MEAT & LIVESTOCK AUSTRALIA

finalreport

Project code: SG.130

Prepared by: Dr. Paul Greenwood

NSW DPI

Date published: August 2008

ISBN: 9781741918359

PUBLISHED BY
Meat & Livestock Australia Limited
Locked Bag 991
NORTH SYDNEY NSW 2059

SheepGENOMICS Muscle Sub-Program Training and Consultancy

In submitting this report, you agree that Meat & Livestock Australia Limited may publish the report in whole or in part as it considers appropriate.

This publication is published by Meat & Livestock Australia Limited ABN 39 081 678 364 (MLA). Care is taken to ensure the accuracy of information in the publication. Reproduction in whole or in part of this publication is prohibited without the prior written consent of MLA.

NSW DEPARTMENT OF
PRIMARY INDUSTRIES

Abstract

This consultancy was conducted to assist with development of phenotyping methods and provision of phenotypic data for muscle from sheep within the SheepGENOMICS Muscle Sub-program. On-site training was provided to Dr Jason White (University of Melbourne) and Victorian Department of Primary Industries technical staff in sheep muscle preparation and freezing technique for use in immunohistological and histochemical staining and *in situ* hybridisation. On-going discussions were held with Dr Jason White regarding appropriate sheep myofibre classification techniques and provision of protocols for staining and classification of myofibres. Muscle samples were prepared and provided to Dr James Kijas (CSIRO Livestock Industries) for genotyping, and data provided from the Carwell sheep experiment conducted by Victorian Department of Primary Industries at Rutherglen. Callipyge and resource flock myofibre characterisation and morphometric measurements were provided to Dr Jason White for the phenotyping unit. Results for Callipyge and non-Callipyge genotypes showed there is a marked increase in the proportion of the total myofibre area comprising type 2X and a marked reduction in type 1, 2C, 2A and 2AX myofibres in affected (hypertrophied) muscles (*semimembranosus and semitendinosus*) of *Callipyge* compared to normal sheep, and little if any change in myofibre characteristics in non-hypertrophied muscles. Furthermore, the extent to which the percentage and size (cross-sectional area) of specific myofibre types is altered differs between affected muscles in *Callipyge* sheep. Results for samples from myostatin-mutant heterozygous and myostatin normal genotypes showed that cross-sectional areas of the more glycolytic myofibre types tended to be greater in the myostatin heterozygote compared to the normal animals. This resulted in an overall tendency for the myostatin-mutant heterozygotes to have myofibres with greater cross-sectional area than the normal myostatin sheep. Other significant effects of myostatin genotype on percent, cross-sectional area or relative area of the myofibre types were not evident. Dr Greenwood attended various SheepGENOMICS planning, progress and annual scientific meetings. The methodologies employed in this contract should continue to be utilised in conjunction with metabolic indicators of muscle characteristics to enhance capacity for prediction of favourable and adverse outcomes of genetic selection using gene markers for commercially important production traits. This work should benefit industry by enhancing understanding of relationships between sheep genotypes and phenotypes. More specifically, it will enhance the capacity for prediction of favourable and adverse outcomes of genetic selection using gene markers for commercially important production traits.

Executive Summary

This consultancy was conducted to assist with development of phenotyping methods and provision of phenotypic data for muscles from sheep within the SheepGENOMICS Muscle Sub-program.

Specifically the following was achieved:

- On-site training was provided to Dr Jason White (University of Melbourne) and Victorian Department of Primary Industries technical staff in sheep muscle preparation and freezing technique for use in immunohistological and histochemical staining and *in situ* hybridisation.

- On-going discussions were held with Dr Jason White regarding appropriate sheep myofibre classification techniques and provision of protocols for staining and classification of myofibres.

- Muscle samples were prepared and provided to Dr James Kijas (CSIRO Livestock Industries) for genotyping, and data provided from Carwell sheep experiment conducted by Victorian Department of Primary Industries at Rutherglen.

- Callipyge and resource flock myofibre characterisation and morphometric measurements were provided to Dr Jason White for the phenotyping unit. Results for Callipyge and non-Callipyge genotypes showed there is a marked increase in the proportion of the total myofibre area comprising type 2X and a marked reduction in type 1, 2C, 2A and 2AX myofibres in affected (hypertrophied) muscles (*semimembranosus and semitendinosus*) of *Callipyge* compared to normal sheep, and little if any change in myofibre characteristics in non-hypertrophied muscles. Furthermore, the extent to which the percentage and size (cross-sectional area) of specific myofibre types is altered differs between affected muscles in *Callipyge* sheep.

