PROCEEDINGS OF THE NUTRITION SOCIETY

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Symposium on 'Regulation of nitrogen retention in farm animals'

Limits and limitations to nitrogen use in farm animals

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We are interested in the limits to N use in farm animals for several reasons. An important one is to know what is an achievable limit, since it allows for realism in efforts to rectify apparent deficiencies, or to modify or control the performance of animals. There is no point in striving to move performance beyond a known, real, limit! Consideration of quantitative limits to N use, and the development of frameworks to allow that quantification, therefore, has real relevance to provide a context within which to study regulatory processes. In theory, 'limits' to the use of all nutrients and their products are set by the nature of the animal, i.e. its genes. These determine the possible maximum rates of formation of gene products. But for the great majority, if not all circumstances, these possible maximum rates are not achieved because the body's various regulatory processes (endocrine, neural, etc.) act to modulate metabolism and allow control. The extent to which genes are expressed will define the effective limits to metabolism, and to N use. Gene expression clearly depends on the internal regulatory systems (which are themselves either gene products or under the influence of gene products) and the provision of resources as substrates. The basic resources either are, or are derived from, nutrients. 'Limitations' on the use of any nutrient will, therefore, be some function of the regulatory systems of the body and the amounts and proportions of nutrients which are supplied for metabolism (Fig. 1).

In practice, the limit to N use for an individual might be said to be that which applies when neither the regulatory processes, nor resource (nutrient) provision, is constrained. In this sense, limits have two dimensions, one being the rate of N use (of mass per unit time, e.g. g/d for growth, lactation, egg or fibre production) and the other the mass efficiency of conversion of substrates into product (g N product/g N substrate). Such limits apply to unconstrained performance, i.e. that which is directed by the animal's genes and not constrained by the qualities of food or environment.

At least in theory, limits to N use can be modified in a number of ways. Genetically-determined limits are readily changed across generations by genetic selection. Within generations, limits can be changed by over-riding genetically-determined controls (i.e. the extent of inherent gene expression) through interventions such as the application of endocrine agents (to stimulate growth, lactation) or by immunological means. Aspects of each of these are dealt with in the present symposium. The object of the present paper is to provide a framework for thinking about limits and limitations as they apply to the use of N by the whole animal.

