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The polyunsaturated fatty acid status of foetal and neonatal ruminants

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1. Information on the fatty acid composition of tissues of foetal calves, neonatal lambs, deer and piglets reported by Payne (1978) has been quantified by the use of an internal standard during analysis, to give concentrations of total polyunsaturated fatty acids (PUFA) derived from linoleic acid ($\omega6$) and linolenic acid ($\omega3$) expressed on a per kg tissue basis. The total concentration of both acids ($\omega6+\omega3$) was similar in all tissues examined except brain. Because muscle, the main constituent of the soft tissues of young animals, contains about 40–50 % of the total body content of these acids, it is considered that muscle concentrations are a reflection of total body status of these acids.

2. Concentrations in muscle of both $\omega6$ derivatives and total PUFA were significantly lower in the neonatal lamb and foetal calf than in the mature animal whereas in pigs and deer the concentrations in the young animal were similar to those in the mature animal. Concentrations of $\omega6$ derivatives and total PUFA in lambs were significantly lower than those in calves; the presence of $\omega3$ derivatives reduced the level of significance for total PUFA. Again, total PUFA content did not differ significantly between the piglet and the young ruminants.

3. There was a substantial placental transfer, with apparently a preferential transfer of $\omega3$ derivatives.

4. In brain the levels of $\omega3$ acids were as high in the foetal and neonatal animals as in mature animals. The levels of $\omega6$ acids were lower in young animals.

5. Calculations of $\omega6$ intake from milk showed that the total deficit of $\omega6$ could be made up within a few days.

6. It was concluded that the extent of deficiency of $\omega6$ in young ruminants raised in a grazing situation, as in New Zealand, is marginal and any feeding to overcome this is unlikely to be of any benefit.

On the basis that newborn lambs have low linoleic acid ($\omega6$) concentrations and a high value for 20:3ω9:20:4ω6 compared with the corresponding mature ruminants, it has been suggested that newborn ruminants are deficient in essential fatty acids (Noble, 1973). It has been accepted that in the ruminant the placental transfer of fatty acids, particularly $\omega6$ acid, is very low (Van Duyne, Parker, Havel & Holm, 1960). Despite the fact that linolenic acid ($\omega3$) may exert effects similar in part, to the response with linoleic acid ($\omega6$) (Burr, Burr & Miller, 1932; Mohrhauer & Holman, 1963; Pudelkewicz, Seuffert & Holman, 1968) no real assessment has been made of the quantities of linolenic acid or its derivatives that are present in ruminant tissues.

Derivatives of linoleic and linolenic acids are:

$$\begin{tikzcd}
\text{Linoleic acid family:} & 18{:}2\omega6 \arrow[rr] \arrow[dr] & & 20{:}3\omega6 \arrow[r] & 20{:}4\omega6 \arrow[d] \\
& & 20{:}2\omega6 \arrow[ur] & & 22{:}4\omega6 \arrow[d] \\
& & & & 22{:}5\omega6 \\
\text{Linolenic acid family:} & 18{:}3\omega3 \arrow[rr] & & 20{:}3\omega3 \arrow[r] & 20{:}4\omega3 \arrow[d] \\
& 22{:}6\omega3 & & 22{:}5\omega3 \arrow[ll] & 20{:}5\omega3 \arrow[l]
\end{tikzcd}$$

It can be seen that in any assessment of these acids all those found in significant quantities in tissues (> 0·3 %) (all derivatives except 22:5ω6, 20:3ω3 and 20:4ω3) should be considered.

In the previous paper it has been shown that combined levels of 20:5ω3, 22:5ω3 and 22:6ω3 acids derived from linolenic acid ranged between 59 and 362 g/kg in the phospholipids of various tissues of foetal calves and neonatal lambs (Payne, 1978). Since these acids cannot be synthesized endogenously (Holman, 1968) they, or a precursor such as linolenic acid must have crossed the placenta.

