*Reprod Nutr Dev* (1997) 37, 493–515



**Original article**

# Quantitative aspects of protein fractional synthesis rates in ruminants

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(Received 15 May 1996; accepted 9 September 1997)

**Summary** – Protein fractional synthesis rate (FSR) is a key-factor in the characterisation of ruminant metabolism. Published data from the literature were collected and statistically analysed to isolate the factors influencing FSR. FSR varied largely depending on the tissue considered, over a range from 1 to 20. FSR, with the plasma as the precursor pool for protein synthesis, was halved compared to that of the intracellular pool. The method for supplying the amino acid also significantly affects FSR since the flooding dose technique gave higher FSR estimates than the constant infusion technique. The choice of the labelled amino acid infused influenced FSR. There is a ranking order depending on the tissue or organ. The protein and energy levels of the diets significantly increased FSR, which raises the question of the body nitrogen requirements. Moreover, FSR values were dependent on the physiological status of the animals. To conclude, FSR values should be determined simultaneously with other biological parameters in order to obtain a realistic quantitative estimate of the nitrogen turnover rates during intermediary metabolism.

**protein / fractional synthesis rate / ruminant / methodology / statistical analysis**

**Résumé – Aspects quantitatifs des taux relatifs de synthèse protéique chez le ruminant.** Le taux de synthèse protéique (FSR) est un élément clé du métabolisme des ruminants. Les variations de FSR ont été étudiées statistiquement à partir de données de la littérature pour en isoler des facteurs explicatifs. FSR varie dans un rapport de 1 à 20 selon les tissus. Si le compartiment précurseur de la synthèse protéique est le plasma, FSR est divisé par deux comparé à l'hypothèse du compartiment intracellulaire comme compartiment précurseur. La technique de surcharge en acides aminés donne des valeurs de FSR supérieures à celles obtenues lors d'une infusion continue. Le choix de l'acide aminé infusé influence aussi significativement FSR de façon variable selon le tissu. La concentration éner-

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This paper is dedicated to the late Dr Bjorn Eggum

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gétique ou protéique de la ration augmente FSR, ce qui pose le problème de l'estimation des besoins protéiques de l'organisme. De plus le stade physiologique de l'animal influence les valeurs de FSR. En conclusion FSR doit être déterminé simultanément avec un ensemble de paramètres du métabolisme afin d'avoir une estimation quantitative fiable des flux d'azote dans l'organisme des ruminants.

**protéines / taux de synthèse protéique / ruminant / méthodologie / statistiques**

## INTRODUCTION

Amino acids and proteins are used with a low efficiency by ruminants in their intermediary metabolism. Even though much is known about the qualitative phenomena behind this, quantitative data are needed to understand the variations in the amino acid fluxes and consequently to reduce nitrogen waste by ruminant animals (MacRae et al, 1996). Amino acid utilisation is evaluated more or less directly by several means: tissue protein synthesis, urea excretion, milk protein synthesis. One way to evaluate these different phenomena is to study the rate of nitrogen utilisation by the different tissues, since this might indicate the extent of the intermediary amino acid metabolism (Gill et al, 1989).

Fractional synthesis rate of protein (FSR, %-day) can be defined as the quantity of protein synthesised per day as a percentage of the protein pool size of the considered compartment. FSR in ruminants is a good measurement for evaluating the quantitative metabolism of proteins and amino acids in the organism. When the size of each protein compartment is multiplied by its FSR, values for the fluxes in protein synthesis can be obtained. Consequently, the contribution of each tissue or organ to the protein synthesis of the whole body can be determined. It should be remembered that the quantity of protein synthesised in the organism each day is between two- and four-fold the protein intake (Lobley, 1990).

This work focused mainly on FSR variations and not on protein fluxes since the compartment sizes were not available in most publications and also because FSR is the nearest measurement we have to tissue protein synthesis. The study of FSR is also important since the utilisation of amino acids by the tissues and organs can affect their availability for other purposes. This is particularly true for milk protein production (Meijer et al, 1995). Therefore, FSR are needed to build mechanistic models explaining amino acid fates in ruminants (Lescoat et al, 1996).

Initially, FSR was studied mainly in rodents. The techniques used were then adapted and used with larger animals (Lobley et al, 1980). The FSR values obtained vary depending on a large number of factors related either to the methodology used or to the characteristics of the animal. For this reason, the FSR measurements are termed 'semi-quantitative' (Lobley et al, 1996) since they provide data on the relative effects of experimental factors on FSR within a given experiment, and are not directly comparable to other experimental results.

A large number of experiments have been undertaken to measure FSR in different nutritional situations, or physiological states or using different methodological approaches. This paper attempts to quantify these effects on FSR. The goal of this work was to propose FSR values in ruminants for each organ or tissue within the whole physiological and experimental environment. Clarification of these values will help to evaluate the amino acid and protein dynamic in intermediary metabolism.

## MATERIAL AND METHODS

The data set used in this paper included observations from lambs, sheep, goats, growing steers and cows. A set of 25 publications including 624 observations were found in the literature (Buttery et al, 1975; Lobley et al, 1980; Davis et al, 1981; Bryant and Smith, 1982a, b; Sinnett-Smith et al, 1983; Schaefer et al, 1986; Bohorov et al, 1987; Attaix et al, 1988a, b; Early et al, 1988; Abdul-Razzaq and Bickerstaffe, 1989; Eisemann et al, 1989; Champredon et al, 1990; Early et al, 1990; Hunter and Magner, 1990; Lobley et al, 1990; Baracos et al, 1991; Attaix et al, 1992; Harris et al, 1992; Lobley et al, 1992; Southorn et al, 1992; Crompton and Lomax, 1993; Lobley et al, 1994; Tauveron et al, 1994). For each observation, the pieces of information collected include the FSR, the physiological status, the animal species, the tissue name, animal body weight (W, kg), crude protein intake (CPI, g/day), metabolisable energy intake (MEI, MJ/day), CPI and MEI divided by the metabolic weight [CPW (g/day/kg$^{0.75}$) and MEW(kJ/day/kg$^{0.75}$)], labelled amino acid used, hypotheses concerning the protein synthesis precursor pool and the amino acid supply technique.

The last three pieces of information were derived from FSR since it is calculated by determining the partition of labelled amino acids between the plasma, the intracellular compartment and the protein pool. Each amino acid can follow several metabolic pathways and therefore different amino acids can generate different FSR. The assumed precursor pool for protein synthesis can be either the plasma (HP) or the intracellular pool (HI) (France et al, 1988). FSR is calculated from the ratio of the specific activities of the labelled amino acids in the protein compartment and in the precursor pool. Therefore the choice of the precursor pool affects FSR since the specific activities in the plasma and intracellular pool are different due to the higher amino acid concentration in the intracellular compartment. The labelled amino acid can either be infused for several hours until it reaches a plateau of specific activity [constant infusion (CI) technique] or mixed with a large amount of the same amino acid 'cold' and quickly injected [flooding dose or large dose (FD) technique], which floods the different pools and therefore makes their specific activities equal. A third method (injection) was also used in lambs. These three methods could influence the values measured for FSR during the experiment, since they might interfere with the amino acid metabolism. It was therefore of interest to quantify their influence.

The main statistical parameters of the variables are presented in table I. Average FSR values are presented in the different sections of table II for tissues or organs, as estimated by precursor pool hypothesis, by amino acid supply technique and by labelled amino acid. In addition, the animal's physiological status and species are specified.

