Exploring the Commercial Potential of Sheep Milk
Exploring the Commercial Potential of Sheep Milk

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August 2012

RIRDC Publication No. 11/179
RIRDC Project No. PRJ-00454
Foreword

Sheep milking was introduced in Australia in the 1960s by farmers seeking to diversify from the traditional production of lambs and wool and to meet a perceived market demand for sheep milk products. Australia imports some $10 Million/year in sheep dairy products, and farm gate returns for sheep milk are seemingly high (Bencini and Dawe 1998). About 8000 tons per year of sheep milk products could potentially find a market in Australia, and 250000 ewes would have to be milked in 100-150 dairies to match this demand (Dawe 1990).

The crisis in the wool industry in the nineties demonstrated that the Australian animal industries could no longer ride solely on the sheep’s back. The deregulation of the dairy industry also induced many dairy farmers to seek opportunities to diversify their production.

Despite the evident advantages of milking sheep, the Australian sheep milking industry is still in its infancy. Consultation with industry resulted in the identification of strategies that will assist in the establishment of the industry.

Farmers embarking in sheep milking need practical methods to select sheep suitable for milking from large unselected flocks. We describe work conducted using behavioural tests developed at The University of Western Australia (Murphy 1999) to provide the industry with a practical instrument to select ewes of suitable behaviour. These tests also allowed us to clarify the link between the temperament of the animals and their subsequent performance as dairy animals, in terms of both milk production and milk composition.

Despite the existing demand, often consumers are unaware of the existence of locally produced sheep milk products so small sheep dairy manufacturers struggle to become established. A viable sheep milking industry could only be established by developing local and export markets for sheep milk products.

The establishment of markets for specialty sheep dairy products could also be encouraged by the production of health enhanced milk and dairy products.

Our previous research has shown that it is possible to produce special health enhanced milk by feeding particular diets to the sheep to increase the milk concentration of desirable compounds such as omega 3 fatty acids and Conjugated Linoleic Acid (CLA). In this report, we describe experimental work that involved feeding canola and brewers grains to dairy sheep to increase the milk concentration of these desirable compounds. As these compounds could affect the processing performance of the milk, we also processed this ‘health enhanced’ milk into cheese and tested the cheese through semi-trained panels to assess its acceptance by potential consumers.

We also describe the production of gourmet sheep milk ice creams through a collaboration with Simmo’s Ice Creamery. Although it was not one of the original objectives of the project, this small trial showed that it might be possible to develop niche markets for sheep milk ice creams to increase the market scope for sheep milk products.

This project was funded from RIRDC Core Funds, which are provided by the Federal Government.

This report, an addition to RIRDC’s diverse range of over 2000 research publications, forms part of our New and Developing Animal Industries R&D Program, which aims to conduct RD&E for new and developing animal industries that contribute to the profitability, sustainability and productivity of regional Australia.

Most of RIRDC’s publications are available for viewing, free downloading or purchasing online at www.rirdc.gov.au. Purchases can also be made by phoning 1300 634 313.

Craig Burns
Managing Director
Rural Industries Research and Development Corporation
About the Authors

Associate Professor Roberta Bencini has 23 years of experience in research on sheep milking. Her career in this area started in 1988 when she commenced her PhD on lactation in sheep at The University of Western Australia. Previously, she had been a tenured lecturer of Dairy Science and Technology at the Professional Institute for Agriculture of Villa Igea, Lodi, Italy and was therefore conversant with the biochemistry of milk and its transformation into dairy products.

The marriage between the dairy technology background and the PhD on the lactation physiology of sheep made her an ideal person to attempt to assist the fledgling Australian sheep milking industry. Shortly after she submitted her PhD she commenced a research project with support from the Rural Industries Research & Development Corporation. Subsequently she successfully directed to completion four research projects with the RIRDC and an Australian Research Council (ARC) Linkage project. Three RIRDC funded projects (UWA 23A and UWA 44A and UWA 66A) and the ARC Linkage project (LP0455194) have been completed and the reports have been published. This report pertains to project UWA 84A.

Associate Professor Bencini has an extensive publication record in the area of sheep dairying and sheep milk processing and has been technical editor of a prestigious book on the nutrition of dairy sheep (Pulina and Bencini 2004). She has been a keynote speaker at the 7th Great Lakes Dairy Sheep Symposium, held in 2001 in Wisconsin USA, and has been actively involved in workshops, public lectures and field days to inform rural communities and the general public on sheep milking and sheep milk processing.

Travis Murray completed his honours project at The University of Western Australia on sheep temperament. He was offered a scholarship to undertake work on the relationship between sheep temperament, their behaviour and their dairy potential. His work in this area has resulted in publications and presentations at conferences.
Acknowledgments

The financial support of the Rural Industries Research & Development Corporation is gratefully acknowledged. This project has been extremely hard work. We have to thank all the people that gave their time, often overworked and underpaid, to assist with this project:

**UWA Staff and students:**

Peter Cowl, Shenton Park Field Station Animal Facilities Manager

Relief Milkers: Brooke Anderton, Brian Chambers, Jenny Cheng, Dianne Mayberry and Megan Meates

Svjetlana Mijatovic, General Assistant and PhD Student

Sarah Pugh, 4th year student

Dominique Blache, for providing co-supervision for Travis Murray and advice on statistical analyses.

**Overseas visitors and collaborators:**

Alice Castagnoli, Exchange student, University of Florence, Italy

Dr Alessandro Mazzette, Visiting Scientist, University of Sassari, Italy

Dr Luisella Sistu, Visiting Scientist, University of Sassari, Italy

Dr Anna Nudda, Visiting Scientist, University of Sassari, Italy

Prof Stefano Rapaccini, University of Florence, Italy

**Other collaborators:**

CHR Hansen kindly donated the starter cultures for our Dairy Products Laboratory

Teresa and Vince Borrello, Borrello Cheese Factory, Byford for processing of bulk milk

Garth Simpson, Simmo’s Ice Creamery, for producing experimental batches of ice cream

Edward Gibson, James & Son Australia, for providing brewers’ grain.

**Industry partners**

YHH Holdings: Graham Daws, Director and George Johnson, Stock Manager

Cambray Sheep Dairy: Jane and Bruce Wilde, Owners.
Contents

Foreword ............................................................................................................................................... iii
About the Authors................................................................................................................................ iv
Acknowledgments.................................................................................................................................. v
Executive Summary............................................................................................................................... x
1 - Introduction ...................................................................................................................................... 1
2 - Objectives.......................................................................................................................................... 4
3 - Methodology ..................................................................................................................................... 5
   3.1 - Location and animals ................................................................................................................ 5
   3.2 - Management of the animals ...................................................................................................... 5
   3.3 - Temperament testing of the sheep ............................................................................................ 6
   3.4 - Measurements of production and composition of milk ............................................................ 8
   3.5 - Processing of sheep milk dairy products .................................................................................. 9
   3.5 - Determination of fatty acids profiles ...................................................................................... 10
   3.6 - Statistitical analyses .............................................................................................................. 10
4 - Experimental .................................................................................................................................. 11
   4.1 - Relationship between temperament, behaviour and milk production in experienced dairy sheep ........................................................................................................................................... 12
      4.1.1 - Introduction ......................................................................................................................... 12
      4.1.2 - Materials and Methods ........................................................................................................ 12
      4.1.3 - Results .................................................................................................................................. 13
      4.1.4 - Discussion ............................................................................................................................ 15
   4.2 – Genetic selection for temperament results in sheep that are easier to milk ...................... 18
      4.2.1 - Introduction ......................................................................................................................... 18
      4.2.2 - Materials and Methods ........................................................................................................ 19
      4.2.3 - Results .................................................................................................................................. 20
      4.2.4 - Discussion ............................................................................................................................ 22
   4.3 – Whole or crimped canola as a food for dairy sheep .............................................................. 24
      4.3.1 - Introduction ......................................................................................................................... 24
      4.3.2 - Materials and methods ........................................................................................................ 25
      4.3.3 - Results .................................................................................................................................. 26
      4.3.4 - Discussion ............................................................................................................................ 31
   4.4 – Brewers’ grain as a food for dairy sheep ................................................................................. 32
      4.4.1 - Introduction ......................................................................................................................... 32
      4.4.2 - Materials and methods ........................................................................................................ 32
      4.4.3 - Results .................................................................................................................................. 33
      4.4.4 - Discussion ............................................................................................................................ 35
4.5 - Processing of health enhanced sheep milk ................................................................. 37
  4.5.1 - Introduction .............................................................................................................. 37
  4.5.2 - Materials and methods ......................................................................................... 37
  4.5.3 - Results .................................................................................................................... 38
  4.5.4 - Discussion ............................................................................................................... 40

4.6 - Gourmet sheep milk ice cream ................................................................................... 41
  4.6.1 - Introduction ............................................................................................................ 41
  4.6.2 - Materials and methods ....................................................................................... 41
  4.6.3 - Results and discussion ....................................................................................... 41

5 - General Discussion .................................................................................................... 43

6 - Implications ................................................................................................................. 44

7 - Recommendations .................................................................................................... 45

8 - Intellectual Property .................................................................................................. 46

9 - References ................................................................................................................ 47
Figures

Figure 3.2.1. The milking platform at Shenton Park Research Station, The University of Western Australia, is a rapid exit parlour that allows a high throughput of ewes per hour. .....................6

Figure 3.2.2. a) The isolation box used to test the temperament of sheep. .................................................7

Figure 3.2.3. Dr Anna Nudda (visiting scientist, University of Sassari, Italy) and relief milker Megan Meates measure the milk production of the sheep with the Tru Test milk meters.........................8

Figure 4.2.1. Average platform loading (top) and unloading scores (bottom) of inexperienced Merino ewes genetically selected for calm (white, n = 23) or nervous (black, n = 12) temperament. ..........................20

Figure 4.2.2. a) Average time taken (seconds) to place milking clusters on the teats of inexperienced Merino ewes genetically selected for calm (white, n = 23) or nervous (black, n = 12) temperament.  
   b) Average number of kicks during milking of inexperienced dairy ewes genetically selected for calm (white, n = 23) or nervous (black, n = 12) temperament. .....................21

Table 4.4.1. Composition (%) of the brewers’ grain supplied by James & Son for the experiment .................32

Figure 4.4.1. Daily bulk milk production of dairy ewes fed either a control diet (black line, n= 22) or a diet containing brewers’ grain (white line, n= 22). ..........................................................34

Figure 4.4.2. Average milk production of dairy ewes fed either a control diet (white, n= 22) or a diet containing brewers’ grain (black, n= 22). The bars represent standard errors.................................35
Tables

Table 4.1.1. Behavioural scores and traits of ewes of calm (n=16) or nervous (n=16) temperaments during milking (mean ± standard error). ................................................................. 14

Table 4.1.2. Daily milk production (g, mean ± standard error) and fat and protein concentrations in the milk (g/100g ± standard error) from calm (n=16) and nervous (n=16) experienced dairy ewes over the first 10 weeks of lactation. ........................................................................ 15

Table 4.2.1. Average milk production (g/day), fat, protein and lactose concentrations of milk produced by ewes genetically selected for calm or nervous temperament during the first six weeks of lactation .......................................................... 22

Table 4.3.1. Milk production and composition (protein, fat and lactose) of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9) ........................................ 27

Table 4.3.2. Fatty acids composition in the milk of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9) ...................................................... 28

Table 4.3.3. Fatty acids composition in the milk of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9) ...................................................... 30

Table 4.4.2. Composition (%) of samples of the brewers’ grain collected throughout the experiment ....... 34

Table 4.4.3. Composition of milk from sheep fed a control diet or a diet supplemented with or brewers’ grain (average of whole period: 6 weeks) ............................................................ 35

Table 4.5.1. Renneting time (r, minutes), rate of curd formation (K20, minutes) and consistency of the curd (A30, mm), of the bulk milk obtained from sheep fed a control diet, or diets supplemented with crimped canola or whole canola and used to produce experimental batches of Fetta cheese ........................................ 38

Table 4.5.2. pH, renneting time (r, minutes), rate of curd formation (k20, minutes) and curd consistency (A30, mm) of the milk of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9) .......................................................... 39

Table 4.5.3. Mean scores awarded by a semi-trained panel to batches of Fetta cheese made with milk from sheep fed a control diet, or diets supplemented with whole or crimped canola. .......... 40

Table 4.6.1. Batches of gourmet ice cream produced at Simmo’s ice creamery (Busselton, WA) and their performance at the 2007 Perth Royal show ........................................................................... 42
Executive Summary

What the report is about

The Australian sheep milking industry is still in its infancy and this work was conducted to assist its establishment. In consultation with industry, we investigated the following strategies.

Selection of sheep suitable for milking

Farmers embarking in sheep milking would benefit from being able to select sheep suitable for milking before they actually milk them. Behavioural tests developed at The University of Western Australia (Murphy 1999) could provide the industry with a practical instrument to select ewes of suitable behaviour. These tests also allowed us to clarify the link between the temperament of the animals and their subsequent behaviour in the dairy as well as the quantity and quality of milk that they produced.

Development of markets for dairy products made with sheep milk

A viable sheep milking industry could only be established by developing local and export markets for sheep milk products.

Our previous research efforts demonstrated that it is possible to produce health enhanced milk by feeding diets to the sheep that promote the translocation of desirable compounds such as omega 3 fatty acids (Kitessa S.M., Peake D., Bencini R. and Williams A.J. (2003). Fish oil metabolism in ruminants. III. Transfer of omega-3 polyunsaturated fatty acids (PUFA) from tuna oil into sheep's milk. Animal Feed Science and Technology. 108, 1-14) and Conjugated Linoleic Acid (Hutton P. (2003). Sunflower oil increases the level of conjugated linoleic acid in the milk of different breeds of sheep. Honours Thesis. The University of Western Australia.) into the milk.

Clearly, the health benefits associated with the consumption of this milk could be an important selling point for these products. However, the effects of these compounds on the processing performance of the milk, and on the flavour and taste of the final dairy products are still largely unknown. In this project, we processed this ‘health enhanced’ milk into cheese to examine its suitability for cheese making. We also tested the cheeses through semi-trained panels to assess the consumers’ acceptance of these specialty dairy products. Results from this work will help the establishment of markets for dairy products made with sheep milk.

Aims/objectives

The aim of this project was to assist the establishment of a viable sheep milking industry in Australia by:

- Investigating the suitability of behavioural tests to select dairy ewes based on their temperament
- Developing feeding strategies to increase the health benefits of sheep milk products, so that these products can be commercialised for their health benefits as well as for their superior qualities
- Researching the commercial potential of sheep dairy products for the domestic and export markets.
**Methods used**

To conduct this research we appointed a PhD student, Mr Travis Murray, with support of the RIRDC, to investigate the relationship between temperament, behaviour and milk production in sheep. We used behavioural tests developed at The University of Western Australia on our industry partners’ sheep and then correlated the results of the tests with their performance in the dairy with the aim of providing the industry with a practical instrument to select ewes of suitable behaviour. We also used two lines of sheep genetically selected for calm or nervous temperaments at The University of Western Australia’s Allandale Research Farm and tested their behaviour, milk production and milk composition in a dairy situation.