Using the results reported in a previous paper (Payne, 1978) an estimate of the comparative placental transfer of essential fatty acids on the basis of body-weight for the different animals was determined and compared with the levels in the corresponding mature animal.

EXPERIMENTAL

Animals and diets. The animals and their feeding were as described by Payne (1978).

Methods. Fatty acids were analysed as described previously (Payne, 1978). During the transmethylation of lipid extracts an internal standard of methyl margarate was added to allow a quantitative assessment of fatty acids based on the wet weight of tissue. Peaks corresponding to acids of the ω6 and ω3 series derived from linoleic and linolenic acids respectively, namely 18:2, 20:3, 20:4, 22:4, 22:5 and 18:3, 20:5, 22:5, 22:6, have been grouped to allow calculation of the total acids of each series.

The differences in levels thus obtained have been tested for significance by Student's *t* test and the approximate *t* test where variance was not homogeneous.

RESULTS

Polyunsaturated fatty acids (PUFA) of tissues

The total amounts of acids of the ω6 and ω3 series contained in muscle phospholipids of foetal calves, newborn lambs, newborn piglets, newborn deer calves and mature cows, sheep, pigs and deer are given in Table 1. Corresponding results are given for brain in Table 2 and for liver, heart, lung and intestine in Table 3.

Comparison of the results for all tissues showed that, apart from brain, the total PUFA concentrations of the tissues were not greater than threefold that of muscle. There were variations in the distribution of ω6 and ω3 derivatives as shown by the ratio ω6:ω3 in calf tissues and lamb tissues. All lamb tissues had a low value for this ratio indicating a preponderance of ω3 fatty acids, whereas calf tissues such as lung, heart and intestine had a high value. Liver and brain were the only tissues in the calf with a value for this ratio of less than 1.

Muscle. This constitutes 27–30% of the total body-weight of newborn ruminants (Fourie, Kirton & Jury, 1970; Kirton & Paterson, 1973) whereas corresponding values for liver, brain and other individual tissues are 2–3, 1 and 1–2 respectively. These values considered in conjunction with the concentrations given in Tables 1–3 indicated that muscle contained approximately 40–50% of the total tissue PUFA. As values for other organs or tissues are much lower than that for muscle, concentrations given for this tissue in Table 1 have been used to compare the relative content of PUFA in various animals.

From results given in Table 1 it can be seen that the amounts of ω6 derivatives were significantly lower in both the lamb and the calf than in the corresponding adult ($P < 0{\cdot}01$). However, the calf had significantly higher levels than the lamb ($P < 0{\cdot}01$). As had been anticipated there was a significant content of ω3 acids. Indeed, in the lamb the amounts of the ω3 acids were much higher than those of the ω6 acids. The total PUFA content was still significantly lower in the young animals than in the adult, but proportionately not nearly as low as that of the ω6 acids alone.

The results for the polyunsaturated animals indicate that much of the increase in ω6 acids is at the expense of ω3 acids, which are reduced to the levels found in the pig.

Table 1. *The mean concentrations of fatty acids (mmol/kg tissue) derived from linoleic acid (18:2ω6) and linolenic acid (18:3ω3) in muscle phospholipids of some foetal, neonatal and adult ruminants and non-ruminants*

Animal	No. of animals	Linoleic derivatives (ω6)	Linolenic derivatives (ω3)	Total (ω6+ω3)	ω6:ω3
Lambs	6	0·62	1·35	1·97	0·46
Sheep	5	2·84	2·15	4·99	1·37
Calves	7	1·88	1·31	3·18	1·59
Cattle	4	3·51	2·02	5·53	1·91
Deer calves	2	3·53	3·25	6·78	1·08
Deer	4	4·37	3·74	8·11	1·18
'Poly' sheep*	2	3·85	0·46	4·31	8·9
'Poly' cattle*	2	4·17	0·26	4·43	14·8
Piglets	6	2·34	0·38	2·72	6·94
Pigs	5	3·27	0·54	3·81	6·34
Pooled SD (sheep, cattle, deer)		0·39	0·52	0·92	0·47
Pooled SD (pigs, 'poly' ruminants)		1·52	0·21	0·92	2·89

* Animals fed high levels of polyunsaturated fatty acids.

