represented by the model. Hence, affinity and inhibition constants and steepness parameters of various transactions, competing for a particular substrate, are often set to result in the best fit of model predictions to the available data.

Compensation through competition

An interesting feature of the metabolic approach is that it includes a compensation mechanism for transactions competing for the same substrate. This mechanism is illustrated by an example.

*Example (**Figure 1**)*

Daily (obligatory) endogenous urinary N-losses are modelled as a drain on the amino acid pool, and depend on live weight (see Chapter 2). If, in a sensitivity analysis, these losses per kg live weight are increased (see Chapter 3), the nitrogen accretion rate in the body is not decreased by a similar amount. This is because other processes, depending on the amino acid concentration (amino acid oxidation, muscle protein synthesis) change too.

As already discussed in Chapter 3, such compensation mechanisms do exist in biology. The extent to which an increase in a particular process can be compensated for by a decrease in another process depends on the processes involved. These examples illustrate the importance of representing biological processes in models like this, and of making careful decisions about the representation of fluxes. When carrying out experiments to measure specific fluxes, one should be aware of the possibility that a flux, measured in vivo, can in fact be the net effect of two or more

processes.

Protein versus amino acid metabolism

The representation of protein metabolism by the model, (see chapter 2, 3 and 4), is based essentially on the N-flows measured in the experiments (chapter 1). An additional feature is that the amino acid balance is checked by calculating and comparing the flow of individual amino acids into and out of the amino acid pool (Chapter 2). Inevitable oxidative amino acid losses are introduced in the model because protein turnover is unlikely to be a 100% efficient process. They are assumed to be a fixed proportion of the total flux of a particular amino acid in the amino acid pool (representing a fixed chance of each amino acid being oxidized when passing the site of oxidation). This flux depends only on body protein pool sizes (at a particular protein intake), because the fractional degradation rates of all protein pools are assumed to be fixed. This leads to behaviour of the simulated utilization of individual amino acids for protein deposition which is different to the simulated utilization of nitrogen. For example, from the simulations described in chapter 4, it can be calculated that the efficiency of utilization of individual amino acids for protein deposition increases with increasing protein deposition rates (Table 1, chapter 4). In contrast, the simulated efficiency of N-utilization decreases with increasing protein deposition rate (Table 1, chapter 4), which is supported by data from the literature (see chapter 4). This contradiction is due to the inadequate representation of the metabolism of individual amino acids. Suggestions to improve model performance on this point include the following.

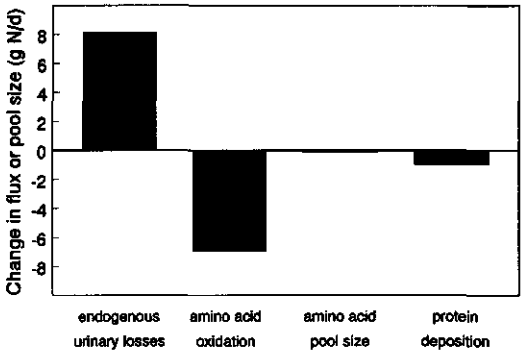

Figure 1. Simulation of increased endogenous urinary nitrogen losses (by 8.14 g N/d), leading to a compensation by decreasing amino acid oxidation (7.0 g N/d) and decreasing amino acid pool size (by 0.14 g N/d), finally resulting in a decreased protein deposition rate by 1.0 g N/d. The simulations are performed at a fixed live weight (160 kg) and fixed nutrient input, and are described in Chapter 3.

- For the sake of simplicity, fractional degradation rates were assumed to be fixed for all protein pools. In Chapter 5, however, it was shown that the

fractional degradation rates of for example muscle protein increased with increasing protein deposition rates. Applying fractional degradation rates, which are dependent on protein deposition rate, and keeping the inevitable oxidation rates of particular amino acids proportional to their flux, would solve the contradiction mentioned above.

- Alternatively, the inevitable oxidative losses could be split into a "maintenance" and a "production" component, the "maintenance" component being related to body protein pool sizes (as currently represented), and the "production" component being related to protein deposition rates.

Incorporation of hormonal regulation of growth into metabolic models

As discussed in Chapter 6, it is difficult to determine whether differences in plasma hormone concentrations actually drive nutrient partitioning or whether they reflect changes in metabolism, directly due to nutrition or to other hormones. It is therefore argued that representation of hormone function in metabolic models is useful only if the hormonal regulation of nutrient partitioning is clearly independent of nutrient intake. This may be the case in any of the following situations:

- In the representation of metabolism of lactating animals (such as the model of Pettigrew et al., 1992), where hormone function is essentially independent of the nutritional situation;
- To represent differences between sexes and genotypes;
- To represent effects of age;

Alternatively, of course, inclusion of hormones in such models can also be a specific objective of the modelling exercise.

Examples of representation of hormone function. In addition to the representation of metabolites, several modellers have included hormone function in metabolic models in a simplified way.

Baldwin et al. (1987), modelling the lactating dairy cow, and Sainz and Wolff (1990), modelling the growing lamb, represented hormone function in terms of changes in glucose concentration ([Gl]). Fluxes under catabolic hormonal control (e.g. lipolysis) and anabolic hormonal control (e.g. glycolysis, fatty acid synthesis) were identified. Catabolic hormone function was simulated by multiplying the v_{max}, while anabolic hormone function was simulated by multiplying the affinity constant K, by: $(\frac{[Gl]_0}{[Gl]})^\theta$, in which $[Gl]_0$ represents the glucose concentration at its reference

level, and θ represents a steepness parameter. A decrease in the glucose, compared with a reference concentration, results in higher fluxes of transactions under catabolic hormonal control (through an increased V_{max}), and in reduced fluxes of transactions under anabolic hormone control (through increased K). In this approach, however, hormonal regulation of nutrient partitioning solely depends on glucose concentration and can therefore not separate the effect of glucose from the effects of catabolic and anabolic hormones.

