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Amino Acid Uptake by the Mammary Gland of the Lactating Ewe

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Abstract

(1) The arterio–venous difference technique, previously used to measure mammary substrate uptake with cows and goats, was used to measure amino acid uptake by the mammary gland of the lactating Merino ewe. Possession of a single large superficial epigastric vein by the Merino ewe makes the Merino breed the most suitable for this type of study.
(2) A method was developed enabling hourly measurement of milk yield without causing undue stress. Milk yield was essentially constant over a 7–8-h period.
(3) Mammary extraction of most non-essential amino acids was low relative to output in milk protein and showed a greater variability with time than that found for the essential amino acids. There was a significant mammary extraction of ornithine and citrulline, neither amino acid being found in ovine milk protein.
(4) Of the essential amino acids valine, isoleucine, leucine and arginine were taken up in excess of their requirement for milk protein synthesis.
(5) On the basis of the extent of mammary extraction, methionine, lysine and leucine were first-, second- and third-limiting to the rate of milk protein synthesis.
(6) Despite fluctuations in arterial amino acid concentrations the arterio–venous differences of the essential amino acids were relatively constant over a 7–8-h period.
(7) The pattern of mammary amino acid uptake in the ewe is contrasted with that found in similar studies carried out with the lactating cow and goat.

Introduction

The arterio–venous concentration (AV) difference technique has been applied to the study of mammary substrate uptake in the lactating dairy cow (Bickerstaffe *et al.* 1974), dairy goat (Mepham and Linzell 1966) and pig (Linzell *et al.* 1969; Spincer *et al.* 1969).

Similar studies with the lactating ewe are of interest because most sheep breeds have not been specifically selected for milk production and contrast with the high yielding, concentrate fed, dairy cow and goat. Previous work with lactating ewes has been restricted to a report of a significant AV difference of acetate across the udder (Costa *et al.* 1976).

Amino acid metabolism by the lactating udder of the sheep has been investigated in a number of *in vitro* perfusion experiments (see Mepham 1971; Verbeke *et al.* 1972; Roets *et al.* 1974). Thus it is of interest to compare amino acid metabolism of the sheep udder *in vitro* with the pattern of amino acid uptake *in vivo*.

This paper describes the development of a method for the hourly milking of lactating ewes and the use of the AV difference technique to measure mammary amino acid uptake.

Methods

Preparation of Animals

Multiparous Merino ewes were used. Each animal had been operated on previously to exteriorize one of the external carotid arteries in a skin-covered loop. Merino ewes were chosen because they possess a single 'milk vein' formed by the confluence of left and right superficial epigastric veins immediately anterior to the udder. Mammary venous blood drains in this vein in a cranial direction. In similarity to the cow and goat, mammary venous drainage also occurs via the external pudic venis (Linzell 1960).

Ewes and lambs were kept at pasture until the day of each experiment when they were restrained indoors in metabolism crates. The carotid artery was cannulated with a 'butterfly' needle (19-gauge) and the 'milk' vein with a 17-gauge plastic cannula 5·0 cm in length (Angiocath, Deseret Pharmaceutical Co., Sandy, Utah, U.S.A.), each cannula being held in position by sutures. The 'milk' vein cannula was more conveniently used with a silicone rubber extension tube tied to the wool on the lateral abdomen.

Teat catheters were inserted as far as the upper teat sinus and held in position with surgical tape. The cathethers were blunted, highly polished 14-gauge syringe needles with side apertures. Each catheter was extended with silicone rubber tubing for collection of milk separately from each udder-half. Between experiments catheters were sterilized by boiling and kept in 70% (v/v) ethanol. Following catheter removal each udder-half was treated with antibiotics to prevent the occurrence of mastitis.

All surgical procedures were carried out within 45 min of placing the ewe in the crate. Lambs were placed in the crates with their mothers, but were prevented from access to the udder by a low wire partition. Proximity of the lamb to ewe was essential to minimize maternal stress.

Experimental Procedures

(i) *Milk yield measurement*

Ewes were milked hourly following intra-arterial injection of 200 m-i.u. oxytocin. Milk flow was facilitated by maintaining light pressure on the udder with the back of the hand while positioning the teat catheter in the teat sinus with thumb and forefinger. Satisfactory milk removal was obtained with two doses (200 m-i.u.) of oxytocin with a 2-min interval between injections, over 90% of the hourly yield being obtained with the first dose. Milking was continued hourly for up to 9 h.

