Body composition and growth in lambs:

The effect of the myostatin g+6723G>A mutation and the

β-adrenergic agonist ractopamine

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A thesis submitted for the degree of Doctor of Philosophy of the University of New England

January 2012

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Abstract

Increasing muscle mass is a key driver to increasing carcass yield in lamb meat production. Greater muscle mass in Australian sheep meat production systems has been achieved by the use of estimated breeding values. Estimated breeding values indirectly select for many genes of small effect to increase muscle mass. Identification of a single known gene to significantly improve muscle mass whilst reducing fatness would enable a greater rate of genetic improvement. Alternatively, exogenous methods, such as β-adrenergic agonists, also contribute to increases in muscle mass by partitioning nutrients to promote skeletal muscle growth whilst reducing fat deposition.

The *myostatin* gene is a known regulator of skeletal muscle during foetal and post-natal development and growth. Complete knockouts or mutations of *myostatin* are attributed to differing forms of the double-muscle phenotype which in cattle have significantly greater muscle mass. In sheep, there is a mutation at the *myostatin* locus which affects muscle mass. The *myostatin* g+6723G>A mutation is found in the 3'UTR region of the *myostatin* locus causing changes in mRNA which alters binding in the RISC complex which is thought to reduce the amount of translated myostatin protein. In the absence or a reduction of myostatin, myoblast cells proliferate at a greater rate, consequently increasing the number of myofibres in skeletal muscle. This thesis investigated the effect of the *myostatin* g+6723G>A mutation on differences in growth, body composition (measured by computer tomography) and muscle fibre characteristics in lambs homozygote (*MSTN* A/A) or heterozygote (*MSTN* A/G) for the mutation compared to wild type (*MSTN* G/G) lambs. The first experiment also considered the use of the β-adrenergic agonist, ractopamine (RAC) into the lambs' finisher diets to increase skeletal muscle mass.

In the first experiment *MSTN* A/G lambs and wild type (*MSTN* G/G) lambs were offered *ab libitum* or restricted access to feed, and treated with or without RAC. The *MSTN* A/G *ab libitum* feed intake lambs were found to perform better than the *MSTN* G/G lambs offered the same diet. The *MSTN* A/G *ab libitum* feed intake lambs where also found to have a lower proportion of type IIX myofibres compared to the *MSTN* A/G restricted intake lambs. Including RAC in the diet of *MSTN* A/G *ab libitum* feed intake lambs had greater total daily carcass growth, compared to the *MSTN* G/G *ab libitum* feed intake lambs. Regardless of genotype, the inclusion of RAC also increased the proportion of type IIC and IIA myofibres and cross-sectional area of type I and IIAX myofibres. The data from this experiment suggests that RAC and the heterozygous *myostatin* g+6723G>A mutation act together to increase growth of muscle on a high plane of nutrition. The experiment also demonstrated that poor nutritional background of lambs heterozygous for the *myostatin* g+6723G>A mutation may negatively influence their growth rates and myofibre characteristics.

The second experiment was designed to determine the basis of differences between lambs with three genotypes for the *myostatin* g+6723G>A mutation. It was postulated that the increase in muscle mass is attributed to inhibition of the myostatin protein. The experiment found that *MSTN* A/A lambs had greater muscle mass, less fat, smaller organ weights and a greater number of muscle fibres than *MSTN* A/G and *MSTN* G/G lambs, yet, the difference in muscle mass was not clear until later in life, at approximately 6 months of age. At slaughter, the *MSTN* A/A lambs were also found to have greater dress percentage and heavier primal meat cuts. The results of this study demonstrate that *MSTN* A/A lambs have greater muscle mass possibly attributable to a reduction in myostatin protein during early development, but at the later stage of development there was a greater amount of myostatin protein in the *MSTN* A/A lambs. It appears that the mechanism that underpins the regulation of myostatin during postnatal development is complex and requires further research.

Declaration

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis and all sources used have been acknowledged in this thesis.

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30 January 2012

Acknowledgements

I would like to acknowledge the financial support provided by the Cooperative Research Centre for Sheep Industry Innovation, University of New England, and Meat and Livestock Australia for my scholarship and research funding. I also appreciate the assistance and support provided by NSW Department of Primary Industries and Department of Primary Industries Victoria.

I'm indebted to my supervisors who I have worked closely with during my candidature. Firstly, I would like to thank my principal supervisor Prof. V. Hutton Oddy for his continued encouragement, probing questions and guided support which has allowed me to evolve into a scientist with intellect and an ability to look at both the big and small picture, something I will value for the my entire career. Secondly, Prof. Paul Greenwood and Dr. Matt McDonagh are both recognised as valuable contributors to my candidature. I'm grateful for your guidance, supervision and support.

Thanks also to all the other fellow scientists and technical staff. Particular thanks to the Ag Research Ruakura Developmental Biology team, Hamilton, New Zealand. Thanks for the opportunity to work in your laboratory Dr. Chris McMahon, Dr. Gina Nicholas and Ms. Carol Berry.