The collected data set represents an unbalanced and non-orthogonal design. The data set was the collection of observations extracted from different experimental designs and therefore had a complex structure. Consequently, although significant statistical differences were obtained, they need to be considered cautiously. Moreover, each measured value was carefully considered to determine if it remained in an acceptable range. An observation was discarded either after a comparison with similar results within an experiment, ie, a repetition of the same treatment in the same study with the same methodological approach, or if the value appeared as an outlying point on the plot of the residuals after a model was applied (Tomassone et al, 1992). Therefore, a step by step approach to the data set was performed. First of all, analyses of variance were performed by tissues or groups of tissues or

**Table I.** Statistical summary of the quantitative variables.

| *Variable* | *Average* | *STD* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|
| Weight (W, kg) | 88.8 | 121 | 4.5 | 628 | 624 |
| Crude protein intake (CPI, g/day) | 182 | 206 | 0 | 904 | 503 |
| Metabolisable energy intake level (MEI, MJ/day) | 19.3 | 21.3 | 0 | 74.2 | 527 |
| Crude protein intake level (CPW, g/day/kg$^{0.75}$) | 8.9 | 4.7 | 0 | 18 | 503 |
| Metabol energy intake (MEW, KJ/day/kg$^{0.75}$) | 772 | 321 | 0 | 1691 | 527 |

**Table II.** (A) FSR values in different locations of the gastro-intestinal tract.

| *Tissue* | *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|---|
| Abomasum | HI | CI | Tyr | lamb | growing | 25.6 | | 23.4 | 27.7 | 2 |
| | | FD | Val | lamb | suckling | 18.7 | 10 | 7.5 | 31.2 | 4 |
| | HP | FD | Val | lamb | growing | 26 | | 23.5 | 28.4 | 2 |
| Caecum | HI | CI | Phe | goat | dry/lactating | 38.6 | | 35.1 | 42.1 | 2 |
| | | FD | Val | lamb | growing | 44.4 | 3 | 40.9 | 46.9 | 4 |
| | | FD | Phe | lamb | growing | 58.4 | | | | 1 |
| | HP | CI | Phe | goat | dry/lactating | 17 | | 15.4 | 18.6 | 2 |
| | | FD | Val | lamb | growing | 27.3 | | 20.4 | 34.1 | 2 |
| | | Injection | Lys | lamb | suckling/growing | 30.5 | 4.7 | 24 | 35 | 4 |
| Colon | HI | FD | Val | lamb | suckling | 40.9 | 5.8 | 35.4 | 47.7 | 4 |
| | HP | FD | Val | lamb | growing | 30.5 | | 26 | 35 | 2 |
| | | FD | Phe | lamb | growing | 51.9 | | | | 1 |
| Duodenum | HI | CI | Leu | steer | growing | 98.5 | | 88 | 109 | 2 |
| | | CI | Phe | goat | dry/lactating | 119 | | 107 | 131 | 2 |
| | | FD | Phe | lamb | growing | 76.6 | | | | 1 |
| | | FD | Val | lamb | suckling | 86.2 | 22.4 | 68.3 | 119 | 4 |
| | HP | CI | Leu | steer | growing | 19.8 | | 18.5 | 21 | 2 |
| | | CI | Phe | goat | dry/lactating | 24.3 | | 21 | 27.5 | 2 |
| | | FD | Phe | lamb | growing | 66.5 | | | | 1 |
| | | FD | Val | lamb | growing | 43.3 | | 41.6 | 44.9 | 2 |
| Ileum | HI | CI | Leu | steer | growing | 67.6 | | 62.4 | 72.8 | 2 |
| | | FD | Phe | lamb | growing | 50.8 | | | | 1 |
| | | FD | Val | lamb | suckling | 64.7 | 12.8 | 46.7 | 77.1 | 4 |
| | HP | CI | Leu | steer | growing | 14.1 | | 13.7 | 14.5 | 2 |
| | | FD | Phe | lamb | growing | 33.6 | | | | 1 |
| | | FD | Val | lamb | growing | 35.6 | | 30.5 | 40.7 | 2 |
| Jejunum | HI | CI | Phe | steer | growing | 78.3 | | 77.6 | 78.9 | 2 |
| | | FD | Phe | lamb | growing | 73.6 | | | | 1 |
| | | FD | Val | lamb | suckling | 78.3 | 14.7 | 60.4 | 93.7 | 4 |

**Table II.** (A) FSR values in different locations of the gastro-intestinal tract (continued).

| *Tissue* | *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|---|
| Jejunum | HP | CI | Phe | steer | growing | 12.4 | | 10.3 | 14.5 | 2 |
| | | FD | Phe | lamb | growing | 61.7 | | | | 1 |
| | | FD | Val | lamb | growing | 42.5 | | 35.7 | 49.3 | 2 |
| Large intestine | HI | CI | Met | goat | dry/lactating | 34.1 | | 24.4 | 43.9 | 2 |
| | HP | CI | Met | goat | dry/lactating | 14 | | 12.2 | 15.8 | 2 |
| | | Injection | Lys | lamb | suckling/growing | 18.5 | 7.7 | 11 | 27 | 4 |
| Oesophagus | HI | FD | Val | lamb | suckling/growing | 21.2 | 8.6 | 12 | 32.8 | 4 |
| Rumen | HI | CI | Leu | sheep | maintenance | 15.8 | 9.7 | 8 | 30 | 4 |
| | | CI | Leu | lamb | growing | 79 | 38 | 26 | 150 | 9 |
| | | CI | Met | goat | dry/lactating | 27.4 | | 23.4 | 31.5 | 2 |
| | | CI | Phe | goat | dry/lactating | 33.7 | | 27.7 | 39.6 | 2 |
| | | CI | Phe | steer | growing | 53.8 | | 51.8 | 55.8 | 2 |
| | | CI | Tyr | cow | dry | 29 | | | | 1 |
| | | CI | Tyr | steer | growing | 52.7 | | 52.2 | 53.2 | 2 |
| | | CI | Tyr | lamb | growing | 31.3 | | 28.8 | 33.8 | 2 |
| | HP | CI | Leu | sheep | maintenance | 9.7 | 3.3 | 5 | 12 | 4 |
| | | CI | Met | goat | dry/lactating | 14.5 | | 13 | 16 | 2 |
| | | CI | Phe | goat | dry/lactating | 19.9 | | 16.4 | 23.3 | 2 |
| | | CI | Phe | steer | growing | 5.7 | | 5.1 | 6.3 | 2 |
| | | CI | Tyr | cow | dry | 14.6 | | | | 1 |
| | | CI | Tyr | steer | growing | 25.7 | | 23.3 | 28 | 2 |
| | | FD | Val | lamb | growing | 28.6 | | 21.9 | 35.3 | 2 |
| | | Injection | Lys | lamb | suckling | 66.5 | | 60 | 73 | 2 |
| | | Injection | Lys | lamb | growing | 24.5 | | 19 | 30 | 2 |
| Small intestine | HI | CI | Met | goat | dry/lactating | 79 | | 61.7 | 96.3 | 2 |
| | | CI | Tyr | lamb | growing | 108 | | 99.2 | 116 | 2 |
| | HP | CI | Met | goat | dry/lactating | 17.6 | | 17 | 18.2 | 2 |
| | | Injection | Lys | lamb | suckling | 30.5 | | 15 | 46 | 2 |
| | | Injection | Lys | lamb | growing | 33 | | 28 | 38 | 2 |