- Results for samples from myostatin-mutant heterozygous and myostatin normal genotypes showed that cross-sectional areas of the more glycolytic myofibre types tended to be greater in the heterozygote compared to the normal animals. This resulted in an overall tendency for the myostatin-mutant heterozygotes to have myofibres with greater cross-sectional area than the normal myostatin sheep. There were no other significant effects of myostatin genotype on percent, cross-sectional area or relative area (percent of total myofibre area) of the myofibre types classified based on MHC characteristics.

- Dr Greenwood attended various SheepGenomics planning, progress and Annual Scientific Meetings.

This work has helped to enable the successful conduct of muscle phenotyping within the SheepGENOMICS Muscle Sub-Program. The methodologies employed in this contract should continue to be utilised in conjunction with metabolic indicators of muscle characteristics to enhance capacity for prediction of favourable and adverse outcomes of genetic selection using gene markers for commercially important production traits.

This work will benefit industry by enhancing understanding of relationships between sheep genotypes and phenotypes. More specifically, it will enhance the capacity for prediction of favourable and adverse outcomes of genetic selection using gene markers for commercially important production traits.

Contents

Page

1 Background 5
2 Project Objectives 5
3 Methodologies...5
4 Results and Discussion 8
5 Success in Achieving Objectives.................... 11
6 Impact on Meat and Livestock Industry 12
7 Conclusions and Recommendations.............. 12
8 Bibliography 12

1 Background

Muscle fibres are the major cell type within muscle and represent the major muscle functional and structural component. Muscle fibre types are associated with growth, body composition, yield and meat quality characteristics.

The successful conduct of the MLA-AWI SheepGENOMICS Muscle Sub-Program required appropriate muscle phenotyping capacity to enable identification and characterisation of animals polymorphic for genes that affect commercial meat production traits.

Conduct of high throughput and more detailed muscle fibre characterisation required establishment of appropriate methodologies for collection and processing of muscle samples and suitable classification and measurement techniques, which was provided by this consultancy. The consultancy also provided muscle tissues from previously phenotyped sheep within a “Carwell” experiment for more detailed genotyping.

2 Project Objectives

- On-site training of Dr Jason White (University of Melbourne) and Victorian Department of Primary Industries technical staff in sheep muscle preparation and freezing technique for use in immunohistological and histochemical staining and *in situ* hybridisation.
- On-going discussions with Dr Jason White regarding appropriate sheep myofibre classification techniques and provision of protocols for staining and classification of myofibres.
- Preparation and provision of muscle samples to Dr James Kijas (CSIRO Livestock Industries) for genotyping, and provision of data from Carwell sheep experiment conducted by Victorian Department of Primary Industries at Rutherglen.
- Myofibre characterisation within phenotyping unit and attendance at SheepGenomics meetings as required.

3 Methodologies

Sample collection and preparation

Muscle samples were collected and prepared as described in Greenwood *et al.* (2006a, 2006b, 2007). They were excised from carcasses following overnight chilling. A block of muscle approximately 1 $cm^2 \times 1.5$ cm was cut from each muscle, ensuring the sampling site was standardised for each muscle. To prepare muscle blocks for histology, subcutaneous fat, fascia and approximately 5 mm of muscle were trimmed from the block, leaving a cube approximately 1 cm^3 for freezing. During sample preparation, the alignment of the sample was maintained to allow for cryosectioning commencing from the cranial end of the tissue block, perpendicular to the length of

the muscle cells. Each muscle sample was mounted using gum tragacanth (Sigma Chemical Company, St Louis, MO: prepared 5% w/v in distilled, deionised H_2O) onto a cork block labelled to show the orientation of the muscle sample, with the muscle fibres running perpendicular to the cork block. Samples were frozen by immersion in iso-pentane cooled to approximately -160°C in liquid nitrogen, prior to storage at -70°C.