In their model of lactating sows, Pettigrew et al. (1992) defined a term "homeorrhetic drive for lactation", representing both the endocrinological milieu and the number of mammary cells. This term, which in fact represents non-nutritional variation in milk yield, was made dependent on the stage of lactation, genetics, litter size and parity number, and was included to modify the v_{max} of several transactions described in the model.

Problems with the representation of hormone function. Hormone function in the examples described above has been included in a simplified way. Representation of detailed hormone function i.e. including hormone interactions, receptor numbers, affinities etc. would make these models very complex and would have to be balanced against the objective of the whole-animal model. The inclusion of hormone pools can also give rise to computational problems. Compared with metabolites, hormones are present at very low concentrations, and multiplication by metabolic volumes to give pool sizes still results in small pools relative to their rate of turnover. In practical terms, this can lead to an unstable model resulting from stiffness problems (see France et al., 1992).

Representation of factors other than nutrition in whole-animal models

In order to make optimal use of animal growth models on a farm level, they should preferably be capable of dealing not only with nutrient intake but also with other factors influencing nutrient partitioning. Model parameters should be selected which are likely to be affected by such factors, and attempts should be made to quantify them. While by no means complete, a number of important factors are discussed below and suggestions are made on their future inclusion in metabolic models.

Environmental factors: ambient temperature and infection. Changes in climatic conditions such as ambient temperature, can have strong effects on nutrient partitioning and thus on animal performance (Bruce, 1993; Schrama et al., 1992). These effects are rarely included in nutrient partitioning models, with the notable exception of the model of pig growth of Black et al. (1986).

The mechanisms by which an animal copes with changes in ambient temperature such as: changing posture, vasoregulation, sweating, modifying feed intake, shivering and improved insulation, are not represented in SIMON. The mechanisms that an animal will actually use, depend on the type and duration of the stress (see Young et al., 1989). Unless these processes are explicitly represented, the extra energy required, expressed in energy units/°C outside the thermoneutral zone, could be modelled as an additional energy-consuming process (i.e. a drain on the acetyl-CoA pool). The energy required would then be provided by oxidation of energy substrates, proportional to their supply. Subsequently, fat, and to a lesser extent, protein deposition rates would be reduced (see for instance Figure 1a, chapter 3). This representation, however, requires knowledge about the determinants of upper and lower critical temperatures (e.g. age, insulation value, thermoneutral heat production, feed intake, see Bruce, 1993; Young et al., 1989). For very young calves, relationships have been developed which could be used to quantify the increased energy requirements in the cold (Schrama et al., 1992, 1993). For calves weighing between 80 and 240 kg, however, no such information is available.

Infections are well known to depress animal performance. The effect of an infection on nutrient partitioning, depends on the type and severity of the infection. The latter is especially difficult to quantify in practice. Medical treatment is often the most obvious strategy, and one may question whether animal growth models should be capable of accounting for the altered metabolism of infected animals. However, if there is no check to feed intake, changes in the feeding strategy may help to control the damage in terms of depletion of body reserves and nitrogen losses, and it may be worthwhile of study.

Depending on the location and type of infection, various processes in the body can be affected. In the case of gastro-intestinal infections, digestibility will certainly be affected. Fever, the rise of the immune response and several repair mechanisms, are all processes that require energy which is consequently unavailable for deposition in affected animals. Urinary N losses have been reported to increase

post-infection. Apart from increased protein catabolism to provide energy, this may result from a specific demand for amino acids, used in response to inflammatory conditions (Reeds et al., 1994). If the energy costs can be quantified (for attempts at this see Baracos et al., 1987; Verstegen et al., 1991; Van Dam et al., 1996), it could probably best be represented as a drain on the acetyl-CoA pool in the model. An increased protein requirement for defense mechanisms could be represented in the model as an additional drain on the amino acid pool. The profile of such proteins has been reported to differ considerably from the body protein profile (Reeds et al., 1994) which can be accounted for in the present construction of the model (chapter 2).

Sex and genotype. The model, described in this thesis, is based on experiments carried out with male Dutch Friesian x Holstein Friesian crossbreds (chapter 1) and is therefore only valid for this type of calves. Various researchers have demonstrated differences in nutrient partitioning both between sexes and breeds. When compared at the same weight, female cattle are generally fatter than castrates, which in turn, are fatter than entire males (Kirchgessner et al., 1993; Robelin, 1986). Also, when compared at a similar weight, genotypes which are heavier at mature weight generally contain less fat in their empty bodies than do animals of smaller mature weight (Campbell, 1988). For example, cattle such as Charolais, Limousin and Simmental are generally leaner at a fixed body weight than Friesian, which in turn, are leaner than British beef breeds such as Hereford and Aberdeen Angus (Robelin, 1986). The distribution of body fat between depots, and the ratios between carcass muscle and bone tissue have also been reported to vary according to genotype (El Hakim et al., 1986; Robelin, 1986). Unfortunately, research on differences between different genotypes of cattle is often rather descriptive in nature (see e.g. Carter, 1975; El Hakim et al., 1986; Kirchgessner et al., 1993; Robelin, 1986). Reported differences in nutrient partitioning between sexes and genotypes are rather large and it therefore deserves attention in the development of animal growth models. The possible effects of sex and genotype on processes represented by the present model, are discussed briefly below.

Differences in digestibility and maintenance energy requirements. Preruminant calves are usually restricted in their feed intake. It is unlikely that nutrient digestibility is affected to a significant extent by sex or genotype. Vermorel et al. (1976), for example, found no differences in nutrient digestibility among

preruminant Friesian, Charolais and double-muscled Charolais calves.

Differences in the maintenance energy requirements between sexes and genotypes can be expected, based on the differences in body composition, described above (Campbell, 1988). Reported differences in maintenance energy expenditure between sexes, are in agreement with this hypothesis. According to the ARC (1980) and Webster (1989) for example, maintenance energy requirements decrease in the following order: entire males, castrates and females. Similarly, for different genotypes, the general view seems to be that the leanest genotypes have the highest maintenance requirements (see Korver et al., 1988; Webster, 1989), although there is no consensus on this point (see Vermorel et al. 1976). Maintenance energy requirements (per $kg^{0.75}$) would then decrease in the following order: Charolais, Friesian, Hereford. Webster (1989) suggested that these differences between genotypes would be too large to be completely explained by differences in body fat content. He suggested that differences in the proportion of (metabolic very active) organs would also play a role. If the changes in maintenance energy requirements are viewed as being a consequence of a changed body composition, the representation of maintenance energy metabolism in this model (see Chapter 2) is appropriate.

