# The polyunsaturated fatty acid status of the twin foetal lamb and the effect of maternal nutrient intake

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1. Restriction of food to ewes bearing twin lambs showed a trend to decrease the level of $\omega$6 fatty acids in muscle phospholipids of the foetus though this was not significant statistically ($P < 0{\cdot}05$).

2. There was a significant increase in the fatty acid ratio, 20:3$\omega$9:20:4$\omega$6 in the twin lambs as compared with single lambs.

3. There were no symptoms of polyunsaturated fatty acid deficiency.

Normal newborn lambs contain low amounts of linoleic acid and related polyenoic acids in comparison with the levels in mature ruminants (Noble, 1973; Payne, 1978*b*). The ratio eicosatrienoic acid:eicosatetraenoic acid (1·5) in muscles and plasma phospholipids is similar to that observed in simple-stomached animals in a state of polyunsaturated fatty acid (PUFA) deficiency (Holman, 1960) where 0·4 is taken as the upper limit of normality. Surprisingly, higher levels of linolenic acid derivatives than linoleic acid derivatives in the lamb at birth were reported also by Payne (1978*b*).

It was feasible that underfeeding, particularly of ewes carrying twin foetuses, could result in yet lower amounts of PUFA being available to the foetus than in fully fed ewes and some signs of linoleic acid deficiency might develop. This investigation reports on the relative fatty acid levels in muscle phospholipids of foetal lambs derived from underfed ewes.

## MATERIALS AND METHODS

Coopworth ewes aged 6 years and averaging 57·7 kg live weight were used; oestrus was synchronized by using progesterone sponges and the ewes were mated to Suffolk rams. The animals were then randomized on a live weight basis into two groups at approximately 45 d post-mating. The groups were grazed differentially until they achieved average weights of 51·4 kg (L−) and 67·2 kg (H−) by day 90 of gestation when they were X-rayed to identify ditocous ewes. They were then shorn and half the ditocous ewes of each group were allocated on a body-weight basis a pelleted diet of 600 lucerne (*Medicago sativa*) meal and 400 barley meal g/kg at average levels of 1·0(−L) or 1·75(−H) maintenance; non-pregnant maintenance was assumed to be 40 g dry matter/kg initial wool-free empty body-weight $(W)^{0{\cdot}75}$. The sheep were fed individually outdoors. To match increasing foetal requirement food was increased weekly in the following manner: 85, 90, 94, 100, 110 and 120% of the average nominal feeding level. The ewes were slaughtered at day 135 of gestation after being shorn and fasted for 18 h. Some of the monotocous ewes of high body-weight were fed the (−H) diet and their progeny constitute a single HH group.

Samples of muscle from hind leg, predominantly vastus lateralis, were obtained from twin foetal lambs from all groups (one per ewe) and from single foetal lambs of HH group and frozen immediately in plastic bags on solid carbon dioxide and stored at −20° until analysed.

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Methods of extraction and analysis of phospholipids for fatty acid composition were as described previously (Payne, 1978*a*).

The results were analysed by an unbalanced analysis of variance.

## RESULTS

Table 1 shows the mean fatty acid composition of the total lipids of muscles of the various nutritional groups. There were few differences between nutritional groups or indeed between single lambs and twin lambs except that there was a decrease in 22:6$\omega$3 with decreased intake of pelleted diet fed individually ($P < 0{\cdot}01$) and an increase in 20:4$\omega$6 with low intake ($P < 0{\cdot}05$) during the initial grazing period. Twin lambs had lower levels of 20:4$\omega$6 than single lambs ($P < 0{\cdot}01$).

The results are expressed on an absolute basis in terms of acids derived from linoleic acid ($\omega$6) and acids derived from linolenic acid ($\omega$3) in Table 2. The latter group did not include linolenic acid itself as this acid was eluted with a larger proportion of eicosenoic (20:1$\omega$9) acid. Exclusion of this fraction had no bearing on the results.

While there was no significant change in the level of $\omega$6 acids as a result of food intake within the twin foetal lambs or between single and twin lambs there was a trend to decrease from HH to LL groups.

The ratio, 20:3$\omega$9:20:4$\omega$6 showed a significant difference ($P < 0{\cdot}01$) between twin lambs and single lambs on a high intake and a slightly greater effect between all twin lambs and the single lambs on high intake though there was no significant effect of feeding *per se*.

There were no significant effects on the levels of $\omega$3 acids though there appeared to be a trend to decrease with lowering of intake particularly when grazing.

Though the mean levels of $\omega$6 and $\omega$3 for the various treatments appeared to be related to mean ewe empty body-weight and hence body reserves of essential fatty acids there was no significant correlation within or between groups for $\omega$6 or $\omega$3 with empty body weight or total lamb weight.

## DISCUSSION

The actual composition differs from the earlier results (Payne, 1978*a*) in slightly lower 22:5$\omega$3 and 22:6$\omega$3 acids and corresponding increases in C16, C18 and C18:1 acids. This may be due to feeding dried lucerne in this pen trial compared with fresh ryegrass (*Lolium perenne*) in the previous trial. It is known that the linolenic acid content of hay as for example used by Moore *et al.* (1968) is lower than observed in fresh grass (Hudson & Karis, 1974). Lucerne has a similar fatty acid composition to grass (Van der Veen & Olcott, 1967) so presumably dried lucerne would have less linolenic acid than fresh grass. Similarly the total acids derived from linolenate were also lower than in the previous report, presumably for the same reason.