The ewes quickly became accustomed to these procedures, milking out being achieved within 2–3 min, with a minimum of disturbance. Milking without catheters, by hand, was a more protracted procedure (5–10 min). Milk samples were stored immediately at −20°C.

(ii) *Blood sampling*

Blood samples were taken only after at least 3 h had elapsed to allow the animals to recover from handling stress and the preparative procedures. Carotid arterial and mammary venous blood samples were taken simultaneously into heparinized tubes. Plasma was prepared immediately and stored at −20°C. Simultaneous AV sample pairs were taken over a period of 5–7 h.

(iii) *Determination of mammary gland weight*

Mammary gland weight was estimated by measuring empty udder volume, by water displacement, at the end of each experiment. The mean of three determinations was taken and corrected to mammary weight taking the specific gravity of the udder as 1·034 (Linzell 1966).

Analytical Methods

(i) *Plasma amino acid analysis*

Plasma samples were prepared for amino acid analysis by mixing 2 ml plasma with 1 ml 6% (w/v) sulphosalicylic acid containing 0·1 μM norleucine. After storage at 4°C for 1 h and centrifugation at 1000 *g* for 10 min the supernatant was rotary evaporated to dryness at 35°C, reconstituted with 0·5 ml lithium buffer (0·3 M), pH 2·2, and stored at −20°C. Any precipitate developing on storage was removed prior to loading by centrifugation of the thawed sample at 1000 *g* for 5 min.

Amino acid analysis was carried out by ion-exchange chromatography using lithium buffers on a Technicon TSM-1 amino acid analyser. Quantitative recovery of amino acids from the basic

column was established using a standard calibration mixture of amino acids. Recovery of basic amino acids was calculated from the norleucine recovery after loading the basic column with a known aliquot of the same sample used for acidic–neutral amino acid analysis. Loss of cystine on storage precluded its quantitative analysis (see Perry and Hansen 1969).

(ii) *Milk protein content*

Milk protein content was measured by an amido black absorption method (Pro-Milk Tester, N. Foss Electric, Hillerod, Denmark) calibrated for ovine milk against Kjeldahl nitrogen determinations.

(iii) *Milk fat content*

Milk fat content was determined by the method of Fleet and Linzell (1964).

(iv) *Milk lactose content*

Milk lactose content was determined by the method of Marier and Boulet (1959).

(v) *Amino acid composition of ovine milk protein*

The amino acid composition of ovine milk protein was determined as described by Davis and Mepham (1974) for guinea pig milk protein.

Results

Milk Yield

Milk yield varied from 60 to 85 ml/h (Table 1) in animals at peak lactation (2–3 weeks), declining to less than 40 ml/h towards the end of lactation. Within an experimental period there was a tendency for yield to decline such that after 8 h of confinement milk yield was 80–90% of the initial yield. This situation is similar to that found with lactating goats where milk yield declines to 90% of its previous level with 8 h of fasting (Linzell 1967).

Table 1. Hourly variation in milk yield and composition

Experiments on sheep A to E were carried out at 14, 10, 8, 21 and 15 days respectively *post-partum*. n.d., not determined

Sheep[A]	Mean milk yield (ml/h)	Gland weight (g)	Milk composition (mean ± s.e.) (g/100 g milk)		
			Fat	Protein	Lactose
A (8)	82±5	970	n.d.	3·96±0·04	n.d.
B (7)	60±2	870	n.d.	3·93±0·09	5·12±0·07
C (8)	69±5	940	n.d.	4·66±0·07	5·05±0·05
D (9)	67±3	930	8·5±0·1	3·96±0·09	4·80±0·07
E (9)	85±3	1100	8·2±0·1	4·06±0·07	5·11±0·06

[A] Numbers in parentheses indicate total length of experimental period (h).

Milk yield over the first hour of measurement was often apparently higher than that achieved subsequently. This was probably due to the inefficient removal of residual milk at the start of each experiment because of stress inhibition of oxytocin activity on the myoepithelial cells (Linzell 1959).

Mature Merino ewes at pasture have been reported to produce 1400–1600 g milk/day (58–67 g/h) at peak lactation (Corbett 1968), in close agreement with the yields obtained in these experiments (Table 1). One ewe (E, in Table 1) with twin lambs had a higher yield at peak lactation than the others with singleton lambs and also possessed the largest udder.