Differences in nutrient partitioning. It is important to determine and quantify the mechanisms responsible for the reported differences in body composition. According to the concept of Whittemore and Fawcett (1976), which is often applied to pigs (see general introduction), a genotype can be characterized by its ability to deposit protein, and by its capacity for lean growth under energy-limiting conditions. As discussed previously, this concept is not valid in preruminant calves. Differences in the capacity for protein deposition, however, may occur between sexes and breeds, and can be the consequence of an increased capacity for protein synthesis, reduced rates of protein degradation or a combination of both. Attempts to characterize the potential of, for example, muscle tissue for protein synthesis by measurement of RNA/protein ratios revealed no large differences between breeds (Lipsey et al., 1978; Van Eenaeme et al., 1989). As reviewed by Simon (1989), there is evidence to suggest reduced fractional muscle protein degradation rates in different genetic lines of chickens. Similarly, Van Eenaeme et al. (1989), found reduced fractional muscle protein degradation rates (3-methylhistidine method) in the Belgian Blue cattle when compared with Friesian Holsteins. Despite the lack of information, this indicates that differences between breeds may be the consequence

of reduced muscle protein degradation, rather than increased muscle protein synthesis rates. Differences in the endocrine system are probably also important. Differences between sexes can be largely attributed to these differences in the endocrine system, in particular the male and female sex hormones. Also, differences between genotypes are, to some extent, undoubtly related to hormonal differences. Plasma hormone profiles, however may not always be sensitive enough to detect these differences. Plasma or serum IGF-1 levels, for example, were not different between three quite different breeds of pigs (Brameld et al., 1996), nor between Friesian Holstein and Belgian Blue bulls (Van Eenaeme et al., 1990). However, Brameld et al (1996) found evidence for breed differences in the production of IGF-1 and growth hormone receptors in muscle tissue. They suggested that muscle growth may be regulated in an autocrine/paracrine manner.

Representation of sex and genotype in metabolic models. Differences in nutrient partitioning can be incorporated in the model in various ways. There are two important issues: which model parameters are likely to be affected by sex and genotype, and how can these parameters be modified to achieve satisfactory model predictions for various genotypes and sexes.

As discussed previously, changes in maintenance energy requirements can be seen as a consequence of altered body composition and are already represented by the model. Differences in initial body composition between sexes and breeds, would have to be derived from the literature. Leaner growth of certain genotypes can be simulated by manipulating any of the parameters involved in protein deposition, which will also affect the fat deposition rate. As discussed earlier, the fractional muscle protein degradation rate is an important candidate. Also, the higher preference of leaner genotypes to use dietary protein for protein deposition may be related to a lower affinity for amino acid oxidation. Furthermore, the regulation of fatty acid and fat metabolism may also be affected. Unfortunately, very little information is available on this subject.

Once choices have been made as to which parameters should be modified, attempts should be made to quantify those parameters. Insufficient quantitative information is available on a metabolic level. Therefore, the quantitative data on body composition, available for different breeds may have to be used to calibrate the model parameters which are likely to be affected by breed or sex.

Another approach, used by Oltjen et al. (1986), in their model for beef cattle, and by Pomar et al. (1991) in their model for growing pigs, was to select model

parameters that were modified for each breed by Taylor's rule (Taylor, 1980). Briefly, this rule is based on the observation that the rates of various functions in animals between species (and by these authors assumed to be valid between breeds as well), are proportional to their mature weight, raised to the power 0.73. These authors use Taylor's rule for modifying the maximum protein deposition capacity (Pomar et al., 1991) and DNA synthesis, protein synthesis, protein degradation and maintenance energy requirements (Oltjen et al., 1986).

CONCLUSIONS AND IMPLICATIONS

Main conclusions from this thesis

In **Chapter 1**, two experiments were described, in which protein and protein-free energy intakes were varied in preruminant calves weighing from 80 to 240 kg. Protein and fat accretion rates were measured by means of chemical body composition analysis. It was concluded that:

- Preruminant calves in this weight range respond to increased protein intake by increasing protein and fat deposition rates.
- Extra protein-free energy intake resulted mainly in extra fat deposition, but also increased the protein deposition rate, even at low protein intake levels.
- In both experiments, the response of protein deposition rate to increased protein intakes was low: about 30% of the extra ingested protein was deposited.
- The calves weighing from 160 to 240 kg LW reached a maximum at a protein deposition capacity of about 39 g N/d.

In **Chapter 2**, a mechanistic model was described, based on the experiments of Chapter 1 and literature data. This model simulates the partitioning of nutrients from ingestion through intermediary metabolism to growth, consisting of protein, fat, ash and water. It was concluded that:

- The model can be used to predict protein and fat deposition rates over a wide range of nutrient inputs, and promises to be a useful tool in the development of feeding strategies in preruminant calves;
- The model also accounts for potential dietary amino acid imbalances, and can be used to predict theoretical amino acid requirements.

In **Chapter 3**, the model, described in Chapter 2 was evaluated. Some important

conclusions were drawn:

- The quantitative importance of the maintenance energy requirements and of protein turnover were highlighted by the model.
- The representation of protein turnover, including its energy costs, enables the investigation of the quantitative importance of hide, bone and visceral protein.
- The model is highly sensitive to changes in the kinetic parameters which describe protein synthesis and amino acid oxidation.
- Simulation of the requirement for a specific amino acid at a given nutrient intake is sensitive to the inevitable oxidative losses, defined for that amino acid. Furthermore, it is sensitive to the content of that specific amino acid in body protein and, to a lesser extent, to the protein turnover rate.
- Comparison with independent data showed that gain of live weight, protein and fat were simulated satisfactorily in the live weight ranges of 55 to 155 and of 180 to 230 kg.

