

Copyright is owned by the Author of the thesis. Permission is given for a copy to be downloaded by an individual for the purpose of research and private study only. The thesis may not be reproduced elsewhere without the permission of the Author.

**ANTI-PARASITIC ACTIVITY
OF
BOVINE MILK**

A thesis presented in partial
fulfilment of the requirements
for the degree
of Master
in Nutritional Science
at Massey University

SHU ER ZENG

2000

ERRATA

Page	Paragraph	Line	Amendment
14	4	4	Should read “sheep and cows than in lambs and calves”
17	3	10	Should read “ of infection with <i>O. ostertagi</i> in cattle (Fox <i>et al.</i> , 1988)”.
18	2	3	Typographical error: “inflammation”
23	2	4	Lambs were between 21 and 25 days-of-age when first infected.
28	1	2	Second antibody was raised at Massey University and used undiluted
29	3	2	Should read “ by a modification of the micro-method of Dorny and Vercruysse (1998)”
30	4	11	Centrifugation was at 10,000 <i>g</i>
33	2	6	Delete “of”
Facing 37	Table 2.3	-	Omasum/body weight for Solid-ex should read 2.8 ± 0.4
Facing 37	Table 2.3	-	Body weight for Solid-ex and Solid-en should read 17.5 ± 1.7 and 15.3 ± 1.3 respectively
39	2	2-3	The height of the papillae (mean for each lamb) were in the range.....
44	2	7	Delete “not”
61	2	2	Replace “development” with “establishment”

ABSTRACT

Previous studies have shown that milk-fed ruminants were less susceptible to gastro-intestinal parasites compared with young animals that were given solid feed in the diet. The present studies were carried out to gain a better understanding of how milk feeding reduces worm establishment in newborn lambs. Two approaches have been taken: an *in vivo* study in which lambs were infected from three weeks of age with third-stage larval *Ostertagia circumcincta* and an *in vitro* examination of direct effects of bovine milk and some of its crude fractions on the motility of *O. circumcincta* larvae.

The *in vivo* experiment was designed to compare parasite establishment in lambs either fed entirely on milk from birth, weaned on to solid feed by three weeks of age, or provided with solid feed from two weeks of age and given a milk feed once a day. To examine whether lack of rumen development was a crucial factor, each diet group consisted of lambs given normal ensheathed third-stage larvae and an equal number of lambs given exsheathed larvae. A total of 24 lambs were included in the study, in six groups each of four lambs. All lambs were infected by tube (into the oesophagus) twice a week with either 1000 exsheathed or ensheathed third-stage *O. circumcincta* larvae. Infection began after the week taken to establish the lambs on their new diet, so that starting on week four of life, the lambs were trickle infected for six weeks.

There were highly significantly lower worm burdens at necropsy in the two milk-fed groups of lambs than in both the other groups, but no difference between the burdens in those completely fed solid food and in lambs receiving a 600 ml milk feed once a day along with solids. Irrespective of the diet, female worms made up half the total number of worms in each lamb, with males and immature stages equally making up the other half. Faecal egg counts in the Milk groups were also very low, three of the eight lambs never providing a faecal sample in which eggs were found. Also consistent with the lower worm burdens were the thinner abomasal mucosa and lower abomasal pH,

although these may also have been affected by the diet. Nodules were visible in the abomasa of all lambs in all groups.

All groups had increased serum gastrin and pepsinogen levels, with considerable variation between animals within all groups.

An important observation was that there was no significant difference between lambs receiving exsheathed or ensheathed larvae for any parameter measured. The immaturity of the reticulo-rumen and omasum does not appear to prevent ensheathed larvae from exsheathing and establishing in the abomasum of lambs with an underdeveloped rumen. The similar worm burdens in the milk-fed lambs given ensheathed and exsheathed larvae therefore does not support the conclusions from an earlier study in calves that lack of rumen function was the reason for lower worm burdens in non-ruminating calves. Instead, it would appear that the milk itself is reducing parasite establishment.

