The importance of protein turnover to metabolism and carcass composition of growing lambs

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ABSTRACT

Carcass composition is determined by the relative partitioning of metabolisable energy intake among protein, fat and heat. Protein deposition is the net difference between rates of protein synthesis and protein degradation in tissues; synthesis and degradation proceeding at rates several times that of deposition. Relatively small changes in rates of protein synthesis or degradation may result in relatively large changes in deposition.

The energy cost of protein synthesis is calculated to account for up to 50% of heat production in growing animals. Increasing rates of protein synthesis may reduce energy available for fat deposition.

The manipulation of carcass fat content may be more readily achieved by increasing lean growth than reducing fat deposition.

Development of accurate methods for measuring protein synthesis should facilitate understanding of the mechanisms by which lean animals are grown and the selection of growth promotants useful in the production of leaner carcasses.

Keywords Protein turnover; growth; sheep; lean lambs; protein synthesis; overfatness; metabolism; carcass composition; leucine.

INTRODUCTION

One of the current objectives of animal production research is to control the partitioning of metabolisable energy (ME) intake such that the ratio of protein:fat deposition is increased. While it would seem logical to search for methods of reducing carcass fat content by reducing the rate of fat deposition, such a manoeuvre may favour lighter carcass weights. However, increasing the rate of protein deposition will both reduce carcass fat content and increase growth rates. The problem of overfat lambs may be more logically viewed as one of underleanness.

Protein Turnover, Energy Metabolism and Carcass Composition

Protein deposition is the net result of two processes, protein synthesis and protein degradation. Tissue proteins are continuously being synthesised and degraded at rates characteristic of the protein and tissue. Protein turnover is the collective term for these processes.

Rates of protein synthesis (and degradation) in tissues are many times greater than deposition rates. For example in 20 kg lambs (Davis *et al.*, 1981) it was calculated that whole-body protein synthesis was 600 g/d (and degradation 580 g/d) when deposition rates were 20 g/d. Clearly only very small changes in the rates of synthesis (or degradation) are required to substantially alter the deposition rates.

The process of protein synthesis is energetically expensive, calculated either on the basis of ATP requirements for peptide bond formation (Waterlow *et al.*, 1978) or on the basis of associated increases in heat production occurring with increasing protein synthetic rate (Pullar and Webster, 1977; Reeds *et al.*, 1980). Protein turnover has been calculated to account for up to 50% of heat production (Webster *et al.*, 1978; Reeds *et al.*, 1980; Davis *et al.*, 1981). Furthermore, it has been suggested by Miller *et al.* (1979) that protein turnover may be an important cycle regulating the partitioning of energy among protein, fat and heat. Therefore, increasing protein synthesis may be a means not only of increasing protein deposition but also of reducing the energy available for fat synthesis.

Evidence supporting this contention is suggested by the observed relationship between protein synthesis and fat deposition. For example, as the rate of protein synthesis decreases with advancing age (Fig. 1), carcass fat content is generally reported to increase (Berg and Butterfield, 1976). Of interest is the observation that the rate of protein synthesis is rapidly declining at about the same age (or weight) at which the animal enters the "fattening phase of growth" (Searle and Griffiths, 1976). Also, there is evidence that when the rate of protein synthesis is higher, as in genetically lean *v* obese rats (Webster *et al.*, 1978), fat deposition is reduced. Presumably, the leaner carcasses observed in these studies would be brought about by decreasing the amount of ME

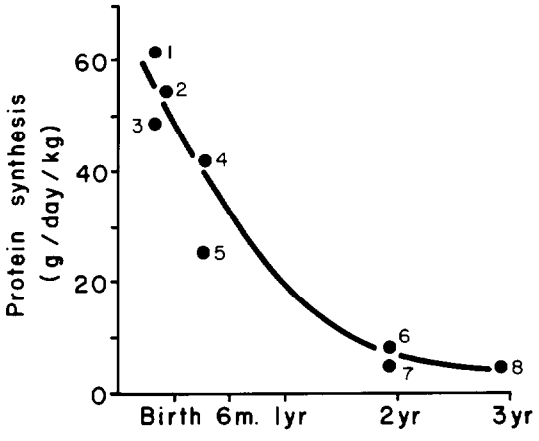

FIG. 1 Whole body protein synthesis changes with age in sheep.
(1) Schaefer and Krishnamurti (1984a); (2) Soltesz *et al.* (1973); (3) Noakes and Young (1981); (4) Jones and Symonds (1982); (5) Davis *et al.* (1981); (6) Buttery *et al.* (1975); (7) Bryant and Smith (1982); (8) Schaefer and Krishnamurti (1984b).

available for synthesis and storage as depot fat and/or by increasing the rate of protein deposition via an increased rate of protein synthesis. The higher heat production, growth rates, and leaner carcasses in bulls *v* steers at the same stage of maturity (Webster, 1977) further illustrates this point.

