
THE PROCEEDINGS OF THE
NEW ZEALAND SOCIETY
OF
ANIMAL PRODUCTION
VOLUME 62 2002

# Characterisation of muscle development in foetuses with and without presumptive double-muscled phenotypes

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## ABSTRACT

Double-muscled (DM) cattle have increased numbers of muscle fibres and most muscles are heavier, although certain muscles are reduced in weight relative to non-double-muscled animals. This study was undertaken to investigate whether this differential muscle growth seen in the adult was apparent during foetal growth.

Foetuses with presumptive DM phenotype were compared to presumptive non-double-muscled controls (NDM), at four gestational ages. Body weight (p-0.001), but not crown rump length was greater in DM foetuses. Both the M. *vastus lateralis* and the M. *vastus medialis* were heavier in the DM (p-0.001). In DM there were fewer type 1 fibres per fascicle in both muscles (p-0.001), and M. *vastus lateralis*, at 210 and 260 days, had smaller type 1 fibre areas (p-0.001).

In conclusion, our data demonstrate differences in muscle development between DM and NDM foetuses, and suggest that the relatively reduced size of M. *vastus medialis* in the DM adult, is attained postnatally.

**Keywords:** double-muscled; cattle; development; fibre area; ATPase.

## INTRODUCTION

Within double-muscled (DM) cattle breeds, all muscles have increased numbers of muscle fibres (Ashmore *et al.,* 1974). This is associated with a generalised increase in muscle size, and a corresponding improvement in meat yield. The hereditary nature of the DM phenotype is well established, but the method of inheritance of the single gene causing the condition has not been agreed upon. The *mh* gene has been mapped to a position on bovine chromosome 2 (Charlier *et al.,* 1995), but as yet has not been cloned.

The extent of the muscular hypertrophy exhibited by DM animals has been shown to be variable between different muscles in a number of DM breeds (Butterfield, 1966, Rollins *et al.*, 1969, Boccard and Dumont, 1974). Muscles with a large surface area tend to be the most enlarged while deeper muscles tend to be reduced in size relative to NDM. As these muscles all have increased muscle fibre numbers, it follows that the reduced muscles have smaller fibre size (Boccard, 1981).

Histochemical fibre typing using myosin ATPase activity allows for the classification of muscle fibre types according to the myosin isoforms which are present. Different myosin isoforms have different contraction speeds and the myosin ATPase reaction allows fibres to be classified on this basis, such that slow fibres are classified as type 1 and fast as type 2. DM cattle have a different composition of histochemical fibre types during both foetal development (Ashmore *et al.,* 1974) and in the postnatal animal (Holmes and Ashmore, 1972), and muscle fibre size is altered in DM cattle according to fibre type.

The current study was undertaken to investigate the development of the M. *vastus lateralis* which is 15% heavier in DM than NDM, and M. *vastus medialis* which is 38% lighter in DM than NDM (Boccard and Dumont, 1974). The objectives of this study were: (i) to determine whether differences in postnatal muscle growth were apparent during foetal growth and were associated with differences in fibre size; and (ii) to determine the contribution of fibre type to differential muscle growth.

## MATERIALS AND METHODS

Foetuses with an expected DM phenotype were produced using standard superovulation and embryo transfer techniques. Donor cows which were pure-bred, phenotypically DM Belgian Blue, were inseminated with semen from pure-bred, phenotypically DM Belgian Blue bulls. Embryos were recovered 7 days after insemination, and were transferred to Hereford x Friesian recipient heifers.

Foetuses with an expected NDM phenotype were produced using either artificial insemination of heifers from the Hereford x Friesian recipient herd, or using embryo transfer into recipients from this herd. Embryos were derived from abbatoir sourced ovaries from beef and/or dairy cows of NDM phenotype, using *in-vivo* production techniques (Lu *et al.,* 1990). Semen used to generate presumptive NDM foetuses was from Friesian bulls.

The recipients were grazed in a single mob, and were slaughtered at 120, 160, 210 and 260 days gestation. Five foetuses were produced at each gestational age for both breeds except for the 210 and 260 day Belgian Blues when 3 foetuses were produced. Foetuses were weighed, crown-rump length measured and M. *vastus medialis* and M. *vastus lateralis* dissected. Muscle samples from the midpoint of the muscle were frozen in isopentane cooled in liquid nitrogen.

Identification of type 1 and type 2 muscle fibres was carried out using histochemical myosin ATPase staining, essentially according to the method of Guth and Samaha (1969), with the exception that following fixation, slides were preincubated for 10 minutes at pH 5.0. This gave an identical staining result to the standard alkaline pre-incubation protocol with pre-incubation at pH 9.4 but histological quality was improved, and staining was more intense.

