



UNIVERSITI PUTRA MALAYSIA

**RESPONSES OF SHEEP TO *HAEMONCHUS.CONTORTUS*
INFECTION WITH RESPECT TO NUTRITIONAL ENHANCEMENT
AND INNATE RESISTANCE**

MOHAMED ALI BEN-GEHSIR

FPV 2000 1

**RESPONSES OF SHEEP TO *HAEMONCHUS. CONTORTUS* INFECTION
WITH RESPECT TO NUTRITIONAL ENHANCEMENT
AND INNATE RESISTANCE**

By

MOHAMED ALI BEN-GEHSIR

**Thesis Submitted in Fulfilment of the Requirements for the
Degree of Master of Science in the
Faculty of Veterinary Medicine
Universiti Putra Malaysia**

July 2000



DEDICATION

*To my parents, brothers, sisters, my wife and my children, Haneen and
Isra, For their moral support and encouragement.*

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in
fulfilment of the requirements for the degree of Master of Science

**RESPONSES OF SHEEP TO *HAEMONCHUS. CONTORTUS* INFECTION
WITH RESPECT TO NUTRITIONAL ENHANCEMENT
AND INNATE RESISTANCE**

By

MOHAMED ALI BEN-GHESHIR

July 2000

Chairman: Associate Professor Dr. Rehana Abdullah Sani PhD.

Faculty: Veterinary Medicine

Gastrointestinal parasitism, in particular caused by *Haemonchus contortus*, is the major source of parasitic gastro-enteritis in tropical countries and is associated with large economic losses. This study was conducted to investigate the enhancement of responsiveness of sheep to *H. contortus* infection by dietary protein supplementation and by selecting and breeding for resistance to *H. contortus*.

In the first experiment, thirty two, 3 month old Dorsimal lambs were used to study the influence of dietary protein supplementation on *H. contortus* infection. Lambs were offered a complete basal ruminant diet (15% crude protein; CP), with or without fish meal as a source of rumen bypass protein (19% CP). Lambs from each dietary treatment group were given either a 7-week trickle infection with *H. contortus* infective larvae or remained uninfected. All lambs were drenched with anthelmintics at week 8 post-infection, then challenged with a single dose of 5000 *H. contortus* L₃ one week later and killed 14 days post-challenge.



Supplementation lower faecal egg counts (FECs) in trickle infected lambs. The non-supplemented, trickle infected lambs had lower packed cell volume (PCV), haemoglobin (Hb) and plasma protein (PP). Although no obvious eosinophilia was observed and peripheral eosinophil and abomasal worm counts were not significantly different among the four groups, supplementation, had significant effect on eosinophil and mast cells in the abomasal mucosa ($P < 0.05$). Significant correlation was recorded between worm burdens and tissue cell counts.

In the second experiment, Santa Ines sheep were selected and bred for resistance to *H. contortus* infection. A foundation population of 123 lambs of 3-4 months of age from two flocks was used. Animals with low FEC (mean < 2725) following naturally acquired infection were deemed as high responder (HR) animals that were resistant to strongly infection, while animals with high FEC (mean > 3675) were classified as low responder (LR) animals that were more susceptible. The HR and LR selected lambs were transferred to UPM and treated to remove the field infection. The lambs were kept indoor and subsequently artificially infected with a single oral dose of 10000 *H. contortus* L₃. At the age of 12 months, HR males were mated to HR females and LR males to LR females. The offspring of these matings were in turn artificially challenged with 10000 *H. contortus* L₃ upon weaning to confirm their responder status. The post-challenge FEC, PCV, PP and body weights of these lambs were monitored.

The FEC of HR animals were significantly ($P < 0.001$) lower than that of LR animals in field and post-challenge. The PCV and PP of LR animals were significantly lower than that of the HR animals. There was a significant, positive

correlation between FECs from field and experimental infections and FECs of the offspring and their sires and dams. This study suggests it is possible to segregate sheep into HR and LR using simple parasitological criteria supported by PCV and PP and that resistance to *H. contortus* is inherited.



Abstract tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagia memenuhi keperluan untuk ijazah Master Sains.