Muscle immunocytochemistry

Muscle immunocytochemistry was performed essentially as described by Greenwood *et al.* (2006a, 2006b, 2007). Cross-sectional, 10-μm-thick, serial sections were cut from each sample using a cryostat microtome (ThermoShandon AS 620 Cryostat SME, Thermotrace Ltd, Noble Park). The sections were air dried and stored at -20°C until commencement of the staining procedures. They were then thawed, fixed in 100% acetone, and recovered in 0.01M phosphate buffered saline, pH 7.2 (PBS) prior to application of blocking solution (10% non-immune serum, Zymed Laboratories, South San Francisco, CA) for 10 min. The blocking solution and subsequent reagents were applied within a well created around each tissue section using a hydrophobic pen. A volume of 50μL of diluted monoclonal antibody against slow or type 1 (clone WB-MHCs, Novocastra, Newcastle upon Tyne, UK; diluted 1:100 in PBS), fast or type 2 (clone MY-32, Sigma Chemical Company, St Louis, MO; diluted 1:1200 in PBS) and types 1, 2B and 2X (clone S5 8H2, see Picard *et al.* (1998), Arguello *et al.* (2001), Reggiani and Mascarello (2004); diluted 1:1000 in PBS) myosin heavy chain (MHC) isoforms were applied to serial tissue sections and incubated for 1 hour at 37°C in a humid chamber. Rabbit anti-laminin, affinity isolated antibody (Sigma Chemical Company, St Louis, MO; diluted 1:500 in PBS) was also included in the solution containing anti-type 1 MHC to allow cellular margins to be delineated. The antibodies were detected using a broad spectrum Labeled-[Strept]-Avidin-Biotin amplification system and the substrate chromagen, diaminobenzidine (Zymed Laboratories, South San Francisco, CA), and the sections dehydrated and cleared using graded ethanols and xylenes and coverslips applied using a xylenes-based mounting medium.

Myofibre classification and morphometry

Stained serial tissue sections were classified and measured as essentially as described by Greenwood *et al.* (2006a, 2006b, 2007). Classification of myofibres into five sub-types (1, 2C, 2A, 2AX and 2X: Figure 1) was based on their staining characteristics for the three antibodies against MHCs as described by Picard *et al.* (1998), except that type 2B and 2AB myofibres were classified as 2X and 2AX. This was done on the basis that these myofibres react to an antibody that recognised type 2B and type 2X MHC (Picard *et al.* 1998; Arguello *et al.* 2001; Reggiani and Mascarello 2004), display variable oxidative capacity and strong glycolytic capacity based on NADH and α-GPD staining, respectively (see Greenwood *et al.* 2006a), and that sheep and other ruminant limb and trunk muscles express type 2X MHC and little, if any, type 2B MHC (Arguello *et al.* 2001; Maccatrozzo *et al.* 2004; Reggiana and Mascarello 2004; Vuoculo *et al.* 2007). Classification of type 2C myofibres was based on positive staining for all three antibodies, albeit at lower staining intensity (Figure 1). Classification of type 2AX myofibres was based on intermediate staining for the type 1, 2B and 2X MHC antibody and negative staining for the type 1 MHC antibody (Figure 1). The classification system also differed from that of Picard *et al.* (1998) due to use of the bright-field antibody detection system described above and bright-field microscopy, rather than fluorescence.

