

THE INFLUENCE OF EARLY EVENTS IN UTERO ON POSTNATAL MUSCLE GROWTH: A REVIEW

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SUMMARY

Animals with more muscle fibres produce more lean meat at slaughter. High muscle fibre number is also correlated with faster and more efficient growth. Understanding the prenatal determination of muscle fibre number will therefore elucidate how postnatal growth potential can be influenced *in utero*. During prenatal muscle development, there is an initial wave of primary myofibre formation. A second wave of muscle fibres, secondary myofibres, then form around the primaries. Primary myofibre numbers are genetically determined and are usually not affected by undernutrition of the mother. However, work in our laboratory on the guinea pig has demonstrated that undernutrition during early pregnancy affects the formation of secondary myofibres in muscles of the fetuses. Growth of the placenta and circulating levels of insulin-like growth factors (IGFs) in both the mother and fetus are implicated in these effects. Supplementary feeding to pregnant sows in early gestation increases the number of secondary myofibres which form in the fetuses. The range of muscle fibre numbers seen within a litter at birth is also reduced by this supplementary feeding - piglets exhibiting very low fibre numbers are reduced. It is suggested that postnatal growth potential can therefore be influenced by prenatal nutritional manipulations.

INTRODUCTION

Muscle mass is determined by muscle fibre number and muscle fibre size. Various factors, including exercise and nutrition can affect the size and types of fibres in postnatal animals, but fibre number is unaffected (Stickland *et al.* 1975; Goldspink and Ward, 1979). In the pig muscle fibre hyperplasia is completed during gestation and is fixed from the time of birth (Staun, 1963; Stickland and Goldspink, 1973). However, fibre number can be affected by conditions *in utero* including maternal nutrition (Wigmore and Stickland, 1983 and innervation (McLennan, 1983). As muscle fibre number is probably the most important determinant of muscle mass (Miller *et al.* 1975) it is evident that prenatal conditions which affect fibre number determinations may have a significant effect on the meat potential of an animal. Although selection for muscle fibre size in the pig can also correlate with an increase in muscle size after two generations (Fiedler, 1988) there is a corresponding decrease in meat quality. Ashmore (1974) showed that the correlation between large fibre size and poor meat quality appeared to be due to a relative increase in fast glycolytic fibres. It would seem, on balance, that high muscle fibre number (which correlates with smaller fibres) is the most relevant parameter in relation to improved muscle growth and quality. This review highlights the importance of muscle fibre number and also explains how this parameter may be affected *in utero* and the consequences of this on postnatal growth. The review will focus on the pig but some reference will be made to work on laboratory animals where appropriate.

MUSCLE FIBRE NUMBER AND MEAT PRODUCTION

Fast-growing strains of pigs and other animals have more muscle fibres than slower-growing strains (Ezekwe and Martin, 1975; Miller *et al.* 1975). This was also found to be true when Dwyer *et al.* (1993) considered pigs within the same strain. Sixty-six Large White x Landrace pigs from seven litters were reared under similar commercial conditions and weighed at monthly intervals from birth to slaughter (at about 80kg). At slaughter the semitendinosus muscle was removed from each pig and the total number of muscle fibres was estimated. It was found that average daily gain (ADG) was significantly correlated with birth weight but not with fibre number for the initial growth period up to 25kg body weight. However, for growth from 25 to 80kg only fibre number was correlated with ADG. Furthermore, during this period, there was also a significant correlation between fibre number and gain/feed ratio (Fig.1). A relationship between birth weight and growth rate to weaning has been shown in other studies (Campbell and Dunkin, 1982).