Milk Composition

The composition of milk secreted over the experimental period showed little change from hour to hour. Milk protein content remained at approximately 4 g/100 g, lactose 5 g/100 g and in two experiments where it was measured milk fat content was approximately 8 g/100 g milk (Table 1). These values are similar to values quoted by Corbett (1968) for the composition of Merino ewe milk.

It has been reported that high doses of oxytocin may produce changes in the ionic composition of milk and a fall in lactose content (Linzell and Peaker 1971). In two experiments where milk Na^+ and K^+ levels were measured throughout, Na^+ concentration varied from 13 to 17 m-equiv/l and K^+ from 22 to 28 m-equiv/l. No trend was apparent in this variation.

Table 2. Mammary amino acid extraction in lactating ruminants

Amino acid[A]	Sheep plasma arterial concn ±s.e. (μg/ml)	Mammary extraction (%) Sheep[B]	Goat[C]	Cow[D]
Asp (7·5)	1·5±0·1	14 (225)	34	36
Asn	2·1±0·1	29 (68)	43	n.v.
Glu (20·8)	15·4±0·9	29 (57)	58	59
Gln	19·6±2·9	13 (258)	25	n.v.
Pro (10·5)	7·8±2·3	8 (239)	40	n.v.
Gly (1·7)	32·8±2·2	0 (5021)	5	6
Ala (3·5)	9·4±0·3	−7 (354)	24	13
Ser (4·6)	6·2±0·3	37 (50)	0·1	36
Cit	21·1±0·8	17 (83)	1·4	10
Orn	7·0±0·5	44 (32)	38	45
Met (2·4)	1·7±0·6	73 (20)	77	58
Ile (4·9)	9·3±0·4	43 (21)	50	39
Leu (9·5)	13·5±0·5	47 (20)	63	42
Val (6·1)	15·7±0·6	33 (32)	38	26
Tyr (4·4)	5·9±0·2	44 (27)	45	42
Phe (4·6)	5·5±0·2	41 (28)	67	40
Lys (7·5)	9·3±0·4	51 (23)	47	59
His (2·8)	7·0±0·2	21 (52)	35	30
Arg (3·4)	14·1±0·4	29 (40)	48	53
Thr (4·3)	10·3±0·6	25 (50)	62	38

[A] Values in parentheses are amino acid contents of ovine milk protein (g/100 g protein). Aspartate and glutamate contents include asparagine and glutamine residues.
[B] Values in parentheses are coefficients of variation of amino acid extraction.
[C] Mepham and Linzell (1966).
[D] Mean of Friesian cows 'J' and 'T' (Bickerstaffe *et al.* 1974). n.v., no value reported.

Mammary Amino Acid Uptake

The mean extractions of amino acids by the mammary gland of the ewe are shown in Table 2. These data were calculated on the basis of analyses made on 48 AV pairs of plasma samples from five sheep. Methionine consistently showed the highest extraction, usually over 70% and sometimes greater than 90%. If the extent of extraction reflects the order of limitation of amino acid supply for milk protein synthesis (Davis and Mepham 1976) then the first three limiting amino acids, in order, are methionine, lysine, and leucine.

Extractions of the non-essential amino acids were, in general, lower than those found for the essential group and invariably there was a net mammary production of alanine. In similarity to the cow and goat there was a net mammary uptake of ornithine and citrulline, neither amino acid being found in milk protein.

In comparison with the cow and goat, mammary amino acid extraction was less in the sheep, the most striking differences being found in the case of threonine which has a 62% extraction by the goat mammary gland but only 25% by the sheep gland (Table 2). Reasons for the variation between the ruminants in the magnitude of mammary extraction of amino acids become apparent following examination of the relative plasma amino acid concentrations in the animals used in these studies (Fig. 1).

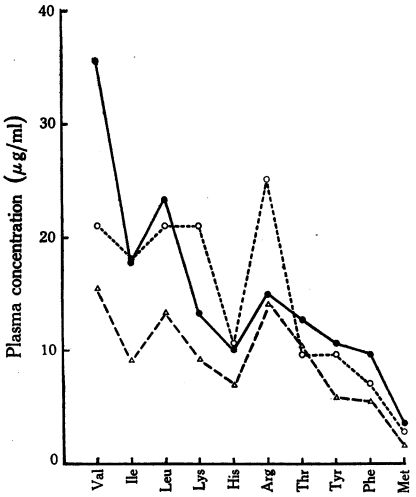

Fig. 1. An aminogram to compare the essential amino acid content of lactating goat (○, Mepham and Linzell 1966), cow (●, Bickerstaffe *et al.* 1974) and sheep (△) plasma.