In **Chapter 4**, application of the model for the development of feeding strategies was illustrated. It was concluded that:

- The model responds well to changes in the quantity and quality of the feed offered, and therefore is considered a useful tool for the development of feeding strategies;
- The model provides a means for estimation of amino acid requirements. More attention should be paid to the minimum oxidative losses of individual, indispensable amino acids. Comparison of the simulated and experimentally derived amino acid requirements indicated that the default value for these losses in the model may be too low for leucine and isoleucine and too high for histidine;
- Predictions at any given time or body weight are not necessarily accurate, due to the fact that the two slaughter experiments on which the model is largely based, were carried out over a rather large live weight range.

Chapter 5 described a study of muscle protein turnover as affected by the experimental treatments of Chapter 1. This was done by means of the measurement of muscle protein deposition and muscle protein degradation. It was concluded that:

- Urinary creatinine excretion is unreliable as an estimator of muscle protein mass in experiments in which a large range of nutrient intakes is applied;
- Increased muscle protein deposition rates, caused by either increasing protein or protein-free energy intakes are accompanied by increased fractional rates of

muscle protein turnover;

In **Chapter 6**, the long-term nutritional regulation of plasma hormone concentration was studied, as affected by the treatments of Chapter 1. The effects on insulin-like growth factor-1 (IGF-1) and thyroid hormones triiodothyronine, T_3; thyroxine, T_4) were studied. It was concluded that:

- Plasma IGF-1 concentration increases with increasing protein intake, but is unaffected by protein-free energy intakes;
- Protein-free energy intake has a major, and protein intake a minor, effect on plasma T_3 concentration;
- Plasma T_4 concentration increases with increasing protein intake, but is unaffected by protein-free energy intakes;
- The small effect of protein intake on plasma T_3 could be a direct effect of the increased amount of T_4, produced by the thyroid gland. In contrast, the effect of protein free energy intake is probably due to peripheral thyroid hormone metabolism, rather than to the activity of the thyroid gland itself.

Implications

Practice. The work described in this thesis, aimed to extend our knowledge at partitioning of nutrients in the body of preruminant calves. The equations, relating protein and fat deposition to protein and protein-free energy intake (Chapter 1), may be used in practice to predict the response of protein and fat deposition rates to nutrient intake. However, care must be taken not to extrapolate beyond the measured range.

The mechanistic model described and evaluated in this thesis, could be used for the development of feeding strategies. The model does not suffer the same limitations as the equations, developed in Chapter 1, because it is based on biological principles. The capability of the model to distinguish between dietary carbohydrates and fats, and to predict amino acid requirements under varying conditions makes it a valuable tool for use in practice. Before applicable in practice, however, a user-friendly interface must be developed.

Research. The model, described in this thesis, can be used as a tool to distinguish the important from the less important issues in research into the nutrition of preruminant calves. Furthermore, it highlights areas in which knowledge is inadequate. From the combination of experimental work and simulations performed

in this thesis, a number of interesting research topics arise:

- From the simulations described in Chapter 4, it appears that exchanging dietary fat for carbohydrates leads to higher simulated protein- and lower simulated fat deposition rates. It would be interesting to test this experimentally.
- The low efficiency of protein utilization and the importance of the minimal oxidative losses in the model estimations of amino aid requirements, stress the need for further investigations. Experiments conducted to determine the needs to replace inevitable oxidative losses of individual amino acids should:
  - include the measurement of N-retention and quantitative measurements of oxidation rates of the amino acid under study;
  - be carried out at several intake levels of the amino acid under study. In this respect, low intake levels are of particular interest. However, zero intake levels should be avoided, as this will specifically stimulate (muscle) protein degradation (Van den Heemel-Grooten, 1996) and consequently increase nitrogen losses due to an amino acid imbalance (Millward et al., 1990).

  Preferably, the role of the amino acid under study in non-protein deposition pathways should be studied and attempts made to quantify them before conducting such study, as this would be important for both the experimental design and the interpretation of the results.

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SUMMARY

INTRODUCTION

Until the sixties, most male calves from dairy herds were slaughtered in the first week of life because of their poor performance in beef production. Some, however, were allowed to suckle their mothers or were given whole milk by bucket for two to eight weeks. These calves produced a pale, tender, exclusive type of meat, called veal. Since the sixties, veal production has increased considerably, with the advent of milk substitutes, in which large quantities of skim-milk powder (available from the EC milk surplus) and whey (from the cheese industry) could be used. By feeding milk substitutes, calves are kept in a pre-ruminant stage until 2-5 months of age. Veal production has always been greatly influenced by agricultural policies and public opinion. Recently, the consumers' demand for lean meat and, to a lesser extent, the necessity to reduce environmental pollution, has forced veal producers to change their management strategy. Whereas maximizing the growth rates of the animals was the objective until the mid-eighties, during the last ten years, it has shifted towards the efficient conversion of feed into lean products. Research in animal nutrition has therefore focused on the development of feeding strategies which can help farmers to achieve this change in objective. Simulation models have proved a helpful tool in the development of feeding strategies in various species (e.g. pigs, beef cattle), but have never been developed for preruminant calves.

The objective of the work described in this thesis, was to gain insight into the partitioning of nutrients in the body of growing, preruminant calves and to develop a simulation model which can be used as a tool for the development of feeding strategies. Attention is focused on male calves from the dairy herd, representing the majority of the calves raised for veal production in The Netherlands. Simulation models need to be based on good hypotheses and reliable experimental results. A prototype model was designed with the following objectives: (i) to predict growth rates and growth composition (i.e. protein and fat deposition rates) from nutrient intakes, and (ii) to gain insight into the nutrient partitioning in the body of preruminant calves.