Though there was a significant increase in 20:4$\omega$6 in groups on reduced intake during the grazing period this is rather difficult to reconcile with a reduced intake of linoleic acid. Since the foetal lambs were only a total weight of 0·6 kg at the end of the grazing period any effect of H− and L− must be due to differences in the ewe in fatty acid storage rather than in the foetus, with a resulting delayed effect on the foetal content of fatty acids. At this stage no explanation is apparent, particularly since continuance on low intake (LL) did not lead to a marked difference from the HH intake.

The trend in total $\omega$6 acid to show decreased values on decreased intakes is what would normally be expected. During the early feeding period such changes must be in fatty acid storage in the ewe with an indirect effect on the foetus. The feeding sequences will not necessarily produce similar levels of $\omega$6 and $\omega$3 acids due to different lengths of the feeding sequences, different composition of the feed, particularly grain, and the indirect effects of early feeding.

Table 1. *The effects of undernutrition and twinning on the fatty acid composition (g/kg ester) of phospholipids of ovine foetal muscle*

(Mean values for no. of animals given in parentheses)

| Group | Feeding regimen | 16 | 16:1 | 18 | 18:1 | 18:2ω6 | 18:3ω3 +20:1ω9 | 20:3ω9 | 20:4ω6 | 20:5ω3 | 22:3ω9 | 22:4ω6 | 22:5ω3 | 22:6ω3 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Twins | HH (5) | 136 | 21 | 116 | 472 | 11 | 9 | 66 | 42 | 9 | 11 | 7 | 42 | 22 |
| | LH (7) | 140 | 21 | 123 | 463 | 12 | 9 | 62 | 49 | 10 | 11 | 9 | 38 | 21 |
| | HL (6) | 148 | 14 | 122 | 488 | 11 | 8 | 58 | 39 | 10 | 7 | 7 | 41 | 18 |
| | LL (5) | 128 | 18 | 117 | 469 | 13 | 9 | 70 | 44 | 11 | 11 | 8 | 46 | 13 |
| Singles | HH (5) | 150 | 22 | 111 | 474 | 13 | 8 | 60 | 55 | 10 | 8 | 8 | 48 | 23 |
| Maximum SE of mean differences | | 10 | 3 | 6 | 13 | 3 | 1 | 6 | 4 | 3 | 4 | 2 | 5 | 3 |
| Statistical significance | | | | | | | | | | | | | | |
| Feeding | H− *v.* L− | — | — | — | — | — | — | — | * | — | — | — | — | ** |
| | −H *v.* −L | — | — | — | — | — | — | — | — | — | — | — | — | — |
| Twinning | | — | — | — | — | — | — | — | ** | — | — | — | — | — |

HH, high grazing intake before day 90, 1·75 maintenance after day 90; LH, low grazing intake before day 90, 1·75 maintenance after day 90; HL, high grazing intake before day 90, 1·00 maintenance after day 90; LL, low grazing intake before day 90, 1·00 maintenance after day 90.

* $P < 0.05$, ** $P < 0.01$.

Table 2. *The effects of undernutrition and twinning on the level of total polyunsaturated fatty acids (mmol/kg tissue) derived from linoleic acid (ω6) and linolenic acid (ω3) and on the ratio, 20:3ω9:20:4ω6 in ovine foetal muscle phospholipids*

(Mean values for no. of animals given in parentheses)

| Group | Feeding regimen | Total lamb wt (kg) | Ewe empty body-wt† (kg) | ω6 | ω3 | 20:3ω9:20:4ω6 |
|---|---|---|---|---|---|---|
| Twins | HH (5) | 7·1 | 47·0 | 0·65 | 0·69 | 1·54 |
| | LH (7) | 6·7 | 38·4 | 0·60 | 0·55 | 1·30 |
| | HL (6) | 6·9 | 40·4 | 0·58 | 0·61 | 1·53 |
| | LL (5) | 5·9 | 29·0 | 0·54 | 0·51 | 1·56 |
| Singles | HH (5) | 4·5 | 51·1 | 0·64 | 0·63 | 1·08 |
| Average SE of mean differences | | 0·4 | 4·9 | 0·09 | 0·10 | 0·16 |
| Statistical significance | | | | | | |
| Feeding | H− *v.* L− −H *v.* −L | • | ** | — | — | — |
| Twinning | | ** | — | — | — | ** |

HH, high grazing intake before day 90, 1·75 maintenance after day 90; LH, low grazing intake before day 90, 1·75 maintenance after day 90; HL, high grazing intake before day 90, 1·00 maintenance after day 90; LL, low grazing intake before day 90, 1·00 maintenance after day 90.

* $P < 0.05$, ** $P < 0.01$.

† Ingesta-free, wool-free, conceptus-free basis.

It is apparent that dividing a decreased intake of ω6 acids between twins with a greater combined weight of tissue than a single lamb has led to an increased ratio, 20:3ω9:20:4ω6 in the muscle of twin foetal lambs. While the results once again verify the earlier suggestion that single foetal lambs have ω6 acid levels and the ratio, 20:3ω9:20:4ω6 similar to those indicative of linoleic acid deficiency in the simple-stomached animal (Noble, 1973), there are no apparent symptoms of any deficiency. It seems that the ruminant has a lower requirement for ω6 acids than is thought to be required for simple-stomached animals.

It has been previously pointed out that the deficit in linoleic acid in the single lamb can be made up within a period ranging from a few days as calculated from muscle concentrations and milk intake (Payne, 1978*b*) and 20 d as assessed from whole body analyses and milk intake (Noble *et al.* 1972). Any additional deficit in linoleic acid in underfed twins represents only a portion of the daily intake from milk so it is likely that levels will be restored within a few extra days of lactation.

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