The essential amino acid levels in sheep plasma are consistently lower than those found in the cow and goat, with the exception of threonine. The most marked differences are to be found in the particularly high levels of valine, isoleucine and leucine in the cow and goat and the high arginine content of goat plasma.

These differences are most likely to reflect a difference in both diet and nutritional status rather than inter-species variation, experiments with the cow and goat cited here being carried out on animals consuming diets supplemented with concentrates, which might be expected to modify the quality and quantity of protein arriving at the duodenum (see Annison 1972).

The magnitude of amino acid extraction is a reflection of the amino acid requirements of the mammary secretory epithelial cells (see Mepham 1976) and once this requirement has been met will decline with increasing rate of mammary blood flow or increasing plasma concentration or both. With the consistently lower essential amino acid content of blood plasma in the lactating ewe (Fig. 1) one might expect, all other factors being equal, a greater mammary extraction of amino acids. However, this is not found to be the case (Table 2).

This paradox may be explained by the fact that methionine, the putative first-limiting amino acid for milk protein synthesis, is at a plasma concentration in the lactating ewe of only 60% of that found in the goat and about half that of the cow.

With the greater blood flow : milk yield ratio that this lower level dictates, extraction of other amino acids must decline. Thus the mammary extraction of essential amino acids may vary with the availability of the first-limiting amino acid.

Pattern of Amino Acid Uptake

There are marked similarities between the pattern of mammary amino acid uptake in the ewe (Fig. 2) and that determined for the cow and goat (see Mepham 1976). On the basis of the uptake : output ratio (see legend to Fig. 2), methionine, phenylalanine, tyrosine, threonine, and histidine show the closest balances between uptake from blood and output in milk protein. In contrast the uptakes of valine, isoleucine, leucine, and arginine are in marked excess of requirements for milk protein synthesis. Similar data have been obtained in other studies with the cow and goat (see Kellaway *et al.* 1974; Clark 1975).

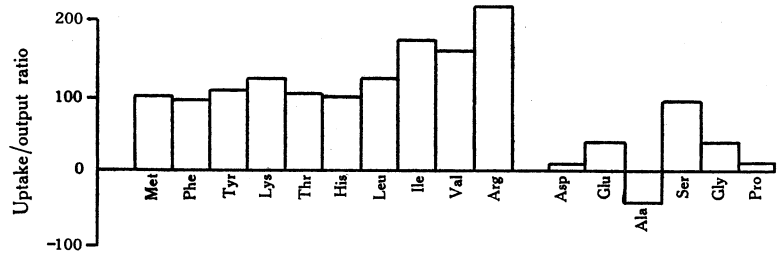

Fig. 2. A comparison of the balance between mammary uptake and output of amino acids across the mammary gland of the ewe. Uptake/output ratios were calculated by division of AV differences for individual amino acids with their content in milk protein (g/100 g; Table 2). Individual ratios are then expressed as multiples of the ratio for the essential amino acid with the lowest value (see Davis 1974; Mepham 1976), i.e. that which expressed the closest balance between uptake and output in milk protein.

Of the non-essential amino acids, mammary uptake, relative to output, is less in the ewe than that found in the cow and goat with the exception of serine. This would imply a greater requirement for the mammary synthesis of non-essential amino acids by ovine mammary tissue, a fact which gains support from relatively greater mammary glucose uptake in the ewe (Davis and Bickerstaffe 1978).

The amino acid composition of ovine milk protein is shown in Table 2. These data are in close agreement with data from a similar study (Williams *et al.* 1976).

Temporal Variation in Amino Acid Uptake

Despite fluctuations in arterial concentrations, amino acid AV differences were relatively constant throughout the experimental period (Fig. 3) declining only to parallel the fall in milk yield. In accord with the data obtained in the goat (Mepham and Linzell 1966; Mepham and Linzell 1974) uptake of non-essential amino acids was variable, as evidenced by the higher coefficients of variation with amino acid extraction for this group (see Table 2). However, rather than reflecting a true variation in uptake it is felt that the low extraction of non-essential amino acids precludes the accurate estimation of AV difference.