TWO EXPERIMENTS

Two experiments were conducted to gather necessary information (**Chapter 1**). In these experiments, 90 preruminant Holstein Friesian x Dutch Friesian calves were used to quantify the effects of protein and protein-free energy intake on protein and fat deposition rates. The two experiments were similar in design, but were performed in two different weight ranges: 80 to 160 kg live weight (LW) and 160 to 240 kg LW in Experiment (Exp.) 1 and 2, respectively. In each experiment, calves were allocated to either an initial slaughter group or to one of 12 dietary treatments (three calves per treatment), which consisted of six protein intake levels at each of two protein-free energy intake levels. The ratio of energy intake from carbohydrates to energy intake from fat was kept constant at around one in both experiments. Calves were slaughtered and analysed for body composition when they had reached the target weight. A balance study was conducted when calves had reached about 120 and 200 kg LW in Exp. 1 and 2, respectively, and apparent faecal nutrient digestibilities were determined. The faecal digestibility of dry matter, energy and fat was high (>94%), and hardly affected by the dietary treatments. Protein digestibility varied between 86 and 96% and increased with increasing protein intake. Average daily gain of the empty body varied between 640 and 1340 g/d and between 420 and 1370 g/d in Exp. 1 and 2, respectively.

Increasing protein-free energy intake by 190 $kJ.LW^{-.75}.d^{-1}$ (averaged over the experimental period) in both experiments resulted in: (i) an increase in the rate of empty body weight gain of 180 and 265 g/d in Exp. 1 and 2, respectively; (ii) an increase in the rate of fat gain of 90 and 140 g/d in Exp. 1 and 2, respectively; and (iii) an increase in the rate of protein gain of 14 and 34 g/d in Exp. 1 and 2, respectively;

Increasing digestible protein intakes from .90 and 2.72 in Exp. 1 and between .54 and 2.22 g $N.LW^{-.75}.d^{-1}$ in Exp. 2 resulted in: (i) a linear increase in the rate of protein deposition from 20 to 40 g N/d in Exp. 1 and a linear increase from 16 to 39 g N/d in Exp. 2 (averaged over protein-free energy intake levels). However, at the high protein-free energy level in Exp. 2, protein deposition rate reached a maximum of 39 g N/d at a protein intake of 1.5 g $N.LW^{-.75}.d^{-1}$; (ii) a linear increase of fat deposition rates of about 25 g/d in Exp. 1 and 70 g/d in Exp. 2 (averaged over protein-free energy intake levels).

In both experiments, the response of the protein deposition rate to increased protein intakes was low: about 30% of the extra ingested protein was deposited.

THE SIMULATION MODEL

The results of these experiments were subsequently used as the basis for further development of the simulation model. The development of a simulation model roughly consists of two equally important phases. First, the actual construction and programming of the model, with fundamental choices of representation of biological processes and parameter estimation. Second, a thorough model evaluation, including a behavioural and sensitivity analysis of the model and a comparison of model predictions with independent, experimental data.

Model construction and parameterization

A metabolic modelling approach was chosen, which has been applied before in several whole-animal models. In these models, the whole animal is represented by metabolite pools and body storage pools. The metabolite pools (usually small and relatively constant in size) serve to partition absorbed nutrients into body storage pools, which increase in size with time, representing the growth of the animal. The model, described in **Chapter 2**, simulates the partitioning of nutrients (protein, lactose, starch and fat) from ingestion through intermediary metabolism to growth, consisting 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.

Simulation of the digestion process is kept fairly simple, because milk replacers are highly digestible in preruminants. Endogenous urinary N-losses, net endogenous protein losses in faeces and losses of scurf are modelled as a drain on the amino acid metabolite pool, the visceral protein pool and the hide protein pool, respectively. Turnover of each protein pool is represented with fixed fractional degradation rates. Fatty acid synthesis and oxidation and turnover of body fat are also represented by the model. Furthermore, the model includes a calculation routine to check possible dietary amino acid imbalance and can be used to predict amino acid requirements on a theoretical basis under various nutritional circumstances. Briefly, an amino acid profile was assigned to each of the body protein pools and to the dietary protein. The supply of individual amino acids (from dietary protein or from body protein degradation) is compared with the demand (for protein synthesis and for replacing inevitable oxidative losses). An improper balance of dietary amino acids will limit protein synthesis and increase amino acid oxidation. Model parameters were estimated partly from literature and partly from the experimental

data. The model simulates the growth of calves on a daily basis, is programmed in a computer simulation language (ACSL) and runs on a VAX computer.

Model evaluation

After the construction of the model, it was evaluated. This evaluation (**Chapter 3**) consisted of (i) a sensitivity and behavioural analysis, and (ii) a test against independent data.

Sensitivity and behavioural analysis. Sensitivity and model behaviour was tested when making changes in the model parameters representing maintenance energy and maintenance protein, protein turnover and energy requirements for tissue deposition. Furthermore, the sensitivity of model predictions to 25% changes in all kinetic parameters was analysed. From these analyses, the model appears sensitive to changes in maintenance energy requirements, indicating their quantitative importance. It is, however, only marginally sensitive to changes in maintenance protein requirements. The representation of protein turnover, including its energy costs, enables the investigation of the quantitative importance of hide, bone and visceral protein. Apart from maintenance energy, protein turnover was identified as the major energy consuming process defined in the model (about 21% of total energy expenditure). Simulation of the requirement for a specific amino acid at a given nutrient intake is sensitive to the inevitable oxidative losses, defined for that amino acid. Furthermore, it is sensitive to the amino acid content of the body protein and, to a lesser extent, to protein turnover rate.

Comparison with independent data. Simulation of two published experiments, not used for development of the model, showed that rates of gain of live weight, protein and fat were simulated satisfactorily in the live weight ranges 55-155 and 180-230 kg.

Model application

Subsequently, simulations were performed to test the suitability of the model for the main purpose dor which it was constructed: the development of a feeding strategy (**Chapter 4**). An optimal feeding strategy depends on the farmers' objective, but always results from the combination of the quality and the quantity of a feed offered. Various simulations are performed, each dealing with an important issue in the development of a feeding strategy. The model responded well to changes in protein and protein-free energy intake. Increasing in the carbohydrate:fat ratio at equal gross energy intakes lead to the simulation of higher protein- and lower fat

deposition rates. The model provides a useful tool for predicting requirements of indispensable amino acids, depending on growth rate and body weight. Comparison of simulated with experimentally derived amino acid requirements showed agreement for most amino acids for calves of around 90 kg live weight. Distributing the same amount of feed differently in the same time span hardly affected model predictions. Reducing daily feed intake and simultaneously increasing the time span markedly reduced performance, when compared at equal total feed intakes. The efficiency of protein utilization, however, increased.