This thesis would not have been possible without the support of my family and friends. I'd like to thank my family and my husband's family for their kind support. I would also like to especially thank my mother-in-law who endured many hours of proof reading. And finally, my husband Rob, your loving and selfless support, understanding, patience and guidance has allowed me to follow my dream, this truly depicts the kind person you are – thank you.

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List of Abbreviations

A	age
ActRIIB	activin type IIB receptor
ADG	average daily gain
ATP	adenosine triphosphate
βAA	β-adrenergic agonists
βAR	β-adrenergic receptors
bp	base pair
cAMP	cyclic adenosine monophosphate
Cdk2	cyclin-Cdk2
Cdks	cyclin-dependent kinases
CIDR	controlled internal drug release
CKIs	cdk-inhibitors
cm	centimetre
COOH	C-terminal
CREB	cAMP response element-binding
CSA	cross-sectional area
C site	fat depth measured 45 mm from the midline of the 12^{th} rib
CT	computer tomography
d	day
°C	degrees Celsius
DNA	deoxyribonucleic acid
DM	dry matter
DMD	dry matter digestibility
DMI	dry matter intake
h	hour
EBV	estimated breeding values
EDTA	ethylenediaminetetra-acetic acid
EMA	eye muscle area

EMD	eye muscle depth
g	gravitational force
g	gram
GDF8	growth and differentiation factor 8
GR	total soft tissue depth measured 110 mm from midline over the 12th rib
HIGH	*ab libitum* feed intake
h	hour
HCW	hot carcass weight
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HRP	horse radish peroxidase
iu	international unit
IMF	intramuscular fat
kDa	kilo Dalton
kg	kilogram
k_m	efficiency of use for ME for maintenance
L	litres
LD	*longissimus dorsi*
LOW	restricted feed intake
LW	liveweight
µ	micro (×10-6)
µg	microgram
µm	micrometer
M	molar
ME	metabolisable energy
MEI	metabolisable energy intake
ME_m	metabolisable energy for maintenance
ME_p	ME available for production
MHC	myosin heavy chain
MJ	megajoules

min	minute
mRNA	messenger Ribonucleic acid
miRNA	microRNA
mm	millimeter
MRF	muscle regulatory factor
MSTN	myostatin
MSTN	*myostatin* gene
***MSTN* A/A**	homozygote for the *myostatin* g+6723G>A mutation
***MSTN* A/G**	heterozygote for the *myostatin* g+6723G>A mutation
***MSTN* G/G**	homozygote wild type
n	number
N	nitrogen
NaCl	sodium chloride
NH2	N-terminal
NO RAC	No ractopamine
OD	optical density
P	probability
PAGE	polyacrylamide gel electrophoresis
PKA	protein kinase A
USA	United States of America
RAC	ractopamine
Rb	retinoblastoma
RE	retained energy
RISC	RNA-induced silencing complex
RNA	ribonucleic acid
pH	hydrogen ion concentration
SDS	sodium dodecyl sulphate
SE	standard error
SM	semimembranosus

SNP	single nucleotide polymorphism
S phase	**s**ynthesis phase
ST	semitendinosus
TBE	tris-borate-EDTA buffer
TGF-β	transforming growth family - β
Type I	slow oxidative myofibres
Type IIC	intermediate or transitional between types I and IIA myofibres
Type IIA	fast oxidative-glycolytic myofibres
Type IIAX	intermediate or transitional between types IIA and IIX myofibres
Type IIX	fast glycolytic myofibres
UTR	untranslated region
W	metabolic weight ($W^{0.75}$)

Thesis structure

This thesis reports two experiments designed to evaluate the effect of the *myostatin* g+6723G>A mutation on production in meat lambs, with emphasis on traits such as: growth, lean meat yield, meat quality and feed efficiency. Chapter 1 provides an introduction and background information on the current knowledge of the function and mechanism of myostatin, results of preliminary research on the *myostatin* g+6723G>A mutation in lambs and the β-adrenergic agonist, ractopamine. Chapters 2, 3 and 4 present the results of the first experiment and Chapter 5 presents the results of the second experiment. Chapter 6 is the general discussion which summarises the major findings and important implications identified during the studies. Chapter 7 is a consolidated reference list. Chapter 8 consists of appendices and is divided into two parts. The first part is a list of published or submitted peer reviewed journal manuscripts and abstracts. The second part is detailed materials and methods of myofibre and computer tomography.

Chapter 2 reports on the effect of heterogenous *myostatin* g+6723G>A mutant lambs on growth, body composition and myofibre characteristics when offered differing feed allowances treated with or without ractopamine. Chapter 3 evaluates the effect of ractopamine on objective meat quality measurements. Chapter 4 evaluates the digestibility of feed and protein, fat and energy retention in the lambs. Chapter 5, the final experiment, evaluates the basis of difference between lambs with three genotypes for the *myostatin* g+6723G>A mutation on growth, body composition, myofibre characteristics and differences in the concentration of myostatin in plasma and muscle during their stages development.

Each experimental chapter is present in a peer reviewed journal format. Chapter 2 has been accepted for publication in the *Journal of Animal Science.* Chapter 3, 4 and 5 have been prepared for submission to the *Journal of Animal Science*.