Investigations also included feeding strategies to increase the unsaturated fatty acids concentration of sheep milk by feeding canola and brewers’ grain. This ‘health enhanced’ milk was processed into cheese to examine its suitability for cheese making. We tested the cheeses through semi-trained panels to assess the acceptance of these dairy products by potential consumers.

Collaboration with a local ice cream producer, Simmo’s Ice Creamery, tested the suitability of sheep milk for the production of gourmet ice creams.

**Results/key findings**

The rapid temperament tests that we used in this project have been the subject of large research projects in the lamb and wool producing sectors of the sheep industry. They had never been tested for their usefulness in sheep dairies. Our work demonstrated that there is a relationship between the temperament of sheep, their behaviour in the dairy, and their subsequent performance as dairy animals. Calm sheep were easier to train to the milking routine, and they also produced more milk but the relationship between the sheep’s temperament and the quality of their milk was unclear.

Feeding canola to the sheep changed the proportion of unsaturated fatty acids in the milk, although not in the expected direction, and did not result in any processing problems in the milk or resulting dairy products. Feeding brewers’ grain resulted in maintaining lactation in sheep that were at the end of their lactation, a finding that warrants further investigation.

Gourmet sheep milk ice creams produced by Simmo’s were entered in the Perth Royal Show dairy products competition, and one of them won an award.

**Implications for relevant stakeholders**

The use of rapid temperament tests could become an important method to screen sheep suitable for milking from large unselected flocks. Since there is a relationship between the temperament of sheep, their behaviour in the dairy and their subsequent performance as dairy animals, the selection of calm animals for milking will bring considerable advantages to sheep dairy producers.

Sheep dairy producers and manufacturers should consider feeding canola or brewers’ grain to their dairy sheep if they wish to commercialise their products for their health benefits to consumers.

Our collaboration with Simmo’s demonstrated that it should be possible to establish markets for gourmet sheep milk ice cream.

**Recommendations**

The rapid temperament test used in this project has been widely tested and adopted in the wool and lamb industries. Sheep dairy producers should also adopt this test to select sheep suitable for milking from large flocks. Selecting calm animals for milking will result in animals that are easier to train and produce more milk. Although the relationship between temperament and milk quality was not completely elucidated by our work, the literature suggests that sheep that eject their milk should
produce milk with greater concentrations of total solids, mainly due to higher concentrations of milk fat (Labussière 1988) and possibly milk protein (Sart S., Bencini R., Blache D.B. and Martin G.B. (2004). Calm ewes produce milk with more protein than nervous ewes. Animal Production in Australia. 25, 307.).

Sheep dairy producers and manufacturers wishing to commercialise their products for their health benefits would have to substantiate these claims by providing additional information in the nutrition information panel of the dairy products in question. They would also have to conduct cost benefit analyses before adopting these strategies, as these were not within the scope of this project.
1 - Introduction

Farmers that wanted to diversify from the traditional productions of lambs and wool pioneered sheep milking in Australia in the 1960s. They were motivated by the possibility of replacing the imported sheep dairy products, worth $10 Million/year (Bencini and Dawe 1998) and by reports by Dawe (1990) that about 8000 tons per year of sheep milk products could find a market in Australia and to match this demand 250000 ewes would have to be milked in 100-150 dairies.

However, many sheep dairies that commenced their operations in those early days no longer exist and many more since then have failed to become established. The failure of those early operations was mainly attributable to the lack of productive breeds of dairy sheep. This changed dramatically in the 1990s when YHH Holdings and the Western Australian Department of Agriculture formed a joint venture to import the Awassi, a specialized breed of dairy sheep, mainly to produce fat tail lambs for the live sheep trade (Lightfoot J. (1987). The Awassi fat tail sheep project. Journal of Agriculture 4, 107-113.). At the same time Awassi and East Friesian sheep were imported into New Zealand, specifically to be milked, and from there they were quickly released into Australia (T. Grant and J. Allison Pers. Comm.).

Our early research efforts concentrated on testing the dairy potential of these newly imported breeds, and their crosses, with local breeds as well as addressing many of the problems that were perceived to hinder the establishment of the industry (Bencini 1999, Bencini 2005, Bencini and Agboola 2003). The sheep dairy manual published by the RIRDC in 2009 was, in part, a result of those research efforts (Stubbs et al. 2009).

Despite these research efforts the Australian sheep milking industry is still in its infancy and it is struggling to become established. Consultation with industry resulted in the identification of the following strategies to assist the establishment of the industry.

Selection of sheep suitable for milking

When we started our project, YHH Holdings, our industry partners and also the major importer of the Awassi sheep, had embarked in a major sheep milking operation. They were milking up to 400 sheep per day at a newly established dairy at Dandaragan, and exported several shipments of sheep milk Fettato the Middle East. They identified the urgent need for research into the development of rapid and efficient methods for the selection of sheep of a suitable temperament. This was because they had very large flocks, with some 400,000 sheep run on several different properties, and did not have the time or the facilities to actually put them through the milking parlour and see how they performed: a simple screening method was highly desirable. Significantly, no work had been done on this aspect of sheep milking, even in the countries that traditionally have established sheep milking industries.

This is why for this project we decided to appoint a PhD student, Mr Travis Murray, in consultation with and the support of the RIRDC, to investigate this topic. We used behavioural tests developed at The University of Western Australia (Murphy 1999) on our industry partners’ sheep and then correlated the results of these tests with their performance in the dairy both in terms of behaviour and quantity and quality of milk produced. The aim of this work was to provide the industry with a practical instrument to select ewes suitable for milking.

These behavioural tests had also allowed the establishment of a temperament flock of Merino sheep that have been genetically selected for calm and nervous temperaments at
The University of Western Australia’s Allandale Research Farm. The temperament flock provided us with the unique opportunity to investigate the effect of genetic selection for temperament on the dairy potential of sheep.

Even among specialised breeds of dairy sheep some animals do not release endogenous oxytocin, the let down hormone, and therefore do not let down their milk. As a consequence, only the milk from the cisterns of the udder can be collected. The resulting milk is poorer in fat because fat is more abundant in the milk retained in the alveolar tissue found in the upper parts of the udder (Labussière 1988). Sheep that do not eject their milk also have lower total lactation yields and shorter lactations because the milk that is left in the mammary glands inhibits further milk secretion (Wilde and Peaker 1990, Bencini et al. 2003). Milk ejection is inhibited by adrenaline, which is released by the animals if they are stressed (Findlay 1970). Clearly there could be a nexus between the temperament of the animals and their incapacity to let down their milk. Our research aimed to clarify this by testing the hypothesis that calm ewes would develop a spontaneous ejection of milk and therefore produce more milk of a better quality, while nervous ewes would continue to release adrenaline and therefore never develop a milk ejection reflex. As a consequence, they would produce less milk, and this milk would be lower in fat.

When selecting ewes it is ideal to be able to predict ewes that are most likely to produce more milk but also be more likely to behave suitably. Although most sheep are initially reluctant to be milked, they soon habituate to the milking routine and become co-operative because the regular exposure of animals to novel experiences enables them to become habituated to non-painful stimuli that had formerly induced a stress response (Reid and Mills 1962). However, some sheep never get used to the milking routine and they eventually have to be removed from the flock as they distress other sheep, may harm milking staff and slow down productivity. Blache and Bickell (2010) demonstrated that the selection of ewes with calmer temperaments decreased their response to stress, and we hypothesised that this type of selection can have further applications in sheep dairying, whereby we can select ewes that will be less stressed by the novelty of the milking procedure and behave more suitably. Although the model that we used was the Merino sheep, notoriously not a good dairy sheep, if calm Merino sheep produced more milk or milk of a better quality, we could reasonably conclude that genetic improvement of temperament is desirable in any breed of sheep.

Development of markets for dairy products made with sheep milk

Although sheep dairy producers might have been attracted to the industry by the idea of replacing the imports of sheep milk products, some of these products, for instance the Roquefort, are DOC (Denomination d’Origine Controllée) and thus protected by legislation in their countries of origin (Bencini 1999). Others like the Pecorino, Fiore Sardo and, again, the Roquefort, are made starting with raw milk, which is not permitted under Australian regulations. Additionally, consumers seeking to purchase imported cheeses are unlikely to shift to local products unless they learn about them. Therefore, a viable sheep milking industry could only be established by developing markets for sheep milk products.

Our previous research efforts resulted in the development new sheep dairy products that were rapidly adopted by local sheep dairies and became very popular among consumers (T. Dennis, Cloverdene Dairy and J. Wilde, Cambray Dairy, Pers. Comm.). We also conducted consumer surveys that have shown that there are large potential markets for these products (Bencini 1999, Bencini and Agboola 2003, Burton and Bencini 2005).

Sheep milk contains twice as much calcium as cows milk (Bencini 2002) as well as higher concentrations of Conjugated Linoleic Acids (CLA), a class of compounds that has attracted much interest due to their beneficial effects on health (Nudda et al. 2004). Our
research has shown that it is possible to further increase the potential health benefits of sheep milk by feeding particular diets to the sheep so that desirable compounds such as omega 3 fatty acids (Kitessa et al. 2003) and Conjugated Linoleic Acid (CLA; Hutton 2003) are translocated into the milk.

Clearly, the health benefits associated with the consumption of this milk would be an important selling point for these products.

Nudda et al. (2005) demonstrated that these compounds vary seasonally in sheep milk due to variations in the diet and are readily found in the resulting dairy products. However, since these compounds are essentially unsaturated fatty acids, their increase above naturally occurring levels could potentially affect the processing performance of the milk or the flavour and taste of the final dairy products. At the time of writing no research had been done in this area and so the consequences of producing this health-enhanced milk for processing were largely unknown. To address this issue we processed this ‘health enhanced’ milk into cheese to examine its suitability for cheese making. We also tested the cheeses through semi-trained panels to assess the acceptance of these specialty dairy products by potential consumers. Results from this work will help the establishment of markets for specialty sheep milk products.
2 - Objectives

This project aimed to assist the establishment of a viable sheep milking industry in Australia by:

• Investigating the suitability of behavioural tests to select dairy ewes based on their temperament.

• Developing feeding strategies to increase the health benefits of sheep milk products, so that these products can be commercialised for their health benefits as well as for their superior qualities.

• Researching the processing properties of this health enhanced milk and the acceptance of the derived dairy products by consumers.

• Exploring the commercial potential of sheep dairy products for the domestic and export markets.

Outcomes, deliverables and outputs of the project

The project aimed to provide the sheep milking industry with:

• Simple techniques to assess the behaviour of sheep before they enter the dairy parlour, so that ewes with a suitable temperament can be selected from large flocks of sheep.

• Practical strategies for feeding dairy sheep to increase the health benefits of sheep milk products.

• Knowledge of the processing and marketing potential of these health enhanced dairy products.

• Apart from this report, the results have been or will be published in peer-reviewed journals and communicated through presentations at conferences.
3 - Methodology

3.1 - Location and animals

3.2 - Management of the animals

Housing and nutrition

During the experimental periods, animals were kept in communal paddocks at the Shenton Park Field Station of The University of Western Australia where they grazed irrigated pasture composed predominantly of kikuyu (*Pennisetum clandestinum*) and subterranean clover (*Trifolium subterraneum*) and had always meadow hay available to them. They also received up to 1kg of concentrate made up of lupins (392 g/kgDM protein, 21 MJ/kgDM energy) and 10% oats. During milking they were given approximately 300g of a mixture of 70% oaten chaff, 30% lupins, and 0.5% hi-cal and salt as contentment food. When experiments were completed and/or the lactation was concluded the sheep were returned to their respective properties of origin.

Lambs that were born to the experimental ewes were housed in an outdoor pen when separated from their mothers and were offered a mixture of 60% oaten chaff, 30% lucerne hay and 10% cracked lupins as creep feed.

Milking

A 12 bay rapid-exit milking platform purchased from Prattley (Temuka, NZ) with funds provided partly by the RIRDC and partly by The University of Western Australia was used to milk the sheep (Figure 3.2.1). Entry and exit ramps for the platform were built at the Combined Workshop of the Faculty of Natural and Agricultural Sciences at The University of Western Australia. When possible the sheep were fed on the platform a few weeks before lambing, so that they learnt that by walking onto the platform they received food. The sheep were milked twice a day with an Alfa Laval milking machine that had a pulsation rate of 120/min and vacuum pressure of 40 kPa. At the end of each milking the teats were disinfected with an iodine-based commercial preparation (Alfadyne Teat Sanitiser, Australia).
Figure 3.2.1. The milking platform at Shenton Park Research Station, The University of Western Australia, is a rapid exit parlour that allows a high throughput of ewes per hour. a) Sheep on the platform b) the feeder is lifted to allow the rapid exit of the sheep (c and d). Photographs by Craig Macfarlane.

3.3 - Temperament testing of the sheep

The temperaments of the ewes were tested using the ‘isolation box test’, which was developed to evaluate temperament in sheep by measuring the effects of isolation on their level of agitation (Murphy 1999, Blache and Ferguson 2005; Figure 3.2.2). During the ‘box’ test, sheep are placed inside a container (approximately 1m³), which sits on rubber mountings with a counter attached to its side. This agitation meter records the number of movements and vocalisations made by the sheep thus providing an independent measure of the level of nervousness of the animal due to isolation.
Figure 3.2.2. a) The isolation box used to test the temperament of sheep. A sheep is placed inside the box, which sits on rubber mountings. An agitation meter attached to its side gives a measure of the level of agitation of the animal by recording the number of movements and bleats that the sheep makes. b) A farmer conducts the test on a flock of sheep. The test only takes one minute to perform, so it has a good throughput of sheep. Photograph from Blache and Ferguson (2005).

Throughout a flock of sheep, temperament scores are normally distributed, so the results allowed the classification of animals by selecting the extremes to categorise our sheep into calm or nervous groups. This method has been used to select flocks of sheep for breeding at Allandale Research Farm (Murphy 1999). The box test was chosen in preference to the arena test as a measure of temperament. The arena test is a motivational choice test that measures the approach and/or avoidance behaviour of the sheep towards humans because, in comparison to isolation, the presence of a human is also a fear-eliciting situation in sheep.
(Vandenheede et al. 1998). In the arena test, the sheep are placed alone into an arena and their attraction to a group of sheep in a pen relative to their avoidance of a human standing within the arena is assessed (Kilgour 1998). The number of approaches or withdrawals of the tested animal from the person is combined with other objective measures such as vocalisations and defecations to produce a score (Murphy 1999). The arena test was not incorporated into this study because this procedure is more complicated and time consuming when compared to the isolation box test, and we wanted to use a method that could be of practical application for the sheep milking industry.

3.4 - Measurements of production and composition of milk

Milk production was measured with Tru Test milk meters (Tru Test Distributors, Auckland, New Zealand). These testers have a valve that diverts a proportion of the milk produced (in our case 58g/kg produced) into a plastic jar (Figure 3.2.3). The jar is then weighed and the weight is multiplied by a constant to calculate the daily production of milk.

Figure 3.2.3. Dr Anna Nudda (visiting scientist, University of Sassari, Italy) and relief milker Megan Meates measure the milk production of the sheep with the Tru Test milk meters. Photograph by Roberta Bencini.