MORE EXPERIMENTAL DATA

The experiments, described in Chapter 1, were also used to measure the long-term effects of nutrient intake on muscle protein turnover and the nutritional regulation of circulating thyroid hormones and insulin-like growth factor-1 (Chapter 5 and 6). Unfortunately, results of these measurements, originally planned to be included in the model, only became available after completion of the model.

The effect of nutrient intake on muscle protein turnover (Chapter 5)

In the two experiments, described previously, muscle protein degradation rates were measured by the urinary excretion of 3-methylhistidine (3-MH). 3-Methylhistidine is an amino acid, formed by the post-translational methylation of histidine during the synthesis of myofibrillar (muscle) proteins. During myofibrillar protein degradation, 3-MH is released, cannot be re-utilized for protein synthesis and is (in ruminants, rats, rabbits and humans) quantitatively excreted with urine. Therefore, it is a measure of muscle protein breakdown. 3-Methylhistidine was analysed in the urine samples, obtained during the balance trials of the experiments (see Chapter 1), which were performed at approximately 120 (Exp. 1) and 200 kg LW (Exp. 2). Correction of 3-methylhistidine excretion for muscle protein mass was performed based either on the urinary creatinine excretion rate or on estimated body composition during the balance trial. The former is often used in literature, and easy to perform, whereas the latter was possible in the present experiments because quantitative body composition analyses were performed. Additionally, fractional rates of muscle protein synthesis (FSR) and degradation (FDR) during the balance trial were calculated. In both experiments, FDRs and FSRs increased with increasing protein intake. FSRs also increased with increasing protein-free energy intake in both

experiments. FDRs tended to increase with increasing protein-free energy intake in both experiments. It was concluded that the increased muscle protein deposition rates, induced by nutrient intake are accompanied by increased muscle protein turnover rates. Furthermore, urinary creatinine excretion was considered unreliable as estimator of muscle protein mass.

The effect of nutrient intake on plasma thyroid hormones and insulin-like growth factor-1 (Chapter 6)

During both experiments (Chapter 1), plasma samples were taken every fortnight, 5-6 hours post-feeding from the jugular vein, and analysed on thyroxine (T_4), triiodothyronine (T_3) and insulin-like growth factor-1 (IGF-1). The objective was to study the long-term nutritional regulation of circulating levels of these hormones. Plasma IGF-1 and T_4 levels increased with increasing protein intake, but were unaffected by protein-free energy intake. In both experiments, plasma T_3 levels were higher at the high protein-free energy intake level and increased slightly with increasing protein intake. It was concluded that the effect of protein intake on plasma T_3 could be a direct effect of the increased amount of T_4, produced by the thyroid gland. The effect of protein-free energy intake on plasma T_3 is probably due to peripheral thyroid hormone metabolism, rather than to the functioning of the thyroid gland itself.

In summary, this thesis quantifies the response of body weight gain and its composition to altered nutrient intakes in preruminant dairy calves, weighing between 80 and 240 kg. The simulation model, described and subsequently tested, promises to be useful for the development of feeding strategies for these calves, and for directing future research. Furthermore, this research shows the additional value of an integrated approach of experimental and modelling research. One of the most important questions, resulting from this thesis is why preruminant calves utilize ingested proteins for protein gain with such a low efficiency.

SAMENVATTING

INLEIDING

Anders dan vroeger streeft de vleeskalverhouder tegenwoordig niet alleen naar het maximaliseren van de groeisnelheid van zijn kalveren. Als gevolg van de vraag naar mager vlees en de mestwetgeving is ook een efficiënte omzetting van voer in vlees belangrijk. Veel veevoedkundig onderzoek is er tegenwoordig op gericht voerstrategieën te ontwikkelen om boeren te helpen deze veranderde doelstelling te realiseren. Inzicht in de groei en groeisamenstelling van kalveren en de respons hiervan op variatie in nutriëntopname is hierbij van belang. Simulatiemodellen kunnen een handig hulpmiddel zijn om bestaande kennis over groeiprocessen te integreren.

De doelstelling van het hier beschreven onderzoek was het vergroten van inzicht in de groei van vleeskalveren en het ontwikkelen van een simulatiemodel waarmee voerstrategieën voor vleeskalveren kunnen worden opgesteld. Als uitgangspunt werden stierkalveren uit de melkveestapel gebruikt, omdat deze het grootste deel van de kalveren vormen die als vleeskalf worden gemest. Er werd een prototype model ontwikkeld. Nadat bleek dat er niet voldoende gegevens beschikbaar waren om te gebruiken voor de ontwikkeling van het model werden eerst twee experimenten uitgevoerd.

TWEE EXPERIMENTEN

Hoofdstuk 1 beschrijft twee experimenten met in totaal 90 zwartbonte vleeskalveren waarin de relatie tussen nutriëntopname (eiwit en energie) en eiwit- en vetaanzet werd gekwantificeerd. De nutriëntopnames in deze experimenten varieërden van extreem laag tot extreem hoog, zodat een grote variatie in groeisnelheid en lichaamssamenstelling werd verkregen.

De dagelijkse gewichtstoename varieerde tussen 530 en 1570 g/d en nam toe met toenemende eiwit en met toenemende energie-opname. Zowel de aanzet van eiwit als die van vet (in gram per dag) namen toe met toenemde eiwitopname. Het was echter opmerkelijk dat de benutting van het verteerde (melk)eiwit laag was. De efficiëntie waarmee het verteerde eiwit werd omgezet in eiwitgroei varieerde van 60% bij lage eiwitopname (langzame groeiers) tot 35% bij hoge eiwitopname (snelle groeiers). Een hogere energie-opname resulteerde voornamelijk in een hogere vetaanzet, maar ook in een hogere eiwitaanzet. Dit laatste (hogere eiwitaanzet bij

hogere energie-opname) is opmerkelijk, en betekent dat kalveren, zelfs bij lage eiwitopname, efficiënter met voereiwit omgaan als ze meer energie krijgen.