In vitro exposure to fresh bovine milk, commercial bovine milk with either 3.3% or 0.2% fat, the milk powder fed to the lambs, whey protein, casein or ultra low heat skim milk powder all reduced the motility of exsheathed third-stage *O. circumcincta* larvae. The effect on motility was concentration and time dependent for all milks. The active component appears to be associated with proteins and not with the lipid fraction and may be non-specific, as both whey and casein were effective. Different components may be responsible for inhibition of larvae by whey and casein proteins. The activity of whey protein increased as the pH increased; the whey was most active at pH 4.5 and above, when it would be in the anion form. In contrast, there was no difference in activity at pH 5.5 and 6.5 for casein. The effect of time of incubation also differed for whey and casein.

A possible explanation for the in vitro and in vivo effects of milk are the attachment of the proteins to the larvae. The lack of effect when milk and solid feed are ingested together may result from the protein attaching to the food particles in preference to the larvae or, alternatively, the milk may have left the abomasum before the larvae were administered. This suggests that practical

applications for milk proteins as anti-parasite agents may be limited in ruminants consuming solid feed.

ACKNOWLEDGEMENTS

I would like to thank Associate Professor Heather Simpson for her optimism, assistance, guidance, encouragement, patience and tolerance during the period of my studentship all of which far exceeded the reasonable expectation of a supervisor.

I wish to express my special gratitude to Samera Khalaf for her help in the laboratory and for her kind friendship during my studentship.

Particularly, I would like to thank Sabine Przemeczek for her invaluable advice, guidance and criticism during the whole experiment.

Special thanks are due to David Simcock, Dr I Scott, and Dr David Lawton for their assistance and guidance in the experiment.

I would like to thank Babara Adlington and Sheila Ramsay for their assistance in the parasitology work involved in my study.

Particularly, I would like to thank Associate Professor Kathy Kitson and Heather McClean for their recommendation, which guided me to be a student of Associate Professor Heather Simpson.

I am grateful to the Institute of Food, Nutrition and Human Health, Massey University, for providing me with the opportunity, facilities and technical support to undertake this study.

I would like to thank the E.& C. Thoms Bequest, which funded my experimental work.

Sincere appreciation and gratitude go to my husband Zhi Peng Ye for his support and encouragement during my study. Particularly, I thank my daughter Shu Fan Ye who was born in the Chinese “Year of the Sheep” and who was dependent on bovine milk during her early childhood, for her understanding and discipline throughout my study.

TABLE OF CONTENTS

TITLE	i
ABSTRACT	ii
ACKNOWLEDGEMENTS	v
TABLE OF CONTENTS	vi
LIST OF FIGURES.....	x
LIST OF TABLES	xii
INTRODUCTION.....	xiv
CHAPTER 1	
LITERATURE REVIEW.....	1
1.1 BOVINE MILK.....	1
1.1.1 Composition of bovine milk	1
1.1.1.1 Carbohydrate.....	2
1.1.1.2 Lipids	2
1.1.1.3 Nitrogenous compounds	3
1.1.1.3.1 Whey protein	4
1.1.1.3.2 Casein Micelles	5
1.1.1.4 Vitamins, minerals and trace elements	5
1.1.2 Hormones and growth factors in bovine milk.....	6
1.1.3 Defence agents in milk.....	6
1.1.3.1 Immunological agents.....	7
1.1.3.2 Anti-tumour activity	7
1.1.3.3 Antibacterial agents	8
1.1.3.3.1 Lysozyme.....	8
1.1.3.3.2 Lactoferrin.....	8
1.1.3.3.3 Lactoperoxidase.....	9
1.1.3.3.4 Lipids.....	9
1.1.4 Antiparasitic activity of milk	9