The pattern of amino acid uptake determined over 12 weeks of lactation in four sheep did not vary significantly with milk yield or stage of lactation.

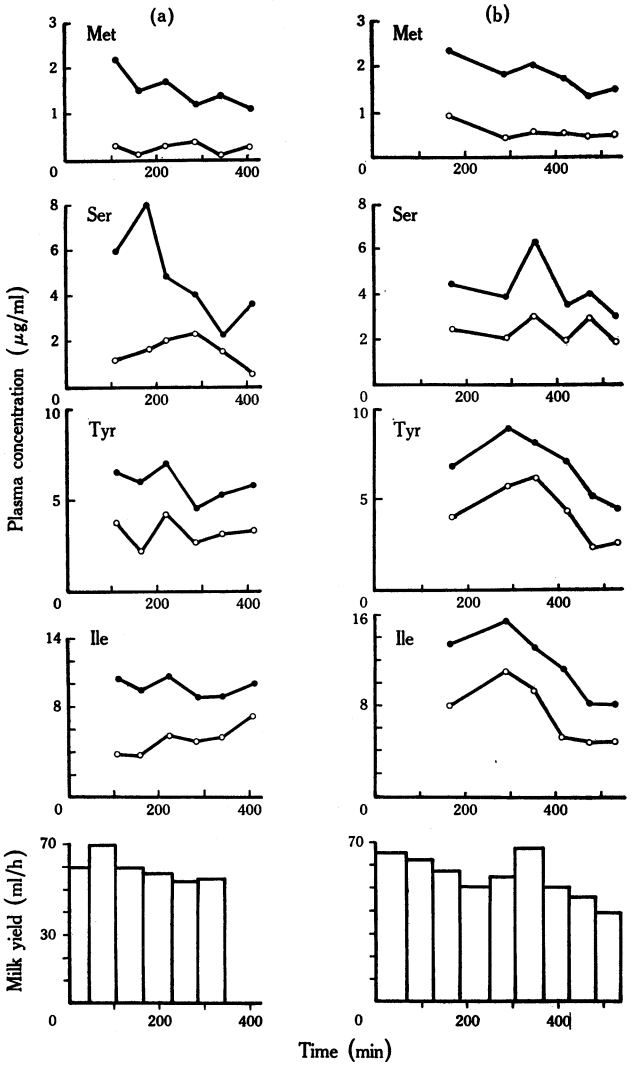

Fig. 3. Temporal variation of amino acid concentrations in arterial (●) and mammary venous (○) plasma in two lactating Merino ewes (*a* and *b*) at peak lactation.

Discussion

Validity of the Method

Assessment of mammary substrate uptake from AV difference measurements in ruminants is both facilitated and complicated by the nature of mammary venous drainage (see Linzell 1974). Incompetence of the valves in the superficial epigastric vein(s) facilitates the sampling of mammary venous blood during lactation, while in some high yielding and older animals, incompetence of valves in the external pudic vein may contaminate blood in the superficial epigastric vein with blood of non-mammary venous origin.

Blood flow in the confluent superficial epigastric vein of the Merino ewes used in these experiments was in a cranial direction in all animals. No attempt was made to determine the direction of flow in the external pudic vein. Valves in this vein may become incompetent in sheep (Linzell 1960) but it is felt that this was unlikely in the animals used here because of the relatively low milk yield of Merino ewes in comparison with the cow and goat. The very high extraction of methionine by the udder in all animals would appear to vindicate this conclusion as contamination of the superficial epigastric vein effluent with blood of non-mammary origin would almost certainly prevent the measurement of extractions greater than 80%. The similarity of the patterns of amino acid uptake in all five ewes would also support the purity of the mammary venous blood sample as it is unlikely that all animals would exhibit valvular incompetence in the external pudic vein. In any event, the trauma induced by manual clamping of the external pudic vein to ensure a lack of back-flow would invalidate any AV difference measurements made.

Assessment of hourly milk yield was greatly facilitated by the use of teat catheters, such that all available milk could be removed from the udder within 2–3 min with a minimum of disturbance. Milk yields from the left and right glands were usually very similar. The dose of oxytocin used (200 m-i.u.) was insufficient to cause changes in the Na^+ and K^+ concentrations in milk, but was essential to the achievement of an accurate measure of hourly milk yield.