Measurements of muscle fibre areas and optical density of ATPase staining were carried out using semi-automated image analysis software (Image, NIH, Bethesda, Maryland). Data was collected from all fibres within each of five entire muscle fascicles for each animal. Three animals were analysed from each group. Individual fibres were defined as either type 1 or type 2 fibres on the basis of optical density and fibre area measurements.

Statistical analysis was carried out by analysis of variance, using age and phenotype as main effects, and testing for age x phenotype interactions. Muscle weight data is presented as back transformed least squares means following covariate adjustment for sex ratio and foetal weight within groups. All other data are mean values with standard errors of the means (sem).

## RESULTS

Body weight increased significantly with age in both phenotypes (p-0.001), and was higher overall in the DM animals (p-0.001) (Figure 1A). Crown rump length also increased significantly with age in both phenotypes (p-0.001), but there was no difference between NDM and DM animals (Figure 1B). Muscle weights for both M. *vastus medialis* and M. *vastus lateralis* were significantly greater in the DM (p-0.001) (Figure 2).

Measurements of muscle fibre area for type 1 fibres showed no difference between DM and NDM in M. *vastus medialis*, but in M. *vastus lateralis*, fibres were significantly smaller in DM than NDM at 210 and 260 days gestation (p-0.001) (Figure 3, Figure 4A). There was no effect of phenotype on the areas of type 2 fibres in either muscle (Figure 3, Figure 4B).

In all muscles there was a significant effect of age on the number of type 1 fibres per fascicle (p-0.001) with the number declining from 120 to 210 days, but then increasing again at 260 days. Also, there were fewer type 1 fibres per fascicle in the DM animals (p-0.001 for both muscles) (Figure 5). Total number of fibres per fascicle was independent of muscle, phenotype and age.

## DISCUSSION

This study has provided no evidence for a reduction in muscle weight or average fibre area in the DM M. *vastus medialis* relative to NDM, and there may be several explanations for this. One possibility is that underlying genotypic differences are not expressed in the foetal period and are only exhibited postnatally. Alternatively, postural dif-

**FIGURE 1:** (A) Body weights of DM (●) and NDM (○) foetuses at four gestational ages. Values are means ± sem. (B) Crown rump length of DM (●) and NDM (○) foetuses at four gestational ages. Values are means ± sem.

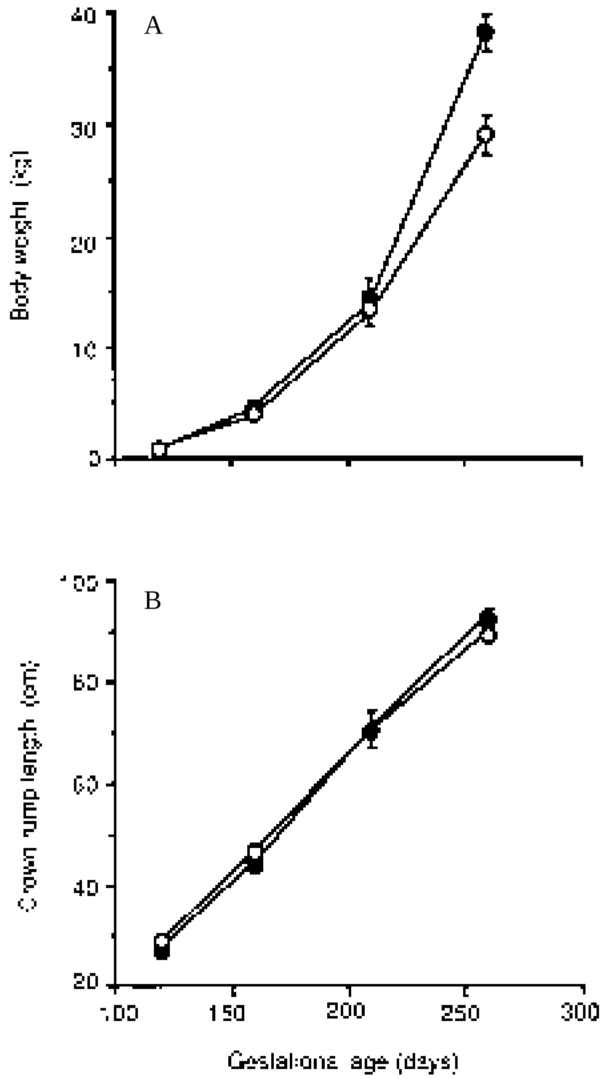



**FIGURE 2:** Weights of M. *vastus lateralis* (□) and M. *vastus medialis* (○) from DM (closed symbols) and NDM (open symbols). Values are least squares means adjusted for body weight and sex ratio within groups ± sem.