Methods for Measuring Protein Synthesis

Assessment of the impact of protein synthesis on metabolism and growth requires accurate measurement of protein synthesis rates. Current methods for determining protein synthesis are based on measuring the rate of amino acid incorporation into protein. The use of radioactive amino acids facilitates such measurements but errors arise in the solution because of uncertainty, or failure, in measuring the specific radioactivity of amino acid in the precursor pool used for protein synthesis. Specifically, there is disagreement as to whether plasma amino acids, whole blood amino acids, intracellular free amino acids, cell membrane amino acids or some mixture of these pools is the most accurate representation of the protein precursor pool. Depending on which pool protein synthesis measurements are based, widely different results will be obtained. This is exemplified by the study of Davis *et al.* (1981) with growing lambs. In this study, whole body protein synthesis calculated from the plasma leucine specific radioactivity (200 g protein/d) was one third the rate calculated from intracellular free leucine specific radioactivity (600 g protein/d).

In view of the significant impact protein synthesis is likely to have on carcass composition, the development of an accurate method for measuring protein synthesis would help clarify the metabolic requirements for lean lamb production. Further, such techniques may be useful in assessing the mechanism of action and possible synergistic relationships among growth promotants.

Numerous attempts have been made to improve methods of measuring protein synthesis, especially with regard to identifying the amino acid precursor pool from which protein synthesis occurs. These procedures are discussed in reviews by Waterlow *et al.* (1978), Garlick (1980) and Waterlow (1984). Theoretically, the most accurate approach is to measure the t-RNA specific radioactivity following the introduction of a radioactive amino acid tracer. This has been accomplished *in vitro* by Airhart *et al.* (1974) and Vidrich *et al.* (1977) with rat liver tissue. The fractional synthetic rate calculated from these data was found to be intermediate to values obtained with either plasma or intracellular free specific radioactivity values. However, t-RNA analysis is technically difficult (Waterlow, 1984) and furthermore, t-RNA specific activity determinations would be prohibitively expensive in terms of isotope costs, to carry out on large animals.

Alternative methods to using t-RNA labelling have, therefore, been sought. A new procedure adopted by Bier and Matthews (1982) has been to make use of the deaminated leucine product, α-keto-isocaproate (α KIC). The tissue fractional protein synthetic rate was calculated from the αKIC labelling, rather than the leucine specific radioactivity, following the infusion of [(^{15}N,1 – ^{13}C)] leucine. The assumption in this procedure is that the labelling of αKIC resulting from leucine catabolism closely reflects that at the site of incorporation into protein, an assumption which may be in error (Schneible *et al.*, 1981). This procedure as used by Bier and Matthews (1982) is dependent, however, on the availability of gas chromatography-mass spectrometry analysis.

Calculation of Protein Synthesis without Direct Knowledge of the Amino Acid Precursor Pool

As previously outlined, the conventional approach to measuring protein synthesis, based on continuous infusion procedures, suffers from the inability to accurately measure amino acid specific radioactivity in the precursor "pool". This is due primarily to significant differences in specific radioactivity among assumed precursor pools. In particular, amino acid specific radioactivity in intracellular tissue pools is considerably less than that of plasma because of intracellular protein degradation.

In an attempt to remedy this situation McNurlan *et al.* (1979) made use of a "flooding" procedure

where a large bolus of non-radioactive amino acid was injected simultaneously with a radioactive tracer. The strategy behind this approach was to bring about equalisation of specific radioactivity in all precursor pools, thereby minimising the uncertainty as to which amino acid pool was most appropriate in the calculation of protein synthesis. However, when extrapolated to large animals the "flooding" technique as used by McNurlan *et al.* (1979) with rats is not appropriate, primarily because the amount of tracer required for such a procedure in a sheep would be prohibitively expensive.