A HELPFUL OVERALL FRAMEWORK

We have found it helpful over the last few years to think about limits and limitations which apply to the performances of farm livestock by using the framework of ideas outlined in Fig. 2.
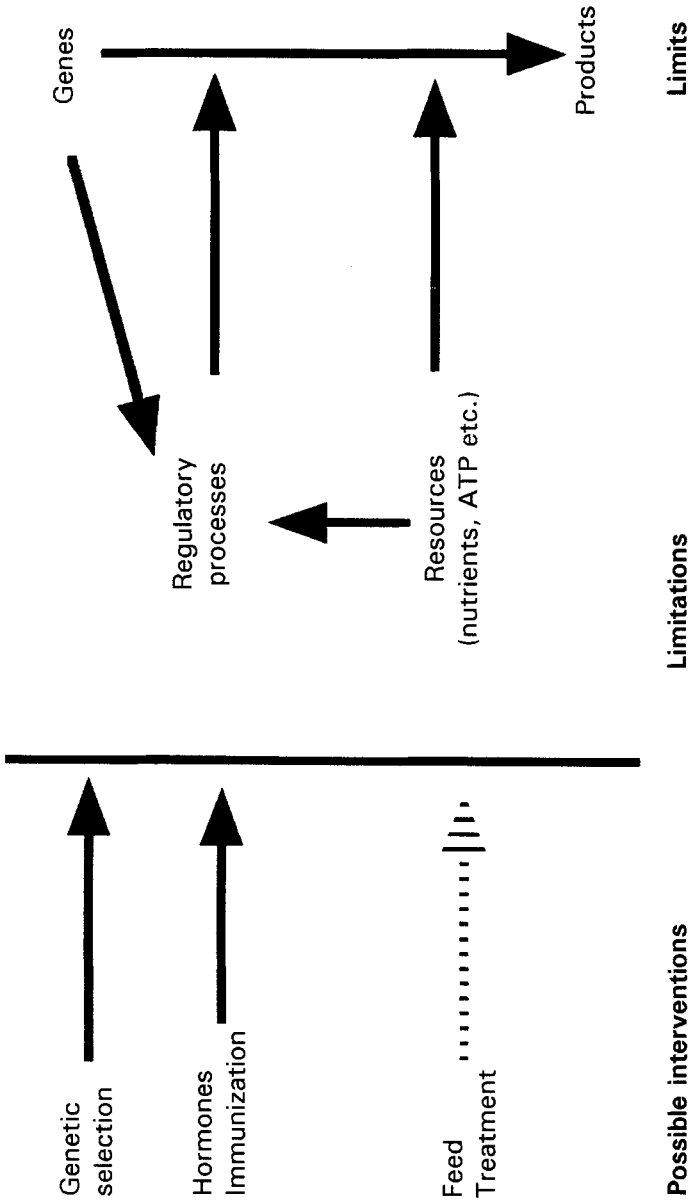
Fig. 1. A schematic representation of the way in which resource supply and internal regulatory processes act as 'limitations' to modify the 'limits' to product formation which, ultimately, are driven by the gene complement of an animal. Some possible 'interventions' are shown which can influence limiting factors at each of the levels of genetic makeup, internal regulation and resource supply.
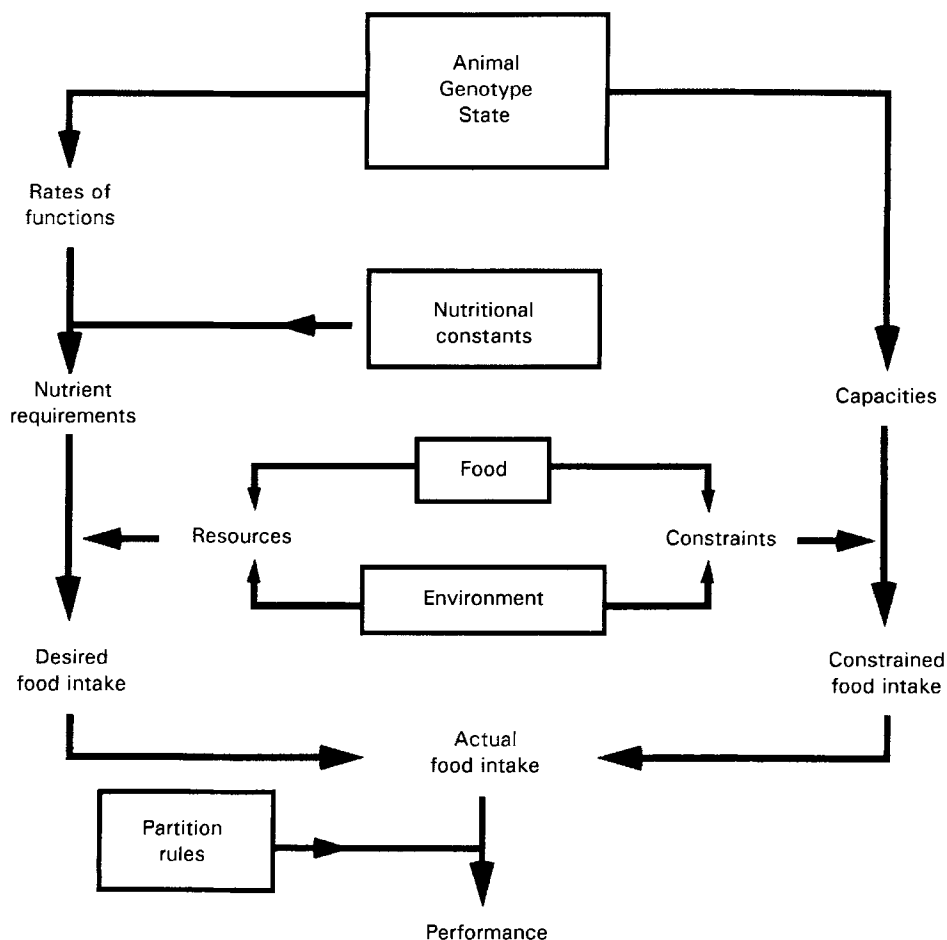
Fig. 2. A schematic framework outlining the concepts which link animal, food and environmental descriptors to food intake and animal performance.

