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Predicting the profile of nutrients available for absorption: from nutrient requirement to animal response and environmental impact

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Current feed evaluation systems for dairy cattle aim to match nutrient requirements with nutrient intake at pre-defined production levels. These systems were not developed to address, and are not suitable to predict, the responses to dietary changes in terms of production level and product composition, excretion of nutrients to the environment, and nutrition related disorders. The change from a requirement to a response system to meet the needs of various stakeholders requires prediction of the profile of absorbed nutrients and its subsequent utilisation for various purposes. This contribution examines the challenges to predicting the profile of nutrients available for absorption in dairy cattle and provides guidelines for further improved prediction with regard to animal production responses and environmental pollution.

The profile of nutrients available for absorption comprises volatile fatty acids, long-chain fatty acids, amino acids and glucose. Thus the importance of processes in the reticulo-rumen is obvious. Much research into rumen fermentation is aimed at determination of substrate degradation rates. Quantitative knowledge on rates of passage of nutrients out of the rumen is rather limited compared with that on degradation rates, and thus should be an important theme in future research. Current systems largely ignore microbial metabolic variation, and extant mechanistic models of rumen fermentation give only limited attention to explicit representation of microbial metabolic activity. Recent molecular techniques indicate that knowledge on the presence and activity of various microbial species is far from complete. Such techniques may give a wealth of information, but to include such findings in systems predicting the nutrient profile requires close collaboration between molecular scientists and mathematical modellers on interpreting and evaluating quantitative data. Protozoal metabolism is of particular interest here given the paucity of quantitative data.

Empirical models lack the biological basis necessary to evaluate mitigation strategies to reduce excretion of waste, including nitrogen, phosphorus and methane. Such models may have little predictive value when comparing various feeding strategies. Examples include the Intergovernmental Panel on Climate Change (IPCC) Tier II models to quantify methane emissions and current protein evaluation systems to evaluate low protein diets to reduce nitrogen losses to the environment. Nutrient based mechanistic models can address such issues. Since environmental issues generally attract more funding from governmental offices, further development of nutrient based models may well take place within an environmental framework.

Keywords: feed evaluation, models, nutrient profiles, rumen fermentation

Introduction

Feed evaluation is the use of methods to describe feedstuffs with respect to their ability to sustain different types and levels of animal performance. Nutritionists and farmers

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in the livestock industry apply feed evaluation systems to match dietary nutrients with those required to support a desired level of production. The practical importance of feed evaluation is obvious with respect to optimising efficiency of feed utilisation, production and financial return to the producer. Provision of inadequate nutrients

relative to the desired production level may compromise production resulting in a negative economic impact on the dairy farm and may have adverse effects on the animal (e.g. ketosis due to glucose shortage). On the other hand, provision of excess nutrients can also have undesirable effects on the animal (e.g. over-conditioning). Moreover, reducing the level of excess nutrients is important in minimising pollution of the environment.

In current feed evaluation systems worldwide, the amounts of energy, protein, vitamins and minerals available for absorption from the gastro-intestinal tract (GIT) are represented, followed by quantitative approaches to describe the required amounts for maintenance and production using a factorial approach. These systems have several drawbacks that are well recognised (e.g. Baldwin, 1995; Dijkstra *et al.*, 2002; Hanigan, 2005). Single energy or protein values for a feed quote static conditions, whereas the energy or protein supply depends on a number of interrelated factors, including site of digestion and level of feed intake. Energy and protein systems have been developed independently, despite vast evidence of the interactions between energy- and protein-yielding nutrients. The level of aggregation hampers inclusion of new concepts and data that arise from experiments conducted at the organ, tissue or cellular levels. Similarly, the current level of aggregation also impedes the integration of concepts that arise from experiments conducted at a higher level of organisation (e.g. climate effects, whole farm aspects, etc.) A major criticism of all energy and protein systems is that they do not predict how performance, in terms of production level and product composition, will change in response to deliberate changes in feeding strategy. Finally, major opportunities to alter milk fat and protein content and composition through nutritional manipulation exist, but its control requires knowledge of delivery of individual nutrients at the mammary gland which is not represented in current systems. To improve upon current prediction schemes, new feeding systems need to be based on mechanisms that govern the response of animals to nutrients, by quantitatively describing metabolite supply at a more detailed level than the current aggregated components.

The problems and challenges in feed evaluation are illustrated by results from an experiment with 16 early lactating dairy cattle (2 to 9 weeks *post partum*) fed glycogenic or lipogenic diets (Van Knegsel *et al.*, 2006). Diets were devised to provide large amounts of glucogenic nutrients (propionic acid and glucose from escape starch) or large amounts of lipogenic nutrients (acetic and butyric acid and long chain fatty acids), but were equal in net energy (NE), protein digested in the small intestine (DVE) and degraded protein balance (OEB) content, calculated according to the Dutch system (Van Es, 1975; Tamminga *et al.*, 1994). Energy balance was measured by indirect calorimetry. Diet did not affect dry-matter intake (DMI) (20.8 *v.* 20.7 kg/day; $P > 0.05$) and thus energy and protein availability did not differ according to the Dutch feeding systems. However,

cows on the lipogenic diet partitioned more energy to milk than cows on the glycogenic diet (1175 *v.* 1073 kJ/kg$^{0.75}$ body weight per day; $P < 0.05$) (Figure 1a) and had a higher milk fat yield (1.89 *v.* 1.67 kg/day; $P < 0.05$). Energy mobilised as body fat tended to be higher in cows fed the lipogenic diet than in cows fed the glycogenic diet (190 *v.* 113 kJ/kg$^{0.75}$ body weight per day; $P < 0.10$). Energy retention as body fat in cows fed the glycogenic diet was positive from week 8 *post partum*, whereas cows fed the lipogenic diet still showed negative energy retention (Figure 1b). The results from this study demonstrate that type of energy (lipogenic or glycogenic) in isocaloric, isonitrogenous diets affects the performance of dairy cattle, which was not expected or could not be predicted with current requirement-based feed evaluation systems.