**Table II.** (B) FSR values in the liver.

| *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|
| HI | CI | Leu | lamb | growing | 47.4 | 21.8 | 24 | 96 | 14 |
| | | Leu | steer | growing | 25.8 | | 24.4 | 27.2 | 2 |
| | | Leu | sheep | maintenance | 28.2 | 5.3 | 22 | 35 | 4 |
| | | Lys | sheep | maintenance | 10.1 | | | | 1 |
| | | Met | goat | dry/lactating | 20.5 | | 15.3 | 25.6 | 2 |
| | | Phe | lamb | growing | 57.2 | | 53.7 | 60.6 | 2 |
| | | Phe | goat | dry/lactating | 28.5 | | 26.5 | 30.6 | 2 |
| | | Phe | steer | growing | 13.8 | | 13.7 | 13.9 | 2 |
| | | Tyr | cow | dry | 60 | | | | 1 |
| | | Tyr | steer | growing | 32.4 | | 26.3 | 38.5 | 2 |
| | | Tyr | lamb | growing | 48.5 | | 45 | 52 | 2 |
| | FD | Phe | lamb | growing | 54.6 | | | | 1 |
| | | Val | lamb | suckling | 115 | | | | 1 |
| | | Val | goat | lactating | 35.3 | 5 | 31.5 | 41 | 3 |
| HP | CI | Leu | lamb | growing | 21.5 | 5.6 | 14.5 | 29.1 | 5 |
| | | Leu | steer | growing | 8.4 | | 8.1 | 8.7 | 2 |
| | | Leu | sheep | maintenance | 18 | 4.2 | 12 | 22 | 4 |
| | | Met | goat | dry/lactating | 8.8 | | 8.6 | 9.1 | 2 |
| | | Phe | lamb | growing | 13.4 | 9.1 | 4.7 | 24.2 | 4 |
| | | Phe | goat | dry/lactating | 11.7 | | 10.7 | 12.6 | 2 |
| | | Phe | steer | growing | 3.3 | | 3.2 | 3.4 | 2 |
| | | Tyr | cow | dry | 7.4 | | | | 1 |
| | | Tyr | steer | growing | 10.4 | | 10 | 10.8 | 2 |
| | FD | Phe | lamb | growing | 33.7 | 9.1 | 26.3 | 49.4 | 5 |
| | | Val | lamb | suckling | 97.8 | | | | 1 |
| | | Val | lamb | growing | 22.3 | | 22.1 | 22.5 | 2 |

**Table II.** (C) FSR values in different locations in the muscle tissues.

| *Tissue* | *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|---|
| Anconeus | HI | FD | Val | goat | lactating | 3.9 | 0.5 | 3.3 | 4.3 | 3 |
| Average | HI | CI | Tyr | lamb | growing | 3.3 | 1.13 | 1.46 | 5.29 | 11 |
| | HP | CI | Tyr | lamb | growing | 2.18 | 0.92 | 1 | 3.48 | 9 |
| Biceps femoris | HI | CI | Leu | sheep | maintenance | 3.75 | 1.16 | 2.1 | 4.6 | 4 |
| | | CI | Leu | lamb | growing | 3.89 | 0.33 | 3 | 4 | 9 |
| | | CI | Leu | steer | growing | 4 | 2.46 | 1.6 | 6.8 | 4 |
| | HP | CI | Leu | sheep | maintenance | 3 | 1.26 | 1.3 | 4 | 4 |
| | | CI | Leu | steer | growing | 1.75 | 1.09 | 0.6 | 3.1 | 4 |
| Diaphragm | HI | CI | Lys | sheep | maintenance | 2.2 | | | | 1 |
| | | CI | Phe | goat | dry/lactating | 3 | | 2.87 | 3.13 | 2 |
| | | CI | Tyr | sheep | maintenance | 2.7 | | 2.6 | 2.8 | 2 |
| | | FD | Val | lamb | suckling | 12.72 | 7.24 | 6.7 | 21.6 | 5 |
| | | FD | Val | goat | lactating | 4.77 | 0.45 | 4.3 | 5.2 | 3 |
| | HP | CI | Phe | goat | dry/lactating | 2.04 | | 1.79 | 2.29 | 2 |
| | | CI | Tyr | sheep | maintenance | 1.45 | | 1.4 | 1.5 | 2 |
| | | FD | Val | lamb | suckling | 18.4 | | | | 1 |
| External | HI | CI | Phe | lamb | growing | 4.68 | | 4.21 | 5.15 | 2 |
| intercostal | | CI | Phe | steer | growing | 2.85 | | 2.56 | 3.14 | 2 |
| | HP | CI | Phe | lamb | growing | 3.36 | | 3.26 | 3.46 | 2 |
| | | CI | Phe | steer | growing | 1.33 | | 1.31 | 1.35 | 2 |
| Gastrocnemius | HI | CI | Leu | steer | growing | 2.3 | | 2 | 2.6 | 2 |
| | | CI | Lys | sheep | maintenance | 1.7 | | | | 1 |
| | | CI | Phe | lamb | growing | 4.13 | | 3.23 | 5.04 | 2 |
| | | CI | Tyr | sheep | maintenance | 2.55 | | 2.3 | 2.8 | 2 |
| | HP | CI | Leu | steer | growing | 1.15 | | 1 | 1.3 | 2 |
| | | CI | Phe | lamb | growing | 3.1 | | 2.79 | 3.41 | 2 |
| | | CI | Tyr | sheep | maintenance | 1.15 | | 1 | 1.3 | 2 |

**Table II.** (C) FSR values in different locations in the muscle tissues (continued).

| *Tissue* | *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|---|
| Hindlimb | HI | CI | Leu | lamb | growing | 2.28 | 0.61 | 1.52 | 3.13 | 8 |
| | | CI | Phe | goat | dry/lactating | 3.79 | | 3.34 | 4.24 | 2 |
| | | CI | Phe | lamb | growing | 2.23 | 0.6 | 1.65 | 2.8 | 4 |
| | HP | CI | Leu | lamb | growing | 2.03 | 0.77 | 1.09 | 3.53 | 18 |
| | | CI | Phe | goat | dry/lactating | 2.03 | | 1.93 | 2.14 | 2 |
| | | CI | Phe | lamb | growing | 1.51 | 0.7 | 0.8 | 3.3 | 14 |
| | | FD | Phe | lamb | growing | 2.39 | 0.59 | 1.73 | 2.99 | 8 |
| Longissimus dorsi | HI | CI | Leu | lamb | growing | 4.12 | 1.68 | 1 .5 | 7 | 16 |
| | | CI | Leu | steer | growing | 4.25 | 2.34 | 2.1 | 6.7 | 4 |
| | | CI | Lys | sheep | maintenance | 1.8 | | | | 1 |
| | | CI | Met | goat | dry/lactating | 1.58 | | 1.34 | 1.83 | 2 |
| | | CI | Phe | lamb | growing | 2.1 | | 1.57 | 2.64 | 2 |
| | | CI | Tyr | cow | dry | 0.93 | | | | 1 |
| | | CI | Tyr | steer | growing | 1.95 | | 1.82 | 2.08 | 2 |
| | | CI | Tyr | sheep | maintenance | 2.05 | | 1.7 | 2.4 | 2 |
| | | CI | Tyr | lamb | growing | 6.54 | 1.31 | 5.38 | 7.96 | 3 |
| | | FD | Val | goat | lactating | 3.37 | 0.21 | 3.2 | 3.6 | 3 |
| | HP | CI | Leu | lamb | growing | 2.41 | 1.2 | 1.08 | 4.9 | 9 |
| | | CI | Leu | steer | growing | 1.8 | 0.9 | 0.9 | 2.8 | 4 |
| | | CI | Met | goat | dry/lactating | 0.64 | | 0.53 | 0.75 | 2 |
| | | CI | Phe | lamb | growing | 1.15 | 0.35 | 0.78 | 1.55 | 4 |
| | | CI | Tyr | cow | dry | 0.84 | | | | 1 |
| | | CI | Tyr | steer | growing | 1.74 | | 1.64 | 1.83 | 2 |
| | | CI | Tyr | sheep | maintenance | 0.9 | | 0.7 | 1.1 | 2 |
| | | CI | Tyr | lamb | growing | 3.77 | 1.23 | 2.46 | 4.89 | 3 |
| | | CI | Tyr | sheep | dry/lactating | 3.27 | 0.31 | 3 | 3.6 | 3 |
| | | FD | Leu | lamb | growing | 2.64 | 0.32 | 2.24 | 3.16 | 12 |
| | | FD | Phe | lamb | growing | 2.42 | 0.33 | 2.09 | 2.71 | 4 |

