# Lamb vigour is affected by DHA supplementation of ewe diets during late pregnancy

R.M. Pickard, A.P. Beard, C.J. Seal, S.A. Edwards
University of Newcastle upon Tyne, School of Agriculture Food and Rural Development,
King George VI Building, Newcastle upon Tyne NE2 7RU, UK. (r.m.pickard@ncl.ac.uk)

## 1. Introduction

The improvement of neonatal viability by modifying maternal nutrition during gestation is of major importance in many livestock species and has also been the subject of biomedical research. Recent investigations have examined the potential of using long chain omega-3 essential fatty acids (EFAs) in maternal diets during late pregnancy. Long-chain omega-3 fatty acids, particularly docosahexaenoic acid (DHA - 22:6 n-3), are required for many specific structural and metabolic functions in the body and are found in high concentrations in brain tissue (Innis, 2000). DHA may be synthesised from its precursor, α-linolenic acid (LNA) via eicosapentaenoic acid (EPA), and is thought to be selectively transported across the placenta (Haggarty *et al.*, 1997; Dutta-Roy, 2000), yet it remains unclear whether these processes are always adequate to completely meet the needs of the developing neonatal brain. Work in monogastric species has shown that feeding an omega-3 supplement during gestation has improved the viability of the neonate (Rooke *et al.*, 2001). However in ruminants, the requirements for dietary long chain EFAs by the dam are uncertain due to both interconversion and biohydrogenation of dietary fatty acids by the rumen microflora. Previous investigations have predominantly used fish oil as the source of long chain omega-3 EFAs in livestock diets but alternative, more sustainable sources are desirable. To date, the effect of period of inclusion of EFAs in gestation diets has not been thoroughly explored. The period of rapid brain growth in the ovine foetus occurs between 10 and 6 weeks prior to birth (Turley *et al.*, 1996), suggesting that this might be the time when a readily available source of DHA would be most beneficial. Therefore, this study sought to explore the effects of feeding an algal (renewable) source of long-chain EFAs, with a relatively high level of DHA, to sheep at different times during pregnancy. The study was structured to determine whether there was a time window in foetal neural development when DHA supplementation would have pronounced effects on subsequent measures of lamb viability.

## 2. Materials and Methods

### *2.1 Animals and housing*

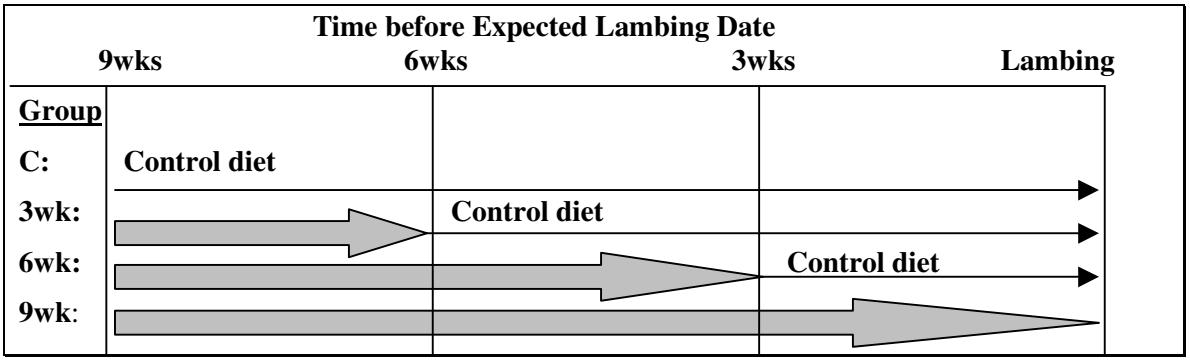
North of England Mule ewes, with known conception date and scanned as carrying twins, were allocated between treatments on the basis of conception date and sire breed. They were housed in an open fronted strawed shed in 4 treatment groups of 12 ewes. At lambing ewes were moved to individual pens, and remained there with their lambs for at least 24h, until lambs were deemed strong enough for the ewe and lambs to be moved into group pens.