The change from a requirement to a response system to meet the needs of various stakeholders requires prediction of the profile of absorbed nutrients and their subsequent utilisation for various purposes. Variation in responses of animals may be related to differences in the profile of nutrients available for absorption or to differences in
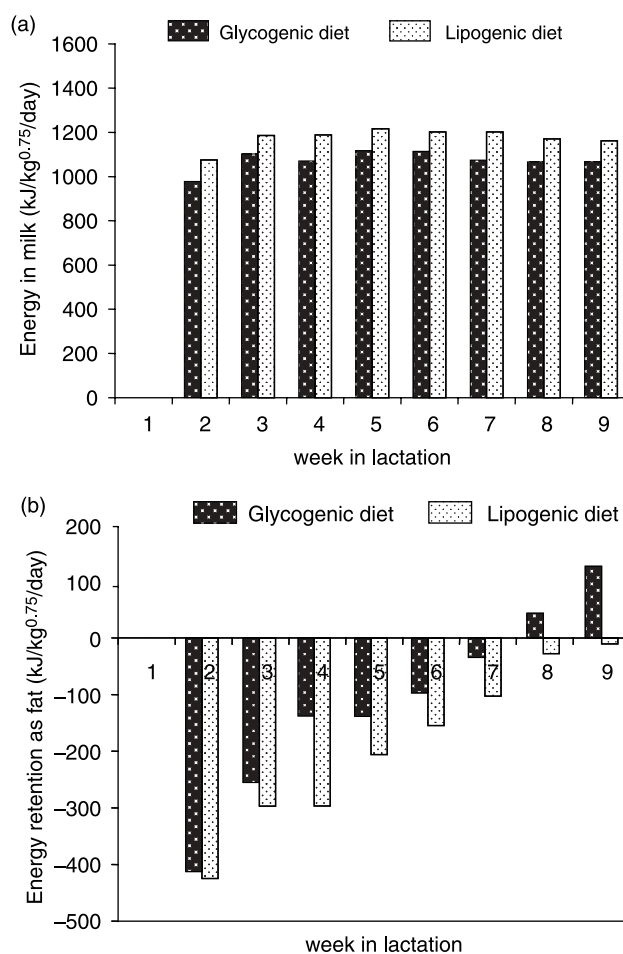

Figure 1 (a) Energy in milk and (b) energy retention as body fat in early lactation dairy cattle fed glycogenic or lipogenic diets (Van Knegsel *et al.*, 2006). Diets were equal in intake and contents of net energy and protein digested in the small intestine.

partitioning absorbed nutrients for various purposes. The partitioning of absorbed nutrients is described in the contribution of Friggens and Newbold (2007) in this issue. The aims of the present paper are to examine the challenges to predicting the profile of nutrients available for absorption in dairy cattle and to highlight future perspectives for further improved prediction with regard to animal production responses and environmental pollution. Although nutrient based models offer good prospects to prevent nutrient imbalances and reduce the occurrences of related metabolic diseases (including rumen acidosis, ketosis and abomasal displacement; Goff, 2006), health and reproduction issues are outside the scope of the present paper. The paper focuses on fermentation processes in the reticulo-rumen (hereafter rumen) as these have a large impact on the profile of nutrients available for absorption. Actual availability of nutrients for production depends on utilisation of absorbed nutrients by the wall of the GIT and the prediction of this utilisation has been reviewed elsewhere (Bannink *et al.*, 2006a). Prediction requires mathematical models and for model classification, development and evaluation the reader is referred to various textbooks (Baldwin, 1995; Dijkstra *et al.*, 2005a; Thornley and France, 2006).

Rumen degradation and passage

Digestion of feed in the rumen (disappearance of feed entities in the rumen) is the result of two competing processes: degradation and passage. Degradation occurs primarily through the activity of micro-organisms in the rumen, although in fresh forages, plant-derived proteinases may contribute to the first stages of protein degradation (Attwood, 2005). Smaller-sized substrates are then fermented to yield volatile fatty acids (VFA) as well as other end-products including carbon dioxide and methane. The passage rate determines the time that feed is retained in various compartments of the GIT for digestive action. These processes have to be integrated and described mathematically to predict digestion of feed and production of VFA and microbial matter in the rumen, and to predict the outflow of undigested feed and microbial matter.

Rumen degradation

Quantitative description of rumen degradation requires appropriate biological data. Information obtained *in vivo* is the most reliable and should be the reference to assess other methods and to evaluate predictions. However, *in vivo* trials cannot be considered routine in most laboratories and cannot be carried out for each feeding situation that occurs in practice. Hence a considerable amount of research on *in vitro* and *in sacco* techniques has been carried out. Current protein evaluation systems as well as extant mechanistic models require feed degradation parameters as an input. Degradation parameters are usually obtained using the polyester or nylon bag technique

(*in sacco* technique) or the gas production technique. In the *in sacco* technique, it is assumed that the washout fraction is rapidly degradable and that a truly undegradable fraction exists that remains after prolonged incubation. Based on gas production profiles, the soluble part of the washout fraction of barley and maize was fermented faster than the insoluble washout fraction (Yang *et al.*, 2005). Although in general little escape of soluble substrate from the rumen occurs, degradation is not necessarily complete (e.g. Volden *et al.* (2002) on outflow of soluble protein and Nocek and Tamminga (1991) on outflow of soluble starch). In most mechanistic models of rumen fermentation, predicted escape of soluble substrates depends upon the concentration of the microbial biomass present as well as the fractional passage rate. A higher microbial biomass will allow increased uptake and metabolism of soluble material, as evidenced by the effect of inoculum concentration on rate of gas production in batch cultures (Rymer *et al.*, 2005). In comparison to current empirical feed evaluation systems in which substrate degradation follows first-order kinetics, the representation of substrate degradation as a second-order kinetic process (degradation being affected by substrate concentration and microbial activity) allows various substrate interactions to be described quantitatively. For example, the rate of degradation of carbohydrates depends in part on the availability of nitrogen (N) sources (Carro and Miller, 1999) through effects on microbial growth.