**Table II.** (C) FSR values in different locations in the muscle tissues (continued).

| *Tissue* | *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|---|
| Masseter | HI | FD | Val | goat | lactating | 6.47 | 0.35 | 6.1 | 6.8 | 3 |
| Sartorius | HI | CI | Phe | steer | growing | 2.6 | | 2.39 | 2.81 | 2 |
| | HP | CI | Phe | steer | growing | 0.63 | | 0.62 | 0.63 | 2 |
| Semi tendinosus | HI | CI | Leu | steer | growing | 1.7 | | 1.5 | 1.9 | 2 |
| | | CI | Phe | steer | growing | 2.82 | | 2.79 | 2.85 | 2 |
| | | CI | Tyr | sheep | maintenance | 1.8 | | 1.4 | 2.2 | 2 |
| | | FD | Val | goat | lactating | 3.57 | 0.31 | 3.3 | 3.9 | 3 |
| | HP | CI | Leu | steer | growing | 0.8 | | 0.7 | 0.9 | 2 |
| | | CI | Phe | steer | growing | 0.64 | | 0.54 | 0.74 | 2 |
| | | CI | Tyr | sheep | maintenance | 0.75 | | 0.6 | 0.9 | 2 |
| | | CI | Tyr | sheep | dry | 3 | | | | 1 |
| | | CI | Tyr | sheep | lactating | 2.25 | | 2.1 | 2.4 | 2 |
| Tensor fascia lataeHI | | FD | Val | lamb | suckling | 12.24 | 7.67 | 6.3 | 22.9 | 5 |
| | HP | FD | Val | lamb | suckling | 20.1 | | | | 1 |
| Trapezius | HI | CI | Leu | steer | growing | 7.85 | | 7.7 | 8 | 2 |
| | HP | CI | Leu | steer | growing | 3.35 | | 3 | 3.7 | 2 |
| Triceps brachii | HI | CI | Leu | steer | growing | 2.5 | | 2.2 | 2.8 | 2 |
| | HP | CI | Leu | steer | growing | 1.3 | | 1.1 | 1.5 | 2 |
| Vastus | HI | CI | Tyr | sheep | maintenance | 7.05 | | 6.5 | 7.6 | 2 |
| intermedius | | CI | Tyr | lamb | growing | 5.41 | 1.93 | 3.62 | 7.46 | 3 |
| | HP | CI | Tyr | sheep | maintenance | 2.65 | | 2.6 | 2.7 | 2 |
| | | CI | Tyr | lamb | growing | 3.56 | 1.54 | 2.31 | 5.28 | 3 |
| Vastus lateralis | HI | CI | Tyr | sheep | maintenance | 2.6 | | 2.1 | 3.1 | 2 |
| | | CI | Tyr | lamb | growing | 6.89 | 0.85 | 5.97 | 7.66 | 3 |
| | HP | CI | Tyr | sheep | maintenance | 1 | | 0.8 | 1.2 | 2 |
| | | CI | Tyr | lamb | growing | 4.07 | 1.08 | 2.86 | 4.92 | 3 |
| | | FD | Leu | lamb | growing | 2.54 | 0.23 | 2.13 | 2.93 | 12 |

**Table II.** (D) FSR values in the skin.

| *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|
| HI | CI | Leu | lamb | growing | 35.9 | 13.9 | 15 | 68 | 11 |
| | | Leu | steer | growing | 13.7 | | 11.3 | 16.1 | 2 |
| | | Met | goat | dry/lactating | 2.4 | | 2.2 | 2.6 | 2 |
| | | Phe | goat | dry/lactating | 8.8 | | 6.4 | 11.1 | 2 |
| | | Tyr | cow | dry | 3.88 | | | | 1 |
| | | Tyr | steer | growing | 6.1 | | 4.8 | 7.4 | 2 |
| | | Tyr | lamb | growing | 19.8 | | 19.1 | 20.5 | 2 |
| | FD | Val | lamb | suckling | 24.1 | | | | 1 |
| HP | CI | Leu | lamb | growing | 8.5 | 2.9 | 5.2 | 11.7 | 4 |
| | | Leu | steer | growing | 4 | | 3.6 | 4.4 | 2 |
| | | Met | goat | dry/lactating | 0.9 | | 0.6 | 1.2 | 2 |
| | | Phe | lamb | growing | 5.3 | 2.1 | 3.3 | 7.6 | 4 |
| | | Phe | goat | dry/lactating | 2.1 | | 1.6 | 2.6 | 2 |
| | | Tyr | cow | dry | 1.42 | | | | 1 |
| | | Tyr | steer | growing | 3.35 | | 3.15 | 3.55 | 2 |
| | FD | Val | lamb | growing | 8.1 | 2.5 | 5.7 | 11 | 4 |
| | | Val | lamb | suckling | 20.1 | | | | 1 |

**Table II.** (E) FSR values in various tissues and organs.

| *Tissue or organ* | *Model* | *Method* | *Amino acid* | *Animal* | *Physiological status* | *FSR (%/day)* | *STD (%/day)* | *Min* | *Max* | *N* |
|---|---|---|---|---|---|---|---|---|---|---|
| Brain | HI | CI | Tyr | lamb | growing | 10.15 | | 10 | 10.3 | 2 |
| Heart | HI | CI | Leu | sheep | maintenance | 3.25 | 0.5 | 2.7 | 4 | 4 |
| | | | Leu | lamb | growing | 8.7 | 2 | 6 | 11 | 9 |
| | | | Lys | sheep | maintenance | 3.2 | | | | 1 |
| | | | Phe | goat | dry/lactating | 7.3 | | 6.4 | 8.2 | 2 |
| | | | Phe | steer | growing | 2.2 | | 2.1 | 2.3 | 2 |
| | | | Tyr | lamb | growing | 7 | | 6.5 | 7.5 | 2 |
| | HP | CI | Leu | sheep | maintenance | 2.9 | 0.8 | 2.2 | 3.8 | 4 |
| | | | Phe | steer | growing | 1.1 | | 1.05 | 1.14 | 2 |
| | | | Tyr | sheep | dry/lactating | 6 | 1.2 | 4.8 | 7.1 | 3 |
| Kidney | HI | CI | Phe | lamb | growing | 46.7 | | 38.3 | 55 | 2 |
| | | | Phe | goat | dry/lactating | 44.7 | | 39.8 | 49.5 | 2 |
| | | | Phe | steer | growing | 13.75 | | 13.7 | 13.8 | 2 |
| | | | Tyr | lamb | growing | 42.4 | | 34.8 | 49.9 | 2 |
| | HP | CI | Phe | lamb | growing | 16.3 | | 15.9 | 16.7 | 2 |
| | | | Phe | goat | dry/lactating | 18.4 | | 16.4 | 20.4 | 2 |
| | | | Phe | steer | growing | 3.8 | | 3.4 | 4.1 | 2 |
| Lung | HI | CI | Phe | goat | dry/lactating | 19 | | 18 | 20 | 2 |
| | HP | CI | Phe | goat | dry/lactating | 8.1 | | 7 | 9.3 | 2 |
| Mammary gland | HI | CI | Phe/Met | goat | dry | 17.4 | | 6.3 | 28.5 | 2 |
| | | | Phe/Met | goat | lactating | 119 | | 107 | 131 | 2 |
| | HP | CI | Phe/Met | goat | dry | 5.7 | | 2.8 | 8.7 | 2 |
| | | | Phe/Met | goat | lactating | 41.6 | | 41.5 | 41.6 | 2 |
| Spleen | HI | CI | Phe | goat | dry/lactating | 31 | | 23.7 | 38.3 | 2 |
| | HP | CI | Phe | goat | dry/lactating | 8.4 | | 8 | 8.8 | 2 |
| Uterus | HI | CI | Phe/Met | goat | dry | 23.2 | | 14.6 | 31.8 | 2 |
| | | | Phe/Met | goat | lactating | 18.6 | | 12.3 | 25 | 2 |
| | HP | CI | Phe/Met | goat | dry | 10.1 | | 6.8 | 13.3 | 2 |
| | | | Phe/Met | goat | lactating | 5.1 | | 4.1 | 6.2 | 2 |

organs with the precursor pool hypothesis (PPH) and the infusion technique (IT) nested within the precursor pool hypothesis as the two explaining factors:

$$\text{model I:} \quad FSR_{ij/ik} = \mu + (PPH)_i + (IT/PPH)_{j/i} + \varepsilon_{ij/ik}$$