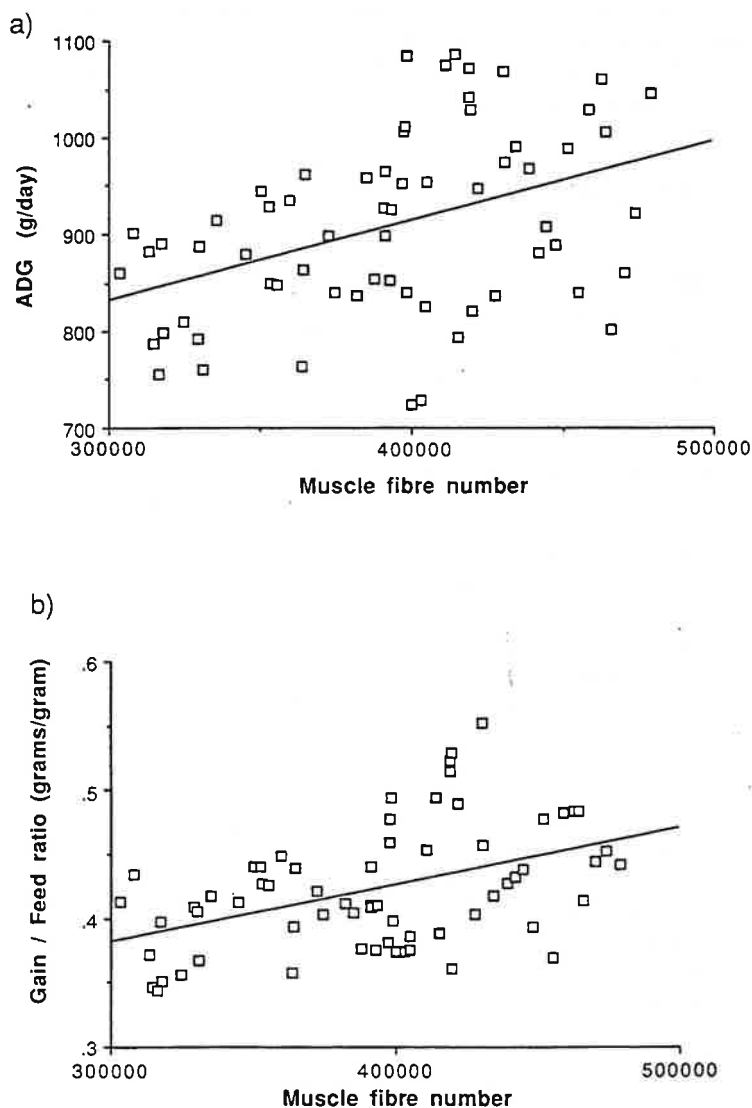


Fig. 1(a) Average daily gain (ADG) from 25 to 80kg liveweight against total fibre number in the semitendinosus muscle ($r = 0.415$, $P < 0.001$); (b) Gain/feed ratio from 25 to 80kg liveweight against total semitendinosus fibre number ($r = 0.419$, $P < 0.001$).

It is probable that small pigs compete less effectively for nutrition. Growth rates can, in fact, be improved by separating piglets into similar weight groups (England, 1974) which suggests that the correlation between birth weight and early growth may be related to feed intake. The later relationship after 25kg between fibre number (not birth weight) and growth rate supports the notion that growth at this stage is more determined by the pig's genotype (Blunn *et al.* 1953). It is known that pigs selected for fast growth are more efficient in feed conversion and contain less fat than unselected pigs (Campbell and Taverner, 1988). Pigs with more muscle fibres also exhibit less fat (Stickland and Goldspink, 1975) and this is consistent with more efficient growth. At equivalent live weights pigs with more fibres also have thinner fibres (Fiedler, 1988); delivery of nutrients to thinner fibres may be more efficient due to smaller diffusion distances. Taken overall, it seems therefore that pigs with high fibre number grow faster and more efficiently than those with a low fibre number.

In order to understand the factors which might affect fibre number determination it is necessary to understand the process of myogenesis in terms of primary and secondary fibre formation.

MYOGENESIS - PRIMARY AND SECONDARY MYOFIBRES

Muscle fibres develop prenatally as two distinct populations. Fibres which form during the initial stages of myoblast fusion are primary myofibres which provide a framework for the formation of the larger population of smaller secondary myofibres. During their early formation secondary fibres have very close mechanical contact with primary fibres. In most porcine muscles the two populations can be distinguished by adenosine triphosphatase activity with primaries reacting as slow fibres and secondaries as fast (Fig.2). In the pig, only primary fibres are present at 38 days' gestation and their number gradually increases to a maximum at about 60 days. Secondary myofibre formation occurs rapidly from about 54 to 90 days' gestation. In the semitendinosus muscle up to 20 secondaries form around each primary myofibre (Wigmore and Stickland, 1983).