c

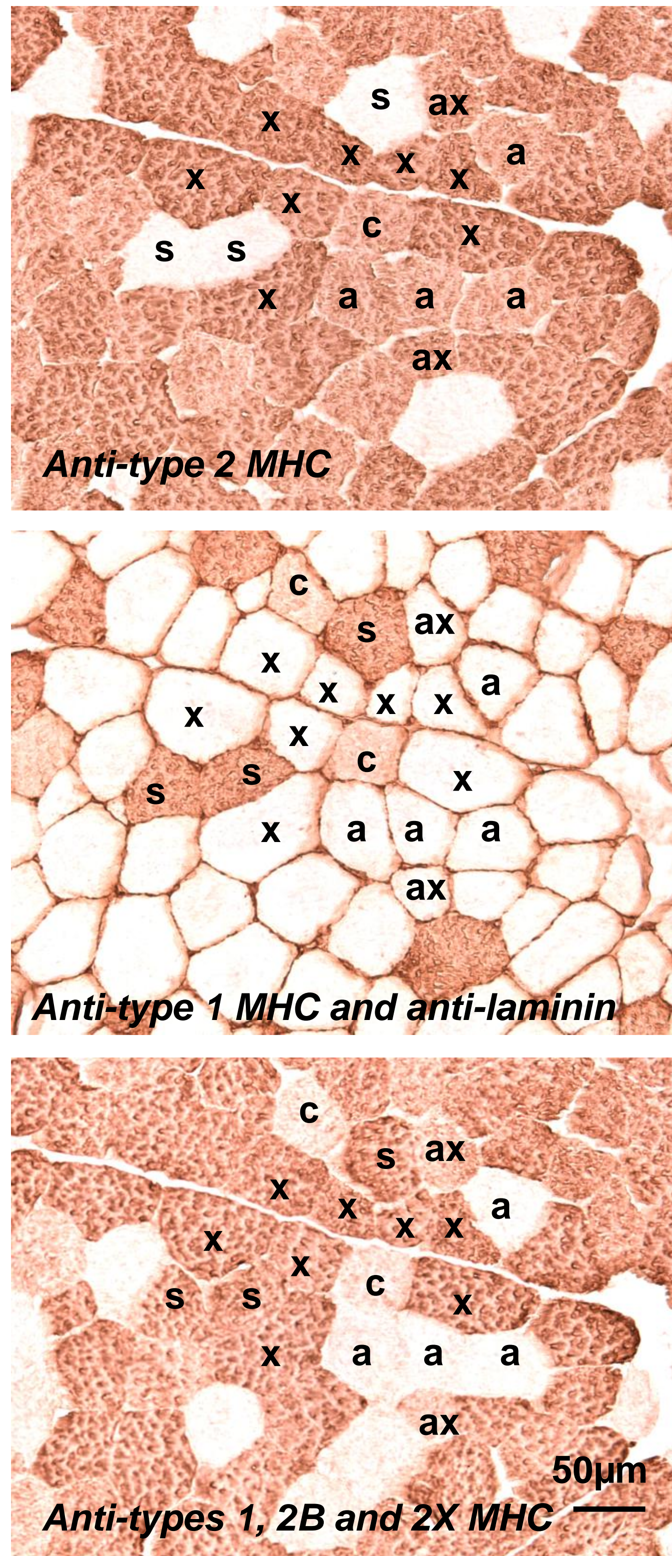

Figure 1. Classification of myofibre types in sheep muscle using immunocytochemical staining of myosin heavy chains.

For each sample classified, the total area of each myofibre type relative to the total myofibre area was calculated from the percentage and average size of the myofibre types. For each sample classified, the total area of each myofibre type relative to the total myofibre area was calculated from the percentage and average size of the myofibre types. The system was calibrated using a stage micrometer, and average cross-sectional area of each myofibre type measured. Myofibres were classified and measured from at least two fields of view, prepared as described above, for each muscle. For each muscle classified, the total area of each myofibre type relative to the total myofibre area, and the overall average cross-sectional area of myofibres, was calculated from the percentage and average size of the myofibre types. Where appropriate, the ratios of fast (type 2) to slow (type 1) myofibres and of more glycolytic myofibres (type 2X) to those with more oxidative capacity (types 1 + 2C + 2A + 2AX: see Greenwood *et al.* 2006b) were also calculated.

4 Results and Discussion

On-site training

On-site training of Dr Jason White (University of Melbourne) and Victorian Department of Primary Industries technical staff in sheep muscle preparation and freezing technique for use in immunohistological and histochemical staining and *in situ* hybridisation was successfully conducted by NSW Department of Primary Industries technical staff.

Provision of samples for genotyping and data from "Carwell" study

Muscle tissue samples and data from the Victorian DPI "Carwell" study were provided to CSIRO Livestock Industries and it is understood that DNA was successfully extracted. Myofibre characteristics from the animals are summarised in Table 1. To date, genotyping results have not been generated to allow for an assessment of their associations with myofibre and other data to be made.