For model I, $FSR_{ij/ik}$ is the FSR estimate of the observation *ij/ik* according to the level *i* of the factor PPH and the level *j* of the factor IT according to the level *i* of the factor PPH; $\mu$ is the intercept of the model; $(PPH)_i$ is the effect of the level *i* of the precursor pool hypothesis. $(IT/PPH)_{j/i}$ is the effect of the level *j* of the factor infusion technique IT within the level *i* of the factor PPH; $\varepsilon_{ij/ik}$ is the residual error of the model.

Since the precursor pool hypothesis was always significant and since within each hypothesis, the infusion technique was nearly always significant, further analyses were performed separately on the HP and HI hypotheses and within the precursor pool by the infusion technique. Moreover, as the data sets obtained were highly unbalanced for the remaining factors, the interactions between these factors were not tested. In a second step, analysis of variance (model II) and covariance (model III) were performed with the physiological status (Phys) and the labelled amino acid used (Aa) as qualitative factors and CPW or MEW as quantitative variables:

$$\text{model II:} \quad FSR_{ijk} = \mu + (Phys)_i + (Aa)_j + \varepsilon_{ijk}$$

$$\text{model III:} \quad FSR_{ijk} = \mu + (Phys)_i + (Aa)_j + \alpha * X_{ijk} + \varepsilon_{ijk}$$

For model II, $FSR_{ijk}$ is the FSR estimate of the observation *ijk* according to the level *i* of the factor Phys and the level *j* of the factor Aa; $\mu$ is the intercept of the model; $(Phys)_i$ is the effect of the level *i* of the animal's physiological status; $(Aa)_j$ is the effect of the level *j* of the factor labelled amino acid; $\varepsilon_{ijk}$ is the residual error of the model.

For model III, $\alpha$ is the slope relating FSR and X, a quantitative covariate; X is either CPW or MEW.

The resulting models are presented in the relevant sections. They are referred to by their roman number. Since the data set was unbalanced, the option LSMEANS in the SAS package was used to obtain corrected estimates of average FSR. The statistical software used was SAS (SAS, 1987) with the GLM procedure.

## RESULTS

FSR varied greatly (table II). A simple statistical approach underlined the large range of averaged values between tissues from less than 1%/day for some muscle tissues to more than 100%/day for some sections of the gastrointestinal tract. However, the values for each tissue or organ varied within a normal distribution, if the influencing factors were taken into account. Therefore, a tissue or a group of tissues, having a common biological basis, were analysed for each factor.

For each tissue or organ with data with the intracellular and the plasma hypothesis, the precursor pool choice was a factor with a major influence (table II). The true protein precursor pool is the $^t$RNA-amino acid compartment (France et al, 1988). However, this compartment has a short half-life of between 2 and 5 s (Martin et al, 1977) and consequently its turnover rate cannot be easily measured. Moreover, as far as the authors were aware, no information is available on this pool size or on its regulation by local nutritional or hormonal factors. Furthermore measurement methodologies have to be improved to study this compartment. Other precursor pools, therefore, were chosen, having much longer half-lifes: either the free amino acid in the cell, ie, intracellular pool hypothesis (HI), or the free amino acid in the 'plasma' (HP). For HP, one can choose several different sampling techniques. The amino acids can be measured in the plasma, in the blood, in the vein or in the artery. The choice influences the FSR values obtained (Lobley et al, 1992). The true value for FSR is usually assumed to be between those given by HP (the minimum) and HI (the maximum) (Lobley et al, 1980). A study of the averaged FSR values by tissues and organs showed a linear relationship between the FSR estimated by HI versus the FSR estimated by HP. The FSR estimated by HI were two to three times higher than by HP except for the aboma-

sum and colon (fig 1). The obtained regression, without abomasum and colon, was:

$$(\text{FSR under HP})_i = 2.07(+-2.0) + 0.35(+-0.038)*(\text{FSR under HI})_i + \varepsilon_i$$

$$n = 16,\ R^2 = 0.85,\ \text{RSD} = 4.23,\ P < 0.01$$

FSR under HP or HI were the average values by tissue or by organ and by precursor pool hypothesis. These results can be explained by the concentration of the labelled amino acid, which is physiologically lower in the intracellular pool than in the plasma pool and also by the fact that FSR increases with the ratio [specific activity in the protein compartment] divided by [specific activity in the precursor pool] according to the equations used to calculate FSR (Garlick, 1980). The abomasum and colon were not taken into account since few values were available and these were only on young growing lambs.

For this reason, statistical analyses were undertaken for both HI and HP.

## Gastro-intestinal tract (GIT)

FSR in the GIT was measured in the different sections of the digestive tract. FSR varied largely between GIT sections from an average of 21%/day for the abomasum to 95.1%/day in the duodenum with HI and from an average of 17%/day for the whole large intestine to 37.6%/day for the colon with HP (table IIA).

The observations were divided into three groups according to their anatomical position: rumen (oesophagus, reticulo-rumen,

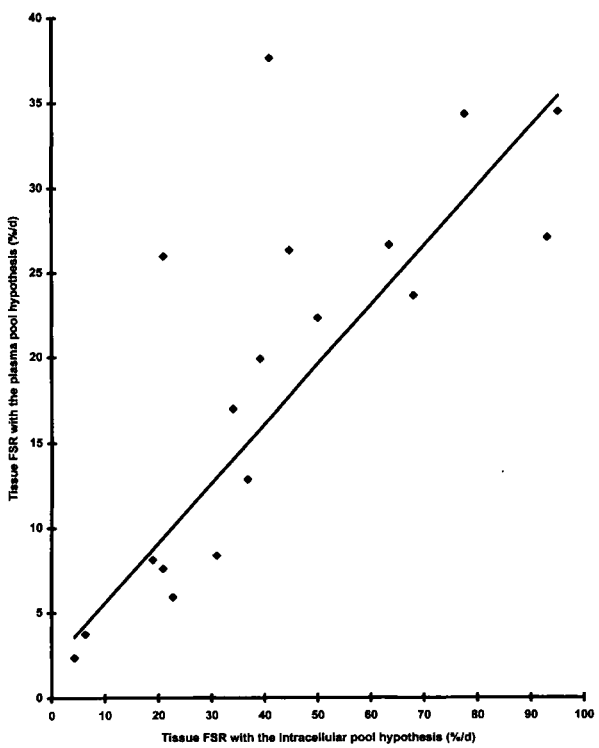


**Fig 1.** Relationship between averaged tissue FSR estimated either with the intracellular or the plasma pool as precursor $y = 2.07(+-2) + 0.35(+-0.038)*x$, $n = 16$, $R^2 = 0.85$, RSD = 4.23.

omasum and abomasum), small intestine (duodenum, jejunum, ileum and small intestine) and large intestine (colon, caecum and large intestine). On the one hand, the grouping factor was tested by an analysis of variance and no significant difference was found within each group for FSR. On the other hand, however, there was a meaningful difference between groups, the small intestine having a higher FSR than the two other groups.