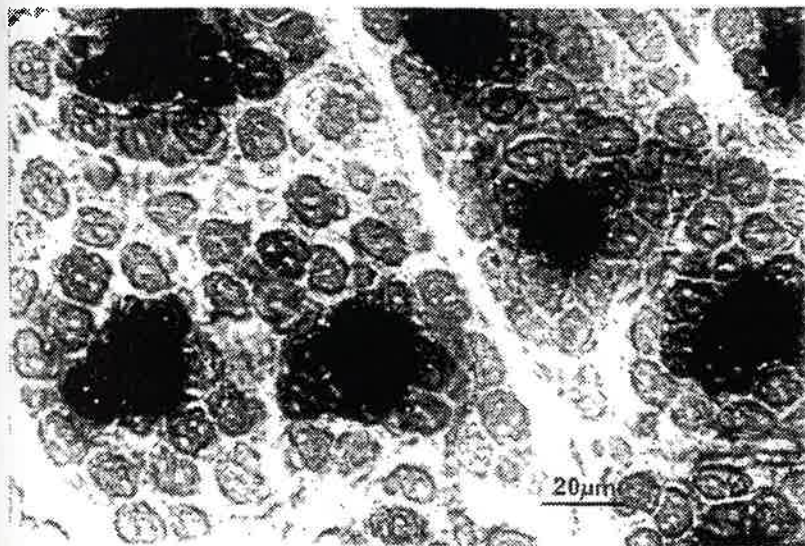


Fig 2. Transverse section of porcine semitendinosus muscle reacted for acid-stable adenosine triphosphatase activity. The large positively reacted fibres (slow) are the primary myofibres and the remainder are secondary myofibres.

During later prenatal development and also during postnatal growth some of the secondaries become slow fibres so that clusters of slow fibres (one of which was a primary myofibre) are surrounded by fast fibres. This arrangement of fibre bundles persists in the postnatal pig so that, even in mature pigs, it is possible to determine the numbers of primaries and secondaries which contributed to the development of a given muscle.

SOURCES OF VARIATION IN MUSCLE FIBRE NUMBER

In a survey of 48 Large White pigs from five litters (Dwyer and Stickland, 1991) it was found that primary fibre number in *m.semitendinosus* was responsible for the variation in total muscle fibre number between litters. Within litters, both primary number and secondary:primary ratio contributed to differences in fibre number. However, when only the largest and smallest extremes of the litters were compared, variation in fibre number was due only to secondary:primary ratio differences; this confirms the results of Handel and Stickland (1987). The smallest pigs in a newborn litter are almost certainly a consequence of undernutrition *in utero*. This is highlighted by the U-shaped distribution of foetal weights in each uterine horn which is believed to be a consequence of differing nutrition (McLaren and Mitchie, 1960; Perry and Rowell, 1969). The development of muscle fibre number was studied in largest and smallest (excluding the runt) littermates during gestation by Wigmore and Stickland (1983). No difference was found in the number of primary fibres but a difference was observed in the secondary fibre population from about 65 days onwards which produced a difference of 17% in the numbers by birth (Fig.3). At the time of rapid secondary fibre hyperplasia primary fibres in the smaller fetuses were smaller in diameter. It was speculated that the small size may restrict the available surface area for secondary fibre formation. However, a difference in myoblast proliferation must also be a contributory factor as the muscles exhibit a difference in DNA content (Wigmore and Stickland, 1984).

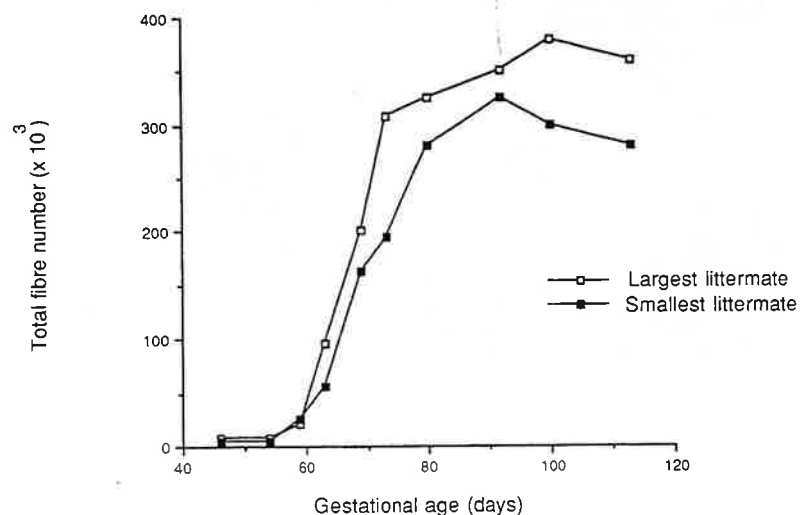


Fig 3. Total number of muscle fibres in the semitendinosus muscle for the largest and smallest littermates against gestational age.