Samples of milk were collected from the Tru Test jars, stored at 1-4°C and analysed within 24 hours with a Milko Scan 133 (Foss Electric, Denmark) calibrated for sheep milk. This is a single-beam infrared instrument, which measures the infrared absorption at wavelengths characteristic of the components to be analysed. When testing for fat concentration with the A filter, it measures the absorption at 5.73µ by the carbonyl group of the ester linkage. If the B filter is used it measures the absorption at 3.5µ by the –CH₂ groups. For protein it measures the absorption at 6.46µ by the amine II groups of the peptide bond and for lactose the absorption at 9.6µ by the hydroxyl group. The instrument automatically calculates total solids by adding protein, fat, lactose and a constant mineral bias of 0.79%.
3.5 - Processing of sheep milk dairy products

Experimental batches of sheep milk Fetta cheese were processed in our Dairy Products Laboratory. The following equipment was used for this project:

- Milko Scan 133 – described above, was used for analysis of milk composition before the milk was transformed into dairy products.

- Lattodinamografo or lactodynamograph (Foss Electric, Italy) - This instrument was used to measure the clotting properties of the milk. These were the renneting time, \( r \), or time it took for the milk to clot, the rate of curd formation, \( k_{20} \), which is the time it took for the clotting milk to reach a standard consistency of 20mm and the consistency of the curd, \( A_{30} \), which is the distance between the two arms of the lactodynamograph output (Bencini 2002).

- Batch pasteuriser - a laboratory batch pasteuriser of 30L capacity built at the workshop of the Faculty of Natural and Agricultural Sciences at The University of Western Australia. This was used to pasteurise the milk prior to processing at a temperature of 72°C for 20 seconds.

- Cheese vat - a stainless steel-jacketed cheese vat of 30L capacity also built at the workshop of the Faculty of Natural and Agricultural Sciences at The University of Western Australia. The vat had a variable speed paddle to mix the milk and temperature adjustment to maintain the milk at the desired temperature.

- Incubator - a thermostated incubator of approximately 80L capacity was used to keep dairy products at the desired temperature.

Other equipment used for the project included pH meter, balances, refrigerator (4°C) and freezer (-20°C).

For each experimental batch of cheese we recorded:

- Composition of the milk (protein, fat, lactose, total solids)

- Initial pH of the milk

- Amount of milk processed

- Clotting properties of the milk

- Processing procedures (e.g. amount of rennet added, type of starter culture used, time in cheese vat, etc.)

- Yield of dairy products from each litre of milk

Cheese taste panels - Semi-trained panels composed mainly of staff members of The University of Western Australia were used to assess experimental batches of cheese.

Tests for common pathogens (Escherichia coli, Coagulase Positive staphylococci, Salmonella and Listeria monoytogenes) were conducted on selected batches of cheese at the Food Hygiene Laboratory of the Western Australian Centre for Pathology and Medical Research (Path Centre) to ensure that the cheese was safe to consume.
3.6 - Determination of fatty acids profiles

Fatty acid profiles were determined at the laboratory of our collaborator Prof. Stefano Rapaccini at the University of Florence, Italy.

To determine the fatty acid profile of milk, samples of milk (2g) were extracted according to Secchiari et al. (2003). Then the extract was methylated with 2 ml of 0.5 mol/L sodium methoxide in methanol (10 min at 50°C). Fatty acids methyl esters (FAME) were extracted using n-hexane with C19:0 as internal standard, as described by Park et al. (2001) and by Kramer et al. (1997).

To separate and quantify the FAME we used a capillary column (Chromopack CP-Sil 88 Varian, Middelburg, the Netherlands: 100 m x 0.25 mm i.d.; film thickness 0.20 μm) and nonadecanoic acid (C19:0) methyl ester (Sigma Chemical Co., St. Louis, MO) as the internal standard. The injector and flame ionisation detector temperatures were respectively 270°C and 300°C. The programmed temperature was 40°C for 4 min, increased to 120°C at a rate of 10°C/min, maintained at 120°C for 1 min, increased to 180°C at a rate of 5°C/min, maintained at 180°C for 18 min, increased to 200°C at a rate of 2°C/min, maintained at 200°C for 1 min, increased to 230°C at a rate of 2°C/min and maintained at this last temperature for 19 min. The split ratio was 1:100 and Helium was the carrier gas with a flux of 1 ml/min.

Individual FAME were identified by comparison with the relative retention time of FAME peaks from samples, with the standards mixture 37 Component FAME Mix (Supelco, Bellefonte, PA). Individual trans9 C18:1, trans11 C18:1, trans12 C18:1, trans13 C18:1 (Supelco), CLA isomers mix standard (Sigma Chemical Co) and published isomeric profile were used to identify CLA and trans C18:1 isomers of interest. Since all methods using peak normalization and expressing results in relative percentage of the area of analysed peaks are subjected to an over-evaluation because areas of small peaks are not considered, the problem was avoided using nonadecanoic acid as the internal standard. All results concerning fatty acid composition are expressed as g/100g of fat.

3.7 - Statistical analyses

Measurements of behaviour were analysed using a generalised linear mixed model and observations measured with arbitrary units were analysed with a log linear model.

Least squares analysis of variance with repeated measures was used for statistical analysis of the milk output and composition in Genstat (Version 8 for Windows). Statistical differences were considered to be significant for all analyses at P < 0.05.

Regression analysis was used to establish relationships between milk composition and yields of dairy products.

Results of the fatty acids profiles were analysed by GML of SAS (1999) using a linear model with repeated measures diet and sampling time, diet x sampling time, ewe (diet):

\[ y_{ijk} = \mu + D_i + T_j + D_i \cdot T_j + E_k(D_i) + e_{ijk} \]

where \( y_{ijk} \) was the observation; \( \mu \) was the overall mean; \( D_i \) the diet (i = 1, 2, 3) and \( T_j \) the sampling time (j = 1 to 3), as fixed factor; \( H_i T_j \) the interaction between diet and sampling time; \( E_k \) the animal as random factor and \( e_{ijk} \) the residual error. For simplicity, only one level of probability (P<0.05) was adopted for the significance of differences between means.

All results are presented as means ± standard errors, unless otherwise indicated.
4 - Experimental

We conducted research work on the main areas identified in the introduction, namely:

• the nexus between sheep temperament, their behaviour in the dairy and their suitability as dairy animals;

• the development of behavioural tests that would allow the rapid identification of stock suitable for milking;

• the development of feeding strategies to increase the health benefits of sheep milk products;

• the processing properties of this health enhanced milk; and

• the acceptance of the derived dairy products by potential consumers.

We conducted a series of experiments to assess the temperament of specialised dairy ewes, of naïve dairy ewes that had not been previously milked, and also of the “temperament flock” Merino ewes, using both nervous and calm sheep that had been genetically selected for these traits.

We also conducted experiments aimed at producing milk with an increased level of unsaturated fatty acids by feeding dairy sheep with canola (either whole seed or in the ‘crimped’ form) and brewers’ grain, a relatively cheap byproduct of the brewing industry that would reduce the feeding costs of sheep dairy enterprises.

We then processed the milk produced by sheep that received these feeding treatments to test its suitability for cheese making and the acceptance of the cheese by potential consumers using semi trained panels. This work is described in detail in the following chapters.
4.1 - Relationship between temperament, behaviour and milk production in experienced dairy sheep

4.1.1 - Introduction

The profitability of sheep dairy enterprises could be increased either by increasing the amount of milk produced or by decreasing the time it takes to milk the ewes (Dawe and Dignand 1992, McKusick et al. 2002, Murray et al. 2009). Therefore, it would be useful for producers to devise tests to select ewes that are suitable for milking, both in terms of amount of milk produced and behaviour during milking as improving these traits would maximise the efficiency of production.

Because the milk ejection reflex, essential for maximal milk collection, is inhibited by stress via the release of catecholamines such as adrenaline and noradrenaline (Barowicz 1979, Silanikove et al. 2000), it is important that animals are not stressed at milking. If the milk ejection reflex does not occur only the cisternal fraction of the milk is removed at milking and the alveolar fraction is left behind as residual milk (Labussière 1988, Labussière et al. 1969, McKusick et al. 2002, Bruckmaier et al. 1997). Ewes that do not let down their alveolar milk produce less milk and their milk is also lower in fat because most of the milk fat is found in the alveolar tissue (Labussière et al. 1969, Marnet et al. 1998, McKusick et al. 2002).

While it would be reasonable to assume that the failure to let down the milk might be linked to the ewe’s temperament, little work had been done to test this hypothesis. For instance, Dimitrov-Ivanov and Djorbineva (2003) observed a significant relationship between temperament during milking and milk ejection, but they did not clarify if temperament assessed before milking was related to milk production and behaviour of the sheep once introduced to the milking routine.

We expected that dairy ewes classified as calm through temperament testing would have a lower stress response to machine milking, and therefore would produce more milk and of a better composition in a dairy environment.

To test this hypothesis, we assessed the temperament of specialised dairy ewes using the box test described in Chapter 3 (Blache and Ferguson 2005), and measured their production of milk, its composition and the behaviour of the sheep during milking.

4.1.2 - Materials and Methods

Ninety-five experienced dairy ewes in their second to fourth lactation were tested for their temperament using the isolation box test three weeks before parturition and then milked as described in Chapter 3 from approximately two weeks after lambing. The behaviour of the ewes was observed during milking twice weekly for seven weeks by an observer who had not been not involved in the temperament testing and did not know the results of the tests. This person gave a score to each ewe based on how easy it was to load and unload them on the milking platform. This was done using a scale ranging from 1 to 4, where (1) indicated that no encouragement was needed and (4) required the handler to physically walk the sheep onto the platform and force it into a stall. The behaviour of the sheep during milking was video recorded twice a week, once during a morning session and once in the afternoon, for seven weeks starting from the second week of lactation. From these recordings, the
same observer counted how many times each sheep kicked, removed the milking cups and the time taken to place the milking clusters onto each sheep’s udder.

Milk production was measured fortnightly over ten weeks as described in Chapter 3 and milk samples were analysed to determine their fat, protein and lactose concentrations using our Milko Scan 133, also as described in Chapter 3. The behaviour during milking, milk production and composition of the milk from the 16 most nervous and the 16 calmest ewes were analysed with repeated measures using the generalised linear mixed model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + e_{ijk}$$

where $Y_{ijk}$ was the $Y_{ijk}$th observation, $\mu$ was the general mean, $\alpha_i$ was the fixed effect of lactation week, $\beta_j$ was the fixed effect of temperament (calm/nervous), $(\alpha\beta)_{ij}$ was the interactions between the fixed effects and $e_{ijk}$ was the residual error $\sim N(0,\sigma^2_e)$.

Parameters measured with arbitrary units were analysed with the log linear model:

$$\ln(F_{ij}) = \mu + \lambda_i^A + \lambda_j^B + \lambda_{ij}^{AB}$$

where $\ln(F_{ij})$ was the log of the expected cell frequency of the cases for cell $ij$ in the contingency table, $\mu$ was the overall mean of the natural log of the expected frequencies, $\lambda_i^A$ the main effect for temperament, $\lambda_j^B$ the main effect for time and $\lambda_{ij}^{AB}$ was the interaction effect.

To compare the milk output and composition we used repeated measures ANOVA in Genstat (9th Edition, VSN International Ltd, United Kingdom).

### 4.1.3 - Results

The temperament scores of the ewes ranged from 16 to 267. The temperament scores of the 16 most calm and 16 most nervous ewes selected for this experiment were at least two standard deviations away from the mean score of the flock and were statistically different (calm: $44.9 \pm 3.64$, nervous: $193.5 \pm 5.25$; $P < 0.05$; Table 4.1.1).

The nervous sheep were harder to load on the platform with an overall average score of $1.55 \pm 0.2$ compared to the average of $1.29 \pm 0.16$ for the calm ewes, although this difference was not significant ($P = 0.15$, Table 4.1.1). This relationship was stronger during the first week of the experiment when the sheep were getting used to the procedure ($P = 0.08$).
Table 4.1.1. Behavioural scores and traits of ewes of calm (n=16) or nervous (n=16) temperaments during milking (mean ± standard error).

<table>
<thead>
<tr>
<th></th>
<th>Calm</th>
<th>Nervous</th>
<th>Significance (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperament score</td>
<td>44.9 ± 3.64</td>
<td>193.5 ± 5.25</td>
<td>&lt; 0.005</td>
</tr>
<tr>
<td>Loading score</td>
<td>1.29 ± 0.049</td>
<td>1.55 ± 0.063</td>
<td>0.15</td>
</tr>
<tr>
<td>Time to attach cups (s)</td>
<td>4.28 ± 0.138</td>
<td>4.72 ± 0.135</td>
<td>0.29</td>
</tr>
<tr>
<td>Number of kicks</td>
<td>1.66 ± 0.234</td>
<td>1.12 ± 0.092</td>
<td>&gt; 0.05</td>
</tr>
<tr>
<td>Unloading score</td>
<td>1.10 ± 0.028</td>
<td>1.02 ± 0.013</td>
<td>&gt; 0.05</td>
</tr>
</tbody>
</table>

There were little and non significant differences in the behaviour of the two temperament groups during milking and average scores for loading and unloading, the time taken to attach the milking cups and the number of kicks were similar for the calm and nervous sheep (P > 0.05, Table 4.1.1).

Although it was more difficult and it took longer for the milkers to place the milking clusters on the nervous ewes (calm: 4.28 ± 0.44 seconds, nervous: 4.72 ± 0.46 seconds), this difference failed to reach significance (P = 0.29, Table 4.1.1).

There was a significant interaction between time and temperament on milk production over the 10 weeks of recording indicating that the evolution of milk production over time was different for the two groups (P < 0.05).

While not statistically significantly different, calm ewes produced on average 462 ± 36g of milk per day and nervous ewes produced 394 ± 33g of milk per day (Table 4.1.2).

There was no effect of temperament or time on the concentration of fat or protein in the milk (P > 0.05), but the concentrations of fat and protein increased over time in both calm and nervous ewes (Table 4.1.2).
Table 4.1.2. Daily milk production (g, mean ± standard error) and fat and protein concentrations in the milk (g/100g ± standard error) from calm (n=16) and nervous (n=16) experienced dairy ewes over the first 10 weeks of lactation.

<table>
<thead>
<tr>
<th>Week of lactation</th>
<th>Milk production (g/day)</th>
<th>Fat (g/100g)</th>
<th>Protein (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calm</td>
<td>Nervous</td>
<td>Calm</td>
</tr>
<tr>
<td>2</td>
<td>250±33</td>
<td>377±56</td>
<td>3.7±0.3</td>
</tr>
<tr>
<td>4</td>
<td>657±105</td>
<td>434±52</td>
<td>5.7±0.6</td>
</tr>
<tr>
<td>6</td>
<td>301±67</td>
<td>171±45</td>
<td>6.4±0.5</td>
</tr>
<tr>
<td>8</td>
<td>581±70</td>
<td>549±93</td>
<td>7.0±0.3</td>
</tr>
<tr>
<td>10</td>
<td>527±53</td>
<td>532±82</td>
<td>7.3±0.3</td>
</tr>
</tbody>
</table>

|                  | P<0.001     | P<0.001     | P<0.001     |
| Time             | P=0.421     | P=0.991     | P=0.536     |
| Temperament      | P=0.043     | P=0.892     | P=0.917     |
| Time x Temperament |             |             |             |

4.1.4 - Discussion

Experienced dairy ewes that had a calmer temperament produced more milk than ewes with a more nervous temperament but only during weeks four, six and eight of lactation, demonstrating a large variability in the daily amount of milk produced. This is consistent with previous observations that milk production in sheep is very variable (Bencini 1999, Bencini and Agboola 2003, Bencini 2005).