Statistical analyses were performed for each group. A graphical and simple statistical analysis of the observation distribution underlined that one ruminal FSR value exceeded the average by two standard deviations. This value of 150%/day was measured on one growing lamb with an infusion of casein, whereas the average of the two other lambs submitted to the same treatment was 35.5%/day (Davis et al, 1981). This observation was, therefore, discarded. This underlined the problem of individual variations that affected the data and which were related either to the animal or to the technique used.

For the rumen, model I was highly significant; however HP and HI tended only to be different ($P = 0.11$). Within HP, the ranking between infusion technique was not significant but FD FSR were higher than CI ones. Within HI, the opposite result was observed: CI gave estimates twice as high as FD. However, since very few data were available using FD and HI and only on young lambs (ie, with a higher FSR), the result should be questioned. The low number of FD and HI observations available was not surprising, since the measurement of the intracellular pool is not required. With FD, the plasma and intracellular pools are flooded with a high dose of unlabelled amino acid together with a labelled one. The aim is to have the same specific activity in all possible protein precursor pools, including amino-acyl-$^t$RNA, and therefore to obtain more realistic FSR (Garlick et al, 1994). This is not the case with CI since the specific activity in the intracellular pool is much lower than in the plasma pool (Garlick, 1980). The higher FSR estimates with FD could be due to a high recycling of labelled amino acids in CI. Therefore, the specific activity is higher with CI and consequently the FSR estimates are lower. This is especially the case in tissues with high FSR such as the GIT (Lobley et al, 1994). The contradictory result observed for HI in the ruminal tissues underlined the need to specify the particular experimental set up including the animal species and physiological status.

For the ruminal tissues with CI and HI, model III was highly significant ($P < 0.01$) with the factors labelled amino acid and physiological status. The levels of tyrosine FSR were significantly lower than those of methionine, leucine and phenylalanine, these latter were not significantly different one from another due to their broad standard deviations. The same result was observed for the maintenance status between values for dry, growing and lactating animals.

For the small intestine, model I was highly significant. HI gave FSR estimates higher than HP. Within HI, FD estimates were lower than CI ones, whereas within HP, FD estimates were higher. The same comment could be made as for the ruminal group.

For the small intestine, with the precursor pool hypothesis HI, the physiological status and the amino acid used partially explained the significant differences in the FSR values obtained ($P < 0.1$) with models II. With the CI technique, lactating animals had higher FSR than growing ones. In addition, methionine tended to provide lower estimates than leucine, phenylalanine and tyrosine. With the FD method, growing animal FSR were higher than suckling ones and valine FSR were higher than phenylalanine ones. When the plasma was used as the precursor pool hypothesis, no model

was significant since too little data were available.

For the large intestine, model I gave the same ranking as for the rumen and small intestine for the precursor pool hypothesis: HI FSR were higher than HP. Within both hypotheses, CI gave lower estimates than FD ones. The only significant values in model II were for HI and FD, where a ranking between labelled amino acids was observed with the phenylalanine FSR higher than the valine ones.

This difference between amino acids, whatever the GIT section, underlined the importance of choosing a particular amino acid for the FSR estimation. Ranking orders between amino acids could result from their particular metabolism. For example, tyrosine is a product of phenylalanine metabolism, while methionine as a precursor of cysteine is extensively utilised in the GIT (Lobley, 1992). Other factors such as the physiological state of the animal could also have an influence. For example, experiments using methionine as a tracer have been carried out comparing lactating or dry goats and there was a significant difference in the resulting FSR values (Champredon et al, 1990).

To conclude, the GIT had high FSR depending on the section of the digestive tract considered. FSR varied with the amino acid used and with the supply method, the CI method usually giving lower estimates. Moreover the physiological state of the animal also influenced the measured FSR.

## Liver

It has been well-documented that the liver is a highly metabolically active organ (eg, Danfær, 1994) with a relatively high FSR: an average of 39.2%/day with HI, and 19.9%/day with HP (table IIB). Model I underlined the usual ranking between HI and HP observed in the other tissues and organs and between CI and FD within precursor pool hypothesis, FD giving higher estimates than CI ($P < 0.01$).

No other qualitative factor was significant. This was understandable since within a labelled amino acid or physiological state, the measured variations were larger than between factors. As an example, for HI and FD and using valine as the labelled amino acid, the FSR were either 35.3 for goats or 115 for lambs. These variations make it virtually impossible to advance hypotheses concerning the possible effects of varying amino acid metabolism.

Quantitatively, within HI and CI, there was a significant effect ($P < 0.05$) for the crude protein intake divided by the metabolic weight. FSR increased with the protein intake. Even though this relationship was significant, it only explained a low percentage of the FSR variations ($R^2 = 0.17$, $n = 27$). This result, however, seemed consistent with previous ones (eg, Grizard et al, 1988).

## Muscle tissues

Numerous FSR measurements were available for the muscle tissues. The average value was 4.29%/day for HI and 2.35%/day for HP. Due to the large number of data, a relationship was fitted between HI and HP values (fig 2):

$$(\text{FSR under HP})_i = 0.03(+\text{–}0.17) + 0.553(+\text{–}0.04)*(\text{FSR under HI})_i + \varepsilon_i$$

$$n = 71,\ R^2 = 0.72,\ \text{RSD} = 0.67,\ P < 0.01$$

This relationship within muscle tissues was globally consistent with the between tissue relationship presented above. While the intercept was not significantly different from zero in both relationships, the slopes were largely different: 0.35 between tissues versus 0.55 within muscle tissues. This result

underlined possible variations in the protein dynamics between tissues and organs.

The 'muscle tissues' themselves include a wide range of tissues with different FSR, as can be seen in table IIC. Moreover, each muscle is not homogeneous from a protein point of view (myosine, sarcosine, enzymes, etc). Muscle FSR is therefore the average of a mixture of individual protein FSR. For example, collagen and non-collagen FSR in growing lambs are not the same (Pell and Bates, 1987). While keeping in mind these limitations, the muscle tissue observations were merged for further analysis.

Model I was significant for the precursor pool hypothesis as illustrated in figure 2. Within HI and HP, CI gave significantly lower FSR estimates than FD. This supports the idea of different fates for each infused amino acid in the protein pools during an infusion of long duration versus a flooding dose procedure.

Model III was applied within the precursor pool hypothesis and infusion technique. Within HI and FD, the physiological state was highly significant. FSR variations in this set of 20 observations could be fully explained by FSR measurements on suckling lambs since these young animals had muscle FSR three times higher than lactating or other growing animals. Moreover, in the same data set and model, FSR were significantly increased by the metabolisable energy intake divided by the animal metabolic weight. Within HI, no other model was significant.

Within HP and CI, two model IIIs were significant with the labelled amino acid as qualitative factor and either the crude protein intake or the metabolisable energy intake divided by the metabolic weight. Crude protein and metabolisable energy linearly increased FSR values as observed in the liver. However, it was not easy to distinguish the crude protein and metabolisable energy effect, since a variation of both factors was mainly related to the total of dry matter intake, and it was not possible, therefore, to discriminate between the two variables. The ranking order between labelled amino acids was the same for the two models: tyrosine > leucine > phenylalanine > methionine, even though the levels of statistical significance varied slightly between models. Within HP and FD, the same results were observed for crude protein and metabolisable energy. A ranking order was also observed between the two infused amino acids, leucine and phenylalanine. However phenylalanine gave significantly higher FSR estimates ($P < 0.06$) than leucine with model III using metabolisable energy as the covariate. The opposite result (leucine FSR higher than phenylalanine, $P < 0.01$) was observed with crude protein as covariate. The FSR result with crude protein could

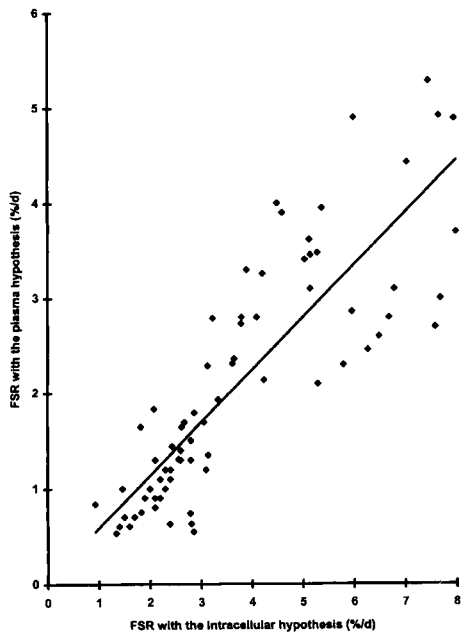


**Fig 2.** Comparison of FSR estimates according to the precursor pool hypothesis in the muscle tissues $y = 0.03(+-0.17) + 0.053(+-0.04)*x$, $n = 71$, $R^2 = 0.72$, RSD = 0.67.

be rejected since a confusion existed between the crude protein level and the labelled amino acid used. As a result the whole data set structure could not discriminate between energy and protein influences on FSR in muscle tissues.