Whilst the *in sacco* technique provides information on degradation rates of various individual organic matter (OM) components, the situation is less clear cut with the gas production technique. For prediction of the profile of absorbed nutrients, information on separate OM components is essential, since in contrast to fibre, any protein, starch and lipid that flow out of the rumen may be digested by host enzymes in the small intestine. Moreover, the type of substrate fermented has a significant impact on the type of VFA formed in the rumen of dairy cattle (Bannink *et al.*, 2006b), necessitating various OM components to be distinguished. However, the gas production technique measures gas that arises from fermentation of all OM components in the substrate incubated, and further techniques are required to obtain data on fermentation of OM components. One approach is to interpret the various phases observed in gas production profiles. The first phase of the gas production profile, in the early stages of incubation, is assumed to be related to an easily fermentable, soluble fraction, whilst the second phase might be related to fermentation of plant cell walls. In contrast, different phases may also occur upon separate incubation of fractionated plant material (Williams *et al.*, 2000) or of pure carbohydrates (Mould *et al.*, 2000), including glucose. In other efforts to distinguish OM into components, a curve subtraction technique has been proposed based on incubation of whole and neutral-detergent fibre (NDF) extracted material (Stefanon *et al.*, 1996). The accuracy of this technique depends, among others, on the assumption

101

that extraction does not affect degradation of the fractions. Yet the fibre structure may be partially disrupted by neutral-detergent extraction, giving rise to elevated fibre degradation rates and invalidating the underlying assumption of the curve subtraction technique (Kennedy *et al.*, 1999; Ruiz *et al.*, 2002). For mechanistic models, the use of the gas production technique may provide valuable qualitative information especially when the technique is used to study more fundamental aspects of fermentation. However, given the above considerations and since the gas production technique is a batch culture process, its potential as a source of quantitative data for mechanistic models, based on individual substrates, may be inherently limited (Dijkstra *et al.*, 2005b).

Rumen passage
In contrast with the extensive amount of data available on substrate degradation rates, availability of data on ruminal retention times is relatively scarce. Compartmental analyses of the *in sacco* (Lopez *et al.*, 1999) and gas production techniques (France *et al.*, 2005) have been applied to derive the link between these methods and actual *in vivo* degradation of nutrients in the rumen. These analyses clearly indicated the significant effect of rate of passage upon calculated extent of degradation. Accounting for the retention time in the rumen is a pre-requisite for many feed evaluation systems (Agricultural and Food Research Council (AFRC), 1993; National Research Council (NRC), 2001; Thomas, 2004). Moreover, the fractional rate of passage is also a major determinant of microbial protein efficiency (discussed in the next section). Passage from the rumen is a complex process that involves mixing, disruption and comminution of various components. The rumen operates as a partially stirred, continuous-flow reactor and different sub-compartments with different flow characteristics may be distinguished: liquid, escapable particles, and retained particles. To estimate passage dynamics of feed particles, often external markers (rare earth and chromium markers) or inert internal markers (acid-insoluble ash and indigestible fibre fractions) are used. Results of passage studies using external markers show that fractional passage rates are feed specific and vary with factors including roughage to concentrate ratio, chemical composition and feed intake level (Colucci *et al.*, 1990; Rinne *et al.*, 1997). For external markers of particle passage, it is assumed that they represent the passage behaviour of the particles ingested with the diet. However, applicability of the results have been criticised because external markers may restrict the digestibility of the particles they are linked to, they may bind preferentially to small particles, and they may migrate to rumen fluid (Uden *et al.*, 1980; Siddons *et al.*, 1985; Combs *et al.*, 1992). The inert internal markers referred to earlier, circumvent these problems as they form intrinsic components of the feed particles. However, these markers are not unique and cannot be distinguished from the bulk material. Hence, information on passage dynamics from the rumen demands knowledge of the pool size and this can only be obtained from rumen evacuations or

slaughter. These are time consuming and labour intensive procedures and require that the animals are in steady-state or that the rumen is evacuated frequently. Therefore, it is desirable to use markers that evade the aforementioned limitations, and that are representative of the *in vivo* situation. Stable isotopes (^{13}C, ^{15}N) appear to be suitable internal markers to meet these conditions (Südekum *et al.*, 1995; Huhtanen and Hristov, 2001). When bound to different feed fractions, isotope markers may also be used to quantify the passage rate of these individual fractions. An example using ^{13}C enriched grass silage (grass was a mixture of ryegrass (*Lolium perenne* L.) and timothy grass (*Phleum pratense* L.; Pellikaan, 2004)) is presented in Figure 2. Faecal excretion of ^{13}C labelled feed components was fitted using the multi-compartmental model described by Dhanoa *et al.* (1985) and goodness-of-fit parameters indicated an accurate fit. As expected, various feed fractions did not behave identical, and fractional passage rate from the rumen of the NDF fraction was lower than that of dry matter (DM). Upon comparison with Cr-mordanted NDF, the internal markers had markedly lower fractional passage rates (Table 1). Such lower fractional passage rates when using internal markers may be related to the fact that in this study, the ^{13}C-labeled grass was cut to a size of 3 to 5 cm before entering the rumen, whilst Cr-mordanted NDF was ground to pass a 0.5-mm sieve. Moreover, mordanting fibre with Cr restricts the digestibility of the labelled particles, thus affecting passage kinetics. In contrast, the internal marker particles are subject to usual fermentation in the rumen and the lower functional specific weight during active fermentation may have retarded their passage out of the rumen (Kaske *et al.*, 1992). Both internal and external markers indicated a higher fractional passage rate at higher intake levels (Table 1). However, the effect on fractional passage of grass regrowth period (6 *v.* 12 weeks, with
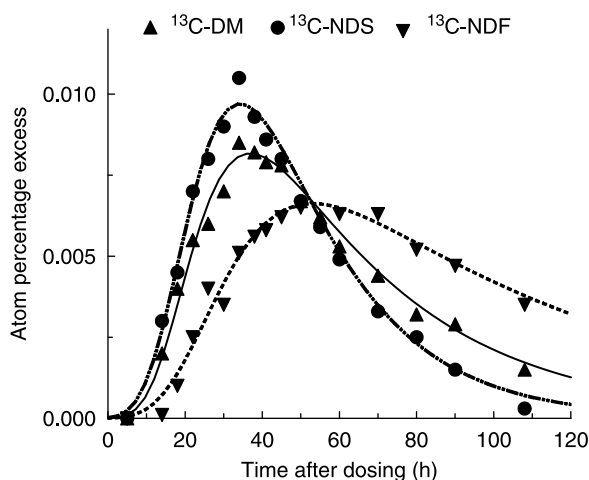

Figure 2 Faecal excretion patterns of ^{13}C-enriched grass silage chemical components (DM, dry matter; NDS, neutral-detergent solubles; NDF, neutral-detergent fibre) in dairy cattle (data from Pellikaan, 2004). Grass was a mixture of ryegrass (*Lolium perenne* L.) and timothy grass (*Phleum pratense* L.). Line fitted using the multicompartmental model of Dhanoa *et al.* (1985).