### *2.2 Experimental Design and Treatments*

The 48 ewes were allocated between 4 treatments, balanced for weight and condition score. Ewes were fed either a control diet based on silage and a commercial ewe concentrate feed, or a similar diet containing algal biomass (spray-dried product from *Crypthecodinium cohnii*) to provide 12g DHA/ewe/day. Ewes were given 7 days to acclimatise to basal forage and concentrate diet before being introduced to the experimental supplements. The 4 treatment

groups were (Fig 1): ewes fed solely on the control diet for 9 weeks prior to lambing (C); ewes fed the DHA diet for the first 3 weeks of the trial (3wk) then returned to control diet; ewes fed the DHA diet for the first 6 weeks of the trial (6wk); and then returned to the control diet, and those receiving DHA diet for 9 weeks up to parturition (9wk). After lambing all ewes received the standard concentrate.

***Figure 1: Diagrammatic representation of treatment groups, showing periods of omega-3 supplementation.*** ⇨ = DHA supplement ⟶ = Control supplement




***2.3 Diets***

Ewes were offerered grass silage *ad libitum* and a daily concentrate supplement was fed according to a scale relating to stage of gestation. Table 1 shows the composition of the ewe concentrate feed, which was mixed on farm. Algal biomass was added to this concentrate for treatment groups, whilst Control diets were supplemented with a source of vegetable oil to equate the lipid content; table 2 shows the major fatty acids present in each supplement.

***Table 1: Composition of the concentrate mix and the Algal Biomass***

| Component | Ewe Concentrate | Algal Biomass |
|---|---|---|
| Ether Extract (%) | 4 | 46 |
| Crude Protein (%) | 20 | 20 |
| Starch and Sugars (%) | 40 | 10 |
| Moisture (%) | 13 | 7 |
| Crude Fibre (%) | 10 | 10 |
| Ash (%) | 5 | 7 |

***Table 2: Major fatty acids of vegetable oil and Algal Biomass***

| Fatty acid | Vegetable Oil (%) | Algal Biomass (%) |
|---|---|---|
| Saturated fats | 0 | 17 |
| Oleic acid (18:1) | 32 | 5 |
| Linoleic acid (18:2) | 34 | 0 |
| α-linolenic acid (18:3) | 33 | 0 |
| DHA | 0 | 19 |

***2.4 Measurements***

Silage feed intake measurements were taken daily during the experimental period by weighing the silage offered, and the amount remaining in the trough prior to the next feed. Samples of silage, ewe-feed and biomass were also taken weekly throughout the trial. Ewe blood samples

were obtained prior to experimental diet inclusion, after experimental diet inclusion and on a 3 weekly basis thereafter. During the expected lambing period ewes were monitored on a 24 hour basis. Lambing assistance was given if necessary, in accordance with good farm practice. The level of assistance required by each ewe at birth was recorded on a scale from 0-3 (no assistance, light, moderate and intensive assistance, respectively). The time of each birth was recorded and a 5 cm sample of umbilical cord was taken and frozen. A colostrum sample was collected from the ewe. Lambs were monitored during the maternal bonding period and the time recorded when each lamb first stood (on all four feet for more than 10 seconds). After standing, lambs were tagged for identification, weighed and blood sampled, then returned to their mother and allowed to suckle naturally. Ewes which had lambed were blood sampled immediately prior to the next concentrate feeding time. At 24 hours after birth, lambs were weighed and blood sampled again. Lamb weights were obtained at 2, 5, and 12 weeks after birth, and again at weaning.

### *2.5 Analytical Procedures*

Fatty acids were extracted from plasma and colostrum samples using a modified version of a published lipid isolation method (Christie, 1982), whereby the fats are purified and split into their component parts using a methyl/toluene solution. After further purification the solvent containing the fatty acids can then be obtained by centrifuging the samples and collecting the supernatant. Fatty acid profiles were obtained using Gas Liquid Chromatography, using a 30m BPX70 capillary column (SGE Europe Ltd. Milton Keynes, UK).