## Skin

The skin accounts for 23 to 26% of total protein synthesis flux in growing lambs (Gill et al, 1989). This is of the same order of magnitude as the GIT contribution. Even though its importance could be lower in large ruminants, the skin's contribution to the whole-body protein synthesis flux should not be neglected.

FSR values varied largely depending on the precursor pool hypothesis with an average of 22.8%/day for HI and 5.9%/day for HP. Moreover, within HI or HP FSR measurements covered a broad range of values apparently depending, at least partially, on the animal's physiological state (table IID). This was not supported by the statistical fitting owing to the fact that the data set was unbalanced between the different factors. In addition, the HI results should be considered cautiously since, as stated by Lobley et al (1992), the extensive dilution of the tissue tracer pool might justify not considering the HI measurements owing to the methodological problems arising from defining the intracellular pool. Therefore, the animal model needs to be modified for a diffuse tissue such as the skin. Such a modification was undertaken by Harris et al (1989) using a new catheterization procedure in sheep.

In conclusion, it can be said that no valid data on skin FSR are currently available.

## Mammary gland

FSR in the mammary gland of ruminants were only examined in a few studies, despite the fact that in lactating goats, the mammary gland contributes up to 46% of the whole-body protein synthesis (Champredon et al, 1990). Moreover, in two of the published works (Champredon et al, 1990; Baracos et al, 1991), these FSR were studied by use of the usual CI technique, and the mammary gland was treated as though it were any other tissue. The only significant effect observed was the influence of being in lactation versus being dry in goats. A tremendous increase in FSR was observed for the lactating animals (table IIE). More specific studies have focused on dividing mammary protein synthesis into endogenous protein and milk protein. As a result, Oddy et al (1988) found that flux estimates for protein synthesis were decidedly greater than the fluxes for milk protein production, which only represents 60% of the synthesised protein. Moreover, they showed that there was an effect of lactation stage on leucine oxidation in the mammary gland. This effect was critical in this case since leucine was used to evaluate the amino acid fluxes in the mammary gland. This underlines the fact that the values of FSR obtained at one stage of lactation should not be extrapolated to other stages. Consequently, the study of FSR in the mammary gland should be included in a wider context aiming at identifying the amino acid fate in these glands. A first proposal for dealing with lactating cows was made by France et al (1995) where a model of $^{13}$C-leucine was fitted in the udder. They clearly discussed the limitations of their model and they proposed it as a tool for ranking the essential amino acids involved in milk protein synthesis. In conclusion, FSR in the mammary gland is only one indicator of the protein metabolism occurring there. This stresses the fact that for specialised organs or tissues the usual FSR measurement approach, if it gives 'semi-quantitative' information (Lobley et al, 1995), cannot be used directly to quantify the actual FSR. Moreover, the variable to be identified is the protein flux, therefore a

measurement of the protein compartment sizes should be carried out simultaneously with the FSR determination.

### Other tissues and organs

FSR values were collected for a wide range of other organs and tissues. Their average values are reported in table IIE. Too few values were available for any one of these other tissues or organs to isolate any particular effect. However, the sum of these protein compartments represents a significant proportion of the whole-body protein synthesis: 8% in the model of Gill et al (1989) and the sizes of the involved protein compartment (lung, uterus, brain, etc) is not negligible. The observed discrepancies between their FSR values underlines the point that the whole body is highly heterogeneous and therefore global estimates should be weighted for an individual tissue or organ metabolism.

## DISCUSSION

FSR have been measured in a large number of experiments. However, their value is questioned (Lobley et al, 1995) since several factors interact with the actual measurements. Moreover, although hormones are known to influence the partition of amino acids between different pathways including protein synthesis, few of these effects have been quantified (Grizard et al, 1988).

The present statistical study enabled quantitative assessments of some of the factors effecting FSR measurement. First of all, the choice of the precursor pool determines the range of FSR values that can be obtained. It is better to choose a precursor pool with a specific activity which is close to the 'actual' precursor pool, ie, the $^{t}$RNA-bounded amino acids (France et al, 1988). Various analytical and physiological problems remain to be addressed even though the given compartment can be specified. For the latter, the roles of erythrocyte-bound amino acids, small peptides or small proteins could account for a large part of the labelled molecules transported in the blood (Lobley et al, 1992). For the former, these molecules are difficult to analyse properly and most of the analyses with labelled molecules are only based on plasma free amino acids (Oddy et al, 1988; France et al, 1995). Moreover, all these compartmental models assumed a uniform behaviour of the protein within each compartment (France et al, 1988). There could be however a gradient of exchanges and concentrations between the artery and the vein (Norwich, 1992). In addition, different levels of enrichment can be attained inside a given protein compartment due to the highly variable half-lives of different proteins (Swick and Song, 1974).

However, if a choice between the two precursor pool hypotheses is to be made, our study could help to support one or the other hypothesis. Since HP and HI results are linearly related whatever the method or the tissue (figs 1 and 2), the easiest measurement should be chosen. Nevertheless, calibration of the relationship between HI and HP should be performed according to the experimental environment because of the varying slopes depending on the tissues. The conclusion should be that one of the two measurements, ie, HI or HP, is sufficient since the other one will not give additional pieces of information about the protein synthesis rates.

A second factor that must be considered concerns the amino acid supply technique. On the one hand, FD seems closer to the actual precursor pool from a specific activity point of view. It should, then, be used preferentially to study acute changes in FSR (Garlick et al, 1994) even though the steady-state conditions need to be verified. Nevertheless, FD is problematic owing to the possibility of disturbing the normal metabolism

of the protein pool in humans, by affecting the transamination or the oxidation rates (Rennie et al, 1994). However, as far as the authors are aware, this kind of effect has not yet been demonstrated in growing or lactating ruminants. On the other hand, if the aim of an experiment is to follow the protein dynamics in a tissue, the CI technique could be useful since, after making certain assumptions, it permits the measurement of the protein degradation rate (Rennie et al, 1994).

Moreover, with FD, flooding levels have to be attained, ie, the specific activities in the true protein precursor pool and all the others should be equivalent. This has been measured in rats by Smith and Sun (1995) where they showed that, depending on the tissue, the flooding was more or less effective. These authors also observed that valine was channelled differently from the plasma pool to protein synthesis depending on the tissue. If there was no flooding, either the $^{t}$RNA-bound and the free intracellular valine pools were in isotopic equilibrium as in cardiac and slow-twitch muscles or there was a difference as in the liver. FD, however, influenced the channelling of the amino acids coming from the degradation of tissue protein and, therefore, the intracellular amino acid pool was not in equilibrium with the protein precursor pool. In ruminants, the flooding conditions have been reduced to a comparison between the intracellular and plasma pool specific activities (eg, Lobley et al, 1992).