As might be expected, particularly when the sows were on a barley ration in which the predominant fatty acid is linoleic acid, the piglets had a high value for ω6:ω3 though the total PUFA content was not significantly different from those in the lamb or the calf. The ω6 acid levels in piglets were significantly higher than in lamb muscle ($P < 0·01$) but not significantly higher than in calf muscle.

The young deer had higher levels of both ω6 and ω3 acids than those in the bovine calf, possibly indicating a greater transfer of these acids across the placenta.

Brain. The differences in PUFA content between the lamb and calf and the corresponding mature ruminants were less distinct in brain (Table 2). There was very little difference between calves and lambs in amounts of ω6 acids. Comparison with the adult levels suggested that the levels of ω6 were significantly lower in both the calf ($P < 0·01$) and the lamb ($P < 0·05$). The concentrations of ω3 acids were very high, being much higher than those of the ω6 derivatives. There was no significant difference in levels of ω3 acids between the calf and the adult.

Piglets appeared to have slightly higher levels of the ω6 acids but lower levels of the ω3 acids than those of young ruminants. The concentrations of both groups of acid were similar in the piglet and the pig. The total concentration of the PUFA in the pig, however, appeared to be lower than that in the ruminant.

Unlike muscle, the PUFA content in brain of the deer calf showed similarities to corresponding ruminant levels. In general, brains of mature ruminants contained higher levels of ω6 acids but only slightly higher levels of ω3 acids than brains of foetal or neonatal ruminants.

Feeding protected PUFA supplements to cattle resulted in quite large increases in the amounts of ω6 acids in brain, but there was no decrease in levels of ω3 acids as in muscle.

Replenishment of PUFA in newborn ruminants by milk intake

With knowledge of the linoleic acid content of milk and the milk intake by the young ruminant it is possible to determine how long it will take to make up the deficit.

Table 2. *The mean concentrations of fatty acids (mmol/kg tissue) derived from linoleic acid* ($18:2\omega6$) *and linolenic acid* ($18:3\omega3$) *in brain phospholipids of some foetal, neonatal and adult ruminants and non-ruminants*

Animal	No. of animals	Linoleic derivatives ($\omega6$)	Linolenic derivatives ($\omega3$)	Total ($\omega6+\omega3$)	$\omega6:\omega3$
Lambs	4	8·4	26·0	34·4	0·37
Sheep	6	13·0	28·6	41·6	0·46
'Poly' sheep*	2	21·3	23·7	45·0	0·98
Calves	7	9·8	23·4	33·2	0·43
Cattle	4	14·7	23·8	38·5	0·62
'Poly' cattle*	3	26·4	26·4	52·8	1·00
Piglets	2	18·4	11·2	29·2	1·92
Pigs	3	17·3	13·0	30·3	1·33
Deer calves	2	7·9	15·0	22·9	0·52
Deer	2	13·9	12·9	26·8	1·07
Pooled SD		3·4	5·1	7·0	0·15

* Animals fed high levels of polyunsaturated fatty acids.

Table 3. *The mean concentration of fatty acids (mmol/kg tissue) derived from linoleic acid* ($18:2\omega6$) *and linolenic acid* ($18:3\omega3$) *in various tissue phospholipids of foetal calves, and neonatal lambs and piglets*