Table 1 *Fractional rumen passage rates (per h) of grass silage in dairy cattle at DM intake levels of 7.0 and 12.3 kg/day and ensiled after a grass regrowth period of 6 or 12 weeks using various external and internal markers. Grass was a mixture of ryegrass (Lolium perenne L.) and timothy grass (Phleum pratense L.). Data from Pellikaan (2004)*

	External markers		Internal markers[†]		
	Co-EDTA	Cr-NDF	^{13}C-DM	^{13}C-NDF	^{13}C-NDS
Intake level:					
7.6 kg DM per day	0.099	0.049	0.023	0.016	0.035
12.3 kg DM per day	0.104	0.069	0.034	0.028	0.040
Regrowth period:					
6 weeks	0.133	0.053	0.036	0.020	0.049
12 weeks	0.105	0.054	0.030	0.017	0.040

[†] ^{13}C-enriched chemical components: DM = dry matter; NDF = neutral-detergent fibre; NDS = neutral-detergent solubles.

corresponding digestible OM contents of 0.798 and 0.717 g/g DM and net energy for lactation contents of 6.4 and 5.5 kJ/g DM, respectively) differed depending upon the use of diverse markers. The internal markers indicated a 20% lower fractional passage rate for the 12-weeks regrowth period compared with the 6 weeks regrowth period of grass, but Cr-mordanted NDF did not indicate such a difference. A major step in predicting the profile of nutrients available for absorption is to recognise that, just as for degradation of individual fractions, the retention time of individual fractions within a feed may differ. The amount of data on passage using internal markers is rather limited at present and this hampers the adoption of such results in feed evaluation systems. Given the significant impact of passage on substrate degradation, microbial efficiency and VFA production, this area requires much more research.

Various feed evaluation systems incorporate empirical equations to predict fractional passage rates, usually of liquid, forages and concentrates (e.g., AFRC, 1993; NRC, 2001; Thomas, 2004). A number of different equations have been developed based on various characteristics including DM intake level, proportion of concentrate or forage in the diet, etc. However, all equations developed to date suffer from low overall predictability. Seo *et al.* (2006) compared equations used in various systems, and the highest R^2 values obtained were only 0.25 (liquid passage), 0.40 (concentrate passage) and 0.39 (roughage passage). Clearly, increases in passage prediction accuracy are required. From a mechanistic viewpoint, particle size and functional specific gravity are the most important factors that determine the probability of particles passing out of the rumen (Kaske *et al.*, 1992). Various chemical components may not represent these physical attributes, and interactions between chemical components may affect the change in these attributes within the rumen. Thus, mechanistic predictions of passage require a description of physical properties and dynamic changes in these

properties in particular, but to date efforts to represent dynamic changes in particle size and functional specific gravity are largely absent.

Efficiency of microbial protein synthesis

Microbial protein synthesis (MPS) in the rumen usually provides the majority of amino acids (AA) available for absorption. The total amount of microbial protein flowing to the small intestine depends on nutrient availability in the rumen and efficiency of use of these nutrients by ruminal micro-organisms (see reviews by Bach *et al.* (2005) and Firkins *et al.* (2006)). Prediction of the extent of degradation was discussed in the previous section. This section will focus on the efficiency of MPS (EMPS).

In protein evaluation systems, EMPS is usually expressed on an OM basis (usually in g microbial protein per kg OM degraded). Various OM components differ in energy yield, however, and EMPS values were more statistically revealing in a number of experimental studies when expressed on a carbohydrate rather than an OM basis (review by Firkins *et al.* (2006)). In general, empirical protein evaluation systems predict MPS using a constant EMPS value. In some systems, EMPS is variable and depends on, e.g., level of feed intake (AFRC, 1993), fractional passage rate (Thomas, 2004) or fractional degradation rate (Russell *et al.*, 1992). Yet there are many mechanistic factors that affect EMPS. Mathematical equations to describe variation in microbial growth and microbial efficiency in open or closed anaerobic systems are well documented in the literature (notably the work of Pirt (1975)) but are applied surprisingly little in empirical protein evaluation systems for ruminants. Basically, substrate consumption by micro-organisms will follow enzyme kinetics represented by rectangular hyperbola, and efficiency depends upon the partitioning of substrate consumed for growth and non-growth purposes. Critical elements in these equations include the maintenance requirement and the amount of substrate required per unit growth once maintenance has been met. Usually, the goal of rumen nutrition is to maximise EMPS to use ruminal degradable N as efficiently as possible and to decrease N losses to the environment. It should be noted that substrate utilised by microbes is partitioned between fermentation end-products (mainly VFA) and microbial biomass formation. Thus an increase in EMPS usually coincides with a decrease in VFA produced per gram of carbohydrate degraded, which reduces the supply of non-protein energy to the animal. The concept of microbial efficiency and production of biomass *versus* production of fermentation end-products is central to many other fermentative systems as well, but the aim in some other systems (e.g. environmental bioremediation systems like water purification) is to waste as much energy and to produce as least biomass as possible and is thus opposite to that in the rumen. Therefore, mathematical equations developed may be applicable to many fermentative situations with widely different ultimate goals.

Maintenance requirement comprises any energy or substrate necessary to the growth of the micro-organism and used in such a way that this energy or substrate is not available for biosynthesis. Thus this includes true maintenance as well as endogenous metabolism and spilling of energy or substrates. The significance of the maintenance requirement value for efficiency of microbial growth is illustrated in Figure 3. Isaacson *et al.* (1975) used chemostat cultures to estimate the maintenance requirements of mixed rumen bacteria and the value derived (0.05 g glucose/g bacterial DM per h) is applied in most mechanistic models. However, maintenance values can vary widely according to the bacterial species involved (Russell and Baldwin, 1979) and chemical conditions in the rumen (e.g. osmotic value; Shi and Weimer (1992)). Given the impact of maintenance requirement on microbial growth efficiency in the usual (low) range of growth rates of microbes in the rumen (Figure 3; maintenance requirement raised from 0.05 to 0.15 g carbohydrate/g microbial DM per h), surprisingly few maintenance requirement values have been reported in literature, and clearly more data are required to improve extant mechanistic models.