It is of interest to note that it is possible to carry out these experiments on such a timorous creature as the ewe where previously only trained animals have been used (Linzell 1967). A prerequisite of such experiments is to minimize stress, and in the ewe it would seem to be relatively easy to accomplish this by placing the lambs in close proximity to the ewes.

Milk Yield

Milk yield per unit weight of tissue is similar in the sheep to that of the other ruminants. Linzell (1972) quotes values of 1·90 ml per gram per day for cows at peak lactation and 1·75 ml per gram per day for goats. In these experiments peak milk yields were the equivalent of 2·11, 1·66, 1·75, 1·72 and 1·85 ml per gram per day. These data are in keeping with the postulate that milk yield is similar per unit weight of mammary tissue in all species (Linzell 1972). The reason that the goat, an animal of similar size to the sheep, gives relatively more milk is a reflection of its udder size and not increased activity of its individual secretory cells (Linzell 1972).

The total solids content of ovine milk is greater than that found in the cow and goat and one might therefore expect a relatively greater blood flow : milk yield ratio. Calculation of mammary blood flow in these experiments on the basis of the Fick principle with methionine AV difference (Davis and Bickerstaffe 1978) indicates a mammary blood flow : milk yield ratio in the ewe of 870 : 1 in comparison with the often quoted value of 500 : 1 for the dairy cow and goat (Linzell 1974).

Mammary Amino Acid Requirements

The pattern of amino acid uptake by the udder of the ewe is similar to that found in the cow and goat. The interpretation of the pattern of amino acid uptake is substantiated by experiments carried out *in vitro* with the perfused udder (see Mepham 1971; Verbeke *et al.* 1972; Roets *et al.* 1974). There is little evidence to suggest

that under normal circumstances the residues of methionine, phenylalanine and threonine undergo catabolism or transformation to any significant extent, i.e. the mammary uptake is incorporated solely into milk protein.

In contrast, there is evidence for the existence of catabolic pathways for other essential amino acids. Valine oxidation has been observed in the isolated perfused goat and guinea pig mammary glands (see Mepham 1971; Davis and Mepham 1976) as has the incorporation of valine carbon into aspartate, glutamate, glycine, alanine, isobutyrate and β-hydroxyisobutyrate by bovine mammary slices (Derrig *et al.* 1973). Data are also available to explain the fate of the isoleucine, leucine and arginine residues taken up in excess of requirements for milk protein synthesis (see Mepham 1976).

The citrulline requirement of lactating sheep mammary tissue may be greater than that of the cow and goat. Data obtained with isolated perfused sheep and goat udders have indicated that citrulline may be metabolized by way of arginine to urea, ornithine and proline. However, the extent of transfer of citrulline carbon to arginine carbon of casein was nearly five-fold greater in the sheep (Roets *et al.* 1974). The greater extraction of citrulline by the udder of the ewe (Table 2) would substantiate the *in vitro* data.

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References

Annison, E. F. (1972). Rumen metabolism and protected nutrients. Proc. 6th Nutrition Conference for Feed Manufacturers. (Eds H. Swan and D. Lewis.) University of Nottingham. (Churchill Livingstone: London.)

Bickerstaffe, R., Annison, E. F., and Linzell, J. L. (1974). The metabolism of glucose, acetate, lipids and amino acids in lactating dairy cows. *J. Agric. Sci.* **82**, 71–85.

Clark, J. H. (1975). Lactational responses to postruminal administration of proteins and amino acids. *J. Dairy Sci.* **58**, 1178–97.

Corbett, J. L. (1968). Variation in the yield and composition of milk of grazing Merino ewes. *Aust. J. Agric. Res.* **19**, 283–94.

Costa, N. D., McIntosh, G. H., and Snoswell, A. M. (1976). Production of endogenous acetate by the liver in lactating ewes. *Aust. J. Biol. Sci.* **29**, 33–42.

Davis, S. R., and Mepham, T. B. (1974). Amino acid and glucose uptake by the isolated perfused guinea pig mammary gland. *Q. J. Exp. Physiol.* **59**, 113–30.

Davis, S. R., and Mepham, T. B. (1976). Metabolism of L-[U-^{14}C]valine, L-[U-^{14}C]leucine, L-[U-^{14}C]-histidine and L-[U-^{14}C]phenylalanine by the isolated perfused lactating guinea pig mammary gland. *Biochem. J.* **156**, 553–60.