Taken as a whole, the results suggest that primary fibre number is a relatively more fixed genetic component than secondary fibre number and is therefore more indicative of the genotype of an animal. This is confirmed in a study by Stickland and Handel (1986) which showed that primary fibre number was the major contributor to the muscle fibre number difference seen between large and small strains of pigs. Secondary fibre number is more vulnerable to environmental factors *in utero*, including nutrition. This is also confirmed by various experiments including those by Ward and Stickland (1991) which showed that undernutrition of pregnant guinea pigs produced a deficit in secondary fibre numbers of the offspring but no affect on primary numbers.

THE EARLY CRITICAL PERIOD IN GESTATION

In a recent experiment by Dwyer *et al.* (1993) it was shown that a 40% nutritional restriction to pregnant guinea pigs from conception to 25 days gestation (and then *ad lib* to term) produced the same 20-28% deficit in muscle fibre number in the neonates as restriction throughout gestation (full term in the guinea pig is about 70 days) (Fig.4).

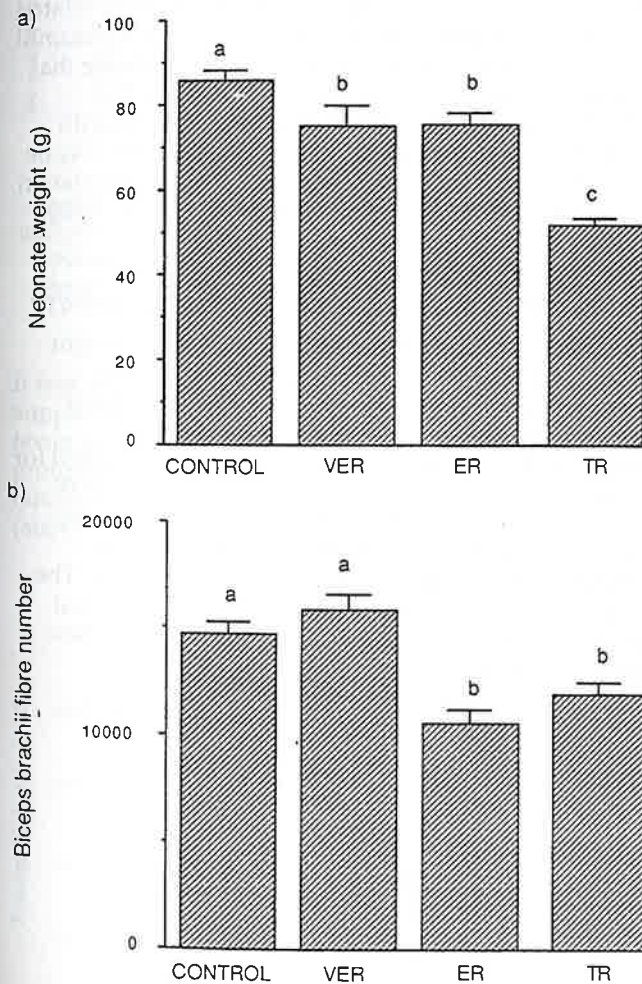


Fig 4.(a) Neonatal body weight and (b) Fibre number in biceps brachii at birth for the progeny of guinea pigs fed either a restricted diet up to 15 days gestation and then *ad lib* (VER), a restricted diet up to 25 days and then *ad lib* (ER), a restricted diet throughout gestation (TR), or *ad lib* throughout (control). Means and standard errors are given. Significant differences ($P < 0.05$) are indicated by differing letters.

Restriction up to 15 days only was not effective in this respect. It would appear therefore that inadequate nutrition at critical periods in early gestation may have a significant effect on the development of muscle fibres which takes place later in gestation (in the guinea pig the maximum rate of secondary myofibre formation occurs between days 35 to 45). From the work already mentioned this deficit in muscle fibres may have a significant effect on growth rate and on growth efficiency. It has been shown that restriction of maternal intake in pregnant sows leads to a reduction in ADG of the progeny from ten weeks postnatal onwards (Pond *et al.* 1985; Pond and Mersmann, 1988). Under conditions of natural undernutrition within a litter runt pigs grow slower and less efficiently than their larger littermates (Powell and Aberle, 1980). The smaller pigs in a litter tend to have fewer muscle fibres (Handel and Stickland, 1987) but interestingly small birth weight pigs with a relatively high fibre number are capable of exhibiting catch-up growth (Handel and Stickland, 1988).