1.2 ABOMASAL NEMATODE PARASITES	10
1.2.1 Life cycle of abomasal parasites	11
1.2.2 Pathophysiology	12
1.2.2.1 Hyperpepsinogenaemia	13
1.2.2.2 Reduced acid secretion	15
1.2.2.3 Hypergastrinaemia	17
1.2.3 Abomasal parasitism in milk-fed ruminants	19
1.3 CONCLUSIONS	21
CHAPTER 2	
EFFECT OF A MILK DIET ON <i>OSTERTAGIA CIRCUMCINCTA</i>	
INFECTION IN LAMBS	22
2.1 INTRODUCTION	22
2.2 METHODS	23
2.2.1 Experimental design	23
2.2.2. Animals	23
2.2.3 Feeding	24
2.2.4 Infection with <i>O. circumcincta</i> larvae	24
2.2.5 Collection of blood samples	25
2.2.6 Collection of faecal samples	25
2.2.7 Necropsy	25
2.2.8 Tissue for pepsinogen estimation	26
2.2.9 Abomasal pH	26
2.2.10 Gross measurements of the reticulo-rumen	27
2.2.11 Histology and morphometry	27
2.2.12 Assays	27
2.2.12.1 Gastrin radioimmunoassay	27
2.2.12.2 Pepsinogen assay	29
2.2.13 Parasitology	31
2.2.13.1 Larval culture	31
2.2.13.2 Counting larvae and assessing viability	32
2.2.13.3 Larval exsheathment	32
2.2.13.4 Faecal floats	32
2.2.13.5 Faecal egg counts	32
2.2.13.6 Postmortem worm counts	33
2.2.14 Statistics	33

2.3 RESULTS	34
2.3.1 Parasitology	34
2.3.1.1 Faecal Egg Counts	34
2.3.1.2 Postmortem worm counts	35
2.3.2 Abomasal pH	36
2.3.3 Tissue pepsinogen	36
2.3.4 Serum pepsinogen	36
2.3.5 Serum gastrin concentration	36
2.3.5 Body weight	37
2.3.6 Organ weights	37
2.3.6.1 Reticulo-rumen	37
2.3.6.2 Omasum	38
2.3.6.3 Abomasum	38
2.3.7 Organ morphology	39
2.3.7.1 Reticulo-rumen	39
2.3.7.2 Abomasum	39
2.3.8 Abomasal histology	39
2.4 Discussion	40
2.4.1 Parasite establishment	40
2.4.2 Abomasal function	41
2.4.3 Development of the stomach	42
2.4.4 Effect of milk in the abomasum	43
CHAPTER 3	
THE EFFECT OF MILK AND MILK COMPONENTS ON THE MOTILITY OF <i>OSTERTAGIA CIRCUMCINCTA</i> IN VITRO	46
3.1 INTRODUCTION	46
3.2 MATERIALS AND METHODS	47
3.2.1 Larval motility assay	47
3.2.2 Test solutions	48
3.2.2.1 Hanks Balanced Salt Solution (HBSS)	48
3.2.2.1 Fresh bovine milk	48
3.2.2.2 Commercial homogenised bovine milk (3.3% fat)	48
3.2.2.3 Commercial low fat bovine milk (0.2% fat)	48
3.2.2.4 Bovine milk replacer for lambs	49
3.2.2.5 Whey protein	49

3.2.2.6 Casein	49
3.2.2.7 Ultra low heat skim milk powder (ULHSMP)	50
3.2.2.8 Soybean trypsin inhibitor.....	50
3.2.3 Statistics	50
3.3 RESULTS.....	51
3.3.1 Larval <i>O. circumcincta</i> motility in fresh bovine milk.....	51
3.3.2 Larval <i>O. circumcincta</i> motility in commercial homogenised bovine milk (3.3% fat).....	51
3.3.3 Larval <i>O. circumcincta</i> motility in commercial low fatbovine milk (0.2% fat).....	52
3.3.4 Larval <i>O. circumcincta</i> motility in bovine milk replacer for lambs	52
3.3.5 Comparison of effect of the bovine milks On larval <i>O. circumcincta</i> motility.....	52
3.3.6 Larval <i>O. circumcincta</i> motility in whey protein solutions	53
3.3.7 Larval <i>O. circumcincta</i> motility in casein solutions.....	53
3.3.8 Larval <i>O. circumcincta</i> motility in ultra low heat skim milk powder (ULHSMP).....	54
3.3.9 Larval <i>O. circumcincta</i> motility in HBSS containing trypsin inhibitor.....	54
3.4 DISCUSSION	55
CHAPTER 4 GENERAL DISCUSSION.....	58
REFERENCES	62