Fig. 2 is intended to suggest that an animal of a given genotype in a given state (body composition, reproductive state, health state) is seeking to eat sufficient of a nutritionally-balanced food to allow it to meet its 'performance' objectives; i.e. the desired food intake of the animal is a function of its target performance. With respect to N use, no growing animal would be expected to deposit or secrete more N endproducts (largely proteins) than its target, unless its internally regulated controls were over-ridden by management (i.e. external) interventions. It would, however, be expected to try to follow a genetically-determined growth path which would direct skeletal, associated muscle and organ development. Divergence from this path would be rectified opportunistically; perhaps under the kind of control which Millward (1995) has referred to in his 'protein-stat' theory of growth regulation.

The framework also recognizes that there are limits to the capacities of animals to deal with constraints which are either characteristics of food or other aspects of the environment. For example, physical properties of food (e.g. water-holding capacity) or the presence of toxins may be accommodated by animals up to a certain limit, beyond which intake is restricted (constrained).

The amounts of nutrients which are yielded to the animal for a particular rate of food consumption are partitioned amongst various metabolic pathways, according to some regulatory profile. If the animal has achieved its desired intake of a perfectly, nutritionally-balanced food, then the partition rules which apply are those which allow the animal to achieve its phenotypically-driven limit. However, if, as is common, intake is constrained or imbalanced, then the challenge for the nutritional scientist is to establish partition rules which will govern the regulation of metabolism for inadequate or imbalanced resources.

These comments are little more than a re-statement of the fundamental nutritional questions: how much food will an animal eat and how will it dispose of nutrients from that food once it has been eaten? The value of the structure (Fig. 2) is to focus on key issues which are likely to help quantify answers to these questions.

An important underlying premise for this logic is that animals will seek to eat sufficient of an appropriately balanced food to allow them to achieve their target, or preferred, performance (i.e. in some practical sense, a phenotypic limit) unless over-ridden by an externally-managed intervention. Is this a reasonable proposition? One approach to test it has been to offer free, *ad libitum*, choices between pairs of feeds to animals to challenge their abilities to select amounts and proportions of foods which allow them to achieve what appear to be limiting rates of performance, or to adapt their feeding behaviour to rectify the consequences of previous imbalances of nutrition.

By offering, to growing pigs, free *ad libitum* choice between pairs of highly-digestible feeds which differ in their protein : energy value, it has been shown that such animals will make dietary choices which allow them to grow protein at what appears to be a maximal rate, and to adjust the compositions of their bodies (lipid : protein value) in consistent ways, according to their current lipid : protein value (Kyriazakis & Emmans, 1991), their genotype (Kyriazakis *et al*. 1993*b*) and their state of growth (Kyriazakis *et al*. 1993*a*). Both pigs and sheep also seem to make selections between digestible feeds of different protein : energy value, such that they retain N in body tissues at what appears to be a maximal rate whilst avoiding surplus intake of N, i.e. intake of N above the limit for 'productive' use is minimized (Kyriazakis *et al*. 1990; Hou *et al*. 1991; Kyriazakis & Oldham, 1993).

Such studies have convinced us that it is a reasonable proposition, at least for the growing animal, to state that animals attempt to eat to satisfy needs which will allow them to grow according to their internally-driven limits.

The picture may be a little different with dairy cows, whose milk production is managed through external intervention in most modern systems of milk production. Milk production is not driven by the combined needs of the cow-plus-calf system but by the mechanically-controlled withdrawal of milk at a frequency dictated by man and not the cow and/or calf. For some feeding systems, at least, dairy cows do not appear to select between foods of different protein : energy yield according to phenotypic milk yield (Tolkamp *et al*. 1996, 1997*a*). Rather, they seem to make dietary choices which can be interpreted as being consistent with the achievement of a satisfactory yield of rumen-degradable protein to support rumen microbial activity (Tolkamp *et al*. 1997*b*). We have some evidence for the same phenomenon in sheep (Cooper *et al*. 1994) and one might speculate that such a strategy is appropriate for ruminants to enable them to achieve satisfactory digestion and, hence, intake of forage, i.e. to achieve targets for energy consumption. The manner in which ruminants are able to recognize the extent to which the rumen environment is 'satisfactory' or not to help the achievement of limiting goals is unclear. Amongst other things, the impact of rumen NH_3 load on regulatory systems may be involved, although the evidence that this is effective for dealing with short-term rather than longer-term 'recognition' is not strong (Kyriazakis & Oldham, 1997).

If there were to be circumstances under which available feeds might, in any combination, readily satisfy needs for rumen-degradable N but differ in their capabilities to supply absorbed amino acids (metabolizable protein; Agricultural and Food Research Council, Technical Committee on Responses to Nutrients, 1992), perhaps differences in phenotype (for milk-protein yield) might then be expecte.' :o lead to differing dietary selections if feed pairs on offer were to be appropriately uifferent. Results to test this idea are not yet available.