The mechanism whereby undernutrition affects fibre number determination has been investigated in the guinea pig. In pregnant guinea pigs restricted by 40% (of an *ad lib* diet) throughout gestation the peripheral labyrinth (the exchange area) of the placenta is reduced by 33% and correlates with fetal weight towards term (Dwyer *et al.* 1992). Undernutrition reduces maternal and fetal serum levels of IGF-1 as well as fetal levels of IGF-2. Cortisol appears to be inversely related to IGF-1 levels but thyroid hormones do not seem to be important until at least late gestation (Dwyer and Stickland, 1992). It is probable that restriction only in early gestation has a permanent effect on the development of the placenta thereby affecting fetal IGF levels with consequent effects on the developing muscle tissue. The IGFs may be important regulators of muscle fibre number as they have been shown to stimulate myoblast proliferation *in vitro* (Ewton and Florini, 1980).

STIMULATION OF MYOGENESIS AND EFFECTS ON POSTNATAL GROWTH

From the foregoing discussions it would appear that lighter weight pigs contain fewer muscle fibres due to undernutrition *in utero*. An experiment was carried out (Dwyer *et al.* 1994) with the aim of increasing the number of fibres in the lighter pigs and thereby increasing postnatal growth rate. Maternal feed intake was doubled for one of three different periods during pregnancy: 25 to 50 days (HE group), 50 to 80 days (HL group) or 25 to 80 days (HT group). The offspring were slaughtered at 5 weeks and their semitendinosus muscles sectioned and analysed. Results are shown in Figure 5. The variability of primary fibre number between the groups meant that total fibre numbers were not significantly different. However, the progeny of all supplemented sows contained a greater mean secondary:primary fibre ratio than control pigs.

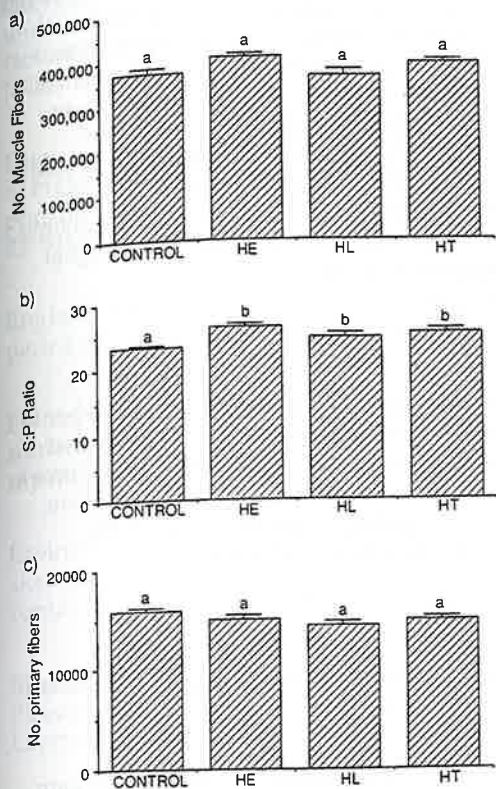


Fig 5.(a) Total muscle fibre number, (b) mean secondary:primary fibre ratio and (c) total primary fibre number in semitendinous muscle of progeny from sows fed either a supplemented diet from 25 to 50 days gestation (HE), 50 to 80 days (HL), 25 to 80 days (HT) or no supplementation (control). Means and standard errors are given. Significant differences ($P < 0.05$) are indicated by differing letters.

It was also evident that the distribution of fibre numbers in pigs from supplemented groups was smaller than controls with significantly fewer pigs with low fibre numbers. A number of pigs from the HT group were grown on to 80kg. These pigs had a faster growth rate (about 10%) from day 70 to slaughter and an increased gain:feed ratio (about 8%) compared with controls (Fig. 6).