The difference between the two temperament groups might be explained by a difference in their stress reactions to the milking procedure. In nervous ewes, the stress of milking could have induced a release of adrenaline that inhibited the release and action of oxytocin, and therefore effectively prevented the milk ejection reflex (Barowicz 1979). Alternatively the calmer ewes might have directed their energy to milk production while the nervous ewes would have allocated part of their energy to their stress response to machine milking. Differences in resource allocation under stress have been proposed to be an evolutionary mechanism, mainly in response to immune challenges, but also to stress induced by predators (McNamara and Buchanan 2005, Parsons 2005).

The temperament of the sheep did not affect the composition of the milk or their behaviour during milking because the average scores for loading and unloading, the time taken to attach the milking cups and the number of kicks were similar for the calm and nervous sheep. Additionally, although it was more difficult and it took longer for the milkers to place the milking clusters on the nervous ewes, the difference between calm and nervous sheep failed to reach significance.

The influence of temperament on behaviour might have been masked by the process of habituation of the ewes and their recognition that the milking procedure was harmless. The careful handling of animals during the adaptation period combined with the positive reinforcement of the food offered on the milking platform possibly resulted in any differences in behaviour being noticeable only when animals were completely novel to the
procedure, or as in the case of this experiment, when they were initially re-introduced to milking for a subsequent lactation.

The nervous ewes were more difficult to load onto the milking platform only in the early stages of the experiment, when more intervention was required when they were entering the milking platform. Loading onto the platform can be a highly stressful procedure for both the animals and handlers when the ewes have not been through the procedure before, or at the beginning of a new lactation when the procedure is still relatively novel (Van Reenen et al. 2002). The ewes used in this experiment were dairy animals that had experienced the milking procedure in previous lactations. However, it is possible that their temperament influenced their ability to cope with the stress associated with milking. As the ewes were milked daily, this enabled them to habituate to the painless milking procedure that had previously induced a stress response (Reid and Mills 1962). During this time of habituation the ewes became familiar with the parlour and learnt that they would get a food reward once on the platform. This would likely have reduced their stress so that they were more relaxed when they entered the platform at the next milking (Dawe and Dignand 1992).

Clearly training is a powerful medium for decreasing stress and improving the behaviour during milking in ewes. However, being a learnt behaviour, it needs to be implemented at every generation and on each new individual, while a genetic solution like selecting animals based on temperament would result in a long-term effect that could help to further enhance the positive influence of training. These behavioural observations, combined with the fact that on average nervous dairy ewes needed more time for the cups to be placed on their udders, indicate that temperament plays a role in the efficiency of the milking operations. The calm ewes had the lowest loading scores and the shortest time taken to attach the milking cups. This would translate into significant advantages if the time saved for each individual sheep being milked was multiplied by the number of sheep milked and the number of milkings per year that occur on a sheep dairy farm. The willingness of the ewes to be milked would also reduce the stress for both the animals and their handlers, which would then result in an improvement in animal welfare as well as working conditions.

The temperament of the calm ewes did not result in better behaviour during milking over the whole milking period in this experiment. Although this might seem surprising, it could be due to the fact that the ewes had been milked in previous lactations, and any sheep that displayed unacceptable behaviours had already been removed from the flock. For these animals milking was also only a change to their daily routine, but it was not fully novel because the sheep had been milked in previous lactations. Our ewes may have been initially frightened when re-introduced to the milking routine, but soon after they would have recognised the procedure as painless and possibly rewarding due to the frequent positive contact with the milking staff and the food that was offered during milking. It is very likely that improved temperament may improve learning capacity, as sheep are able to remember situations for extensive periods of time (Kendrick et al. 2001) and the perception of stress can influence the ability of an animal to retain information (Diamond et al. 1996). In fact, the calm ewes displayed similar behaviours over the whole period of milking suggesting that they remembered their previous experiences with the milking procedure. In support of this, we have personally observed that sheep remembered the location of a feed bin and how to remove its lid upon returning to the field station after a period of six months on the industry partner’s farm.

In addition, the nervous ewes used in this experiment may not be considered “truly” nervous because they had not been genetically selected on their temperament and they been handled extensively before. Long-term periods of handling can improve temperament scores and change the animal’s response to stressful situations (Boissy and Bouissou 1995). Our dairy ewes might have a reduced fearfulness of humans because they were previously
exposed to the intensive milking environment where the interaction between animal and handler was frequent, as in all dairy systems.

The hypothesis that calm ewes would produce more milk than their nervous counterparts was not fully supported, but the evolution of milk over time was different between temperaments. It appears that the production of milk, which can be affected by factors such as nutrition (Haenlein 1996), breed and age (Bencini and Pulina 1997), can also be influenced by temperament. The physiological mechanism by which temperament affects milk production is still unclear. In this experiment we did not test if the milk ejection reflex was affected by temperament, because we did not have electronic milk meters that would have allowed us to plot milk flow over time during each milking to detect the presence of a double peak of milk ejection. However, the fact that milk production and fat concentration were not higher in calm sheep may be a consequence of the partial separation of the ewes from their lambs during the early stages of lactation. In mixed management systems, where ewes are milked while lambs are also allowed to suckle at night, only the cisternal fraction of milk is obtained during the machine milking of the ewes (McKusick et al. 2002, Marnet and McKusick 2001, Marnet and Negrao 2000) because milk ejection does not occur while the ewes remain in partial contact with their lambs (McKusick et al. 2002). This may have resulted in a confounding effect because effectively the ewes may not have ejected their milk due to the fact that they were nursing their lambs instead of the stresses associated with milking.

In conclusion, the selection on temperament, using a simple one-minute behavioural test is a practical, heritable and repeatable technique. Other studies have assessed temperament during milking (Dimitrov-Ivanov and Djorbieva 2003), but this offers little to producers who want to eliminate poor producers and flighty animals before they actually start milking them. The fundamental application of this work was to predict behaviour and production before milking, in order to offer producers a means of selecting for temperament to improve these traits. As a result, the ‘isolation box test’ may offer producers a means of selecting sheep more likely to yield more milk throughout their lactation by potentially reducing their perceived stress and hence improving welfare, and this trait could be included when breeding or culling dairy sheep.

Our results indicate that there is need for further research to eliminate the effects of experience on behaviour and the maternal bond on milk ejection. The behaviour of inexperienced ewes with no previous experience in a milking environment should be investigated to determine the role of temperament in modulating the response of sheep to novel environments and the rate at which they adapt and habituate to milking. This was investigated in our next experiment, described in Chapter 4.2.
4.2 – Genetic selection for temperament results in sheep that are easier to milk

4.2.1 - Introduction

Milking can be a highly stressful procedure and animals may fail to meet their genetic potential because of an inability to cope with the dairy environment (Dobson and Smith 2000). This is particularly evident in ewes that are still inexperienced and not yet familiar with the milking routine. The dairy is a noisy unfamiliar place and animals are required to do unnatural things such as entering the parlour and finding a place in the stalls. Stalls that immobilise the heads of the sheep can be even more stressful and yet they are commonly used in the Mediterranean countries. As a result, when sheep are first introduced to the milking procedure they are initially reluctant to be milked and they may display undesirable behaviours. While most animals will gradually habituate to the procedure over time (Reid and Mills 1962), a small number of ewes never habituate and have to be removed from the flock because they could potentially injure themselves, their flock mates, or their handlers (Dawe and Dignand 1992).

The selection of ewes based on their temperament, defined as the reactivity of an animal in response to humans or novel environments (Murphy 1999), has the potential to provide producers with ewes that are easier to manage. The temperament of an animal interacts with other biological and environmental factors to alter the probability that a given behavioural outcome will occur (Lyons et al. 1988), and under uniform conditions, it is reasonable to expect that ewes selected for a calm temperament will be more manageable in the dairy and will be able to adjust to the milking procedure more rapidly.

At The University of Western Australia’s Allandale Research Farm, Merino ewes have been genetically selected for either calm or nervous temperaments over eighteen generations using the isolation box test and the arena test, two methods of temperament assessment that can be used in selection to influence the sensitivity of sheep to potential stressors and produce differences in their behaviour (Murphy 1999, Gelez et al. 2003). When introduced to a dairying system, ewes selected for calm or nervous temperament should display behavioural differences during the introductory period of milking and provide evidence that selection on temperament can improve the efficiency of the milking operations, while reducing the amount of stress imposed on the animals. Therefore we expected ewes of calm temperament to be easier to load and unload from the milking platform, to kick less during milking and forcefully remove their milking cups less often, to require less time to place the milking clusters onto their udders, and in general, to be easier to manage compared to the nervous sheep. This is a direct result of stress impairing learning in nervous ewes, particularly hippocampal dependant forms of learning such as spatial memory tasks (Diamond et al. 1996). Therefore, nervous animals would be more likely to make negative judgements about events such as milking and interpret this novel stimulus to be adverse.

As nervous ewes are more likely to experience stress during milking, and hence release hormones that inhibit the milk ejection reflex (Silanikove et al. 2000), we also expected that calm ewes would produce more milk and the evolution of milk yield would be different between temperaments (Murray et al. 2009).

The blockage of the milk ejection reflex reduces milk production as it allows only the removal of the cisternal milk. As the high fat alveolar fraction remains as residual milk within the udder the milk collected is also lower in fat (McKusick et al. 2002, Bruckmaier et al. 1997). This residual milk left in the udder also inhibits further milk secretion and
ultimately this results in shorter lactations, which further reduce the productive output of the sheep (Bencini et al. 2003).

To test these hypotheses we investigated whether Merino ewes that had been genetically selected for calm temperament would habituate to the milking procedure faster and be easier to manage than ewes selected for nervous temperament by observing their behaviour once they were introduced to the milking routine. We also measured the production and composition of the milk to test whether calm ewes would produce more milk and/or milk with a higher fat concentration.

4.2.2 - Materials and Methods

Thirty-five twin-bearing Merino ewes genetically selected for calm (23) and nervous (12) temperaments with the box and arena behavioural tests over eighteen generations (Murphy 1999) were introduced to the milking procedure for the first time approximately two weeks after lambing. They were milked once a day and their behaviour during machine milking was video recorded for the first twelve consecutive days of milking to document the specific behaviours of the ewes when milked. Milk production and its composition were also measured weekly for the first six weeks of milking.

The ewes had never been milked before and were in their second lactation. They were pregnancy tested as bearing twins by ultrasound at approximately sixty days of pregnancy. Before lambing, ewes were confined in single, straw laden pens within a sheep shed. After lambing they were moved out into communal paddocks where the same pasture and feed were available as described in Chapter 3.

The ewes were milked in a mixed management system, whereby they were separated from their lambs in the morning (06:30) and were rejoined with them after the afternoon milking and allowed to nurse their lambs at night (15:30). During the separation lambs were kept in an outdoor pen and provided with creep feed consisting of milk replacement pellets (Independent Lab Services: 24% crude protein, 12MJ ME/kg/DM) and long fibre hay. The ewes were milked once a day for the first two weeks of lactation, and twice daily thereafter.

The same behaviours were observed as in the previous experiment. However, the behaviour of the ewes was also videorecorded on the first twelve consecutive days of milking. From the footage we recorded the occurrence of kicks, deliberate removal of the milking cups, stamping and the time taken to attach the milking clusters to the teats. An objective score of the difficulty to load and unload each ewe from the platform was also recorded as in the previous experiment.

The milk production of individual sheep was measured weekly over the first six weeks of milking. Milk samples, preserved with 10% potassium dichromate, were also analysed weekly to determine their fat, protein and lactose concentrations.

The measurements of behaviour were analysed with repeated measures using a generalised linear mixed model and observations measured with arbitrary units were analysed with a log linear model. Least squares analysis of variance with repeated measures was used for statistical analysis of the milk output and composition in Genstat (9th Edition, VSN International Ltd, United Kingdom). All analyses included temperament as an independent variable and statistical differences were considered to be significant for all analyses at P<0.05.
4.2.3 - Results

For the first three days the calm ewes were easier to load onto the platform but overall there was no difference between the two temperament lines as the difference disappeared over time (C= 1.66±0.06, N= 1.81±0.12; Figure 4.2.1).

![Graph showing platform loading and unloading scores](Figure 4.2.1. Average platform loading (top) and unloading scores (bottom) of inexperienced Merino ewes genetically selected for calm (white, n = 23) or nervous (black, n = 12) temperament. The scores are objective arbitrary units.)

The nervous ewes were also more difficult to remove from the milking platform (C= 1.24±0.10, N=1.51±0.24; objective arbitrary unit. P<0.001, Figure 4.2.1).

Calm ewes also required less intervention by the handlers during milking and it took significantly less time (seconds) to place the milking cups on their teats (C= 5.94±0.23, N=6.80±0.24; P<0.05, Figure 4.2.2 a).
Figure 4.2.2. a) Average time taken (seconds) to place milking clusters on the teats of inexperienced Merino ewes genetically selected for calm (white, n = 23) or nervous (black, n = 12) temperament.

b) Average number of kicks during milking of inexperienced dairy ewes genetically selected for calm (white, n = 23) or nervous (black, n = 12) temperament.

Although the nervous sheep kicked more during milking, the difference in the number of kicks between lines was not significant overall (C = 1.29 ± 0.10, N = 1.60 ± 0.12; P > 0.05, Figure 4.2.2 b).

The calm sheep also produced more milk but this difference was not significant. Over the six weeks of milk collection and analysis calm ewes only produced an average of 208g ± 26.9 per day, while nervous produced an overall average of 194g ± 37.1 per day (P > 0.05, Table 4.2.1). There was also no difference in the concentrations of fat, protein or lactose observed in the milk of calm and nervous ewes (P > 0.05, Table 4.2.1).
Table 4.2.1. Average milk production (g/day), fat, protein and lactose concentrations of milk produced by ewes genetically selected for calm or nervous temperament during the first six weeks of lactation.

<table>
<thead>
<tr>
<th>Week of Lactation</th>
<th>Milk Production (g/day)</th>
<th>Fat (g/100g)</th>
<th>Protein (g/100g)</th>
<th>Lactose (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Calm</td>
<td>Nervous</td>
<td>Calm</td>
<td>Nervous</td>
</tr>
<tr>
<td>1</td>
<td>155±13</td>
<td>122±16</td>
<td>3.06±0.22</td>
<td>3.07±0.30</td>
</tr>
<tr>
<td>2</td>
<td>149±17</td>
<td>126±13</td>
<td>3.53±0.29</td>
<td>3.54±0.22</td>
</tr>
<tr>
<td>3</td>
<td>274±36</td>
<td>243±43</td>
<td>5.65±0.37</td>
<td>5.41±0.38</td>
</tr>
<tr>
<td>4</td>
<td>230±29</td>
<td>201±31</td>
<td>6.20±0.46</td>
<td>5.88±0.53</td>
</tr>
<tr>
<td>5</td>
<td>176±36</td>
<td>193±31</td>
<td>6.84±0.47</td>
<td>6.59±0.51</td>
</tr>
<tr>
<td>6</td>
<td>262±30</td>
<td>253±39</td>
<td>5.41±0.37</td>
<td>5.31±0.48</td>
</tr>
</tbody>
</table>

4.2.4 - Discussion

The results of this experiment supported our hypothesis that inexperienced ewes that had been selected for calm temperament would be easier to milk than their nervous counterparts. Our results show that ewes of calm temperament were significantly easier to load during days 1 to 3 of recording and to unload from the platform throughout the experimental period. It also took significantly less time for the milkers to place the milking cups upon their teats. This indicates that nervous ewes were possibly more stressed by the milking routine and this resulted in more undesirable behaviours.