However, environmental influences on N metabolism can lead to modifications in feeding behaviour under other circumstances. When sheep have been challenged with intestinal nematodes which are known to increase endogenous N loss into the gastrointestinal tract (Poppi *et al.* 1986), and which will also evoke an immunological response, their choices between pairs of feeds which differ in N : metabolizable energy value adjusts to conserve N intake even though total food intake is suppressed somewhat (Kyriazakis *et al.* 1994, 1996).

QUANTIFYING LIMITS: GROWTH

A framework for describing the unconstrained growth of animals to maturity has been described (Emmans & Fisher, 1986; Emmans & Oldham, 1987; Emmans, 1989). A Gompertz description of the growth curve (Hancock *et al.* 1995) defines three variables (initial size (or protein mass), mature size (or protein mass) and the rate of maturing). These together allow the estimation of rates of protein deposition at any particular degree of maturity; the variables can be used to define a genotype, and the approach has been applied to poultry (Hancock *et al.* 1995), pigs (Ferguson & Gous, 1993*a,b*) and sheep (Friggens *et al.* 1997).

Poultry provide a good test of ideas about the possible influences of genetic selection on limits and limitations to N use in growing animals, because selection for growth performance in this species has been the most extreme for any farm species. In broad terms, mature weight (A) and rate of maturing (Gompertz rate parameter; B) vary inversely with each other in unselected species (Table 1 illustrates the relationship for mammals, but there would be expected to be a similar pattern for birds).

Options for genetic selection to modify limits to N use might be thought of, therefore, schematically (Fig. 3), i.e. selection could be for increased mature size, whilst accepting an associated reduction in scaled rate of maturing, an increased rate of maturing with no change in mature size, or something intermediate. It is generally unlikely (for food-producing animals) that genetic selection would be for reduced mature size.

The performances of male broilers over the last few decades (Table 2) show that both mature protein mass and scaled rate of maturing have increased, genetically, during that

Table 1. *Mature weights (A), Gompertz rate parameters* (B) and maximum growth rates (g/d) across unselected mammals*

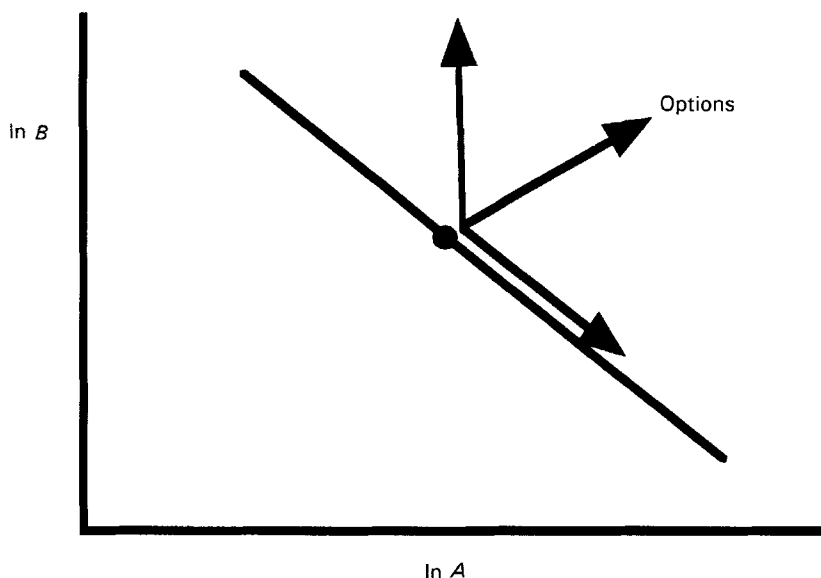| A (kg)	B (/d)	Maximum growth rate (g/d)
0·04	0·087	1·3
4	0·025	37
400	0·0072	1060

* See Hancock *et al.* (1995).

Fig. 3. An illustration of possible options to change, through genetic selection, limits to growth performance by exploiting or modifying the general relationship between mature weight (*A*; kg) and the Gompertz rate parameter (*B*; /d; Hancock *et al.* 1995) which holds across unselected species.