Growth of 6 month pigs

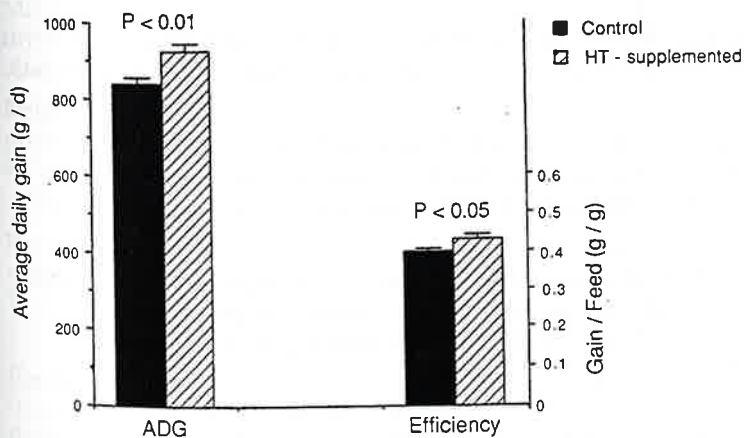


Fig 6. Average daily gain (ADG) from 70 to 130 days and gain/feed ratio for progeny of nutritionally supplemented sows (HT) compared with controls. Means and standard errors are given. Significances of differences are indicated.

The vulnerability of the early stages of gestation to fibre number determination is also shown in a recent experiment by Rehfeldt *et al.* (1993). Administration of porcine somatotropin to pregnant sows during days 10 to 24 resulted in 27% more fibres in the semitendinosus muscles of the progeny at birth. This increase was due solely to an increase in the secondary:primary fibre ratio (Rehfeldt, personal communication). Also of interest is the fact that the hormone treated sows produced piglets with significantly increased levels of serum IGF-1 at birth. This may indicate faster postnatal growth as Buonomo *et al.* (1987) found that fast-growing strains of pigs contained higher serum IGF-1 levels than slow-growing pigs.

CONCLUSIONS

This review has highlighted the importance of muscle fibre number to postnatal growth rate and feed conversion efficiency. It is clear that levels of maternal nutrition, particularly in early gestation, may have a very significant effect on muscle fibre number development *in utero*. Sows given extra feed during early gestation produce pigs with improved growth rate and greater feed conversion efficiency.

References

- Ashmore, C.R. (1974). Phenotypic expression of muscle fibre types and some implications to meat quality. *J. Anim. Sci.*, 38: 1158-1164.
- Blunn, C.T., Baker, G.N. and Hanson, L.E. (1953). Heritability of gain in different growth periods in swine. *J. Anim. Sci.*, 12: 39-50.
- Buonomo, F.C., Lanterio, T.J., Baile, C.A. and Campion, D.R. (1987). Determination of insulin-like growth factor-1 (IGF-1) and IGF binding protein levels in swine. *Domest. Anim. Endocrinol.*, 4: 23-31.
- Campbell, R.G. and Dunkin, A.C. (1982). The effects of birth weight and level of feeding in early life on growth and development of muscle. *Anim. Prod.*, 35: 185-192.
- Campbell, R.G. and Taverner, M.R. (1988). Genotype and sex effects on the relationship between energy intake and protein deposition in growing pigs. *J. Anim. Sci.*, 66: 676-686.
- Dwyer, C.M., Fletcher, J.M. and Stickland, N.C. (1993). Muscle cellularity and postnatal growth in the pig. *J. Anim. Sci.*, 71: 3339-3343.
- Dwyer, C.M., Madgwick, A.J.A., Crook, A.R. and Stickland, N.C. (1992). The effect of maternal undernutrition on the growth and development of the guinea pig placenta. *J. Dev. Physiol.*, 18: 295-302.
- Dwyer, C.M., Madgwick, A.J.A., Ward, S.S. and Stickland, N.C. (1993). The effect of maternal undernutrition in early gestation on the development of fetal myofibres in the guinea pig. *J. Dev. Physiol.*, 20: 111-117.
- Dwyer, C.M. and Stickland, N.C. (1991). Sources of variation in myofibre number within and between litters of pigs. *Anim. Prod.*, 52: 527-533.