The improvement of these aspects of sheep handling within dairying systems would result in a reduction in the time and effort required to train the animals, and would therefore result in improved animal welfare. Sheep genetically selected for temperament behave similarly under normal conditions, but once a stressor is applied their responses to that challenge are different. This suggests that the perception of stress by the animal is strongly influenced by an innate component and that this plays an important role in moderating the manner in which the sheep respond to a challenge (Blache and Bickell 2010).

Our results suggest that the milking procedure is, in fact, stressful to ewes, particularly during the initial phase of learning and habituation. Stress during milking is difficult to measure because blood sampling to measure cortisol, the major hormone that dramatically increases due to stress (Beausoleil et al. 2008), can induce stress and confound results.

In this experiment it appears that the ewes were habituating to the procedure with repeated exposure as the time required to attach the cups gradually decreased over the experimental period. The amount of time required to attach the milking cups onto the teats depends on the reaction of the animal to handling within the milking stall and we expected that the seconds required would decrease over time as the ewes habituated to the procedure and became more accepting of the milking cups. Through positive reinforcement ewes gradually habituated to milking and their stress response was reduced with time. By the end of recording both calm and nervous ewes had made improvements in the amount of time it took for milkers to place the milking cups on their teats and their loading scores (Figure 4.2.2a). Even though the ewes habituated and showed improved behaviour within
approximately three days, the first 3-4 days are important because during this time animals that misbehave can cause injuries to the milkers, to themselves or to other sheep. If milking flocks are large the delays caused by animals that misbehave can result in long shifts and fatigued workers. Throughout our four research projects we have personally witnessed several instances were animals fell off the platform and on two instances sheep had their legs broken. On several occasions our milkers had their wrists kicked or stamped on.

The nervous ewes also did not kick more than the calm ones at the beginning, but gave more kicks at the end of the monitoring period. This could be due to an initial “freeze” response to the novel procedure resulting in calm and nervous sheep behaving similarly at the beginning (Schulkin et al. 2005).

During the initial period, when the ewes were new to milking, they needed to learn the routine and become aware of the relative harmlessness of entering the platform and being milked. We expected that improved temperament would increase learning capacity as the perception of stress can influence the ability of an animal to retain information (Diamond et al. 1996). However, this was not observed in this case as both calm and nervous sheep appeared to habituate overtime to the milking routine.

This study did not identify a relationship between milk production or its composition with the temperament of the ewes. This is most likely due to the use of low producing ewes in this experiment given that there is a negative relationship between milk yield and its composition (Bencini and Pulina 1997). These ewes were sourced from the genetically selected flocks of calm and nervous sheep bred at The University of Western Australia. All these ewes were Merinos, a sheep selected for wool rather than milk production (Bencini et al. 1992), and as a result their milk production was extremely low and hence observing differences in milk output and its composition between groups was unsuccessful.

It is possible that the ewes were not only producing minimal milk, but also failed to eject their milk during machine milking, regardless of temperament, because they were allowed to nurse their lambs. Milk ejection may not occur while the ewes remain in partial contact with their lambs, and oxytocin release during machine milking may not have occurred (McKusick et al. 2002). This would have resulted in a failure to let down the milk and in the accumulation of the alveolar milk fraction within the udder due to the removal of only the cisternal fraction of the milk (McKusick et al. 2002, Marnet and McKusick 2001, Marnet and Negrao 2000).

Our results for milk composition support this, as the concentration of fat in the milk was about half of its normal values at the first two recordings and then rose to normal values towards the end of the study and corresponded to a significant increase in the daily production of milk.

In conclusion, our results suggest that the genetic selection of ewes based on their temperament can positively influence their behaviour and ability to cope with the stress associated with the novelty of the milking system. This should, in turn, result in time saving for sheep milk producers and an improvement in welfare for the animals.
4.3 – Whole or cramped canola as a food for dairy sheep

4.3.1 - Introduction

Over the last decade there has been rapidly mounting interest in the metabolism and biological activities of conjugated linoleic acids (CLA), which are positional and geometric isomers of linoleic acid (18:2). There are two volumes dedicated to advances in CLA research and its analytical methods (Yurawecz et al. 1999, Sebedio et al. 2003). There are several review articles on the general biological activity of CLA (Parodi 1994, 1999, Pariza et al. 2001) and its specific role in milk fat reduction (Bauman and Grinari 2003, Grinari and Bauman, 2003).

The isomer of CLA abundantly found in sheep milk (cis9, trans11-Octadecadienoic acid; 18:2c9, t11 CLA; Parodi 1994) has been reported to have beneficial properties for human health (Nudda et al. 2004). It has been shown to be a suppressant of tumour growth in human cancer tissues (Lavillonniere and Bougnoux 1999, Pariza et al. 2001) and chemically induced tumours in laboratory animals (Ip et al. 1991, Lavillonniere and Bougnoux 1999).

In previous research we demonstrated that the concentration of CLA in the milk of sheep can be increased by feeding them sunflower oil (Hutton 2003).

There are also claims for the health benefits of consuming the n-3 polyunsaturated fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). These include reduced risk of cardiovascular disease (Sheard 1998), enhanced development of brain and visual acuity in infants (Hoffman et al. 1993), modulation of autoimmunity (Calder 1998, Grimble 1998), and modulation of inflammatory disorders (Kinsella 1986, 1987, Simopoulos 1991, Calder, 1998). The cardio-protective role of EPA and DHA is of major significance, as cardiovascular disorder is one of the most important causes of death in the developed nations. However, the consumption of n-3 fatty acids in most developed nations is less than the recommended daily intake of 650 mg (Simopoulos et al. 1991). Part of the problem is likely to be that n-3 PUFA are not abundant in food items that are traditionally consumed daily in developed nations, i.e. meat and milk from ruminants.

Various avenues have been pursued to increase the levels of n-3 PUFA in the western diet. For instance, many forms of vegetable oils and dairy spreads of high polyunsaturated fatty acid content, but not necessarily of high n-3 content, have entered the food market in several countries. Previously our studies focused on production of n-3 PUFA-enriched milk of goats (Kitessa et al. 2001) and sheep (Kitessa et al. 2003) through supplementation of protected tuna oil. This approach aims to value-add to milk while retaining its other nutritional and health benefits. This approach also provides an option for natural incorporation of EPA and DHA into dairy products as opposed to post-harvest chemical manipulations that are less acceptable to consumers. Although the results of those studies were promising, it is unlikely that farmers would spend the time and money to mix sunflower oil into a pelleted diet or molasses and fishmeal to feed to their sheep.

We were interested in other potential sources of beneficial compounds that would help the commercialisation of dairy products made with sheep milk as products beneficial for human health. If we could develop simple and cheap feeding techniques, for instance based on relatively inexpensive feedstuff such as canola or even by-products such as brewers’ grain, to increase the concentrations of these beneficial compounds in the milk, then it would be possible to commercialise the milk and derived dairy products for their health benefits.
Canola may provide these benefits because it contains linoleic acid that can be transformed in the rumen to produce Conjugated Linoleic Acids (CLA). The main source of CLA for humans is from ruminant milk and meat. Ruminants produce CLA through ruminal biohydrogenation of linoleic acid and through endogenous synthesis by the enzyme Delta(9)-desaturase. During the biohydrogenation process linoleic acid from the feed is hydrogenated to CLA. This CLA can be absorbed in the small intestine and be subsequently incorporated into milk and meat or it can be further hydrogenated to trans vaccenic acid (TVA). TVA is important for the endogenous synthesis of CLA. TVA is either absorbed in the small intestine and then incorporated into tissue and converted to CLA by the Δ°9 desaturase enzyme or it is further hydrogenated to stearic acid (Bauman and Griinari 2003).

The production of CLA in ruminants is not sufficient to result in products that will produce health benefits for consumers. Banni and Martin (1998) reported that an average person would need to consume 3.5g of CLA per day to enjoy the health benefits, and this equates to 3kg of cheddar cheese. Sheep produce milk with the highest amount of fat, 6-7% compared to 3.6% in cow’s milk (Bencini 1999), and the highest concentration of CLA, approximately 27 mg compared to about 7 mg in cow’s milk (Banni and Martin 1998). By eating cheese made from sheep’s milk people would need to consume 1.5kg of cheese, which is still too much for a person to consume on a daily basis. This has sparked an interest in sheep because by increasing the amount of linoleic acid fed to the animal, the biohydrogenation process in the rumen could be manipulated to increase the concentration of CLA in the milk.

Canola provides a source of linoleic acid that can be fed to ruminants. There are two types of canola that could be used for this purpose, whole canola and crimped canola. Whole canola contains 100% whole seeds while crimped canola contains 43% whole seeds and 57% cracked seeds. Crimped canola was used in experimental diets at Vasse Research Station, in the south west of WA, in an attempt to increase the concentration of unsaturated fatty acids in cow’s milk (Debski 2004). That study only used crimped canola because it was thought that the whole seeds were too small to be digested by cows. However, as sheep are smaller than cows they might be able to chew and digest smaller feed particles than cows and could be capable of digesting whole canola seeds. A short term feeding experiment conducted by one of our students (Pugh 2004) confirmed that sheep were able to digest whole canola seeds. That study also showed that feeding canola, crimped or whole, to dairy sheep for only seven days did not affect milk production or its composition.

In this experiment we examined whether whole and crimped canola could be fed for longer periods and used to increase the concentration of CLA in sheep’s milk. We expected that crimped canola would increase the concentration of CLA in sheep’s milk more than whole canola because in the crimped canola the seeds are more available for digestion by the rumen microbes.

4.3.2 - Materials and methods

Our flock of dairy sheep was split into three groups of 21 sheep each stratified for breed, stage of lactation, production of milk, parity and number of lambs in order to have three comparable groups. The sheep grazed in the communal paddocks described in Chapter 3 and also had meadow hay constantly available to them. In addition, one group (control) was fed the concentrates usually given to our sheep, consisting of up to 1kg of lupins plus 10% oats/head/day. The other two groups were fed the treatment diets containing either whole or crimped canola. The amount of canola to be included in the diet to replace some of the lupins was calculated so that the diets remained isoenergetic. To ensure that we studied the long-term effects of these diets the feeding continued for 6 weeks.
At fortnightly intervals we tested the milk production of the sheep and collected milk samples to analyse for fat, protein, lactose, total solids and for its profile in unsaturated fatty acids (including Omega-3 and CLA).

Samples of milk were analysed for their content of unsaturated fatty acids at the University of Florence by our Italian collaborator, Prof Stefano Rapaccini, following the methodology reported in the methods section. However, due to a shortage of funding our collaborators decided to analyse samples from only nine sheep per group instead of the original 21 and to conduct the analyses only for three of the sampling weeks.

4.3.3 - Results

There were non-significant differences between the production of milk and the milk composition for the three groups (Table 4.3.1) and only minimal differences in the fatty acids profile (Table 4.3.2). There was, however, a significant decrease in the concentration of cis9, trans11 CLA in the milk of sheep fed the crimped canola (Table 4.3.3).
Table 4.3.1. Milk production and composition (protein, fat and lactose) of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9).

### Milk Production (g/day)

<table>
<thead>
<tr>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/9/05</td>
<td>609.1</td>
<td>553.31</td>
<td>506.35</td>
<td>78.06</td>
</tr>
<tr>
<td>23/9/05</td>
<td>592.3</td>
<td>502.69</td>
<td>467.69</td>
<td>78.06</td>
</tr>
<tr>
<td>7/10/05</td>
<td>625.6</td>
<td>543.72</td>
<td>460.87</td>
<td>78.06</td>
</tr>
</tbody>
</table>

### Protein (g/100g)

<table>
<thead>
<tr>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/9/05</td>
<td>5.65</td>
<td>5.99</td>
<td>5.57</td>
<td>0.20</td>
</tr>
<tr>
<td>23/9/05</td>
<td>5.45</td>
<td>5.89</td>
<td>5.33</td>
<td>0.20</td>
</tr>
<tr>
<td>7/10/05</td>
<td>5.96</td>
<td>6.09</td>
<td>5.52</td>
<td>0.20</td>
</tr>
</tbody>
</table>

### Fat (g/100g)

<table>
<thead>
<tr>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/9/05</td>
<td>7.78</td>
<td>7.42</td>
<td>7.78</td>
<td>0.42</td>
</tr>
<tr>
<td>23/9/05</td>
<td>7.43</td>
<td>8.02</td>
<td>7.78</td>
<td>0.42</td>
</tr>
<tr>
<td>7/10/05</td>
<td>6.95</td>
<td>7.64</td>
<td>7.34</td>
<td>0.42</td>
</tr>
</tbody>
</table>

