

New Zealand Society of Animal Production online archive

This paper is from the New Zealand Society for Animal Production online archive. NZSAP holds a regular annual conference in June or July each year for the presentation of technical and applied topics in animal production. NZSAP plays an important role as a forum fostering research in all areas of animal production including production systems, nutrition, meat science, animal welfare, wool science, animal breeding and genetics.

An invitation is extended to all those involved in the field of animal production to apply for membership of the New Zealand Society of Animal Production at our website www.nzsap.org.nz

[View All Proceedings](#)

[Next Conference](#)

[Join NZSAP](#)

The New Zealand Society of Animal Production in publishing the conference proceedings is engaged in disseminating information, not rendering professional advice or services. The views expressed herein do not necessarily represent the views of the New Zealand Society of Animal Production and the New Zealand Society of Animal Production expressly disclaims any form of liability with respect to anything done or omitted to be done in reliance upon the contents of these proceedings.

This work is licensed under a [Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License](http://creativecommons.org/licenses/by-nc-nd/4.0/).



You are free to:

Share— copy and redistribute the material in any medium or format

Under the following terms:

Attribution — You must give [appropriate credit](#), provide a link to the license, and [indicate if changes were made](#). You may do so in any reasonable manner, but not in any way that suggests the licensor endorses you or your use.

NonCommercial — You may not use the material for [commercial purposes](#).

NoDerivatives — If you [remix, transform, or build upon](#) the material, you may not distribute the modified material.

<http://creativecommons.org.nz/licences/licences-explained/>

β -lactoglobulin expression in bovine mammary tissue

C.A.FORD, M.B.CONNETT¹ AND R.J.WILKINS

AgResearch, Ruakura Agricultural Centre, Private Bag 3123 Hamilton, New Zealand.

ABSTRACT

The levels of the A and B variant forms of β -lactoglobulin were measured in the milk of 70 heterozygous cows. On average, 1.2 times more A than B variant protein was found in the milk. Measurements of mRNA levels in lactating tissue from four of these animals indicated that approximately 60% more A allele than B allele mRNA was present. We conclude that differences in the two alleles, either at the level of transcription or mRNA stability, are probably responsible for the different rates of production of the corresponding protein variants.

Keywords: β -lactoglobulin, bovine, alleles, gene expression.

INTRODUCTION

In this investigation we investigate the molecular mechanism(s) which control the levels of the A and B β -lactoglobulin protein variants in bovine milk. Beta-lactoglobulin is a major constituent of cows milk, making up some 10% of total protein. Two major protein variants are found in New Zealand herds, A and B, and these differ by two amino acids; the asparagine at position 64 and valine at position 118 in the A variant protein are substituted by, respectively, glycine and alanine in the B variant. As a consequence of these substitutions, the two variants have distinct physicochemical properties which have an economic impact on a number of milk processing protocols. Manipulating the levels of one or the other of the β -lactoglobulin variants in milk would have distinct economic advantages. The first step in doing this is to pinpoint the molecular factors which control β -lactoglobulin expression. In the present study we have chosen to look at heterozygous cows in which the relative levels of expression of the A and B variants of β -lactoglobulin can be accurately monitored in individual animals.

METHODS

Proteins

Foremilk samples were collected from 70 A/B heterozygotes in a mixed Jersey-Friesian Ruakura herd on 18 consecutive mornings in March-April 1992. One ml samples of whole milk was centrifuged for 12 min at 15,000 rev/min in a benchtop microfuge and whey carefully removed from the clear middle layer between the fat and a fluffy casein pellet. Two ml samples of this whey were mixed with loading dye and then separated by native polyacrylamide gel electrophoresis and stained with Coomassie blue. The relative levels of the β -lactoglobulin A and B proteins were determined with a laser densitometer (Ford, 1993). Control experiments were done with purified A and B β -lactoglobulin proteins (Sigma Chemical Company) to ensure that equal loadings of protein actually

gave equal laser signals and also with whey prepared by traditional acid precipitation methods to ensure that the observed A to B protein ratios were not an artifact of the preparation method.

RNA

Total RNA was isolated from lactating mammary tissue obtained either at autopsy or by biopsy. The relative levels of mRNA for β -lactoglobulin variants A and B were determined by two independent methods:

Northern Blotting/Allele Specific Probes

RNA samples were electrophoresed and blotted to duplicate membranes which were probed with ³²P-labelled oligonucleotide 24mer probes (5' AGGCACTGGCAG-A or G-CCAGGCTTTGCT 3') specific for, respectively, the A or B allele of β -lactoglobulin. Stringent washing conditions were used to preferentially remove mismatched probe (Wilkins, 1993). The autoradiographs were scanned with a laser densitometer and the relative levels of A and B mRNA determined from the allele specific levels of hybridisation (Ford 1993).