An additional problem with the measurement techniques is the uniqueness of the FSR measurement due to the slaughtering of the animal to obtain the corresponding tissue enrichment. To partially overcome this limitation, it is possible to perform biopsies. The stress involved to the animal with this technique could, however, influence the results (Lobley et al, 1992).

In conclusion, FD and CI could be used in different situations depending on the goals of the experiment. For FSR measurement, FD is preferable in ruminants since it is less time and isotope consuming. For integrated measurements of several fluxes, CI can provide an interesting data set by studying the balance of several fluxes in the intermediary metabolism.

A third factor is that the choice of the labelled amino acid used could have an influence on the resulting FSR measurements. Significant models were proposed above for different tissues and organs with varying ranking orders between the amino acids depending on the tissues. These results need to be supported by the simultaneous study of several amino acids in the same experiment, as was undertaken in the rat by Obled et al (1989). They stated that the relationship between the specific activity of the true precursor pool and the extra- and intracellular specific activity varies with the different amino acids and that the turnover rate for tissue protein varies according to the amino acid used because of the heterogeneity of the protein pool. The same explanations can be used to justify the observed results of our quantitative study. Consequently, different tracer amino acid fluxes have to be measured simultaneously in order to calculate a weighted protein synthesis. For example, protein exportation and oxidation have to be accounted for in organs such as the liver (Southorn et al, 1992) or mammary gland (France et al, 1995). Therefore, a simultaneous kinetic with different amino acids known for their varying fates in the studied tissue or organ would be needed to obtain more realistic FSR values.

A fourth factor effecting FSR measurements is the physiological state of the animal. Hormones relating to the animal's status could have an effect but this was not demonstrated in the available data. A global qualitative ranking order could be given for the different physiological states from the highest FSR values in most tissues of suckling young animals to the lowest values

found in adult animals at maintenance. However, this first ranking order does not help to discriminate between the age and the physiological state effect. FSR is observed to decrease with age as in monogastrics (Tesseraud, 1995). Nevertheless, at a given age, a discrimination could be performed between maintenance and dry status with low FSR on the one hand and growing and lactating status with higher FSR on the other. To summarize, the whole body FSR might be related to the production of proteins either for internal use (muscle protein synthesis) or for exportation (milk protein synthesis) as in a 'pull' system. However, this whole-body conclusion should be tested for each tissue or organ since a given ranking order (eg, FSR higher in the mammary glands of lactating versus dry goats) can be inverted in different tissues (eg, FSR is lower in the muscle tissues of lactating versus dry goats; Baracos et al, 1991).

A fifth factor is that an increased level of feeding intake of either metabolisable energy or crude protein increases the FSR in most of the tissues studied. These results support the conclusions of Grizard et al (1988), who proposed that diet in ruminants has the same influence as is observed in monogastrics; that an increase of FSR occurs with both the energy and protein intake. This positive influence of the protein supply on FSR underlined the possibility of a 'push' effect of the circulating protein on the tissue FSR and probably the protein turnover rate. The more amino acids that are available for the tissue, the higher the FSR. However, the linear increase with crude protein intake might have a plateau which was not underlined in our study since the influencing factors hide these possible effects. A higher energy supply also increases FSR. The mechanisms involved are not isolated. Furthermore, in the available data set there was a strong correlation between energy and crude protein intake and consequently there might be a confusion of effects between energy and protein intake. These diet effects have to be studied for their influence on FSR together with such other key-fluxes as protein degradation or oxygen consumption in order to generate a global picture. An initial study has been carried out in this direction with the steer hind limb by Boisclair et al (1993) where they followed a number of metabolites together with the protein synthesis flux. New models should be fitted to each organ or tissue. An interesting set-up was proposed by Neutze et al (1997a, b) with multicatheterised lambs in which absolute and fractional synthesis and degradation fluxes in the small intestine have been measured simultaneously and they therefore managed to demonstrate a positive effect of the intake level on FSR. Since each of these models are more focused on one group of organs or tissues, they could be more informative. As an example, the protein metabolism in the mammary gland is regulated differently than it is in other organs and tissues (Grizard et al, 1988). Its metabolism should, therefore, be studied using specific animal models such as the one used by France et al (1995). Tissue and organ FSR are directly derived from the whole-body scale, in a similar way as for rodents; however, owing to their large size, ruminant animal FSR experiments have to be more regional.

In our study, variations in FSR measurements were underlined. Moreover several methodological factors were isolated. Nevertheless, FSR also varied with the physiological state of the animal. This is important since FSR multiplied by their related compartment sizes produced the absolute protein fluxes. Therefore, variations in FSR influenced these fluxes as can be observed in figure 3 from the data of Baracos et al (1991) and Champredon et al (1990). Within HP and CI, flux values were different for dry or lactating goats. In addition, the labelled amino acid choice changed the ranking order between the protein fluxes. Consequently, even though interacting factors were isolated, they need to be evaluated more since

their use in a given experiment can influence the biological conclusions.

## CONCLUSION

The main objective of this work was attained since it was possible to propose FSR estimates for the different tissues and organs with interacting factors (table II).

When FSR results are used qualitatively, the precursor pool has to be defined to avoid unjustified comparisons. The amino acid supply technique has an influence on the FSR measurements. However, both FD and CI could be used if the assumptions involved are clearly defined. The particular amino acid used as tracer highly influences the resulting FSR measurements. The choice of tracer amino acid is linked to the protein and/or amino acid metabolism and the composition of either the whole body or the specific tissue studied. FSR is also influenced by the feeding level of protein and/or energy and by the physiological status of the animal. However, possible mediating factors such as hormones were not isolated. In this work, quantitative FSR estimates were given (table II); however, these averages have to be used cautiously. Within-experiment and if possible within-animal measurements might be preferable owing to the influence of the experimental set-up and the individual variations.

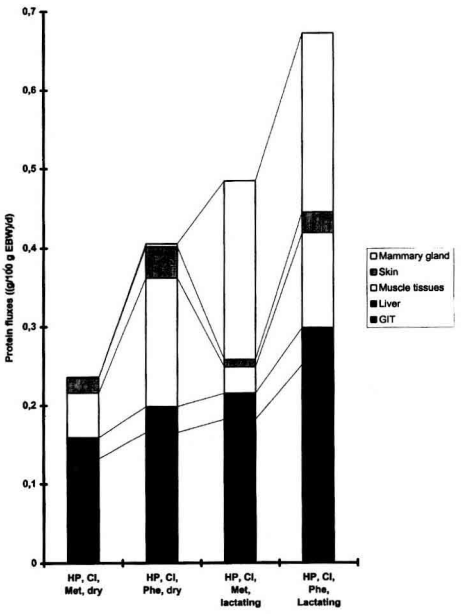


**Fig 3.** Absolute protein synthesis fluxes depending on the labelled amino acid chosen and on the physiological status.

Finally, to understand protein metabolism more precisely, it would be useful to simultaneously study the FSR, the fractional protein degradation rate and the oxidation ratio of the amino acids. Furthermore, to have a quantitative picture of the individual amino acid metabolism, the compartment size and the amino acid profiles of each pool have to be evaluated. In addition, experiments should focus on increasing understanding of the physiological status, and in particular, the stage of lactation, on the protein metabolism since the current available results are hampered by methodological variations. Therefore, both animal and mathematical models could be interesting ways to overcome the limitations of the usual 'one tracer at the whole-body level giving one measurement' approach.

## ACKNOWLEDGMENTS

We thank P Williams, director of Research and JC Robert, director of Ruminant Research and Development at Rhône-Poulenc Animal Nutrition for their financial and scientific support, and for their helpful advice during this project. The anonymous reviewers are also warmly acknowledged for their helpful comments. In addition, the authors acknowledge E Thompson for correcting our written English.

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