Energy spilling may form a significant part of the maintenance requirement. Conceptually, energy spilling can be regarded as energy decoupling between anabolic and catabolic functions of the cell through 'futile cycles'.
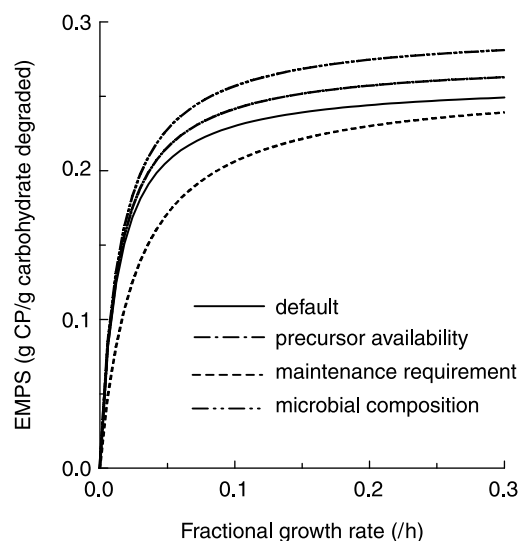

Figure 3 Microbial yield (g of microbial crude protein (CP) per g of carbohydrate) predicted by the double reciprocal equation of Pirt (1975) as affected by maintenance requirement, availability of precursors for microbial growth, and microbial composition. Default situation calculated according to Dijkstra *et al.* (1992) assuming a maintenance coefficient of 0.05 g carbohydrate per g microbial dry matter (DM) per h, a microbial crude protein content of 0.6 g/g microbial DM, and ammonia and preformed amino acids to deliver 0.80 and 0.20 g nitrogen (N) per g required N. Changes in assumptions: (i) maintenance requirement, maintenance requirement raised to 0.15 g carbohydrate per g microbial DM per h; (ii) precursor availability, ammonia and amino acids to deliver 0.40 and 0.60 g N per g required N, respectively; (iii) microbial composition, microbial CP content raised to 0.80 g/g microbial DM at the expense of storage polysaccharides in microbial DM.

The term futile cycles describes the concept where the microbial cell has both an irreversible pathway for a chemical transformation process in one direction and a different and irreversible mechanism for the reverse process. If one or the other functions at a different time, metabolism is not wasteful, but if they act simultaneously, there might be no or a small net transformation and the result would be that cellular energy is dissipated, thus reducing EMPS. In the rumen, energy availability is usually the determinant most limiting microbial growth and energy spilling would be of minor importance. However, in certain situations (particularly immediately after feeding a diet high in easily degradable carbohydrates), the availability of nutrients other than energy may limit microbial growth, and spilling of energy in the excess of carbohydrate availability may occur.

In many cases, maintenance activity still remains a relatively fuzzy concept in biological terms but for prediction purposes, representation of the phenomenon seems satisfactory. Pirt (1982) proposed to account mathematically for non-growth requirements by including a constant maintenance energy term independent of the specific growth rate and a term which decreases with increases in specific growth rate. Evaluation of this representation showed good accuracy when compared with observed data, but application to predict EMPS in the rumen would require quantitative data for parameterisation and these are not available. Baldwin (1995) and Dijkstra *et al.* (1992) used a different representation and adopted a Michaelis-Menten relationship in which the fermentation of carbohydrates to VFA for non-growth purposes was limited by increased availability of N-sources. A similar representation can be applied for other nutrient sources that may impair the biosynthetic use of energy in carbohydrates. The pH value might also affect the apparent maintenance requirement, as *in vitro* EMPS decreases at lower pH values (review by Firkins *et al.* (2006)). However, Bach *et al.* (2005) performed a meta-analysis to asses the effect of pH on EMPS, and found no relationship between pH and EMPS. Ruminal pH may obviously have a large impact on the degradation of OM (especially fibre). In this respect, to predict MPS in dairy cattle accurately, representation of pH effects on nutrient degradation is of greater importance than representation of pH effects on microbial maintenance requirements. Synchronisation of energy from degraded carbohydrate and various N sources will theoretically increase EMPS by proper matching of nutrients and avoidance of energy spilling, but benefits of synchronisation *in vivo* are equivocal (Firkins *et al.*, 2006). Recycling of urea-N to the rumen and temporary storage or depletion of intracellular polysaccharides will assist in overcoming instantaneous deficiencies (Dijkstra *et al.*, 2002). Overall, the practical impact of energy spilling and the need to account for it in models of rumen metabolism seems to be limited in most situations in dairy cattle nutrition.

104

In current protein evaluation systems, the source of N (preformed AA or peptides versus ammonia) usually does not affect MPS or EMPS. However, substrate degradation and bacterial growth have been shown to increase with addition of AA or peptides in fibrolytic and amylolytic bacteria (Bach *et al.*, 2005). In various models, the theoretical improvement in EMPS when all N required is provided by AA rather than ammonia varied widely: 19% (Russell *et al.*, 1992), 59% (Dijkstra *et al.*, 1992) and 77% (Baldwin, 1995). A major part of the variation in these values can be explained by differences in the units in which EMPS is expressed (i.e. microbial protein per unit OM or per unit carbohydrates degraded). Incorporation of preformed AA or peptides directly into microbial protein, rather than synthesis of AA from ammonia and a carbon skeleton, enables more carbohydrate to be used for other purposes and consequently results in more efficient growth. In Figure 3, the effect of a change in ratio of N sources used for protein synthesis (default, 0.80 and 0.20 g N per g N required delivered by ammonia and AA, respectively; alternative, 0.40 and 0.60 g N per g N required delivered by ammonia and AA, respectively) is presented. Current mechanistic models include the effect of N source by representation of various N-containing substrate pools, and this representation allows for some variation in predicted EMPS.