HET SIMULATIEMODEL

De resultaten van de hiervoor beschreven experimenten werden gebruikt als basis voor het simulatiemodel. Het ontwikkelingstrajekt van een simulatiemodel bestaat grofweg uit 2 fasen: de eerste fase bestaat uit het ontwerpen, programmeren en parameteriseren van het model (Hoofdstuk 2). Tijdens deze fase wordt beslist welke biologische processen in het model worden weergegeven en worden gegevens uit literatuur en eigen experimenten gebruikt om parameterwaarden te bepalen. De tweede fase (Hoofdstuk 3) bestaat uit het testen van het gedrag van het model bij variërende input en bij variërende parameterwaarden (gevoeligheidsanalyse). Tijdens deze fase moeten modelvoorspellingen ook worden vergeleken met onafhankelijk verkregen experimentele waarden. In Hoofdstuk 4 worden toepassingen van het model besproken.

Ontwikkelen en parameteriseren van het model (Hoofdstuk 2)

Voor het ontwikkelen van het model is gekozen voor een mechanistische benadering. De groei van het hele kalf wordt hierbij verklaard uit wat er gebeurt op weefselnivo. Ten behoeve van het simulatiemodel wordt het kalf opgesplitst in een aantal "pools". Er zijn twee soorten pools te onderscheiden: metabolietenpools en lichaamspools. De metabolietenpools zijn in de regel klein en zijn voor het verdelen van opgenomen nutriënten naar de lichaamspools. De omvang van deze pools verandert in de loop van het groeitrajekt dan ook nauwelijks. Nutriënten komen uiteindelijk terecht in de lichaamspools. Deze pools nemen dus in grootte toe naarmate het kalf zwaarder wordt. Het model bestaat uit 10 pools, waarvan 4 metabolietenpools (aminozuren, vetzuren, glucose en acetyl-coenzyme A) en 6 lichaamspools. De lichaamspools (orgaaneiwit, huideiwit, spiereiwit, boteiwit, vet en anorganische stof) vormen samen de droge massa van het gesimuleerde kalf.

Het model wordt gestuurd door nutrientaanbod: bekende hoeveelheden nutriënten worden in het model ingevoerd. Vervolgens berekent het model hoeveel van deze nutriënten voor het dier beschikbaar komen, hoe deze over de metabolietenpools worden verdeeld en hoeveel er uiteindelijk in de lichaamspools terechtkomen. De volgende processen zijn in het model weergegeven: de behoefte

aan energie en eiwit voor onderhoudsprocessen, turnover van eiwit en vet, vetzuursynthese, vetzuuroxidatie, glycolyse en aminozuuroxidatie. Verder bevat het model een berekeningsmethode waarmee een eventueel tekort aan een specifiek essentieel aminozuur kan worden vastgesteld. Het model kan dan ook worden gebruikt om in elke gewenste situatie de behoefte aan een bepaald aminozuur te simuleren.

Het model is dynamisch (simulaties worden op een van-dag-tot-dag basis uitgevoerd) en rekent in stappen van ±15 minuten. Het is geprogrammeerd in een simulatietaal (ACSL) en draait op een VAX computer.

Het testen van het model (Hoofdstuk 3)

Het testen van het model bestond uit een gedrags- en gevoeligheidsanalyse en een vergelijking met onafhankelijke gegevens. Hieruit bleek onder andere dat het model goed reageert op veranderingen in nutriëntinput, en dat onderhoudsprocessen en eiwitturnover de belangrijkste energieverbruikende processen zijn. Verder bleek het model in staat in verschillende situaties de behoefte aan individuele essentiële aminozuren te simuleren. Hierbij moet echter worden opgemerkt dat er op dat gebied weinig gegevens beschikbaar waren om de kwaliteit van de simulaties mee te vergelijken. Gezien de gevoeligheid van deze simulaties voor een aantal aannames dat in het model gedaan is verdient het aanbeveling met gericht experimenteel onderzoek het model op dit gebied te verbeteren. Meer informatie over de oxidatieve verliezen van individuele aminozuren bij vleeskalveren en betrouwbaarder gegevens over de aminozuursamenstelling van lichaamsweefsels kunnen leiden tot betrouwbaarder schattingen van aminozuurbehoeftes.

Er waren weinig gegevens voorhanden waarmee modelvoorspellingen konden worden vergeleken. Simulatie van twee gepubliceerde experimenten liet zien dat gewichtstoename, eiwit en vetaanzet naar tevredenheid konden worden voorspeld.

Toepassen van het model voor de ontwikkeling van voerstrategieën (Hoofdstuk 4)

Behalve in het onderzoek kan het model ook gebruikt worden voor de ontwikkeling van voerstrategieën voor vleeskalveren. Bij het ontwikkelen van een voerstrategie is niet alleen de voersamenstelling van belang, maar ook de hoeveelheid voer die in de loop van het mesttrajekt aan de dieren wordt verstrekt. In hoofdstuk 4 wordt aan de hand van enkele simulaties toegelicht op welke manier het model kan worden gebruikt voor de ontwikkeling van een voerstrategie.

Uit deze simulaties bleek onder andere dat het (isocalorisch) vervangen van vetten in het voer door koolhydraten leidt tot simulatie van een hogere eiwitaanzet

en een lagere vetaanzet. Het verschillend verdelen van een bepaalde hoeveelheid voer in hetzelfde tijdsbestek had nauwelijks effekt op de door het model voorspelde waarden. Het verdelen van eenzelfde hoeveelheid voer over een langere mestduur leidde tot lagere voorspelde groeicijfers, maar tot een hogere voorspelde efficiëntie van eiwitbenutting.