### Lactose (g/100g)

<table>
<thead>
<tr>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>9/9/05</td>
<td>4.82</td>
<td>4.68</td>
<td>4.69</td>
<td>0.09</td>
</tr>
<tr>
<td>23/9/05</td>
<td>4.73</td>
<td>4.70</td>
<td>4.54</td>
<td>0.09</td>
</tr>
<tr>
<td>7/10/05</td>
<td>4.79</td>
<td>4.71</td>
<td>4.78</td>
<td>0.09</td>
</tr>
</tbody>
</table>
Table 4.3.2. Fatty acids composition in the milk of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9). Means with different superscripts were significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Date</th>
<th>9/9/05</th>
<th></th>
<th></th>
<th></th>
<th>23/9/05</th>
<th></th>
<th></th>
<th>7/10/05</th>
<th></th>
<th></th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Whole canola</td>
<td>Crimped canola</td>
<td>SEM</td>
<td>Control</td>
<td>Whole canola</td>
<td>Crimped canola</td>
<td>SEM</td>
<td>Control</td>
<td>Whole canola</td>
<td>Crimped canola</td>
</tr>
<tr>
<td>C6</td>
<td>0.58 a</td>
<td>2.57 b</td>
<td>1.40 c</td>
<td>0.32</td>
<td>2.90 a</td>
<td>2.58 a</td>
<td>1.07 b</td>
<td>0.44</td>
<td>1.07 a</td>
<td>1.40 a</td>
<td>2.08 b</td>
</tr>
<tr>
<td>C8</td>
<td>0.97</td>
<td>0.11</td>
<td>0.12</td>
<td>0.10</td>
<td>1.15</td>
<td>1.05</td>
<td>1.00</td>
<td>0.11</td>
<td>1.08</td>
<td>1.04</td>
<td>1.07</td>
</tr>
<tr>
<td>C10</td>
<td>3.12 a</td>
<td>3.62 a</td>
<td>3.90 b</td>
<td>0.26</td>
<td>3.80</td>
<td>3.50</td>
<td>3.33</td>
<td>0.44</td>
<td>3.72</td>
<td>3.51</td>
<td>3.40</td>
</tr>
<tr>
<td>C11</td>
<td>1.14</td>
<td>1.19</td>
<td>1.18</td>
<td>0.02</td>
<td>0.19</td>
<td>0.16</td>
<td>0.15</td>
<td>0.02</td>
<td>1.18</td>
<td>1.15</td>
<td>1.17</td>
</tr>
<tr>
<td>C12</td>
<td>1.74 a</td>
<td>2.47 b</td>
<td>2.48 b</td>
<td>0.24</td>
<td>2.43 a</td>
<td>2.24 ab</td>
<td>2.03 b</td>
<td>0.19</td>
<td>2.52 a</td>
<td>1.98 b</td>
<td>2.18 ab</td>
</tr>
<tr>
<td>C13</td>
<td>0.06</td>
<td>0.08</td>
<td>0.09</td>
<td>0.01</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
<td>0.01</td>
<td>0.08</td>
<td>0.07</td>
<td>0.07</td>
</tr>
<tr>
<td>C14 iso</td>
<td>0.07</td>
<td>0.08</td>
<td>0.08</td>
<td>0.01</td>
<td>0.08</td>
<td>0.08</td>
<td>0.07</td>
<td>0.01</td>
<td>0.08</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>C14</td>
<td>7.06 a</td>
<td>8.48 b</td>
<td>7.38 ab</td>
<td>0.51</td>
<td>8.28 a</td>
<td>7.28 b</td>
<td>7.36 b</td>
<td>0.63</td>
<td>7.57 a</td>
<td>7.16 a</td>
<td>8.18 b</td>
</tr>
<tr>
<td>C15 anteiso</td>
<td>0.42</td>
<td>0.43</td>
<td>0.47</td>
<td>0.03</td>
<td>0.47</td>
<td>0.44</td>
<td>0.41</td>
<td>0.03</td>
<td>0.43</td>
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</tr>
<tr>
<td>C14-1</td>
<td>0.12</td>
<td>0.14</td>
<td>0.13</td>
<td>0.01</td>
<td>0.14</td>
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<tr>
<td>C15</td>
<td>0.65</td>
<td>0.67</td>
<td>0.86</td>
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<td>0.80</td>
<td>0.72</td>
<td>0.65</td>
<td>0.01</td>
<td>0.73</td>
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<td>0.67</td>
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<tr>
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<td>0.17</td>
<td>0.16</td>
<td>0.17</td>
<td>0.01</td>
<td>0.18</td>
<td>0.16</td>
<td>0.16</td>
<td>0.01</td>
<td>0.16</td>
<td>0.17</td>
<td>0.17</td>
</tr>
<tr>
<td>C16</td>
<td>18.14</td>
<td>21.18</td>
<td>20.29</td>
<td>1.03</td>
<td>22.13</td>
<td>18.94</td>
<td>18.54</td>
<td>1.07</td>
<td>19.34</td>
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</tr>
<tr>
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<td>0.72</td>
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</tr>
<tr>
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<td>0.32</td>
<td>0.37</td>
<td>0.39</td>
<td>0.03</td>
<td>0.40</td>
<td>0.33</td>
<td>0.34</td>
<td>0.02</td>
<td>0.36</td>
<td>0.37</td>
<td>0.35</td>
</tr>
<tr>
<td>C17-1</td>
<td>0.12</td>
<td>0.14</td>
<td>0.14</td>
<td>0.01</td>
<td>0.14</td>
<td>0.13</td>
<td>0.12</td>
<td>0.01</td>
<td>0.13</td>
<td>0.13</td>
<td>0.13</td>
</tr>
<tr>
<td>C18</td>
<td>11.75</td>
<td>11.57</td>
<td>11.99</td>
<td>0.89</td>
<td>13.04</td>
<td>10.97</td>
<td>11.30</td>
<td>0.92</td>
<td>10.53</td>
<td>12.77</td>
<td>12.01</td>
</tr>
<tr>
<td>C18:1 trans 6-8</td>
<td>0.06</td>
<td>0.04</td>
<td>0.03</td>
<td>0.003</td>
<td>0.03</td>
<td>0.05</td>
<td>0.05</td>
<td>0.004</td>
<td>0.05</td>
<td>0.06</td>
<td>0.03</td>
</tr>
<tr>
<td>C18:1 trans 9</td>
<td>0.24</td>
<td>0.22</td>
<td>0.22</td>
<td>0.01</td>
<td>0.23</td>
<td>0.24</td>
<td>0.22</td>
<td>0.01</td>
<td>0.22</td>
<td>0.22</td>
<td>0.24</td>
</tr>
<tr>
<td>C18:1 trans 10</td>
<td>0.32</td>
<td>0.23</td>
<td>0.23</td>
<td>0.03</td>
<td>0.23</td>
<td>0.27</td>
<td>0.28</td>
<td>0.03</td>
<td>0.23</td>
<td>0.28</td>
<td>0.27</td>
</tr>
<tr>
<td>C18-1 trans 11</td>
<td>0.56 a</td>
<td>0.58 a</td>
<td>0.80 b</td>
<td>0.07</td>
<td>0.65</td>
<td>0.67</td>
<td>0.62</td>
<td>0.08</td>
<td>0.73 a</td>
<td>0.69 a</td>
<td>0.52 b</td>
</tr>
<tr>
<td>C18-1</td>
<td>0.27</td>
<td>0.26</td>
<td>0.22</td>
<td>0.03</td>
<td>0.26</td>
<td>0.22</td>
<td>0.28</td>
<td>0.03</td>
<td>0.23</td>
<td>0.27</td>
<td>0.26</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>13,14</td>
<td>C18-1c9</td>
<td>C18-1c12</td>
<td>C18-2n-6 cis</td>
<td>C18-3n-6</td>
<td>cla tot</td>
<td>C20-1</td>
<td>C21</td>
<td>C20-4n6</td>
<td>C22</td>
</tr>
<tr>
<td>-----------------</td>
<td>------</td>
<td>-------</td>
<td>--------</td>
<td>----------</td>
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<tr>
<td>trans</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C18:1 trans</td>
<td>0.32</td>
<td>0.33</td>
<td>0.44</td>
<td>0.03</td>
<td>0.41</td>
<td>0.37</td>
<td>0.36</td>
<td>0.03</td>
<td>0.41</td>
<td>0.40</td>
<td>0.34</td>
</tr>
<tr>
<td>C18-1c9</td>
<td>20.28</td>
<td>22.13</td>
<td>21.07</td>
<td>0.46</td>
<td>24.22 a</td>
<td>23.34 a</td>
<td>19.92 b</td>
<td>0.45</td>
<td>18.97</td>
<td>22.01 b</td>
<td>22.49</td>
</tr>
<tr>
<td>C18-1cis11</td>
<td>0.82</td>
<td>1.06</td>
<td>1.02</td>
<td>0.44</td>
<td>1.01</td>
<td>1.10</td>
<td>0.89</td>
<td>0.45</td>
<td>0.81</td>
<td>0.73</td>
<td>0.74</td>
</tr>
<tr>
<td>C18-1c12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
<td>0.01</td>
<td>0.13</td>
<td>0.12</td>
<td>0.12</td>
<td>0.01</td>
<td>0.12</td>
<td>0.12</td>
<td>0.12</td>
</tr>
<tr>
<td>C18-2n-6 cis</td>
<td>1.34</td>
<td>1.32</td>
<td>1.31</td>
<td>0.09</td>
<td>1.44</td>
<td>1.31</td>
<td>1.22</td>
<td>0.09</td>
<td>1.39</td>
<td>1.33</td>
<td>1.25</td>
</tr>
<tr>
<td>C18-3n-6</td>
<td>0.18</td>
<td>0.17</td>
<td>0.22</td>
<td>0.01</td>
<td>0.23</td>
<td>0.18</td>
<td>0.16</td>
<td>0.02</td>
<td>0.16</td>
<td>0.22</td>
<td>0.19</td>
</tr>
<tr>
<td>cla tot</td>
<td>0.39</td>
<td>0.39</td>
<td>0.38</td>
<td>0.03</td>
<td>0.34</td>
<td>0.40</td>
<td>0.41</td>
<td>0.03</td>
<td>0.43</td>
<td>0.36</td>
<td>0.37</td>
</tr>
<tr>
<td>C20-1</td>
<td>0.03</td>
<td>0.02</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.01</td>
<td>0.03</td>
<td>0.04</td>
<td>0.04</td>
</tr>
<tr>
<td>C21</td>
<td>0.05</td>
<td>0.06</td>
<td>0.06</td>
<td>0.01</td>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
<td>0.01</td>
<td>0.06</td>
<td>0.06</td>
<td>0.05</td>
</tr>
<tr>
<td>C20-4n6</td>
<td>0.11</td>
<td>0.12</td>
<td>0.11</td>
<td>0.01</td>
<td>0.12</td>
<td>0.11</td>
<td>0.11</td>
<td>0.09</td>
<td>0.11</td>
<td>0.11</td>
<td>0.12</td>
</tr>
<tr>
<td>C22</td>
<td>0.18</td>
<td>0.19</td>
<td>0.19</td>
<td>0.01</td>
<td>0.23</td>
<td>0.18</td>
<td>0.15</td>
<td>0.01</td>
<td>0.15</td>
<td>0.19</td>
<td>0.21</td>
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<tr>
<td>C23</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.01</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>C24</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
<td>0.04</td>
<td>0.03</td>
<td>0.03</td>
<td>0.03</td>
</tr>
</tbody>
</table>
Table 4.3.3. Fatty acids composition in the milk of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9). Means with different superscripts were significantly different (P<0.05).

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>9/9/05 g/100 g CLA</th>
<th>23/9/05 g/100 g CLA</th>
<th>7/10/05 g/100 g CLA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Whole canola</td>
<td>Crimped canola</td>
</tr>
<tr>
<td>6/8tt</td>
<td>0.35 a</td>
<td>0.20 b</td>
<td>0.25 b</td>
</tr>
<tr>
<td>7/9ct</td>
<td>9.31 a</td>
<td>10.06 a</td>
<td>7.65 b</td>
</tr>
<tr>
<td>7/9tt</td>
<td>0.77</td>
<td>1.02</td>
<td>1.04</td>
</tr>
<tr>
<td>8/10tc</td>
<td>1.63 a</td>
<td>1.22 b</td>
<td>1.60 a</td>
</tr>
<tr>
<td>8/10tt</td>
<td>1.26 a</td>
<td>1.57 b</td>
<td>1.37 a</td>
</tr>
<tr>
<td>9/11ct</td>
<td>70.20 a</td>
<td>76.89 b</td>
<td>77.99 b</td>
</tr>
<tr>
<td>9/11tt</td>
<td>1.93 a</td>
<td>2.42 b</td>
<td>2.12 a</td>
</tr>
<tr>
<td>10/12cc</td>
<td>4.02 a</td>
<td>0.77 b</td>
<td>1.12 b</td>
</tr>
<tr>
<td>10/12ct</td>
<td>4.82 a</td>
<td>0.35 b</td>
<td>0.54 c</td>
</tr>
<tr>
<td>10/12tt</td>
<td>0.77</td>
<td>0.81</td>
<td>0.88</td>
</tr>
<tr>
<td>11/13cc</td>
<td>1.47 a</td>
<td>3.24 b</td>
<td>1.14 c</td>
</tr>
<tr>
<td>11/13ct</td>
<td>1.23 a</td>
<td>0.72 b</td>
<td>1.12 a</td>
</tr>
<tr>
<td>12/14tt</td>
<td>1.74 a</td>
<td>1.10 b</td>
<td>1.45 c</td>
</tr>
<tr>
<td>13/15tt</td>
<td>0.51 a</td>
<td>1.21 b</td>
<td>0.98 c</td>
</tr>
</tbody>
</table>
4.3.4 - Discussion

Our results indicate that the fatty acids profile of the milk samples was affected by the treatment but not in the expected direction as sheep fed the crimped canola produced milk with significantly lower concentrations of C9 trans 11 CLA. This is surprising because in the crimped canola the oils are more readily available than in the whole canola, and the only possible explanation is that the CLA absorbed in the small intestine was further hydrogenated to TVA instead of being translocated into the milk. The production and gross composition of the milk were also unaffected by the feeding treatments. This suggests that the processing performance of the milk was not negatively affected by the changes in the concentration of unsaturated fatty acids and is consistent with previous short-term feeding experiments (Pugh 2004).

The fact that the production of milk was unaffected by the different diets is not surprising as the diets were designed to be isoenergetic and therefore we did not expect an increase in milk production. The levels of production were also consistent with the stage of lactation of the animals. However, because only nine samples per group and only three of the sampling weeks were selected for the statistical analyses, it is difficult draw any firm conclusions from these results.

The initial choice of 21 sheep per treatment was based on the application of power analysis to the coefficient of variation of milk production and composition in sheep. This revealed that to detect a 20% difference at the 5% level of significance a minimum of 20 sheep per treatment was required (Bencini 1993). Clearly the decision made by our collaborators to analyse only some of the samples may have potentially affected the validity of our results but this problem cannot be addressed at this stage.

The content of oleic acid (C18:1 cis9) tended to increase in both of the diets containing canola, a fact that is not surprising as this fatty acid is present in high concentrations in canola.

The fact that the milk produced by sheep fed the control diet and the diet containing whole canola had a greater concentration of cis9, trans11 CLA suggests that its biohydrogenation to stearic acid (C18:0), by rumen microorganisms was more efficient when they had crimped canola as a substrate. As the cis9, trans11 CLA is the biologically active isomer known to be beneficial for human health (Nudda et al. 2004) this finding would warrant further investigation.

In any case we can conclude that, similarly to results obtained previously in dairy cows (Debski 2004) and in a short term feeding experiment in dairy sheep (Pugh 2004), feeding canola did not have any negative effects on the health of the animals and on the production and composition of their milk. However, the potential benefits to consumers of the milk and derived dairy products obtained from sheep fed canola still remains to be investigated.
4.4 – Brewers’ grain as a food for dairy sheep

4.4.1 - Introduction

Brewers’ grain has been reported to be beneficial when fed to farm animals (DePeters et al. 2000) and has been reported to increase milk production in dairy cows (Valentine and Wickes 1982, Polan et al. 1985, Phipps et al. 1995). This feed has been reported to be a better supplement for growing sheep than cassava waste (Adeneye and Sunmonu 1994) but to our knowledge there are no published detailed studies on the effects of feeding brewers’ grain to dairy sheep, particularly on the milk production and composition.

Being a by-product of the production of beer, brewers’ grain is also readily available to Australian farmers at a relatively low cost. This, as well as other industry by-products, could not only save money to sheep dairy farmers but might also result in milk of favourable composition (e.g. higher in unsaturated fatty acids) that would therefore be beneficial to consumers. However, their composition and therefore nutritional value can be highly variable (Mirzaei-Aghsaghali and Maheri-Sis 2008).