Table 2. *Mature protein weight* (P_m; *kg*), *Gompertz rate parameters* for protein growth (B;/d) and rates of protein gain at market weight (g/d) for poultry (male broilers) in commercial populations over the last 40 years*

Year...	1955	1975	1995
P_m	0·8	1·1	1·4
B	0·025	0·03	0·045
Protein gain at market wt (g/d)	7	12	21

* See Hancock *et al.* (1995).

time, with enormous changes in daily rates of protein gain at market weight. This is a very powerful illustration of the magnitude of effects which genetic selection can have in changing limiting rates of performance. By comparison, the rates of change in limits to N accretion, even in highly-selected pig populations, are modest and, in the ruminant populations, almost trivial, even allowing for the extreme effects of breed substitution, especially with cattle.

Such modifications to the rates of limiting N retention carry with them some penalties (e.g. problems with the development of bone structures and functionality of internal organs; the latter, for example, leading to potentially disastrous health problems such as ascites in poultry; Julian, 1993) but also secondary advantages, such as reduced N wastage for improved environmental control.

QUANTIFYING LIMITS TO NITROGEN USE: LACTATION

To describe unconstrained limits to N use in lactating animals presents more challenges than there are with growth. Litter size in suckling animals, or the frequency of milk removal in animals which are milked artificially, are each likely to influence the rates of

synthesis of milk in the mammary gland by changing the rates of removal of autocrine feedback regulators which accumulate in milk (Wilde *et al.* 1995). As previously stated, therefore, the upper limit to milk N secretion is more a function of environment (management) than of genetic control. The definition of an unconstrained environment for a lactating animal, therefore, must include some definition of 'unconstraining management'. Also, we should consider the manner in which N use in body tissues (including the mammary gland) would develop through single and sequential lactation cycles under unconstrained conditions. This is an issue of N partition between milk and tissue which might be treated in a way analogous to that which has been applied in poultry to deal with the partition of N between eggs and feather protein (Emmans, 1989).

Although these issues are clearly important, no established framework exists with the same completeness as that for growth. A preliminary outline was presented some time ago (Oldham & Emmans, 1988), but a more thorough treatment is overdue. It is an interesting debate as to whether the biochemically-based estimates of synthetic limits in models such as those proposed by Baldwin *et al.* (1987) can satisfactorily describe limits to N use which are helpful in this regard.

It is currently our view that, without an effective framework to quantify limits to N use in lactating animals in the terms implied in Fig. 2, the degree of success which may be achieved in overcoming apparent limitations to N use in lactation will be difficult to quantify, at least with the same precision as is possible with growing (especially non-ruminant) animals.

NUTRITIONAL LIMITATIONS

A restricted supply of N substrates will limit N retention. More challenging issues concern the role of nutritional imbalances (malnutrition) on N use. Amino acid imbalances, and protein : energy-yielding substrates (protein : energy value) are two things which have particular significance in affecting the efficiency of N use.

An ideally-balanced mixture of amino acids, by definition, will be used with maximal mass efficiency for net protein synthesis in a particular situation, provided that other support resources (particularly energy) are abundant. Not all amino acids in an ideal mixture need necessarily be used with the same mass efficiencies for protein synthesis if there are obligatory routes of metabolism, other than protein synthesis, which differ between individual amino acids.

Fuller *et al.* (1989) have provided estimates for the efficiencies of use of different essential amino acids for net protein retention in growing pigs. These show that, under N-limiting conditions, the efficiencies of use of amino acids in an ideal mixture are high. Similarly high estimates can be found for the efficiencies of use of amino acids in ideal mixtures for growth or egg production in poultry (Oldham, 1987).

These high efficiencies apply only when other resources which are needed to support the use of amino acids for protein synthesis are adequate. In particular, when energy supply is limited, but ideal protein supply is sufficient for 'unconstrained' N use, it may be expected that there will be partial catabolism of protein to restore a protein : energy value which allows efficient use of resources. This proves to be the case. Improved pig genotypes (Large White × Landrace crosses) use ideal protein for protein retention with a high efficiency (0·82) when metabolizable energy : ideal protein (E : P) exceeds 72 kJ/g (Kyriazakis & Emmans, 1995). Below this value the efficiency of use falls at a rate which is consistent with expectations that part of the ideal protein is catabolized to release energy for the remaining protein to be used for net protein retention. A genotype which has not undergone intensive selection for growth characteristics (Chinese Meishan; Kyriazakis

et al. 1994) behaves in the same way with respect to N use under protein- and E : P-limiting conditions.