Davis, S. R., and Bickerstaffe, R. (1978). Mammary glucose uptake in the lactating ewe and the use of methionine arterio–venous difference for the calculation of mammary blood flow. *Aust. J. Biol. Sci.* **31**, 133–9.

Derrig, R. G., Clark, J. H., and Davis, C. L. (1973). Metabolism of arginine, ornithine and valine by bovine mammary gland. *J. Dairy Sci.* **56**, 651.

Fleet, I. R., and Linzell, J. L. (1964). A rapid method for estimating fat in very small quantities of milk. *J. Physiol. (London)* **175**, 15–17p.

Kellaway, R. C., Ranawana, S. S. E., Buchanan, J. H., and Smart, L. D. (1974). The effect of nitrogen source in the diet on milk production and amino acid uptake by the udder. *J. Dairy Res.* **41**, 305–15.

Linzell, J. L. (1959). Physiology of the mammary glands. *Physiol. Rev.* **39**, 534–76.

Linzell, J. L. (1960). Valvular incompetence in the venous drainage of the udder. *J. Physiol. (London)* **153**, 481–91.

Linzell, J. L. (1966). Measurement of udder volume in live goats as an index of mammary growth and function. *J. Dairy Sci.* **49**, 307–11.

Linzell, J. L. (1967). The effect of very frequent milking and of oxytocin on the yield and composition of milk in fed and fasted goats. *J. Physiol. (London)* **190**, 333–46.

Linzell, J. L. (1972). Milk yield, energy loss in milk and mammary gland weight in different species. *Dairy Sci. Abstr.* **34**, 351–60.

Linzell, J. L. (1974). Mammary blood flow and methods of identifying and measuring precursors of milk. In 'Lactation'. (Eds B. L. Larson and V. R. Smith.) (Academic Press: London.)

Linzell, J. L., and Peaker, M. (1971). Mechanism of milk secretion. *Physiol. Rev.* **51**, 564–97.

Linzell, J. L., Mepham, T. B., Annison, E. F., and West, C. E. (1969). Mammary metabolism in lactating sows. Arteriovenous differences of milk precursors and the mammary metabolism of [^{14}C]glucose and [^{14}C]acetate. *Br. J. Nutr.* **23**, 319–32.

Marier, J. R., and Boulet, M. (1959). Direct analysis of lactose in milk and serum. *J. Dairy Sci.* **42**, 1390–1.

Mepham, T. B. (1971). Amino acid utilisation by the lactating mammary gland. In 'Lactation'. pp. 297–315. (Ed. I. R. Falconer.) (Butterworths: London.)

Mepham, T. B. (1976). Amino acid supply as a limiting factor in milk and muscle synthesis. In 'Cattle Production'. (Eds H. Swan and W. Broster.) (Butterworths: London.)

Mepham, T. B., and Linzell, J. L. (1966). A quantitative assessment of the contribution of individual plasma amino acids to the synthesis of milk proteins by the goat mammary gland. *Biochem. J.* **101**, 76–83.

Mepham, T. B., and Linzell, J. L. (1974). Hour-to-hour variation in amino acid arteriovenous concentration differences across the lactating goat mammary gland. *J. Dairy Res.* **41**, 95–100.

Perry, T. L., and Hansen, S. (1969). Technical pitfalls leading to error in the quantitation of plasma amino acids. *Clin. Chim. Acta* **25**, 53–8.

Roets, E., Verbeke, R., Massart-Leen, A. M., and Peeters, G. (1974). Metabolism of [^{14}C]citrulline in the perfused sheep and goat udder. *Biochem. J.* **144**, 435–46.

Spincer, R., Rook, A. F., and Towers, K. G. (1969). The uptake of plasma constituents by the mammary gland of the sow. *Biochem. J.* **111**, 727–32.

Verbeke, R., Roets, E., Massart-Leen, A. M., and Peeters, G. (1972). Metabolism of [U-^{14}C]-L-threonine and [U-^{14}C]-L-phenylalanine by the isolated perfused udder. *J. Dairy Res.* **39**, 239–50.

Williams, A. P., Bishop, D. R., Cockburn, J. E., and Scott, J. E. (1976). Composition of ewe's milk. *J. Dairy Res.* **43**, 325–9.

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