- Dwyer, C.M. and Stickland, N.C. (1992). The effects of maternal undernutrition on maternal and fetal serum insulin-like growth factors, thyroid hormones and cortisol in the guinea pig. *J. Dev. Physiol.*, 18: 303-313.
- Dwyer, C.M., Stickland, N.C. and Fletcher, J.M. (1994). The influence of maternal nutrition on muscle fiber number development in the porcine fetus and on subsequent postnatal growth. *J. Anim. Sci.*, 72: 911-917.
- England, D.C. (1974). Husbandry components in prenatal and perinatal development in swine. *J. Anim. Sci.*, 38: 1045-1049.
- Ewton, D.Z. and Florini, J.R. (1980). Relative effects of the somatomedins, MSA, and growth hormone on myoblasts and myotubes in culture. *Endocrinol.*, 106: 577-583.
- Ezekwe, M.O. and Martin, R.J. (1975). Cellular characteristics of skeletal muscles in selected strains of pigs and mice and the unselected controls. *Growth*, 39: 95-106.
- Fiedler, I. (1988). Leistungsfrüherkerming von Fleischstutz und Fleischbeschaffenheit durch Muskelfasermerkmale. *Tagber. Akad. Landwirtsch. Wiss. Berlin*, 268: 187-196.
- Goldspink, G. and Ward, P. (1979). Changes in rodent fibre types during postnatal growth, undernutrition and exercise. *J. Physiol.*, 296: 453-469.
- Handel, S.E. and Stickland, N.C. (1987). Muscle cellularity and birth weight. *Anim. Prod.*, 44: 311-317.
- Handel, S.E. and Stickland, N.C. (1988). Catch-up growth in pigs: a relationship with muscle cellularity. *Anim. Prod.*, 47: 291-295.
- McLaren, A. and Mitchie, D. (1960). Control of prenatal growth in mammals. *Nature*, 187: 363-365.
- McLennan, I.S. (1983). Neural dependence and independence of myotube production in chick hindlimb muscles. *Dev. Biol.*, 98: 287-294.
- Miller, L.R., Garwood, V.A. and Judge, M.D. (1975). Factors affecting porcine muscle fiber type, diameter and number. *J. Anim. Sci.*, 41: 66-77.
- Perry, J.S. and Rowell, J.G. (1969). Variations in foetal weight and vascular supply along the uterine horn of the pig. *J. Rep. Fertil.*, 19: 527-534.
- Pond, W.G. and Mersmann, H.J. (1988). Comparative responses of lean or genetically obese swine and their progeny to severe feed restriction during gestation. *J. Nutr.*, 118: 1223-1234.

- Pond, W.G., Mersmann, H.J. and Yen, J-T. (1985). Severe feed restriction of pregnant swine and rats: Effect on postweaning growth and body composition of progeny. *J. Nutr.*, 115: 179-189.
- Powell, S.E. and Aberle, E.D. (1980). Effects of birth weight on growth and carcass composition of swine. *J. Anim. Sci.*, 50: 860-868.
- Rehfeldt, Ch., Fiedler, I., Weikard, R., Kanitz, E. and Ender, K. (1993). It is possible to increase skeletal muscle fibre number in utero. *Biosci. Rep.*, 13: 213-220.
- Staun, H. (1993). Various factors affecting number and size of muscle fibres in the pig. *Acta Agric. Scand.*, 13: 293-322.
- Stickland, N.C. and Goldspink, G. (1973). A possible indicator muscle for the fibre content and growth characteristics of porcine muscle. *Anim. Prod.*, 16: 135-146.
- Stickland, N.C. and Goldspink, G. (1975). A note on porcine skeletal muscle parameters and their possible use in early progeny testing. *Anim. Prod.*, 21: 93-96.
- Stickland, N.C. and Handel, S.E. (1986). The numbers and types of muscle fibres in large and small breeds of pigs. *J. Anat.*, 147: 181-189.
- Stickland, N.C., Widdowson, E.M. and Goldspink, G. (1975). Effects of severe energy and protein deficiencies on the fibres and nuclei in skeletal muscles of pigs. *Brit. J. Nutr.*, 34: 421-428.
- Ward, S.S. and Stickland, N.C. (1991). Why are slow and fast muscles differentially affected during prenatal undernutrition? *Muscle and Nerve*, 14: 259-267.
- Wigmore, P.M.C. and Stickland, N.C.A. (1983). Muscle development in large and small pig fetuses. *J. Anat.*, 137: 235-245.
- Wigmore, P.M.C. and Stickland, N.C. (1984). DNA, RNA and protein in skeletal muscle of large and small pig fetuses. *Growth*, 47: 67-76.