Table 1. Relative[1] areas of myofibre types in *m. longissimus lumborum* samples of 7 months-old lambs provided for genotyping to SheepGENOMICS (from Greenwood *et al.* 2006a)

		Myofibre type				
	n	1	2C	2A	2AX	2X
Sire (R)						
One	20	7.4	0.5	24.1c	3.0	65.2a
Two	28	8.0	0.7	20.2b	3.1	68.6ab
Three	25	8.7	0.9	18.3ab	3.1	69.1abc
Four	24	6.7	0.6	17.4a	2.7	72.9c
SED		1.07	0.33	1.73	0.57	2.13
Sex (S)						
Female	47	7.3	0.5	20.5	2.8	69.1
Castrate	50	8.1	0.8	19.5	3.1	68.8
SED		0.71	0.21	1.15	0.38	1.42
Nutrition (N)						
Pasture	38	5.7a	0.8	19.6	3.2	70.8
Protected	31	8.9b	0.8	21.0	2.8	66.9
Unprotected	28	8.3b	0.4	19.4	2.8	69.2
SED		0.91	0.28	1.48	0.49	1.82
Interactions		R×N	-	-	-	-

[1]As percentage of total myofibre area

Type 1, type 1 myosin heavy chain (MHC) ≡ slow oxidative; Type 2A, type 2A MHC ≡ fast oxidative-glycolytic; Type 2X, type 2X MHC ≡ fast glycolytic; Type 2C, type 1 -type 2A intermediate; Type 2AX, type 2A-type 2X intermediate. CSA, cross-sectional area.

Development of phenotyping capacity

Detailed myofibre phenotyping was successfully developed for the SheepGENOMICS Muscle Sub-Program.

Results for samples from Callipyge and non-Callipyge genotypes are presented in Tables 2 to 4. In summary, there is a marked increase in the proportion of the total myofibre area comprising type 2X and a marked reduction in type 1, 2C, 2A and 2AX myofibres in affected (hypertrophied) muscles (*semimembranosus and semitendinosus*) of *Callipyge* compared to normal sheep, and little if any change in myofibre characteristics in non-hypertrophied muscles. Furthermore, the extent to which the percentage and size (cross-sectional area) of specific myofibre types is altered differs between affected muscles in *Callipyge* sheep.

Table 2. Myofibre characteristics in *semimembranosus* muscle, determined using myosin heavy chain (MHC) antibodies, in normal (n = 3) and Callipyge (n = 3) sheep at 72-78 days of age

Variable	Normal (NN)	Callipyge (CN)	s.e.d.	*P*
Myofibres per mm^2 of muscle CSA	499	263	85.3	0.008
Percent of myofibres				
Type 1	9.7	8.5	1.68	0.494
Type 2C	1.9	0.8	0.51	0.028
Type 2A	18.2	3.6	2.09	<0.001
Type 2AX	4.9	0.7	1.46	0.004
Type 2X	65.2	86.4	2.67	<0.001
Cross-sectional area of myofibres (μm^2)				
Type 1	1512	1468	135.1	0.749
Type 2C	1041	1070	342.2	0.932
Type 2A	1111	1696	147.6	<0.001
Type 2AX	1305	1757	683.6	0.509
Type 2X	1845	3514	483.6	<0.001
Overall	1636	3227	368.4	<0.001
Percent of total myofibre area				
Type 1	9.1	3.9	0.88	<0.001
Type 2C	1.26	0.31	0.27	<0.001
Type 2A	12.4	1.9	1.07	<0.001
Type 2AX	4.3	0.2	1.45	0.005
Type 2X	73.0	93.7	1.93	<0.001

Type 1, type 1 myosin heavy chain (MHC) ≡ slow oxidative; Type 2A, type 2A MHC ≡ fast oxidative-glycolytic; Type 2X, type 2X MHC ≡ fast glycolytic; Type 2C, type 1 -type 2A intermediate; Type 2AX, type 2A-type 2X intermediate. CSA, cross-sectional area.

Table 3. Myofibre characteristics in *semitendinosus* muscle, determined using myosin heavy chain (MHC) antibodies, in normal (n = 3) and Callipyge (n = 4) sheep at 72-78 days of age