Crude protein (CP) content of the microbial material flowing to the duodenum varies widely (between 300 and 660 g/kg bacterial DM; Clark *et al.* (1992)). Although some of these differences in composition may be attributed to methodology, major variation in composition related to diet and time after feeding exists when the same techniques are used. Faster fractional microbial growth rates coincide with higher RNA and lower DNA and microbial cell wall contents. In addition high availability of carbohydrates relative to N sources gives rise to storage of polysaccharides, and N concentrations in bacteria from cows consuming slowly fermentable carbohydrates are higher than those from cows consuming rapidly fermentable carbohydrates (Bach *et al.*, 2005). The stoichiometry of synthesis of microbial cell components is well established and EMPS calculated theoretically varies significantly with changes in microbial composition (Hespell and Bryant, 1979). Thus, actual expenditure of available OM or energy by micro-organisms to synthesise protein can be very different, depending upon microbial composition. An example is presented in Figure 3. The EMPS increases upon a decrease in storage polysaccharide content (polysaccharide content from 0.2 to 0.0 g/g microbial DM) and corresponding increase in CP content (from 0.6 to 0.8 g/g microbial DM). In contrast, current protein evaluation systems usually assume a fixed total CP content of microbial DM. Moreover, individual AA composition observed in various experiments is not constant and again varies with growth rate and with microbial subpopulation (Martin *et al.*, 1996), whereas current protein evaluation systems assume a constant AA profile of rumen microbial matter. In addition, mechanistic models also do not include a representation of variation in AA composition, likely due to paucity of data. Thus, appreciation of dietary factors affecting abundance of individual groups of micro-organisms and their distinct AA compositions is desired to estimate microbial AA flow accurately. Such variation in composition led Firkins *et al.* (2006) to conclude that improved procedures and data for measuring and predicting microbial AA flow to the duodenum are required to make real progress in efficient use of protein for production purposes.

Faster fractional microbial growth rates will increase EMPS by reducing the non-growth requirements. Many mathematical functions are available to estimate growth parameters (including fractional growth rate and lag time) in batch (closed) systems (Lopez *et al.*, 2004). In open systems, such as continuous cultures, fractional rate of passage is amongst the most important determinants of fractional microbial growth rate, as in (quasi-) steady state and when no other clearance routes of microbial matter occur, these should be equal (Pirt, 1975). Whilst most empirical protein evaluation systems assume a constant EMPS, some indeed relate EMPS to rate of passage or feed intake level. The Cornell net carbohydrate and protein system and some systems derived from the gas production technique assume fractional microbial growth rate to be related to fractional degradation rate of substrate. However, although such a relationship may be valid for batch cultures, the development of models to describe microbial metabolism shows that this relationship is not directly applicable to repeatedly fed and continuous cultures (reviewed by Dijkstra *et al.* (2005b)) which are more representative of the *in vivo* situation. For example, Oba and Allen (2003) found *in vivo* that EMPS was negatively related to the fractional degradation rate of starch, but positively related to the fractional passage rate of starch.

It is important to note that fractional growth rate of micro-organisms is not exactly equal to fractional passage rate. Another major determinant of net growth, largely ignored in predicting EMPS, is recycling of microbial material within the rumen. The major contribution to microbial turnover is likely to be related to protozoal activities, since protozoa engulf large amounts of bacteria and since a considerable amount of protozoal biomass is recycled within the rumen (Dijkstra *et al.*, 1998). Defaunation of the rumen almost invariably increases EMPS, although MPS is not necessarily increased due to a possible simultaneous reduction in OM degradation upon defaunation (Jouany *et al.*, 1988). On a variety of diets, simulated recycling of microbial N in the rumen ranged from 35 to 70% whilst observed recycling, measured using isotope dilution techniques, ranged from 20 to 90% (Dijkstra *et al.*, 1998). Recycling of microbial protein wastes energy, although the VFA production may well be increased due to recycling. The released AA may be deaminated and this gives rise to further wastage of N as urea in urine.

Although recycling has a large impact on EMPS, quantitative data are very limited and mathematical

representation of recycling has not received much attention. The limited knowledge about rumen protozoal metabolism is mostly because of their dependence on live bacteria, which confounds their *in vitro* culture (Dehority, 2003). A requirement for live bacteria appears to be manifested in particular in culture periods longer than 2 to 4 days. Moreover, a widely accepted marker to measure the protozoal fraction of microbial protein separate from the bacterial fraction is lacking. New molecular techniques such as real-time polymerase chain reaction (PCR), targeting the gene encoding 18S rDNA to quantify the amount of protozoal biomass (Sylvester *et al.*, 2004), may be promising tools to obtain more quantitative data on protozoa. Protozoal predation is usually represented by rectangular hyperbola, assuming that at low concentrations of microbes, protozoa search thoroughly to secure an adequate uptake of microbial matter, whereas at high microbial concentrations, protozoa reduce their search efforts because of satiation. On a wide range of predator species, the rectangular hyperbola has been found to provide a good description of experimental data (Brown and Rothery, 1993), and this relationship was also central to the levels of predicted recycling mentioned previously (Dijkstra *et al.*, 1998). Information on preferential uptake of certain bacterial species does not reveal a consistent pattern (review by Coleman (1989)). The engulfment of solid feed particles by protozoa is well known and this is also expected to result in engulfment of bacteria attached to these particles. On the other hand, the converse hypothesis of protection against engulfment by means of attachment, has been supported by observations that the fluid-phase, non-fibrolytic bacteria increase to a far higher extent upon defaunation than do the attached bacteria (Weimer, 1992). However, it should be noted that the preference of protozoa for starch and sugars, rather than fibre, will result in a relatively higher availability of starch and sugars compared to fibre upon defaunation and hence a smaller increase in attached, fibrolytic bacteria compared to non-attached bacteria. Interestingly, Rasmussen *et al.* (2005) reported that some bacterial pathogens appear to be resistant to degradation in vacuoles of mixed rumen protozoa. Survival of several types of Salmonella in vacuoles, in particular multi-resistant types, results in higher intestinal cell invasion capacity. On the other hand, toxin-producing Escherichia coli were not engulfed by or attached to mixed ciliate protozoa (Burow *et al.*, 2005). Representation of uptake and digestion of bacteria by protozoa is further complicated because of adaptive mechanisms that bacteria develop to survive protozoal predation. Adaptive mechanisms to increase the survival of bacteria under predation pressure include changing cell surface properties, modification of swimming speed, secretion of bioactive metabolites, or formation of micro-colonies (Matz and Kjelleberg, 2005). Given the large impact of microbial turnover on microbial efficiency and the small amount of experimental data, it appears that mathematical representation of selective engulfment and digestion of bacteria by protozoa may well

be improved further. Quantification of microbial recycling will help to establish more accurate estimates of microbial protein yield factors to be applied in protein evaluation systems.