### *2.6 Statistical Analysis*

Treatment means were compared using Analysis of Variance. Level of assistance at birth was included in the general linear model to identify the effects of treatment on lamb latency to stand.

## 3. Results

Ewe silage intakes averaged 3.9 kg of fresh weight per day during the 9 weeks prior to parturition, with no indication of effects of AB inclusion. Gestation length was approximately 3 days longer in ewes fed DHA diet for 9 weeks, compared to C ewes (Table 3). There were no significant differences in lamb birth weight between groups, either with or without the inclusion of gestation length as a covariate. Latency to stand was reduced in lambs from ewes receiving prolonged DHA supplementation. There was an overall trend for lambs born from ewes with longer gestation lengths to stand more quickly, however this was not significant (P=0.08). Lamb growth rates to 5 weeks of age did not differ significantly between treatments.

***Table 3: Effects of the different feeding treatments on gestation length and measures of lamb viability***

| | **C** | **3wk** | **6wk** | **9wk** | **SEM** | **Sig** |
|---|---|---|---|---|---|---|
| Gestation Length (d) | 145.2 | 147.1 | 147.5 | 148.0 | 0.8 | 0.08 |
| Birth wt (kg) | 5.2 | 5.4 | 5.2 | 5.3 | 0.19 | NS |
| Time to stand (min) ‡ | 31.0 | 27.9 | 21.6 | 22.6 | 2.8 | <0.05 |
| Wt gain in first 24h (kg) | 0.26 | 0.21 | 0.09 | 0.29 | 0.08 | NS |
| DLWG in first 5 weeks | 0.43 | 0.47 | 0.45 | 0.46 | 0.02 | NS |

‡ with adjustment for level of assistance (P<0.005)

There were no differences in ewe plasma fatty acid composition between treatment groups, prior to feeding the experimental supplements. The level of DHA in plasma collected from ewes at lambing was elevated in proportion to the length of time that the DHA supplement had been fed (Table 4). In accordance with plasma fatty acid levels, DHA and EPA concentrations in colostrum also showed graded increases in ewes fed DHA. Lambs born to ewes from the 9wk group had elevated levels of DHA and EPA in their plasma at birth (Table 5), but this was not as pronounced as the increase measured in plasma collected from the ewes themselves.

***Table 4: Effects of treatment on fatty acid composition of ewe blood plasma and colostrum***

| | | C | 3wk | 6wk | 9wk | SEM | Sig |
|---|---|---|---|---|---|---|---|
| **Plasma at birth (g/kg)** | LA (18:2) | 1.04 | 0.73 | 0.80 | 0.53 | 0.120 | <0.05 |
| | LNA (18:3) | 0.16 | 0.17 | 0.16 | 0.16 | 0.025 | NS |
| | AA (20:4) | 0.20 | 0.12 | 0.11 | 0.05 | 0.019 | <0.001 |
| | EPA (22:5) | 0.13 | 0.13 | 0.15 | 0.22 | 0.025 | <0.05 |
| | DHA (22:6) | 0.22 | 0.38 | 0.50 | 0.74 | 0.086 | <0.001 |
| **Colostrum (g/kg)** | LA (18:2) | 0.38 | 0.49 | 0.37 | 0.38 | 0.069 | NS |
| | LNA (18:3) | 0.27 | 0.34 | 0.32 | 0.29 | 0.057 | NS |
| | AA (20:4) | 0.06 | 0.04 | 0.03 | 0.03 | 0.008 | NS |
| | EPA (22:5) | 0.03 | 0.05 | 0.09 | 0.12 | 0.017 | <0.005 |
| | DHA (22:6) | 0.07 | 0.22 | 0.29 | 0.77 | 0.060 | <0.001 |

LA: Linoleic Acid (n-6), LNA: α-linolenic Acid (n-3), AA: Arachidonic Acid (n-6), EPA: Eicosapentaenoic Acid (n-3), DHA: Docosahexaenoic Acid (n-3).