Variable	Normal (NN)	Callipyge (CN)	s.e.d.	*P*
Myofibres per mm^2 of muscle CSA	405	300	70.3	0.134
Percent of myofibres				
Type 1	11.5	5.4	2.57	0.018
Type 2C	1.2	0.3	0.28	0.001
Type 2A	19.3	6.1	2.11	<0.001
Type 2AX	3.3	1.7	0.78	0.044
Type 2X	64.8	86.6	4.54	<0.001
Cross-sectional area of myofibres (μm^2)				
Type 1	1811	1282	220.2	0.016
Type 2C	1530	2038	1006	0.614
Type 2A	1562	2001	545.1	0.422
Type 2AX	1789	1791	326.8	0.996
Type 2X	2664	2990	487.5	0.504
Overall	2314	2808	457.7	0.281
Percent of total myofibre area				
Type 1	9.2	2.5	2.36	0.004
Type 2C	0.8	0.2	0.16	<0.001
Type 2A	13.0	4.0	1.76	<0.001
Type 2AX	2.5	1.2	0.55	0.015
Type 2X	74.5	92.3	3.69	<0.001

Type 1, type 1 myosin heavy chain (MHC) ≡ slow oxidative; Type 2A, type 2A MHC ≡ fast oxidative-glycolytic; Type 2X, type 2X MHC ≡ fast glycolytic; Type 2C, type 1 -type 2A intermediate; Type 2AX, type 2A-type 2X intermediate. CSA, cross-sectional area.

Table 4. Myofibre characteristics in *supraspinatis* muscle, determined using myosin heavy chain (MHC) antibodies, in normal (n = 4) and Callipyge (n = 4) sheep at 72-78 days of age

Variable	Normal (NN)	Callipyge (CN)	s.e.d.	*P*
Myofibres per mm^2 of muscle CSA	383	389	129.1	0.969
Percent of myofibres				
Type 1	33.4	36.6	8.22	0.695
Type 2C	5.4	4.0	2.65	0.616
Type 2A	10.1	9.0	0.90	0.219
Type 2AX	5.2	4.0	1.13	0.260
Type 2X	45.9	46.4	9.43	0.958
Cross-sectional area of myofibres (μm^2)				
Type 1	1931	2014	490.9	0.865
Type 2C	1333	1178	380.7	0.684
Type 2A	1556	1640	404.9	0.836
Type 2AX	1728	2001	504.8	0.590
Type 2X	2160	2602	568.9	0.437
Overall	2018	2212	445.4	0.664
Percent of total myofibre area				
Type 1	34.5	32.6	10.85	0.861
Type 2C	3.6	2.6	2.13	0.625
Type 2A	7.6	6.5	0.31	<0.001
Type 2AX	4.4	3.5	0.99	0.390
Type 2X	49.9	54.8	12.76	0.701

Type 1, type 1 myosin heavy chain (MHC) ≡ slow oxidative; Type 2A, type 2A MHC ≡ fast oxidative-glycolytic; Type 2X, type 2X MHC ≡ fast glycolytic; Type 2C, type 1 -type 2A intermediate; Type 2AX, type 2A-type 2X intermediate. CSA, cross-sectional area.

Results for samples from myostatin-mutant heterozygous and myostatin normal genotypes showed that cross-sectional areas of the more glycolytic myofibre types tended to be greater in the heterozygote compared to the normal animals (Table 5). This resulted in an overall tendency for the myostatin-mutant heterozygotes to have myofibres with greater cross-sectional area than the normal myostatin sheep. There were no other significant effects of myostatin genotype on percent, cross-sectional area or relative area (percent of total myofibre area) of the myofibre types classified based on MHC characteristics.

Table 5. Myofibre characteristics (mean ± SEM) in *longissimus* muscle (n = 39), determined using myosin heavy chain (MHC) antibodies, in normal and mutant myostatin heterozygote sheep.