These measurements of critical E : P values for the efficiency of ideal protein use are important. They provide a strong rule, which applies across genotypes, to predict the use of a limiting amino acid supply both when energy supply is adequate, and when it is not. When combined with a knowledge of expected performance under non-limiting nutrition this rule provides an effective way to help predict the partition of N for growth.

There is a widespread view (Reeds & Davies, 1992) that the value for protein synthesis : net protein retention in the whole animal increases in animals as they grow to maturity. There is no doubt that protein synthesis is maintained even under the most strenuous and challenging conditions in mature adults (Stroud *et al.* 1996); thus, it is inevitable that synthesis : accretion will rise as net accretion falls to zero, i.e. mature (protein) size is reached. As there may be presumed to be some failure to recapture amino acids which have been released on the degradation of proteins (Neale & Waterlow, 1983), it may be thought that the efficiency of use, even for ideally-balanced amino acid mixtures, would reduce with maturity. This seems not to be the case. In cattle of different weights offered different amounts of the same food, the incremental efficiency of conversion of dietary N to N balance was relatively high and did not change with age (Blaxter *et al.* 1966). Similarly in growing pigs (Quiniou *et al.* 1996), there was no evidence of a reduced efficiency of the conversion of ideal protein into net protein retention over the weight range of 45–100 kg. Such observations suggest that the process of protein 'turnover' need not influence net use of N under 'normal' conditions, although interference with major regulatory processes (Grizard *et al.* 1995) can be associated both with effects on net use of N and on the component processes of protein synthesis and degradation.

These principles of efficiency of N use will apply equally to ruminants and to non-ruminants. Data such as those reported by, for example, Storm & Orskov (1984) and Rulquin & Vérité (1993), give sufficient evidence to confirm that the broad principles of amino acid nutrition apply to ruminants in the same ways as they do to non-ruminants. However, there are difficulties in applying reliable control of nutrient supply to ruminant animals through feeding (for reasons of the imprecision and inaccuracy of prediction of the endproducts of rumen fermentation and digestion which remain despite continued attempts over many years to do better). Consequently, there is no reliable set of information to confirm values for the limiting efficiencies of use of amino acids in an ideal mixture, nor for the influence of E : P value on these efficiencies in ruminants.

Such evidence as there is (Oldham, 1987, 1995) would seem to suggest that, at least for lactating cows and goats, the mass efficiency of use of a first-limiting amino acid for milk production can be high (approximately 0·85), and rather similar to values that might be found for growth in non-ruminants, or for egg production in poultry. There are really no good data to allow us to state a position on this, reliably, for growing ruminants, although there have been suggestions that efficiencies of conversion of absorbed amino acids (metabolizable protein) for tissue protein retention in growing ruminants often appear to be rather low (Agricultural and Food Research Council, Technical Committee on Responses to Nutrients, 1992).

The influence of protein : energy value on the efficiency of use of (ideal) amino acid mixtures is still largely conjectural in ruminants for the reasons already stated, relating to the difficulties of getting reliable quantification of nutrient supplies for particular dietary situations. The often low marginal efficiencies of use of (estimated) increments of metabolizable protein for milk protein synthesis may sometimes be a reflection of protein–energy balance as a limitation on N use (Oldham, 1995) in this situation.

CONCLUSION

Whilst a great deal of research effort is geared, rightly, to the acquisition of new knowledge about how the principles and processes through which the conversion of N substrates into N products (a) occur and (b) are regulated, the application of such knowledge into nutritional practice will frequently depend on having quantitative frameworks to express such knowledge. Indeed, it could be said that the most stringent tests of the value of new understanding in this area (and in many others) are those which require quantification of issues in terms that can be applied to, and the consequences measured in, whole animals. If improvements in understanding of the elements of nutritional science cannot be applied with measurable effect in living subjects (human or animal) are they improvements? To this end we have geared our thinking about the subject in ways which emphasize quantification, and which are 'animal-centred' rather than 'process-centred'. The identification of 'limits' to N use, and the 'limitations' to their achievement as presented here are, therefore, in this context and lie as a general backdrop to the more detailed accounts of processes which follow.

The simple framework presented in Fig. 2 is one which we have found particularly useful in developing concepts about the manner in which animals pursue a state of nutrition which allows them to achieve internally-driven targets, and what needs to be known in order to predict how they perform when they cannot achieve these targets.

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