***Table 5: Effects of treatment on lamb blood plasma fatty acid composition before suckling***

| | | C | 3wk | 6wk | 9wk | SEM | Sig |
|---|---|---|---|---|---|---|---|
| **Pre-suckling plasma fatty acid (g/kg)** | LA (18:2) | 0.02 | 0.02 | 0.03 | 0.04 | 0.007 | NS |
| | LNA (18:3) | 0.03 | 0.03 | 0.03 | 0.03 | 0.007 | NS |
| | AA (20:4) | 0.07 | 0.05 | 0.05 | 0.06 | 0.006 | NS |
| | EPA (22:5) | 0.01 | 0.02 | 0.03 | 0.06 | 0.005 | <0.01 |
| | DHA (22:6) | 0.20 | 0.22 | 0.23 | 0.49 | 0.061 | <0.005 |

## 4. Discussion

The present experiment examined the effects of feeding DHA from an algal source to gestating ewes, on measures of lamb viability. Although the efficiency of DHA passage through the rumen remains unclear, the effects of dietary treatment on extending gestation length, and elevating ewe plasma and colostrum EPA and DHA levels, confirm that significant amounts of omega-3 EFAs from the algal biomass were available to the maternal tissues. Cooper *et al.* (2002) looked at the effects of feeding a 50:50 mix of DHA-rich algae and fish oil to wether lambs, and found plasma DHA and EPA concentrations were increased to a greater extent than by feeding fish oil alone, but did not give quantitative data on DHA intake or transfer. Levels of EPA and DHA in sheep colostrum (Capper *et al.*, 2003) and milk (Kitessa *et al.*, 2003) have also been significantly increased by supplementing the diet with fish oil, and in one previous study by feeding algal biomass for a three week period before lambing (Pickard *et al.*, 2004). In previous work, DHA supplementation from fish oil has reduced milk yield and lamb growth rate

(Capper *et al.,* 2003). However, this was not the case in the present study, where lipid supplementation ceased at the time of parturition rather than extending into lactation.

Ewe plasma linoleic acid (LA) levels at birth were significantly lower for ewes from the 9wk treatment group, which is thought to be attributable to the provision of approximately 17g/ewe/day LA via the vegetable oil compared to 0g/ewe/day via the algal biomass. The elevated level of LA in the groups receiving vegetable oil (C, 3wk and 6wk) was associated with significantly higher arachidonic acid (AA) levels in the ewes. This concurred with work by Elmes *et al.* (2004) who fed maternal diets differing in LA (3g/ewe/day vs 0.16g) and significantly increased ewe and lamb plasma AA at the end of gestation with the higher inclusion. However in contrast to Elmes *et al.* (2004) this had no impact on AA levels in lamb plasma at birth, which agrees with the findings from Rooke *et al.* (1999) that showed maternal plasma n-6 fatty acids to be poorly correlated with foetal umbilical cord blood plasma in newborn pigs.

Extension of gestation length by feeding long-chain polyunsaturated fatty acids, particularly DHA, has been found in previous studies in sheep (Capper *et al.*, 2003; Pickard *et al.*, 2004) and other species, and results from the inhibition of eicosanoid synthesis from AA (20:4 n-6). Ewes fed algal biomass for 9 and 6 weeks gave birth to lambs that stood more quickly after birth than those born to ewes in the 3wk and C groups. A similar effect was suggested, but not statistically demonstrated, by the results of Capper *et al.* (2003), who fed a fish oil supplemented diet for 6 weeks prior to gestation. The mechanism responsible for this effect requires elucidation. Whilst fatty acid composition of neonatal tissues such as brain were not determined in this experiment, a difference in lamb plasma DHA levels at the time of birth indicates that transplacental transfer had taken place, although the magnitude of treatment difference was less than that observed in maternal plasma. Latency to stand was not significantly decreased by the 3wk treatment in this experiment, or by supplementation for the 3 weeks immediately prior to lambing in an earlier experiment (Pickard *et al*., 2004), suggesting that this limited period of DHA supplementation was inadequate, even when focussed on a likely developmental window.