Animal	No. of animals	Tissue	Linoleic derivatives ($\omega6$)	Linolenic derivatives ($\omega3$)	Total ($\omega6+\omega3$)	$\omega6:\omega3$
Calf	2	Liver	3·42	3·99	7·41	0·82
	3	Lung	3·09	1·27	4·36	2·34
	2	Heart	2·80	1·74	4·54	1·69
	2	Intestine	3·39	1·41	4·80	2·52
Lamb	2	Liver	1·27	4·29	5·56	0·29
	2	Lung	1·70	3·56	5·20	0·49
	2	Heart	1·89	4·92	6·81	0·48
	3	Intestine	1·52	4·30	5·82	0·36
Piglet	5	Liver	3·17	1·01	4·18	4·10
	3	Lung	3·63	0·61	4·24	5·78
	5	Intestine	3·28	1·03	4·31	3·40
Pooled SD			0·77	0·85	1·35	0·84 calf 0·10 lamb 1·66 piglet

Bovine milk fat contains about 20 g linoleic acid/kg (Patton, McCarthy, Evans & Lynn, 1960; Parodi, 1970) therefore 1 l of milk contains about 1 g linoleic acid on the basis of 5% milk fat. For a 30 kg calf which consumes about 4 l milk/d (Roy, 1971) the intake of linoleic acid is about 4 g. If as shown in Table 1 the difference in linoleic acid content between the calf and the adult is about 500 mg/kg tissue, then the total deficit is about 11 g (on the basis that 75% of body-weight is soft tissue). Allowing for an ever increasing requirement of 1 g linoleic acid/kg tissue growth, the calf would still need only approximately 3 d to make up any deficit. Though Noble, Steele & Moore (1970) have reported the content of linoleic acid in sheep milk to be about 7 g/kg milk fatty acids in the first few days after birth, sheep milk 24 h after birth in New Zealand has been found to contain 20–30 g/kg milk fatty acids (Payne, unpublished results). Hence, there is about 2 g linoleic acid/l milk on the basis of 80 g milk fat/l milk. For a lamb of 4 kg there is a total requirement of about 1·5–2·0 g linoleic acid to achieve concentrations in tissue phospholipids comparable to those in the mature sheep. On the basis of a milk intake of 400 ml/d this could be replaced within 2–3 d. Even if the content of linoleic acid was as low as 7 g/kg milk fatty acids only 7–10 d would be required.

DISCUSSION

In general, it was evident from a consideration of the total concentration of all PUFA of the $\omega3$ and $\omega6$ series that mature animals are not markedly different in their tissue concentrations, whilst the foetal or neonatal calves and lambs contain between 0·5 and 0·8 of the levels in the adult. On this basis it is proposed that, though the foetal and newborn ruminants have very low concentrations of linoleic acid, suggestive of essential fatty acid deficiency, the increased content of PUFA derived from linolenic acid, particularly C_{22} acids, substitutes at least in part for linoleic acid and any deficiency as compared with the levels in mature animals is only marginal.

Though it has been maintained that the criterion for essential fatty acid deficiency in non-ruminants is a value for $20:3\omega9:20:4\omega6$ of greater than 0·4 (Holman, 1960; Noble, 1973) values calculated from results given in a previous paper (Payne, 1978) are: calf 0·3, lamb 1·5, piglet 0·06, deer calf 0·19. Thus, only the value for the lamb exceeded this criterion for essential fatty acid deficiency and was similar to that found in newborn sheep by Noble, Steele & Moore (1972). Since their sheep were given hay, which may contain lower levels of linolenic acid than fresh grass (Moore, Noble & Steele, 1968), and concentrates, it is feasible that the levels of $\omega3$ derivatives were lower in their lambs. Hence the level of $20:3\omega9$ may reflect inversely the level of linoleic acid but not necessarily indicate the essential fatty acid status of ruminants.

Perhaps the most significant observation to arise from the present study is that placental transfer of PUFA is significant as shown by the concentrations present in the foetal and neonatal ruminants. In particular, the apparent preferential transfer of linolenic acid or its derivatives suggests that this acid is actually more essential to foetal development than linoleic acid.