Reverse Transcriptase of RNA and Amplification

RNA samples were reversed transcribed with a β -lactoglobulin primer (5' TCACCTAGATGTGGCA CTGCTCCT 3') and amplified by 20 cycles of a polymerase chain reaction (PCR) by adding a second primer (5' ACCTGGAGATCCTGCTGCAGAAATG 3') end labeled with ³³P to give a DNA fragment of 336 base pairs. Restriction with HaeIII enzyme was used to specifically cleave the cDNA from the B allele at an additional restriction site and so enable it to be distinguished from the A allele cDNA on a sequencing gel. The relative levels of the two bands was measured with a laser densitometer, and by liquid scintillation counting, in order to determine the relative levels of A and B β -lactoglobulin mRNA in the RNA sample.

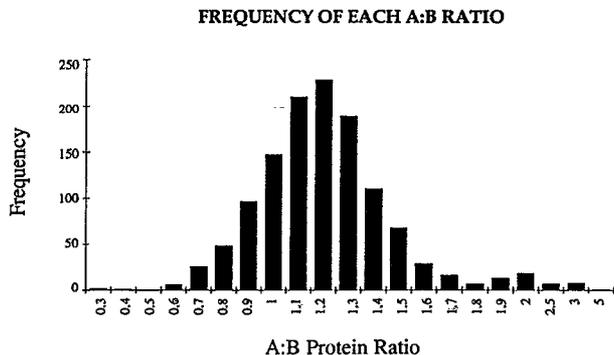
¹Department of Biological Sciences, University of Waikato, Hamilton, New Zealand.

RESULTS

The seventy heterozygous cows in the herd produced, on average, 1.2 times as much of the A as the B variant of the β -lactoglobulin protein. This average value was obtained by analysing foremilk collected from these animals over 18 consecutive days. The histogram of this data is shown in Figure 1. It is clear that individual values for the ratio of the A to B variant protein can vary markedly, from about 0.3 to 5:1. These data are in agreement with that of Graml *et al.*, (1989) although in their case the average ratio (over 2 seasons for Simmental and Brown Alpine cows) was nearer 1.5 and they also showed that, on average, the AA homozygotes also produce about 50% more β -lactoglobulin protein than BB homozygotes.

When our data was broken down with respect to breed, age and individual animal, no clear deviation from the mean was seen for 68 of the cows. When analysed with respect to day of sampling, there was a slight hint of fluctuations in some animals, with a periodicity of a few days. Although many cows occasionally gave milk samples with unusually high or low ratios (see Figure 1) most of these turned out to be isolated instances with repeat samplings being within the normal range. We have no explanation for these aberrant values.

FIGURE 1: Histogram of the frequency of each β -lactoglobulin protein A:B ratio observed during the period studied



Two animals in the herd consistently produced low amounts of the B variant of β -lactoglobulin with the result that the A:B ratios were significantly higher than normal, 1.75 ± 0.1 and 2.7 ± 0.25 respectively. Moreover, the same high ratios were seen in milk produced in the following season (6 months later).

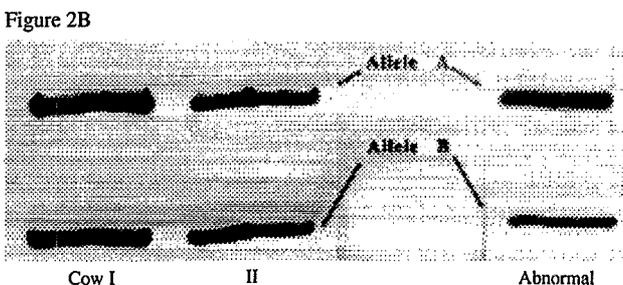
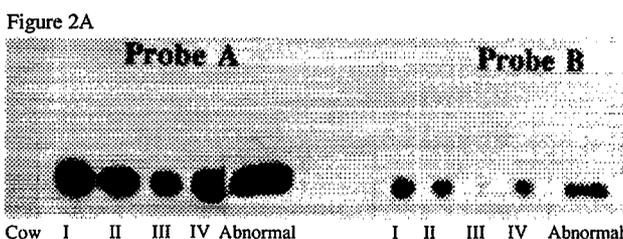
The relative levels of mRNA coding for the A and B variant proteins were measured in total RNA which had been extracted from mammary tissue samples obtained from "normal" animals at autopsy or "abnormal" animals by biopsy. The analysis was by either Northern blotting or reverse transcription-polymerase chain reaction amplification of the mRNA. Both analyses depend on the fact that the A allele of the β -lactoglobulin mRNA could be distinguished from the B allele by two point mutations in the nucleotide sequence (Wilkins and Kuys, 1992).