So far no work has been completed on the effects of feeding brewers’ grain to dairy sheep. Therefore, we conducted an experiment where we integrated the diet of dairy sheep with brewers’ grain sourced from a local beer manufacturer, and compared their milk production and composition to those of sheep fed the control diet.

4.4.2 - Materials and methods

Our flock of dairy sheep was split into two groups of 22 ewes each stratified for breed, stage of lactation, production of milk, parity and number of lambs in order to have two comparable groups.

In addition to the usual grazing in communal paddocks and meadow hay, one group was fed the control concentrates usually given to our sheep, consisting of up to 1 kg of lupins plus 10% oats/head/day. The other group was gradually introduced to the treatment diet, which was supplemented with 200g/head (DM) of brewers’ grain containing 22% dry matter, 24% crude protein (Table 4.4.1) and sourced from the local Swan Brewery and delivered by James and Son (Perth, Western Australia). This was achieved by feeding an extra 50 g/head/day of wet brewers’ grain until the desired quantity of 200g DM/day was reached.

To ensure that we studied the long-term effects of these diets the feeding continued for six weeks. During this period the wet brewers’ grain were placed in plastic bags, stored in a cool room at 4°C and sampled fortnightly. The samples were later analysed by our collaborators at the University of Sassari for dry matter, crude protein and ash. To conduct these analyses samples were oven dried at 60°C for 48 h and ground to pass through a 1-mm screen with a Wiley mill. Dry matter, crude protein and crude ash, were analyzed according to methods 934.01, 976.05, 920.39 and 942.05, respectively, of the AOAC (1990; http://www.aoac.org/). The neutral detergent fibre, NDF, was determined with a heat stable amylase and expressed exclusive of residual ash (Van Soest et al. 1991).

Table 4.4.1. Composition (%) of the brewers’ grain supplied by James & Son for the experiment. Data also supplied by James & Son (Perth, Western Australia).

<table>
<thead>
<tr>
<th>Dry Matter (DM)</th>
<th>Crude Protein (%DM)</th>
<th>MJ ME/kg DM</th>
<th>NDF % DM</th>
<th>OIL % DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>22</td>
<td>24.0</td>
<td>11.0</td>
<td>55</td>
<td>12.0</td>
</tr>
</tbody>
</table>
The bulk milk produced by the two groups was weighed daily and kept separate. It was kept in a walk-in freezer at -20°C with the aim of processing the milk collected from each individual day of the experiment into batches of cheese. Unfortunately the freezer malfunctioned and the milk started to defrost. While we were unable to process the milk into individual batches we were able to send the milk to the Borrello Cheese Factory (Byford, WA) where it was processed in bulk.

At weekly intervals we tested the milk production of the sheep and collected milk samples to analyse them for fat, protein, lactose and total solids.

Because the mean milk production from the two groups differed at the start of the experiment, the data for milk production, fat, protein and lactose were converted to a percentage of the pre-experimental milk production. In separate analyses, daily milk yield, and the concentrations of fat, protein, and lactose in the milk, were used as the within-subjects factors, and compared using the respective error term. Total milk yield (6 weeks) and the average rate of decline in milk production were analysed using ANOVA, with treatment as the between-subjects factor. Homogeneity of variance was checked for each set of data, and no transformations were applied. Where appropriate, post hoc comparisons were made using least significant differences.

4.4.3 - Results

All the food offered was consumed without digestive disturbances or metabolic disorders. No visible moulding or heating were observed in the brewers’ grain for the whole duration of the experiment and the composition of the food remained constant throughout (Table 4.4.2).

Although the two groups of sheep were stratified for their milk production, based on the results of the measurement of milk production that immediately preceded the experimental period, when the experiment began, the control sheep were producing more milk than the treatment sheep. Within 2 weeks from the beginning of feeding their milk production increased slightly and then remained constant, so that by the end of the experiment the two groups were producing the same amount (Figure 4.4.1).
Table 4.4.2. Composition (%) of samples of the brewers’ grain collected throughout the experiment. Samples were collected fortnightly and analysed by Dr A. Mazzette, from the University of Sassari, Italy.

<table>
<thead>
<tr>
<th>Sample number</th>
<th>Dry Matter (DM)</th>
<th>Crude Protein (%DM)</th>
<th>Ash</th>
<th>NDF % DM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>23.0</td>
<td>23.9</td>
<td>4.07</td>
<td>72.9</td>
</tr>
<tr>
<td>2</td>
<td>22.8</td>
<td>26.5</td>
<td>4.13</td>
<td>72.2</td>
</tr>
<tr>
<td>3</td>
<td>22.6</td>
<td>25.7</td>
<td>4.13</td>
<td>72.6</td>
</tr>
<tr>
<td>4</td>
<td>22.6</td>
<td>22.8</td>
<td>4.10</td>
<td>73.2</td>
</tr>
<tr>
<td>Mean</td>
<td>22.8</td>
<td>24.7</td>
<td>4.11</td>
<td>72.7</td>
</tr>
<tr>
<td>SEM</td>
<td>0.09</td>
<td>0.83</td>
<td>0.014</td>
<td>0.21</td>
</tr>
</tbody>
</table>

This was reflected in the average weekly milk productions, which started with the control sheep producing more milk than the treatment group at the beginning and the same amount at the end (Figure 4.4.2).

Figure 4.4.1. Daily bulk milk production of dairy ewes fed either a control diet (black line, n= 22) or a diet containing brewers’ grain (white line, n= 22).
Figure 4.4.2. Average milk production of dairy ewes fed either a control diet (white, n= 22) or a diet containing brewers’ grain (black, n= 22). The bars represent standard errors.

There were no significant differences between the treatment and control groups in the concentrations of fat and protein in the milk (Table 4.4.3). Time (week) affected mean daily milk yield (P ≤ 0.00) but there were no interaction between time and treatment (P ≤ 0.622).

Table 4.4.3. Composition of milk from sheep fed a control diet or a diet supplemented with or brewers’ grain (average of whole period: 6 weeks)

<table>
<thead>
<tr>
<th>Milk components</th>
<th>Treatment</th>
<th>S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>Brewers’ Grain</td>
</tr>
<tr>
<td>Fat</td>
<td>7.16</td>
<td>7.53</td>
</tr>
<tr>
<td>Protein</td>
<td>5.89</td>
<td>6.06</td>
</tr>
<tr>
<td>Lactose</td>
<td>4.67</td>
<td>4.47</td>
</tr>
</tbody>
</table>

The bulk milk produced by the two groups of sheep was processed into Fetta cheese at the Borrello Cheese Factory (Byford, WA). No processing problems were reported and the cheese was considered of good quality (V. Borrello, Pers. Comm.).

4.4.4 - Discussion

Before the start of the experiment the sheep were allocated to treatments based on their milk production on a test day in which milk production was measured. However, at the following measurement of milk production conducted just after the experiment was started, the average milk production of the sheep in the treatment group had decreased more than that of the sheep in the control group and therefore the two groups were no longer balanced. This could be due to the late stage of lactation in which the experiment was conducted when production can decrease dramatically as the weather gets warmer. This resulted in the necessity to compare milk production and composition as percentages of the pre-experimental period, and resulted in non-significant differences between the two groups.

This is surprising because an examination of daily production clearly shows that brewers’ grain restored lactation in end-of-lactation sheep, and this is something that was not previously believed to
be possible. This result warrants further research into the use of brewers’ grain as feed for dairy sheep because the length of lactation is a crucial factor in sheep milk production (Bencini and Pulina 1997) and brewers’ grain might be able to prolong lactation and restore productivity in end of lactation ewes.

The physiological mechanism behind this unexpected lack of decrease in milk production towards the end of lactation is not clear, but Miyazawa et al. (2007) reported that in dairy cows fed brewers’ grain the concentration of acetic acid produced in the rumen increased within 5 hours of feeding the brewers’ grain. This might simply mean that brewers’ grain provides an immediate energy boost that allows dairy animals to support their milk production, and this could be the reason why our sheep maintained their milk production when it should have declined.

This study demonstrated that sheep adapted rapidly to the addition of large amounts of brewers’ grain to the diet. Results from this experiment suggest that dry matter intake, milk yield, and milk composition of dairy ewes fed diets containing 20% of dietary dry matter as brewers’ grain were not modified in comparison to the control diet. Dhiman et al. (2003) reported similar results in dairy cow fed with brewers’ grain representing 15% of the dietary dry matter. Also Murdock et al. (1981) using cows in mid lactation, found no differences in milk yield, and milk composition of cows fed brewers’ grain at 15 and 30% of the dietary dry matter when brewers’ grain replaced soybean meal and barley in diets based on alfalfa hay and corn silage. Hoffman and Armentano (1988) observed no change in feed intake, milk yield, and milk composition of cows fed diets containing 23.5% brewers’ grain. However, milk yield and feed intake were reduced when brewers’ grain replaced soybean meal at more than 20% of dietary dry matter in cows fed diets based on corn silage, but in this case the treatment diets were not isonitrogenous (Davis et al. 1983) and this may partly explain the reduction in milk yield and dry matter intake.

The storage of wet brewers’ grain for a lengthy time is always a concern, particularly in warm weather. For this reason use of this feedstuff is restricted to farms located in areas that are close to a brewery. The brewers’ grain in this experiment were delivered fresh and stored in a cool room at 4°C for 6 weeks. The use of the cool room allowed us to preserve the food for the whole duration of the experiment, preventing the formation of moulds and the onset of anomalous fermentations. This result demonstrates that wet brewers’ grain can be kept for prolonged periods if a cool room is available. At ambient temperature wet brewers’ grain have a shelf life of only four to five days.

Due to their high moisture content of 70% water by weight of total product, the product is expensive to transport and is thus usually economically viable within a 200 km radius from the ethanol production facility. These facts are important as they affect both profitability and logistic issues.

The use of cool rooms would allow farmers to conserve the product for long periods and source it less often, therefore reducing the cost of transport. However, the reduced cost of transport would have to be weighed against the increased cost of storage in cool rooms.
4.5 - Processing of health enhanced sheep milk

4.5.1 - Introduction

Nudda et al. (2005) found that CLA is readily transferred from the milk of dairy sheep into the resulting dairy products, which therefore reflect the natural variation in the concentration of CLA in the milk due to the seasonal variation in the food available to the animals. However, the increase in unsaturated fatty acids above naturally occurring levels resulting from any feeding strategies aimed at increasing the health benefits for consumers may reduce the melting point of the milk fat (Thomson and van der Poel 2000). This may result in altered clotting properties, particularly a lower curd consistency (Bencini 2002) and, as a consequence, might affect the processing performance and the outcome of the cheese (Baer et al. 1996).

Producing milk with beneficial compounds would only be useful if there are no negative effects on its processing performance and if consumers are prepared to buy these health enhanced products and pay higher prices for them. Therefore it was important to investigate the clotting properties of milk that has these desirable fatty acids and to transform this milk into dairy products to ensure that no off-flavours or other undesirable attributes were found in the resulting dairy products.

It was also important to test these products with potential consumers to establish if consumers readily accepted and were willing to buy these products.

4.5.2 - Materials and methods

The milk samples obtained from the canola experiment described in Chapter 4.3 were analysed for their clotting properties at the University of Florence. Additionally, the milk from the three groups of sheep that were fed the crimped canola, whole canola and control diets were collected separately for the duration of the experiment. We tested the clotting properties of the milk and then used it to make 24 batches of Fetta cheese following standard protocols developed in previous projects (Bencini 1999, Bencini and Agboola 2003).

Briefly, the milk was pasteurised at 72°C for 20 seconds, filtered and transferred into a cheese vat where it was cooled to the desired coagulation temperature of 32°-33°C. Mesophilic lactic starter cultures (F-DVD, Hansen) were added (0.1g/L) to the milk, which was then left at 32°-33°C for five minutes. Calf rennet (Hansen, 0.8ml/L of milk), was used to coagulate the milk and the curd was ready for cutting after approximately 50 minutes.

The curd was cut with curd knives into 2-3cm cubes, and left for another 50 minutes in order to expel part of the whey. The whey was separated, and the curd was weighed and transferred into a stainless steel Fetta mould, covered with a lid and incubated at 37°C for approximately 24 hours.

The next day the cheese was removed from the mould and cut into slices, weighed and transferred into plastic buckets with a 10% NaCl brine into a refrigerator at 4°C to complete the maturation (seven days).

Selected batches of the cheese were tested for the common pathogens Escherichia coli, Coagulase Positive staphylococci, Salmonella and Listeria monocytogenes at the Food Hygiene Laboratory of the Western Australian Centre for Pathology and Medical Research (Path Centre) to ensure that the cheese was safe to consume.

We conducted semi-trained panel evaluations of each batch of cheese in a blind trial in order not to bias the respondents. For each batch the assessors were asked to taste the cheeses and evaluate them
for aroma, colour, flavour and texture on a scale from 1 to 7 where 1 meant very bad and 7 meant excellent. They were also asked if they would buy the cheese (yes or no) and to make any additional comments. Comparisons between treatments (control, crimped canola or whole canola) for these taste panel results were conducted by one way ANOVA.

4.5.3 - Results

There was no difference in the clotting properties of the milk used to process the experimental batches of Fetta cheese or in the clotting properties of the milk samples that were analysed at the university of Florence, Italy (Tables 4.5.1 and 4.5.2).

Assessors gave scores of 4.6 and above for texture, flavour, colour and aroma to all batches of cheese. There were no significant differences in scores for texture, flavour, colour, aroma or the respondents’ willingness to buy the cheeses (Table 4.5.3) even though the batches made with the milk of sheep that were fed the whole canola scored marginally lower on all characteristics than the other cheeses.

Table 4.5.1. Renneting time (r, minutes), rate of curd formation (K20, minutes) and consistency of the curd (A30, mm), of the bulk milk obtained from sheep fed a control diet, or diets supplemented with crimped canola or whole canola and used to produce experimental batches of Fetta cheese.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of batches</th>
<th>r   (minutes)</th>
<th>K20  (minutes)</th>
<th>A30  (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>7</td>
<td>10.8 ± 0.92</td>
<td>1.4 ± 0.08</td>
<td>61.4 ± 4.11</td>
</tr>
<tr>
<td>Whole canola</td>
<td>5</td>
<td>11.7 ± 1.16</td>
<td>1.4 ± 0.09</td>
<td>64.4 ± 4.87</td>
</tr>
<tr>
<td>Crimped canola</td>
<td>11</td>
<td>12.4 ± 0.78</td>
<td>1.6 ± 0.06</td>
<td>63.5 ± 3.28</td>
</tr>
</tbody>
</table>
Table 4.5.2. pH, renneting time (r, minutes), rate of curd formation (k20, minutes) and curd consistency (A30, mm) of the milk of sheep fed either a control diet (n=9), a diet containing whole canola (n=9), or crimped canola (n=9).