Variable	Normal	Myostatin heterozygote	*P*
Cold carcass weight (kg)	18.98 ± 0.75	19.67 ± 1.06	0.167
Percent of myofibres			
Type 1	6.84 ± 0.64	7.27 ± 0.96	0.953
Type 2C	2.27 ± 0.35	1.64 ± 0.39	0.357
Type 2A	17.27 ± 1.04	19.08 ± 1.56	0.636
Type 2AX	9.92 ± 1.17	10.40 ± 1.75	0.889
Type 2X	63.76 ± 1.61	61.71 ± 2.41	0.764
Fast to slow	14.91 ± 1.96	13.82 ± 2.93	0.858
Glycolytic to oxidative	1.93 ± 0.13	1.77 ± 0.15	0.932
Cross-sectional area of myofibres (μm^2)			
Type 1	1468 ± 105	1415 ± 158	0.740
Type 2C	902 ± 174	752 ± 210	0.719
Type 2A	1169 ± 79	1216 ± 118	0.158
Type 2AX	1145 ± 68	1192 ± 102	0.103
Type 2X	1572 ± 83	1751 ± 124	0.094
Overall	1437 ± 73	1540 ± 109	0.096
Percent of total myofibre area			
Type 1	6.93 ± 0.56	6.48 ± 0.84	0.414
Type 2C	1.53 ± 0.41	0.77 ± 0.61	0.542
Type 2A	13.74 ± 0.91	15.02 ± 1.36	0.918
Type 2AX	7.77 ± 0.75	8.29 ± 0.83	0.902
Type 2X	69.96 ± 1.41	69.70 ± 2.11	0.700
Fast to slow	15.07 ± 1.75	14.58 ± 2.63	0.588
Glycolytic to oxidative	2.46 ± 0.17	2.44 ± 0.26	0.631

Type 1, type 1 myosin heavy chain (MHC) ≡ slow oxidative; Type 2A, type 2A MHC ≡ fast oxidative-glycolytic; Type 2X, type 2X MHC ≡ fast glycolytic; Type 2C, type 1 -type 2A intermediate; Type 2AX, type 2A-type 2X intermediate. CSA, cross-sectional area.

These findings for the myostatin mutant heterozygote and the normal sheep will also allow for relationships between myofibre and other carcass and muscle characteristics to be assessed by the Phenotyping Unit.

5 Success in Achieving Objectives

All objectives were successfully achieved.

However, results of genotyping for the “Carwell” study for which tissue and samples have been provided have not been completed by CSIRO Livestock industries.

6 Impact on Meat and Livestock Industry

This work has helped to enable the successful conduct of muscle phenotyping within the SheepGENOMICS Muscle Sub-Program.

This work will also enhance the capacity for prediction of favourable and adverse outcomes of genetic selection using gene markers for commercially important production traits.

7 Conclusions and Recommendations

The methodologies employed in this contract should continue to be utilised in conjunction with metabolic indicators of muscle characteristics to enhance capacity for prediction of favourable and adverse outcomes of genetic selection using gene markers for commercially important production traits.

Results of genotyping for the "Carwell" study for which tissue samples and data were provided should be completed by CSIRO Livestock industries.

8 Bibliography

Arguello A, Lopez-Fernandez JL, Rivero JL (2001) Limb myosin heavy chain isoproteins and muscle fibre types in the adult goat (*Capra hircus*). *Anatomical Record* 264, 284-293.

Picard B, Duris MP, Jurie C (1998) Classification of bovine muscle fibres by different histochemical techniques. *Histochemical Journal* 30, 473-479.

Greenwood PL, Davis J, Gaunt GM, Ferrier GR (2006a) Influences on the loin and cellular characteristics of the *m. longissimus lumborum* of Australian Poll Dorset-sired lambs. *Australian Journal of Agricultural Research* 57,1-12.

Greenwood PL, Gardner GE, Hegarty RS (2006b) Lamb myofibre characteristics are influenced by sire estimated breeding values and pastoral nutritional system. *Australian Journal of Agricultural Research* 57, 627-639.

Greenwood PL, Harden S, Hopkins, DL (2007) Myofibre characteristics of ovine *longissimus* and *semitendinosus* muscles are influenced by sire breed, gender, rearing type, age, and carcass weight. *Australian Journal of Experimental Agriculture* 47, 1137-1146

Maccatrozzo L, Patruno M, Toniolo L, Reggiani C, Mascarello F (2004) Myosin heavy chain 2B isoform is expressed in specialized eye muscles but not in trunk and limb muscles of cattle. *European Journal of Histochemistry* 48, 357-366.

Reggiani C, Mascarello F (2004) Fibre type identification and functional characterization in adult livestock animals. *In* 'Muscle Development of Livestock Animals. Physiology, Genetics and Meat Quality.' (Eds MFW te Pas, ME Everts, HP Haagsman) pp. 39-68. (CAB International: Wallingford)

Vuocolo, A, Byrne K, White J, McWilliam S, Reverter A, Cockett NE, Tellam RL (2006) Identification of a gene network contributing to hypertrophy in Callipyge skeletal muscle. *Physiological Genomics* 28, 253-272.