In the case of Northern blotting, duplicate blots which contained RNA lanes from four normal, one abnormal and

controls of "pure" A and B allele RNA were probed with 32 P-labelled oligonucleotides homologous to either the A or B variant mRNA. The "A" probe formed a perfect match with the A allele mRNA while the "B" probe gave a one base mismatch; the situation was vice versa for the B allele mRNA. By washing the blots under stringent conditions (Wilkins 1993) it was possible to preferentially wash off the mismatched probe so that, in the RNA samples from heterozygous animals which are a mixture of A and B allelic mRNA, the levels of the two alleles can be established from the relative hybridisation signals obtained with the two probes. The autoradiographs from such an experiment are shown in Figure 2a. In practice, it is necessary to make corrections for loadings and cross-hybridisation of probes (Ford 1993). When this is done, ratios for the four normal animals averaged 1.65 ± 0.2 to one whereas for the abnormal animal it was 2.8 to one (this was the animal which gave a ratio of 2.7:1 for the A to B protein variants in the milk).

In the second approach to measuring the ratios of allelic mRNA, a primer was used to reverse transcribe cDNA copies of the β -lactoglobulin mRNA and then a second, 32 P end labelled primer added and a PCR reaction initiated in order to amplify up a fragment of cDNA. There were actually two types of fragments in this cDNA, one derived from the A allele mRNA which did not contain a HaeIII restriction site 195 base pairs from the 32 P-labelled end, and one derived from B-allele mRNA which does. Thus, when the PCR product is electrophoresed on a sequencing gel after incubation with HaeIII restriction enzyme, and autoradiographed, two products are seen - one of 237 bp which is derived from A allele mRNA and one of 195 bp which is derived from B allele mRNA (Figure 2b). The ratio of the product in the two bands gives the ratio of A to B mRNA in the original sample. This analysis was carried out for two RNA samples from normal cows, giving ratios of 1.4 and 1.8 to one. For the one abnormal animal analysed, the ratio was 2.4 to one.

FIGURE 2: Detection of specific β -lactoglobulin mRNA alleles by (a) Northern blotting total RNA samples with oligonucleotide probes specific for either the A or the B allele and (b) restriction enzyme analysis of RT-PCR amplified mRNA; the upper band of 237 bp corresponds to the A allele and the lower band of 195 bp to the B allele.



Overall then, it appears that in the mammary gland of "normal" lactating cows, the levels of mRNA for the A allele of β -lactoglobulin are about 60% higher than for the B allele and, in the one "abnormal" animal tested, about 140 to 180% higher.

DISCUSSION

These data suggest that differences in expression of the two alleles of the β -lactoglobulin gene may be responsible for the differential production of the A and B variants of the protein in milk. The preliminary experiments reported here do not allow us to ascertain the exact reasons for the mRNA levels being different, but we intend to look at both the transcription rates from the two alleles, and the stability of the two mRNAs. If the differences do indeed result from subtle differences in the structures of the two genes, and additional mutations can alter the expression of the B allele even further (as would seem the case in the "abnormal" animals) it may be possible to select for altered levels of β -lactoglobulin proteins

by either screening herds for rare mutants or, eventually, by genetic manipulation.

ACKNOWLEDGEMENT

We thank the staff of the Dairying Research Corporation for assistance with milk and tissue collection.

REFERENCES

- Ford, C.A. 1993. Gene expression of the A and B variants of β -lactoglobulin. MSc Thesis, The University of Waikato, New Zealand.
- Graml, R., Weiss, G., Buchberger J. and Pirschner F, 1989. Different rates of synthesis of whey protein and casein by alleles of the β -lactoglobulin and α s1-casein locus in cattle. *Genetics Selection Evolution*, **21**: 547-554
- Wilkins R.J. and Kuys, Y.M., 1992. Rapid β -lactoglobulin genotyping of cattle using the polymerase chain reaction, *Animal Genetics*, **23**: 175 - 178.
- Wilkins, R.J. (1993). Allele specific oligonucleotide probes for mRNA. *Biotechniques*, **14**: 352-356.