## 5. Conclusion

Dietary supplementation of ewe diets during late gestation with a source of DHA can improve lamb vigour, as reflected by a reduced time taken by lambs to stand after birth. There appears to be a threshold level of supplementation for this effect and the mechanism by which EFA supplementation influences vitality requires further investigation.

## Acknowledgements

We thank the staff at Cockle Park Farm and the CEGS laboratory for technical assistance, and ABN (Europe) Ltd and the Yorkshire Agricultural Society for financial support.

## References

Capper JL, Wilkinson RG, Sinclair LA, Pattinson SE & Mackenzie AM (2003) The effect of long-chain polyunsaturated fatty acid and vitamin E supplementation of ewes on neonatal lamb vigour, lamb growth and colostrum parameters. *Proceedings of the British Society of Animal Science*, 7.

Christie WW (1982) *Lipid Analysis*, 2nd ed. Oxford: Pergamon Press.

Cooper SL, Sinclair LA, Wilkinson RG, Chikunya S, Hallett K, Enser M & Wood JD (2002) Rumen biohydrogenation of polyunsaturated fatty acid sources and their effect on plasma fatty acid status in sheep. *British Society of Animal Science*, 177.

Dutta-Roy AK (2000) Transport mechanisms for long-chain polyunsaturated faty acids in the human placenta. *Am J Clinical Nutrition* **71**, 315S-322S.

Elmes M, Tew P, Cheng Z, Kirkup SE, Abayasekara DRE, Calder PC, Hanson MA, Wathes DC & Burdge GC (2004) The effect of dietary supplementation with linoleic acid to late gestation ewes on the fatty acid composition of maternal and fetal plasma and tissues and the synthetic capacity of the placenta for 2-series prostaglandins. *Biochimica et Biophysica Acta (BBA) - Molecular and Cell Biology of Lipids* **1686**, 139-147.

Haggarty P, Page K, Abramovich DR, Ashton J & Brown D (1997) Long-chain polyunsaturated fatty acid transport across the perfused human placenta. *Placenta* **18**, 635-642.

Innis SM (2000) The role of dietary n-6 and n-3 fatty acids in the developing brain. *Developmental Neuroscience* **22**, 474-480.

Kitessa SM, Peake D, Bencini R & Williams AJ (2003) Fish oil metabolism in ruminants: III. Transfer of n-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. *Animal Feed Science and Technology* **108**, 1-14.

Pickard RM, Beard AP, Cain PJ, Madgwick S, Hawken PAR & Edwards SA (2004) Supplementation of ewe gestation diets with algal DHA and effects on neonatal lamb viability. *Proceedings of the 3rd Euro Fed Lipid Congress and Expo*, 312.

Rooke JA, Bland IM & Edwards A (1999) Relationships between fatty acid status of sow plasma and that of umbilical cord, and tissues of newborn piglets when sows were fed on diets containing tuna oil or soyabean oil in late pregnancy. *British Journal of Nutrition* **82**, 213-221.

Rooke JA, Sinclair AG & Edwards SA (2001) Feeding tuna oil to the sow at different times during pregnancy has different effects on piglet long-chain polyunsaturated fatty acid composition at birth and subsequent growth. *British Journal of Nutrition* **86**, 21-30.

Turley S, Burns D, Rosenfeld C & Dietschy J (1996) Brain does not utilize low density lipoprotein-cholesterol during fetal and neonatal development in the sheep. *J. Lipid Res.* **37**, 1953-1961.