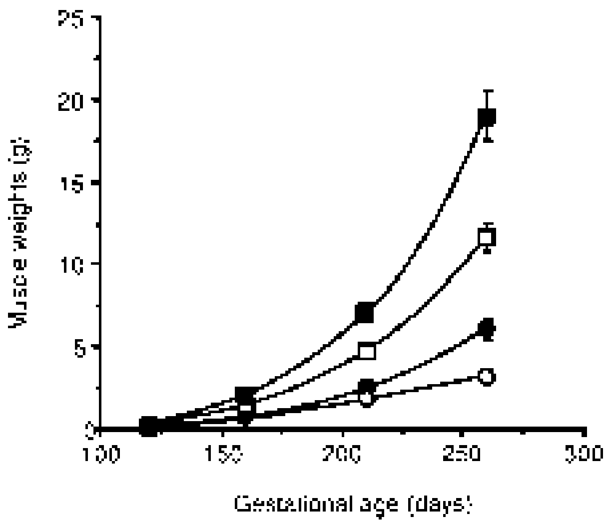
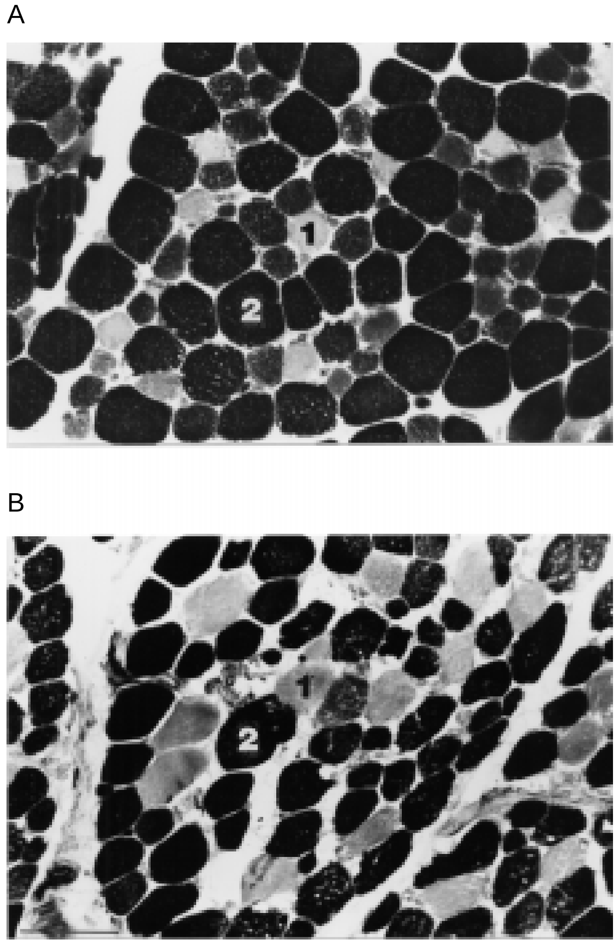


**FIGURE 3:** Cryosection of 260 day (A) DM M. *vastus lateralis* and (B) NDM M. *vastus lateralis* stained for myosin ATPase. 1 = type 1 fibre, 2= type 2 fibre. Bar = 50µm.




ferences may be responsible for the atrophy of the M. *vastus medialis*, as has been described in humans (Speakman and Weisberg, 1977). A further option is that the difference in muscle weights may be the result of altered composition of non-muscle tissues such as connective tissue, but this is not supported by existing literature which indicates less connective tissue in DM phenotypes (Boccard, 1981).

We have shown a decrease in the area of type 1 fibres in DM M. *vastus lateralis* at 210 and 260 days gestation, relative to NDM. This data extends the time periods covered by previous studies in M. *semitendinosus* which showed no difference in the size of type 1 muscle fibres during foetal development up to a crown rump length of 54 cm (approximately 180 days gestation) (Ashmore *et al.*, 1974) or at slaughter at 66 weeks, while in postnatal DM calves from 4 to 26 weeks of age, type 1 fibre size was reduced relative to NDM (Holmes and Ashmore, 1972).

The similarity in the size of type 2 muscle fibres between DM and NDM which we have described is consistent with a previous report for such a comparison during development of foetuses up to approximately 180 days gestation (Ashmore *et al.*,1974).

Muscle fibres derived from primary and secondary myotubes are characterised by the expression of specific

**FIGURE 4:** (A) Average area of type 1 muscle fibres (µm²) of M. *vastus lateralis* (□) and M. *vastus medialis* (○) from DM (closed symbols) and NDM (open symbols). Values are means ± sem. (B) Average area of type 2 muscle fibres (µm²) of M. *vastus lateralis* (□) and M. *vastus medialis* (○) from DM (closed symbols) and NDM (open symbols). Values are means ± sem.

