

**Body composition and growth in lambs:  
The effect of the myostatin g+6723G>A mutation and the  
 $\beta$ -adrenergic agonist ractopamine**

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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  $\beta$ -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  $\beta$ -adrenergic agonist, ractopamine (RAC) into the lambs' finisher diets to increase skeletal muscle mass.

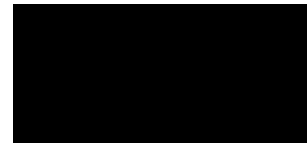
In the first experiment *MSTN* A/G lambs and wild type (*MSTN* G/G) lambs were offered *ab libitum* or restricted access to feed, and treated with or without RAC. The *MSTN* A/G *ab libitum* feed intake lambs were found to perform better than the *MSTN* G/G lambs offered the same diet. The *MSTN* A/G *ab libitum* feed intake lambs were 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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## List of Abbreviations

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

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

<b>min</b>	minute
<b>mRNA</b>	messenger Ribonucleic acid
<b>miRNA</b>	microRNA
<b>mm</b>	millimeter
<b>MRF</b>	muscle regulatory factor
<b>MSTN</b>	myostatin
<b><i>MSTN</i></b>	<i>myostatin</i> gene
<b><i>MSTN</i> A/A</b>	homozygote for the <i>myostatin</i> g+6723G>A mutation
<b><i>MSTN</i> A/G</b>	heterozygote for the <i>myostatin</i> g+6723G>A mutation
<b><i>MSTN</i> G/G</b>	homozygote wild type
<b>n</b>	number
<b>N</b>	nitrogen
<b>NaCl</b>	sodium chloride
<b>NH<sub>2</sub></b>	N-terminal
<b>NO RAC</b>	No ractopamine
<b>OD</b>	optical density
<b><i>P</i></b>	probability
<b>PAGE</b>	polyacrylamide gel electrophoresis
<b>PKA</b>	protein kinase A
<b>USA</b>	United States of America
<b>RAC</b>	ractopamine
<b>Rb</b>	retinoblastoma
<b>RE</b>	retained energy
<b>RISC</b>	RNA-induced silencing complex
<b>RNA</b>	ribonucleic acid
<b>pH</b>	hydrogen ion concentration
<b>SDS</b>	sodium dodecyl sulphate
<b>SE</b>	standard error
<b>SM</b>	semimembranosus

<b>SNP</b>	single nucleotide polymorphism
<b>S phase</b>	synthesis phase
<b>ST</b>	semitendinosus
<b>TBE</b>	tris-borate-EDTA buffer
<b>TGF-<math>\beta</math></b>	transforming growth family - $\beta$
<b>Type I</b>	slow oxidative myofibres
<b>Type IIC</b>	intermediate or transitional between types I and IIA myofibres
<b>Type IIA</b>	fast oxidative-glycolytic myofibres
<b>Type IIAx</b>	intermediate or transitional between types IIA and IIX myofibres
<b>Type IIX</b>	fast glycolytic myofibres
<b>UTR</b>	untranslated region
<b>W</b>	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  $\beta$ -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*.