In order to overcome these problems we have investigated the use of continuous infusion rather than single-injection of unlabelled amino acid (leucine) tracer simultaneously with leucine tracer. By incremental increases in the infusion rate of tracer it was postulated that the specific radioactivity of leucine in plasma and tissue pools would converge as plasma leucine increasingly exchanged with tissue pools. From curve analysis and extrapolation it was hoped to determine the point at which leucine specific radioactivity in plasma and tissue pools was equal. Data supporting this strategy have been obtained previously by Fennessy (1976) with duodenal methionine infusions in sheep.

This was accomplished in the present study with a continuous intravenous infusion of non-radioactive L-leucine (0, 1, 2, 3 g/h; 3 sheep per treatment) administered simultaneously with L-[4,5 – ^{3}H]-leucine. As evident from a series of experiments conducted on wether sheep the specific radioactivity of intracellular leucine in muscle and liver converges with that of plasma as the infusion rate of unlabelled leucine was increased to 3 g/h (Fig. 2).

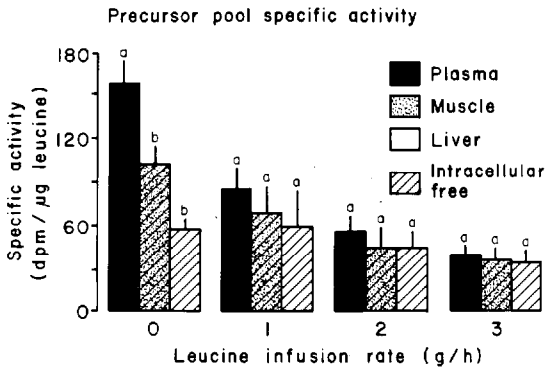

FIG. 2 Leucine specific activity in protein synthesis precursor pools in adult sheep infused intravenously with 0,1,2, or 3 g/h L-leucine.

Preliminary calculation of protein fractional synthetic rate in liver indicates that estimates based on plasma or intracellular leucine specific radioactivity also converge as leucine infusion rates increased (Fig. 3). Further analyses of other tissues are continuing, but this approach would seem to have potential as a technique for measuring protein synthesis in large animals.

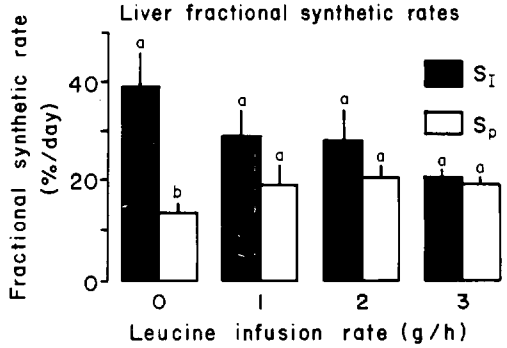

FIG. 3 Tissue fractional protein synthetic rates in liver tissue from adult sheep calculated from either intracellular free (SI) or plasma (Sp) leucine specific activities following the intravenous infusion of 0,1,2 or 3 g/h L-leucine.

CONCLUSIONS

Protein synthesis has been calculated to be energetically expensive (Waterlow, 1984) and is highly correlated with heat production (Pullar and Webster, 1977; Reeds *et al.*, 1980). However, the inadequacy of methods used to assess protein synthesis has led to a variety of viewpoints and assessments as to the demands of protein metabolism on energy metabolism (Kielanowski, 1976; Waterlow *et al.*, 1978; Reeds *et al.*, 1980; Webster, 1980).

Such disagreement may be resolved by the use of amino acid "flooding" procedures either by bolus or incremental infusion outlined above. However, many of the estimates of protein synthesis in animal tissues (or whole-body) currently in the literature are based on inadequate knowledge of the extent of intracellular recycling of amino acids and are thus likely to be underestimates of the true rate. Nonetheless, the data indicate that protein turnover proceeds at several times the rate of deposition and therefore only relatively small changes in (for example) protein synthesis may result in substantial changes in protein deposition. Further, increasing rates of protein synthesis is likely to reduce the energy available for fat deposition.

The accurate assessment of protein synthetic rates in the tissues of large animals is essential to understand the metabolic requirements associated with the production of lean carcasses, and the mechanism of action of growth promotants and hormones.

Finally, as discussed by Webster (1980) the production of lean carcasses is less likely to be

achieved by developing techniques to simply reduce fat deposition without consideration of protein metabolism. Factors which promote protein deposition (increasing synthesis, decreasing degradation) will produce larger leaner carcasses and may even reduce energy available for fat deposition.

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