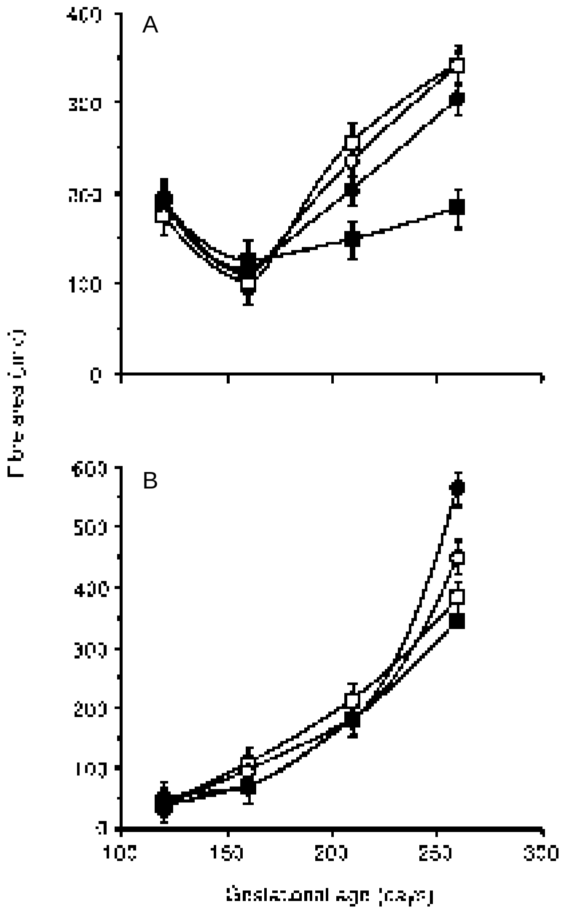
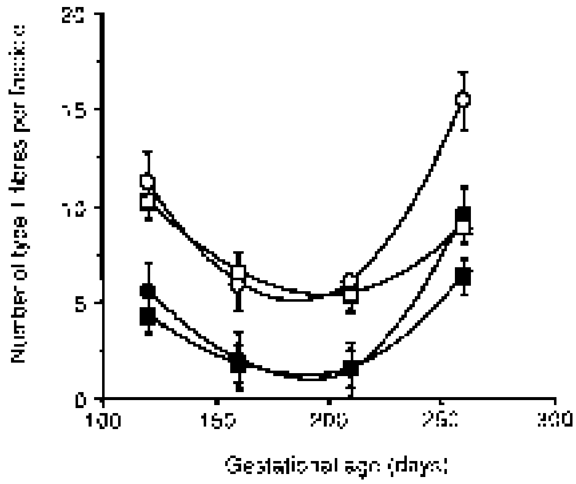



**FIGURE 5:** Total number of type 1 muscle fibres per fascicle M. *vastus lateralis* (□) and M. *vastus medialis* (○) from DM (closed symbols) and NDM (open symbols). Values are means ± sem.




myosin ATPase isoforms, and this study examined muscle fibre type using myosin ATPase histochemistry as a means of characterising stages of fibre development. Initially, primary muscle fibres are type 1 on the basis of a very low level of myosin ATPase activity following alkaline preincubation, and secondary fibres are type 2 which give a positive ATPase reaction. No attempt has been made in this study to distinguish sub-types of type 2 fibres. Development of alkaline myosin ATPase activity in bovine foetuses has been previously reported as occurring at between 144 and 152 days gestation (Robelin *et al.*, 1991), but our protocol has shown substantial activity at 120 days.

In this study, where muscle fibre number per fascicle was constant throughout, the observed changes in fibre type composition are likely to be due to switching from type 1 to type 2 fibres. Ashmore *et al.*, (1974) have suggested that the decreased percentage of type 1 fibres found in their study was due to a more rapid production of type 2 fibres, but we are unable to reconcile this with our constant fibre number per fascicle. From 210 days of gestation, we have shown that the number of type 1 fibres per fascicle increases in all muscles, suggesting that a number of type 2 fibres have been transformed to type 1, and this is supported by Maier *et al.*, (1992) who have also observed a switch from fast (type 2) to slow (type 1) fibre types which commenced at E110 in the sheep.

In conclusion, differential muscle growth of the vastus muscles occurs postnatally in DM animals, and differences in muscle fibre development between DM and NDM are regulated according to fibre type and muscle.

## ACKNOWLEDGEMENTS

The authors wish to thank Bruce Worsnop for his very generous gift of Belgian Blue donor cows and semen and Ross Lasenby and Trevor Watson for care of the donor animals. Dr Neil Cox carried out the statistical analyses. This study was funded by the New Zealand Foundation for Research, Science and Technology.

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