<table>
<thead>
<tr>
<th>pH</th>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9/9/05</td>
<td>6.45</td>
<td>6.03</td>
<td>6.40</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>23/9/05</td>
<td>6.54</td>
<td>6.52</td>
<td>6.45</td>
<td>0.18</td>
</tr>
<tr>
<td></td>
<td>7/10/05</td>
<td>6.35</td>
<td>6.38</td>
<td>6.29</td>
<td>0.18</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>R (reenneting time, minutes)</th>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9/9/05</td>
<td>9.71</td>
<td>10.75</td>
<td>9.75</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>23/9/05</td>
<td>10.38</td>
<td>10.31</td>
<td>10.14</td>
<td>0.58</td>
</tr>
<tr>
<td></td>
<td>7/10/05</td>
<td>11.67</td>
<td>9.84</td>
<td>9.11</td>
<td>0.69</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>K20 (rate of curd formation, minutes)</th>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9/9/05</td>
<td>1.26</td>
<td>1.42</td>
<td>1.33</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>23/9/05</td>
<td>1.45</td>
<td>1.35</td>
<td>1.32</td>
<td>0.07</td>
</tr>
<tr>
<td></td>
<td>7/10/05</td>
<td>1.35</td>
<td>1.30</td>
<td>1.38</td>
<td>0.08</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>A30 (curd consistency, mm)</th>
<th>Date</th>
<th>Control</th>
<th>Whole canola</th>
<th>Crimped canola</th>
<th>SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9/9/06</td>
<td>58.61</td>
<td>59.11</td>
<td>57.30</td>
<td>4.01</td>
</tr>
<tr>
<td></td>
<td>23/9/05</td>
<td>64.24</td>
<td>65.17</td>
<td>57.71</td>
<td>4.01</td>
</tr>
<tr>
<td></td>
<td>7/10/05</td>
<td>68.45</td>
<td>72.53</td>
<td>63.42</td>
<td>4.81</td>
</tr>
</tbody>
</table>
Table 4.5.3. Mean scores awarded by a semi-trained panel to batches of Fetta cheese made with milk from sheep fed a control diet, or diets supplemented with whole or crimped canola. Willingness to buy was based on a yes or no response, and for statistical analysis yes was given a score of 2 and no a score of 1. The average scores of 1.5 and above thus indicate that more people were willing to buy the cheeses than not to buy them.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Texture</th>
<th>Flavour</th>
<th>Colour</th>
<th>Aroma</th>
<th>Willingness to buy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5.1 ± 0.17</td>
<td>5.0 ± 0.23</td>
<td>5.2 ± 0.21</td>
<td>4.8 ± 0.28</td>
<td>1.7 ± 0.08</td>
</tr>
<tr>
<td>Whole canola</td>
<td>4.9 ± 0.20</td>
<td>4.6 ± 0.28</td>
<td>4.8 ± 0.23</td>
<td>4.6 ± 0.27</td>
<td>1.5 ± 0.09</td>
</tr>
<tr>
<td>Crimped canola</td>
<td>5.0 ± 0.17</td>
<td>4.7 ± 0.23</td>
<td>5.2 ± 0.18</td>
<td>4.7 ± 0.26</td>
<td>1.6 ± 0.09</td>
</tr>
</tbody>
</table>

4.5.4 - Discussion

The results of this work indicate that feeding canola does not result in negative effects in the clotting properties or the processing performance of the milk.

The semi-trained panel assessor gave scores above 4.6 to all the batches of cheese, and this also seems to indicate that there were no negative effects of the canola diets on the texture, flavour, colour and consumer’s willingness to buy the cheese.

Even though the cheese made with the milk from the sheep fed the whole canola had marginally lower scores than the other batches, this difference was not significant and the reduced willingness to buy the product could well be offset if consumers are made aware of the potential health benefits of consuming cheese enriched in unsaturated fatty acids. Because we did not want to bias our assessors, blind tasting was used, so that assessors were not influenced in their willingness to buy the cheese by the fact that it contained an increased concentration of unsaturated fatty acids.

Although the fatty acid profiles of the cheeses were not analysed, these results and those from the analysis of the samples reported in Chapter 4.3 strongly suggest that the cheese batches made with the milk produced by the sheep fed the crimped canola were higher in unsaturated fatty acids. We suggest this because the individual milk samples had significantly different fatty acids profiles and also because the semi-trained panel assessor were able to detect a difference, albeit slight and non significant, in the cheeses.

Additionally, Nudda et al. (2005) demonstrated that unsaturated fatty acids found in the diet of dairy sheep are translocated into the milk and from the milk into the dairy products derived from it. Although the fatty acids studied by Nudda et al. (2005) were naturally present in the milk and were due to the seasonal variation in the pasture consumed by the dairy sheep, it is reasonable to assume that all fatty acids, including those resulting form the feeding of canola, are translocated into the cheese.

The results of the semi trained panel and our previous research (Bencini 1999, Burton and Bencini 2005) suggest that consumers are prepared to purchase sheep dairy products. Whether this willingness to buy sheep milk products would increase if consumers were informed of potential health benefits to be gained remains to be investigated.
4.6 - Gourmet sheep milk ice cream

4.6.1 - Introduction

Our previous research indicated that there would be little or no advantage in producing sheep milk ice cream because it might not achieve higher prices than cow’s milk ice cream (Bencini 1999). In our first project experimental batches of sheep milk ice cream developed by Peters & Brownes were rated poorly by assessors from Peters & Brownes but highly by staff from The University of Western Australia. At the time we concluded that possibly both groups of assessors were biased (Bencini 1999).

This initial work discouraged us from pursuing this line of research until Mr. Garth Simpson, owner of the gourmet ice creamery Simmo’s (Busselton, WA) approached us because he wanted to produce gourmet ice creams made with sheep milk and enter them into the Perth Royal Show. Simmo’s is famous for the use of traditional recipes and ingredients to produce unusual gourmet ice creams, which include even a black licorice ice cream.

Although this was not originally planned for this project and was not part of our original objectives, we eagerly accepted to provide a small amount of milk and 1 kg of Fetta cheese as this would generate publicity for the sheep milking industry. The possibility of value adding sheep milk through producing high price fetching gourmet ice cream would provide another avenue for the sale of sheep milk and we considered that this developmental work would ultimately benefit the sheep milking industry.

4.6.2 - Materials and methods

Approximately 80L of sheep milk were processed at the Simmo’s ice creamery (Busselton, WA) into four different batches of ice cream. These were entered into the Perth Royal Show in the category “Class 14 – Products made from other than cows milk” (Table 4.6.1).

The recipes used in this developmental work were classified as commercial-in-confidence and cannot be reported here.

A small number of ice creams were returned to UWA where they were enthusiastically consumed by our colleagues.

4.6.3 - Results and discussion

One of the ice cream batches, the “Berry Swirl” flavoured with vanilla and blueberries received the second silver prize in its category at the Royal Show (Table 4.6.1).
Table 4.6.1. Batches of gourmet ice cream produced at Simmo’s ice creamery (Busselton, WA) and their performance at the 2007 Perth Royal show.

<table>
<thead>
<tr>
<th>Name of batch</th>
<th>Flavour</th>
<th>Colour</th>
<th>Placement</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vanilla Bean</td>
<td>Plain vanilla</td>
<td>Cream</td>
<td>-</td>
</tr>
<tr>
<td>Green Tea</td>
<td>Green tea</td>
<td>Green</td>
<td>-</td>
</tr>
<tr>
<td>Berry Swirl</td>
<td>Vanilla and blueberries</td>
<td>white/purple</td>
<td>Silver (second)</td>
</tr>
<tr>
<td>Fetta and olive oil</td>
<td>Unusual</td>
<td>White</td>
<td></td>
</tr>
</tbody>
</table>

Previous developmental work conducted in collaboration with Peters & Brownes was limited to plain vanilla ice cream that would be hard pressed to compete with cheaper cow’s milk ice cream. Gourmet ice creams are different because they are always sold at high prices regardless of ingredients used.

The fact that assessors from Peters & Brownes rated the ice creams very poorly discouraged us from pursuing the development of sheep milk ice cream further even though we concluded that their assessment was possibly biased but because it contrasted with that of staff members from The University of Western Australia (Bencini 1999).

Significantly, Garth Simpson did not report any processing problems or negative reactions from his personnel towards the sheep milk ice creams, suggesting that they had no bias due to the fact that they were made with sheep milk. This seems to confirm that previous negative assessment of sheep milk ice creams by personnel at the Peters & Brownes Group might have been biased.

The results of this small trial suggest that niche markets could be developed for sheep milk ice creams and may provide yet another avenue for the commercialisation of sheep milk.
5 - General Discussion

Our work on sheep temperament described in Chapters 4.1 and 4.2 demonstrated the existence of a relationship between the temperament of sheep, their behaviour in the dairy and their subsequent performance as dairy animals. If sheep dairy farmers could include in their breeding strategies the selection of calm animals for milking this would bring about advantages both in terms of ease of handling and time taken to train and milk the sheep. Therefore, the use of the rapid temperament tests developed at The University of Western Australia and tested within this project could become an important method to screen sheep suitable for milking from large unselected flocks. Significantly, lamb producers have already adopted these tests on a large scale and thousands of lambs are now routinely tested under the auspices of the Meat and Livestock Australia (MLA, D. Blache Pers. Comm.).

Surprisingly, we did not find a clear relationship between the temperament of the sheep and the composition of their milk. The milk ejection reflex can be blocked by the adrenaline produced by sheep that are under stress, and this should have resulted in the collection of only the cisternal milk, which has a lower concentration of fat (Labussiére 1988). Therefore we expected that the nervous sheep would produce milk with a lower concentration of fat. It is possible that our results were confounded by the presence of lambs, which also prevent the release of the alveolar milk, and would have prevented it in both calm and nervous sheep (McKusick et al. 2002). Further research on the effects of temperament on the patterns of milk ejection and milk composition in sheep that are not nursing their lambs could clarify this.

The work described in Chapters 4.3 and 4.5 demonstrated that feeding canola to the sheep can change the composition of sheep milk without affecting its processing performance and that the resulting dairy products are likely to contain unsaturated fatty acids that are potentially beneficial to consumers. Sheep dairy producers seeking to commercialise their products for their health benefits to consumers could feed canola to their dairy sheep, but should undertake a cost benefit analysis before adopting this practice.

Our work has shown that the presence of unsaturated fatty acids in the milk does not result in processing problems, and a semi trained panel found the resulting cheeses quite palatable. However, further research should be conducted on groups of consumers to gauge their willingness to pay higher prices for these products, especially in relation to their potential health benefits (Bencini 1999, Bencini and Agboola 2003, Burton and Bencini 2005).

The results of Chapter 4.4 showed that the administration of brewers’ grain did not cause health problems and resulted in sheep continuing to lactate longer than expected. This is an extraordinary finding that warrants further investigation because the length of lactation is an important determinant of sheep milk production (Bencini et al. 2010). If this is confirmed, sheep dairy farmers positioned near breweries could use this relatively cheap by-product not only to increase the range of feedstuff available, but also to increase the persistency of lactation in their flocks. Costs of transport and storage would have to be carefully investigated through a cost benefit analysis for farmers located further away from breweries.

Our collaboration with Simmo’s ice creamery resulted in the production of prize-winning ice creams as reported in Chapter 4.6. Although this was only a small trial it suggests that it should be possible to develop niche markets for sheep milk ice creams, and thus increase the market scope for sheep milk products.
6 - Implications

The work described in this report has important implications for the Australian sheep milking industry. Since our work has demonstrated that there is a relationship between the temperament of sheep, their behaviour in the dairy and their subsequent performance as dairy animals, the selection of calm animals for milking will bring advantages to sheep dairy producers such as sheep that are easier to handle, train and milk and reduced stress to sheep and operators alike. The use of rapid temperament tests could be adopted routinely to screen sheep suitable for milking from large unselected flocks. Although it takes only one minute to milk a sheep, which is the same time it takes to test it for its temperament, milking a sheep only once is not sufficient to establish its behaviour and performance in the long term. It also requires that the sheep has lambed and is in an appropriate stage of lactation. By contrast, the box test allows the rapid screening of large numbers of sheep before they are lactating, and it does not require the animal to be in any particular physiological stage to be used. Nowadays animal welfare is becoming important and it can influence consumers’ choices (Martin and Kadokawa 2006). In this context, the selection of calm sheep will be beneficial to their welfare and it could therefore become a selling point for the sheep milking industry.

This work has also demonstrated that the composition of sheep milk can be changed to produce dairy products potentially beneficial to consumers, and this could also be an important selling point for the marketing of sheep dairy products. Sheep dairy producers and manufacturers could feed canola or brewers’ grain to their dairy sheep if they wish to commercialise their products for their health benefits to consumers. It would be necessary, however, to support any health claims by providing specific information on the content in these beneficial compounds in the nutritional information panel of the dairy products in question.

The fact that some of these feedstuffs are by-products makes them even more attractive because they are relatively cheap. In particular, the administration of brewers’ grain resulted in sheep continuing to lactate and maintain milk production longer than expected. The physiological reasons for this are unclear and could generate a number of interesting hypotheses to be tested. As milk production in sheep is highly dependent on the length of lactation (Bencini et al. 2010), the feeding of brewers’ grain should be further investigated.

Further research would also need to be conducted on other by-products, such as olive pomace, or citrus pulp, that could potentially increase the unsaturated fatty acids concentration of sheep milk.

The production of gourmet sheep milk ice creams could also be investigated in further research to examine the possibility of developing niche markets for sheep milk ice creams.
7 - Recommendations

The use of rapid temperament tests should be adopted to screen dairy sheep suitable for milking from large unselected flocks. Since there is a relationship between the temperament of sheep, their behaviour in the dairy and their subsequent performance as dairy animals, the selection of calm animals for milking will bring advantages to sheep dairy producers by increasing animal welfare and improving working conditions for the milkers. As animal welfare issues become increasingly important and influence consumers’ choices (Martin and Kadokawa 2006), we recommend promoting the sheep dairy industry as a clean, green and ethical farming method as a strategy to promote the industry.

Sheep dairy producers and manufacturers should consider feeding canola or brewers’ grain to their dairy sheep if they wish to commercialise and promote their products for their health benefits to consumers. However, any claims on the health benefits of the milk and dairy products derived from it should be backed up by the addition of information on the concentration of unsaturated fatty acids in the nutritional information panel for the dairy products in question.

The use of brewers’ grain could potentially decrease feeding costs, but farmers should evaluate the economical implications of transporting and storing the product before adopting this strategy. In the case of brewer’s grains it could also increase milk production as well as the persistency of lactation in sheep and should be considered as a supplement for lactating dairy sheep.

Sheep dairy producers willing to test any of the above strategies would have to undertake a careful analysis of the costs and benefits associated with their adoption.

There are many industry by-products, such as olive pomace, or citrus pulp, that could potentially increase the unsaturated fatty acids concentration of sheep milk as well as reducing the costs of purchasing feed for dairy sheep.

Further research on the use of these by-products, as well as on the potential health benefits of sheep milk, would greatly benefit the sheep milking industry.
8 - Intellectual Property

The intellectual property of this project resides with the Rural Industries Research & Development Corporation and The University of Western Australia.
9 - References


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Exploring the Commercial Potential of Sheep Milk

By Roberta Bencini and Travis Murray
Pub. No. 11/179

In consultation with industry the research investigated the suitability of behavioural tests to select dairy ewes based on their temperament, as well as the development of feeding strategies to increase the health benefits of sheep milk products, so that these products can be commercialised for their health benefits as well as for their superior qualities.

The commercial potential of sheep dairy products for the domestic and export markets was also explored.

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