Finally, it is essential to note that virtually all of our present quantitative knowledge on microbial metabolism in the rumen and consequently its mathematical representation is based on culturable rumen microbial species. Recent molecular techniques indicate that understanding on the presence of various microbial species is far from complete though. For example, Edwards *et al.* (2004) showed that only 11% of the diversity of the rumen microbial ecosystem is represented by cultured isolates. Hence the information on biochemical properties used to develop mechanistic models may represent only a small fraction of the properties of the total microbial population in the rumen. Molecular techniques may give a wealth of information, but to include such findings in systems predicting the nutrient profile requires quantitative rather than qualitative data as well as close collaboration between molecular scientists and mathematical modellers on interpreting and evaluating data. Novel cultivation techniques have been described that have shown success in the isolation and cultivation of previously unculturable organisms (Kaeberlein *et al.*, 2002) but none of these systems is suitable for application to anaerobic ecosystems. Application of such novel cultivation approaches to the rumen ecosystem will contribute directly to increasing the understanding of microbial metabolic and fermentation processes by recovering in culture previously uncultivable organisms, and may lead to further improvements in the mechanistic representation of MPS.

Environmental pollution

Usually, feed accounts for the major part of all costs on a dairy farm. Numerous studies have demonstrated that nutrition has a major impact on milk production and thus on farm profitability. Milk production responses to increased nutrient intake usually show a curvilinear pattern that follows the law of diminishing returns. Dietary intake or nutrient density at which efficiency of production (output divided by input) is maximised is by and large different from that which maximises financial profits (VandeHaar and St-Pierre, 2006). As a consequence, surpluses of nutrients that may negatively affect the environment have increased dramatically with increased production levels. Nutrients causing environmental concern are in particular those containing excessive nitrogen (N), phosphorus (P) and potassium (K) (Tamminga, 2003). Nitrogen surpluses are associated with losses from the farm system through ammonia volatilisation, nitrate leaching, and dissipation as N_2, N_2O, NO and NO_2 which negatively affect the quality of surface and groundwater and air. P wastes from dairy farms are associated with eutrophication in streams and lakes. Another major environmental concern is global

warming. Enteric fermentation in cattle gives rise to methane excretion which is a potent greenhouse gas. Thus nutrition has a large impact on profitability as well as on environmental sustainability, and a proper balance is sought by farmers and governments.

Given the environmental concerns, there is increasing interest in investigating the potential of specific on-farm measures and of government policies to reduce wastes (Tamminga, 2003; Rotz, 2004). The formulation of strategies requires first of all accurate estimates of excretion of waste, with monitoring surveys indicating the actual state of emissions. Secondly, analyses of management strategies to mitigate emissions under specific farming circumstances are needed. Such strategies require not just data and knowledge on an animal level, but also on other subsystems of the farm, including crops and soil, and therefore whole-farm models are developed.

A common characteristic of these whole farming approaches is that static, empirical representations are used for the individual subsystems with presumed values for transfer rates between these subsystems, such as the efficiency of feed conversion into milk and manure, emission rates and fertilising value of manure, and losses from soil. Such models are relevant for recognition of the relationship between whole farm performance and management within the individual subsystems. However, they have limited applicability to individual farms. Index values are derived from registered farm performance or from general values in databases. These models cannot relate to the level of detail involved with farmer experience and the precise conditions met at a specific farm, nor can they be applied to enhance innovation within subsystems (Sterk *et al.*, 2006). Proper integration of the subsystems into a whole farm model requires appreciation of variation within a subsystem and the interactions between subsystems. For example, reductions in the amount of fertiliser-N may increase N efficiency on the soil-crop subsystem, but at the same time it may result in grass characterised by a lower protein content and degradability and in lower feed intake and milk production at the animal sublevel (Valk *et al.*, 2000). Rotz *et al.* (2005) developed such an integrated beef herd model with the animal subsystem based on nutritional requirements using the Cornell net carbohydrate and protein system. In this model, the response of animals to changes in the diet was achieved through iterative solutions using linear programming techniques. The recognition of variation within the animal subsystem is an important element in this model. However, the drawbacks of current empirical energy and protein systems described in the previous sections also limit prediction of animal response and subsequently excretion of unused nutrients. Another example is the whole-farm model with five subsystems (animal, feed, manure, soil, crop) of Schils *et al.* (2005) to estimate methane emissions. In the animal submodel, methane production is a linear function of milk production assuming a fixed conversion factor of 0.01 kg methane produced per cow per year per kg milk. Equally,

the Intergovernmental Panel on Climate Change (IPCC) recommends calculation of methane emissions based on a fixed conversion value of gross energy (GE) to methane (for dairy cattle: 6% of GE intake, except for diets with a large quantity of grain; IPCC (1997)). However, diet intake and diet composition changes that may result from management changes within the non-animal subsystems, actually cause large variations in the amount of methane per kg energy intake or milk produced (review by Kebreab *et al.* (2006)). Thus, empirical models, including current feed evaluation systems, largely lack the biological basis necessary to evaluate mitigation strategies to reduce excretion of waste. Mechanistic models are important tools for assessing mitigating options and for directing experimental research towards options most likely to result in significant reduction of emissions. It is also important that mitigation options take other policies into account. For example, mitigation strategies to reduce methane may impact on nitrate leaching in the soil and ammonia volatilisation into the air. A first attempt to integrate emissions of various key environmental pollutants (N, P and methane) from dairy cattle within a mechanistic, nutrient-based feed evaluation system was presented by Kebreab *et al.* (2004).

At present, prediction of surpluses excreted into the environment is based on models originally developed to predict nutrient utilisation by the animal. The increased importance of environmental issues in dairy cattle production will likely open up new opportunities to develop and improve nutritional models, be it from a different perspective. For example, current mechanistic models to estimate methane emission in cattle are based on nutritional models developed to predict the type of nutrient available for absorption (Mills *et al.*, 2001). In these mechanistic models, the stoichiometry of VFA formation is essential as the type of VFA absorbed is usually categorised into glucogenic or lipogenic, and the ratio between glucogenic or lipogenic nutrients affects milk production and composition as well as energy balance of dairy cattle (Van Knegsel *et al.*, 2005). At the same time the production of methane is also related to the type of VFA formed. Consequently, models of rumen fermentation developed to predict the profile of nutrients available for absorption were subsequently applied to predict methane production. Further improvements in VFA stoichiometry were partly driven by interest in methane mitigation options. Bannink *et al.* (2005) included the effect of ruminal pH on VFA stoichiometric parameters related to type of substrate fermented, which indicated the profound effect of type of substrate fermented as well as of rumen pH on predicted methane yield (Table 2). Some mitigation strategies to reduce methane emissions, including the addition of fats (in particular unsaturated fatty acids and lauric and myristic acids; Giger-Reverdin *et al.* (2003)) and of organic acids (including fumarate and malate; Castillo *et al.* (2004)), are not yet covered by mechanistic models, although they affect the profile of nutrients available for absorption. Similarly, reduction in methane emissions from the rumen may be