TERUG NAAR DE EXPERIMENTEN

De eerder beschreven experimenten hadden tot doel kwantitatieve informatie te leveren die als basis voor het -op dat moment nog te ontwikkelen- simulatiemodel kon worden gebruikt. In deze experimenten zijn extra metingen aan de kalveren verricht met als doel de restultaten hiervan te gebruiken in het model. Helaas kwamen de resultaten van deze metingen te laat beschikbaar om ze nog in het model te kunnen verwerken. De resultaten van deze metingen zijn beschreven in hoofdstuk 5 en 6.

Het effekt van nutriëntopname op spiereiwitturnover (Hoofdstuk 5)

Ongeveer de helft van het lichaamseiwit zit in spierweefsel. De aanzet van spiereiwit tijdens de groei is het verschil tussen de hoeveelheid die gevormd wordt (synthese) en de hoeveelheid die afgebroken wordt. De afbraak van myofibrillair eiwit, het belangrijkste spiereiwitbestanddeel, kan bij kalveren in vivo worden gemeten. In de experimenten van hoofdstuk 1 is spiereiwitafbraak bepaald door het meten van de uitscheiding van 3-methylhistidine met urine. Om spiereiwitturnover tussen dieren te kunnen vergelijken moet echter gecorrigeerd worden voor de spiermassa van de dieren op het tijdstip van meten. Deze correctie is op twee manieren verricht, welke later met elkaar zijn vergeleken: (i) gebaseerd op de creatinine uitscheiding met urine. Deze is eenvoudig te meten en wordt verondersteld nauw gerelateerd te zijn aan de spiermassa; (ii) gebaseerd op de chemische karkasanalyses, beschreven in hoofdstuk 1. Het doel van deze metingen was om te testen of spiereiwitafbraak afhankelijk is van nutriëntopname en of de twee manieren van schatten van spiereiwitafbraak hetzelfde resultaat opleveren.

Er werd geconcludeerd dat de toenemende eiwitaanzet, veroorzaakt door toenemende eiwit- of energie-opname gepaard gaat met een hogere turnoversnelheid (dus zowel hogere synthese- als hogere afbraaksnelheid) van spiereiwit. Verder bleek creatinine niet betrouwbaar als schatter voor de spiereiwitmassa.

Het effekt van nutriëntopnames op de schildklierhormoon- en insulin-like growth factor-1-spiegels (Hoofdstuk 6)

Verscheidene hormonen spelen een belangrijke rol in de verdeling van nutriënten in het groeiende lichaam. Enkele belangrijke zijn: groeihormoon, mannelijke en vrouwelijke geslachtshormonen, insuline, insuline-like growth factor-1 (IGF-1) en schildklierhormonen. Bij een aantal hormonen is het gehalte in het bloed gedurende de dag niet constant, hetgeen het meten van deze hormoonspiegels in langdurige groeiproeven tot een ingewikkelde aangelegenheid maakt. Schildklierhormonen (triiodothyronine, T_3 en thyroxine, T_4) en IGF-1 zijn wel redelijk constant gedurende de dag, en konden in de experimenten worden gemeten zonder dat dat ingrijpend was voor de kalveren. Het doel van dit onderzoek was de relatie tussen nutriëntopname en IGF-1, T_4 en T_3 spiegels te bestuderen. Hiertoe werd elke 14 dagen, 5 tot 6 uur na het voeren een bloedmonster genomen dat op deze hormonen geanalyseerd werd.

Uit het onderzoek bleken plasma IGF-1 en T_4 gehaltes toe te nemen met toenemende eiwitopname, maar onafhankelijk te zijn van energie-opname. T_3 echter, werd vooral beïnvloed door energie-opname en nam slechts in lichte mate toe met toenemende eiwitopname. Deze lichte toename kan het gevolg zijn van de toename in substraat (T_4) met toenemende eiwitopname. De hogere T_3 spiegels bij kalveren, gevoerd op het hoge eiwitvrije energie-opnamenivo kunnen het gevolg zijn van een verbeterde omzetting van T_4 in T_3 door de lever en andere weefsels.

In dit proefschrift wordt de relatie tussen de opname van macro-nutriënten (eiwit, koolhydraat, vet) en groeisamenstelling bij vleeskalveren gekwantificeerd. Hiertoe is een simulatiemodel ontwikkeld, dat voor een belangrijk deel is gebaseerd op twee uitgebreide slachtproeven. Het simulatiemodel dat in dit proefschrift wordt beschreven en getest, kan worden gebruikt in het onderzoek naar de voeding en fysiologie van vleeskalveren, maar ook voor de ontwikkeling van voerstrategieën. Verder toont dit proefschrift de meerwaarde aan van het combineren van experimenteel en modelonderzoek. Een van de belangrijkste overblijvende vragen is waarom de benutting van hoogwaardige (melk)eiwitten door snelgroeiende vleeskalveren zo laag is.

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CURRICULUM VITAE

Walter Jan Jozef Gerrits werd geboren op 24 oktober 1964 in het brabantse Rips. In 1983 ging hij, na het behalen van het VWO diploma aan het Elzendaalcollege te Boxmeer, Zoötechniek studeren aan de Landbouwuniversiteit te Wageningen. In 1989 studeerde hij af met Veevoeding, Bedrijfseconomie en Proceskunde als afstudeervakken. Aansluitend werkte hij 2 jaar, onder meer als vervangende dienstplicht, aan een laboratoriumautomatiseringsprojekt bij de vakgroep Veevoeding van de Landbouwuniversiteit. In september 1991 werd hij bij diezelfde vakgroep aangesteld als assistent in opleiding (AIO), waarvan u de resultaten in dit proefschrift kunt lezen. Een deel van het onderzoek (4 maanden) werd uitgevoerd op Institute of Grassland and Environmental Research in Engeland. Na deze AIO-aanstelling werd hij 5 maanden aangesteld als toegevoegd onderzoeker bij de vakgroep Veevoeding en vanaf september 1996 werkt hij de komende twee jaar als onderzoeker, twee dagen per week bij de vakgroep Veevoeding, en twee dagen per week bij TNO-ILOB te Wageningen.