The levels of PUFA found are only indicative of the minimal transfer of PUFA as no account has been taken of the metabolism of these acids. The results of Noble *et al.* (1972) indicate that in the neonate linoleic acid metabolism is low. The findings of Pace-Asciak (1976) also indicate that in the lamb foetus the capacity for prostaglandin catabolism in tissues such as liver, lung and kidney, the normal sites for rapid catabolism in the adult, is low so presumably synthesis is also low. Hence, over-all PUFA levels are probably reasonably indicative of the total $\omega6$ transferred.

From a knowledge of the relative concentrations of linoleic acid in the plasma free fatty acids of ruminants and pigs (Leat, 1966) one may have expected piglets to have levels of $\omega6$ derivatives closer to maternal levels. This is probably the result of the pig possessing an epitheliochorial placenta with more layers of cells to impede fatty acid transfer than in the syndesmochorial placenta of the ruminant (Arey, 1954).

In relation to the preferential transfer of linolenic acid, this is most unusual in that there is a higher concentration of linoleic acid than linolenic acid in lipid fractions of most maternal bovine and ovine plasma. Whilst it has been accepted that most fatty acid transfer was from the free fatty acid fraction, cholesterol esters and phospholipids having been shown to be practically impermeable in toto (Goldwater & Stetten, 1947; Popjak & Beeckmans, 1950; McBride & Korn, 1964); it may well be that there is a lipoprotein lipase or phospholipase acting preferentially to liberate linolenic acid at the surface of placental blood vessels.

Another interesting observation is the fact that linolenic acid exists in the foetal animal as the higher PUFA derivatives only and, even in the adult, there is a preponderance in these forms.

It appears that the higher PUFA derivatives are vital to membranes of certain cells and there is a system for preferential placental transfer of the precursor, linolenic acid, or the higher acids. This is especially marked with respect to the brain. In the brain very high levels of $22{:}6\omega3$ are apparently required and the foetal brain has a similar concentration to that in adult brain. This tissue is also marked by the absence of significant quantities of linoleic acid which is apparently not required as such.

The high levels of linoleic and linolenic acid derivatives in the lamb brain indicate that this tissue has preferential use of these fatty acids and the synthesis of higher PUFA must be very efficient. This contradicts the possibility raised by Sinclair (1973) that the conversion of lower PUFA derivatives to the C_{22} PUFA is slow, and limiting to such an extent that ingestion of those C_{22} acids would be beneficial.

In tissues other than brain there is a decrease in these higher PUFA as the intake of linoleic acid increases. This reaches its maximum in the animals given diets containing protected PUFA where the levels of $22\omega3$ poly-acids are low, as mentioned in the previous paper (Payne, 1978). In brain there is little change in $\omega3$ derivatives due to the feeding of protected fats containing linoleic acid, indicating that in brains of mature animals there is a very small turnover of $\omega3$ fatty acids.

The levels of both groups of PUFA in the deer calf were higher than in the bovine indicating an increased placental transfer for both types of acids.

Noble *et al.* (1972) reported that the value for trienoic:tetraenoic acids decreased to less than 0·4 by 10 d after birth and that there appeared to be quantitative retention of linoleic acid up to 30 d of age. The present calculations for making up any deficiency are in general agreement with this finding, particularly as no results were given by these workers for intermediate times between birth and 10 d. Although, on milk feeding, the main part of any inadequacy in linoleic acid should be made up within a few days, there may be quantitative retention of linoleic acid for a longer period with some substitution for linolenic acid derivatives. Studies using $[^{14}C]$linoleic acid has shown quantitative retention of ^{14}C 2 weeks after administration to 1-week-old calves (Payne, unpublished results).

Over all, it appears that though newborn ruminants have low linoleic acid contents at birth, at least in New Zealand and other countries where grass feeding is the rule, the lack of total PUFA is not great and the level of linoleic acid is readily rectified by milk intake over the first few days. Thus, it is anticipated that administration of polyunsaturated oils to the newborn ruminant will be of little value.

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