Table 2 *Methane production from various substrates based on volatile fatty acid stoichiometry derived without variation in rumen pH (pH not considered as independent variable) and with rumen pH variation (pH included as independent variable) in diets composed mainly of roughages (R) or concentrates (C) (adapted from Bannink* et al. *(2005). When pH was included, values are given for pH 5.5 and pH 6.5*

	Methane yield (kJ/g substrate fermented)					
	Without rumen pH variation		With rumen pH variation			
			Rumen pH 5.5		Rumen pH 6.5	
Substrate	R	C	R	C	R	C
Starch	2.40	2.02	1.95	1.69	2.49	2.11
Soluble sugars	3.68	2.92	2.68	1.95	3.98	3.18
Hemi-cellulose	2.53	3.07	2.36	3.38	2.36	3.38
Cellulose	2.70	3.74	2.30	3.10	2.30	3.10

partly offset by increased emissions from hindgut fermentation or subsequently in slurry methanogenesis (Hindrichsen *et al.*, 2006), which for predictions on the animal sublevel require models of hindgut fermentation. Research into such strategies may attract more funding from governmental offices than research with production oriented goals. Thus further development of nutrient based models may well take place within an environmental framework.

Current empirical and mechanistic models to predict the profile of nutrients available for absorption at the animal sublevel generally do not contain sufficient detail to provide input for the crop and soil sublevels. The environmental impact of N excreted in faeces and urine depends on a number of factors, including the manure N and OM composition, in turn largely affected by diet composition and intake level. Increasing the level of dietary CP not only increases total excretion of N in faeces and urine, but generally gives a proportionally larger rise in urinary N excretion (Castillo *et al.*, 2000), which has been associated with increased ammonia volatilisation. Other manure characteristics, including pH, may affect ammonia volatilisation though (Misselbrook *et al.*, 2005). Mineralisation of organic N in slurry is negatively related to fibre content of the slurry, and the uptake of N from slurry by plants and losses from the soil also depends on the form of faecal N and the ratio between C and N in the faeces (Powell *et al.*, 2006). Urea in faeces is rapidly converted into ammonia and potentially immediately available for plant uptake. Undigested feed N in faeces is relatively recalcitrant in soils and mineralises slowly, and is therefore unavailable to plants over the short term. Availability to plants of faecal organic N arising from endogenous secretions in the GIT and from undigested microbial protein is intermediate to that of ammonium-N and undigested feed N. Hence the prediction of N excretion in faeces and urine of dairy cattle and its subsequent fate in plants, soil, water or air depends on the composition of faeces and urine, and requires

mathematical description of the fermentation and digestion processes in the gut. An example of further development of a nutritional model to predict faecal and urinary composition was presented recently by Reijs *et al.* (2006). They expanded the mechanistic rumen model of Dijkstra *et al.* (1992) with static equations to describe digestion processes in the intestines and to describe excretion of various urinary components. The model predicts OM, C and N excretion of various faecal and urinary components. Based on the C:N ratio of faecal and urinary components, N excretion was partitioned into three fractions according to potential availability to crops, viz. an immediately available fraction (N_M), easily decomposable fraction (N_E) and a resistant fraction (N_R). Simulations were performed for four different ryegrass silage types with a high or low fertilisation rate (350 and 175 kg N per ha per year) and an early or late cutting regime (3000 or 4500 kg DM per ha). Some results are presented in Figure 4. The results indicate a wide variation in total N excretion, C:N ratio of faecal DM, and in contribution of N_M, N_E and N_R to total N excretion. Thus management strategies to adjust silage characteristics have a large impact on simulated total N excretion and excreta composition that may affect plant and soil use of N and impact on the environment. Animal manure should be treated as a commodity rather than a waste in whole-farm systems (Tamminga, 2003). Such modelling efforts also indicate that further improvements in representation of post-ruminal processes are required to predict manure composition. As before, these improvements may help improve the prediction of nutrient availability to the animal as well, although from a different angle. It is important to note that variation at the plant level has a significant bearing upon response at the animal sublevel, and in whole farm evaluation of mitigation strategies this interaction should not be ignored (Rotz, 2004).
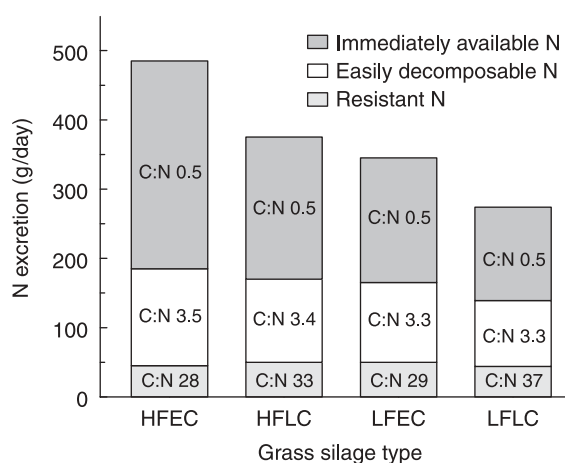

Figure 4 Simulated faecal and urinary N excretion as various N fractions and corresponding C:N ratio for different diets based on four grass silage types (HFEC = high fertilisation and early cut, HFLC = high fertilisation and late cut, LFEC = low fertilisation and early cut, LFLC = low fertilisation and late cut). Total C:N ratio was 4.1 (HFEC), 5.9 (HFLC), 5.7 (LFEC) and 8.0 (LFLC). Adapted from Reijs *et al.* (2006).

Conclusions

In dairy cattle, a change from a requirement to a response system to meet the needs of various stakeholders necessitates prediction of the profile of absorbed nutrients and its subsequent utilisation for various purposes. Integration of new experimental research findings requires mechanistic models that can incorporate new concepts and data. This contribution highlighted a number of key issues in further development of models that predict the profile of nutrients available for absorption, including fractional passage rates out of the rumen and variation in rumen microbial efficiency. Increasing interest in environmental issues will stimulate further development of nutrient based mechanistic models within an environmental framework. Strategies to mitigate waste emissions at the whole-farm level need to include variation at the animal sublevel.

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