LIST OF FIGURES

	Facing page
Figure 2.1 Faecal egg counts (mean \pm s.e.m.) in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	34
Figure 2.2 Faecal egg counts in individual lambs following infection with <i>O. circumcincta</i> larvae.	34
Figure 2.3 Worm counts at necropsy in individual lambs following infection with <i>O. circumcincta</i> larvae.	35
Figure 2.4 Abomasal pH (mean \pm s.e.m.) at necropsy in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.5 Abomasal pH at necropsy in individual lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.6 Tissue pepsinogen content (mean \pm s.e.m.) at necropsy in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.7 Tissue pepsinogen content at necropsy in individual lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.8 Serum pepsinogen concentration (mean \pm s.e.m.) in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.9 Serum pepsinogen concentration in individual lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.10 Serum gastrin concentration (mean \pm s.e.m.) in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.11 Serum gastrin concentration in individual lambs following infection with <i>O. circumcincta</i> larvae.	36
Figure 2.12 Reticulo-rumen weight (mean \pm s.e.m.) at necropsy in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	37

Figure 3.1 Percentage of immotile *O. circumcincta* larvae (mean \pm s.e.m.) in different concentrations of milk for incubation periods of 1, 24 and 48 hours. 51

Figure 3.2 Percentage of immotile *O. circumcincta* larvae (mean \pm s.e.m.) in different concentrations of whey proteins and pH from 2.5 to 6.5 for incubation periods of 1, 24 and 48 hours. 53

LIST OF TABLES

	Facing page
Table 1.1 Compartments and their components in mature bovine milk.	1
Table 1.2 Lipid class composition of mature bovine milk during lactation.	2
Table 1.3 Amino acid composition of bovine milk.	3
Table 1.4. Protein composition of mature bovine herd milk.	4
Table 1.5 The concentrations of vitamins in bovine milk.	6
Table 1.6 Hormones and growth factors in bovine milk.	6
Table 2.1 Experimental groups. Sex and body weight of lambs when assigned to groups (at 14 -18 days of age).	23
Table 2.2 Abomasal worm counts (mean \pm s.e.m.) at necropsy in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	35
Table 2.3 Body, reticulo-rumen, omasum and abomasum weight and organ/body weight (mean \pm s.e.m.) at necropsy in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae	37
Table 2.4 Thickness of abomasal mucosa (mean \pm s.e.m.) at necropsy in 6 groups of lambs following infection with <i>O. circumcincta</i> larvae.	39
Table 3.1 Percentage of immotile larval <i>O. circumcincta</i> (mean \pm s.e.m) in different concentrations of fresh bovine milk for incubation periods of 1, 24 and 48 hours.	51
Table 3.2 Percentage of immotile larval <i>O. circumcincta</i> (mean \pm s.e.m) in different concentrations of commercial homogenised bovine milk (3.3% fat) for incubation periods of 1, 24 and 48 hours.	51
Table 3.3 Percentage of immotile larval <i>O. circumcincta</i> (mean \pm s.e.m) in different concentrations of commercial low fat bovine milk (0.2% fat) for incubation periods of 1, 24 and 48 hours.	52
Table 3.4 Percentage of immotile larval <i>O. circumcincta</i> (mean \pm s.e.m) in different concentrations of bovine milk	52

replacer for lambs for incubation periods of 1, 24 and 48 hours.

Table 3.5 Percentage of immotile larval *O. circumcincta* (mean \pm s.e.m) in different concentrations of whey protein and pH from 2.5 to 6.5 for incubation periods of 2, 4 and 24 hours. 53

Table 3.6 Percentage of immotile larval *O. circumcincta* (mean \pm s.e.m) in different concentrations of casein protein and pH from 5.5 to 6.5 for incubation periods of 2, 4 and 24 hours. 54

Table 3.7 Percentage of immotile larval *O. circumcincta* (mean \pm s.e.m) in different concentrations of ULHSMP and pH from 5.5 to 6.5 for incubation periods of 2, 4 and 24 hours. 54