**TINDAKBALAS BEBIRI TERHADAP INFEKSI *HAEMONCHIS*
CONTORTUS DARI SEGI PEWARISAN KERINTANGAN AND
PENAMBAHAN NUTRISI**

Oleh

Mohamed Ali Ben-Gheshir

Julai 2000

Pengerusi: Profesor Madya Rehana Abdulla Sani, Ph.D.

Fakulti: Perubatan Veterinar

Parasit gastrousus, terutamanya *Haemonchus contortus*, adalah punca utama gastroenteritis berparasit di negara-negara tropika dan ianya berhubungkait dengan kerugian besar pada ekonomi. Kajian ini dijalankan untuk menyiasat peningkatan tindakbalas bebiri terhadap jangkitan *H. contortus* dengan pemberian tokokan diet berprotin dan dengan memilih dan membiak bebiri untuk ketahanan terhadap *H.ontortus*.

Dalam eksperimen pertama, 32 ekor anak bebiri berumur 3 bulan telah digunakan untuk mengkaji pengaruh tokokan diet berprotin ke atas jangkitan *H. contortus*. Anak bebiri telah diberi diet ruminan asas yang sempurna (15% protin kasar) bersama atau tanpa campuran meal ikan sebagai sumber protin pintasan rumen (19% CP). Anak bebiri daripada setiap kumpulan perlakuan diet telah diberi jangkitkan titisan dengan larva infektif *H. contortus* selama 7 minggu ataupun dibiarkan tanpa jangkitan. Kesemua anak bebiri diberi antihelminik pada minggu ke-8 selepas jangkitan dan dicabarkan dengan satu dos yang mengandungi 5,000 L₃ *H. contortus* seminggu kemudian, dan dibunuh pada hari ke-14 selepas dicabar.

Pemberian tokokan diet mengurangkan kiraan telur di tinja (FEC) pada anak bebiri yang dijangkit fistisan. Anak bebiri tanpa diberi tokokan diet yang dijangkit titisan mempunyai kadar isipadu sel padat (PCV), haemoglobin (Hb) dan protin plasma (PP) yang lebih rendah. Walaupun eosinophilia yang ketara tidak diperhatikan dan kiraan eosinophil periferal dan bilangan cacing tidak berbeza dengan signifikan di antara keempat-empat kumpulan tersebut, tokokan diet ada kesan signifikan eosinophil dan sel mast dalam mukosa abomasal ($P < 0.05$). Korelasi yang signifikan dicatat antara bebanan cacing dan kiraan sel tisu.

Pada eksperimen kedua, bebiri baka Santa Ines telah dipilih dan dibiak untuk ketahanan terhadap jangkitan *H. contortus*. Satu populasi asas berjumlah 123 ekor, berumur di antara 3-4 bulan daripada dua kelompok telah digunakan Haiwan dengan kiraan FEC yang rendah (purata $< 2,725$) akibat jangkitan semulajadi dianggap sebagai responder (HR) yang tahan terhadap jangkitan strongly, manakala haiwan dengan kiraan FEC yang tinggi (purata $> 3,675$) diklaskan sebagai responder (LR) yang mudah dijangkiti. HR dan LR anak bebiri yang telah dipilih dipindah ke ladang UPM dan dirawat untuk basmikan jangkitan daripada padang ragut. Anak bebiri itu disimpan dalam kandang dan kemudiannya dicabarkan secara tiruan dengan satu dos sebanyak 10000 L_3 *H. contortus*. Pada umur 12 bulan, jantan HR dikawankan dengan betina HR sementara jantan LR dikawankan dengan betina LR. Anak-anak yang terhasil pula dicabarkan secara tiruan apabila diceraikan susu supaya status tindakbalasnya boleh ditetapkan. FEC, PCV, PP dan haiwan-haiwan berat badan ini selepas cabaran dimantau.

FEC haiwan HR adalah kurang secara signifikan ($P < 0.001$) berbanding dengan haiwan LR di padang dan selepas cabaran. PCV dan PP haiwan LR adalah



lebih rendah dengan signifikan berbanding dengan haiwan HR. Terdapat korelasi positif yang signifikan di antara FEC daripada jangkitan yang berlaku di padang dan yang dilakukan secara ujian, dan di antara FEC anak-anak dengan FEC induk jantan dan betina. Kajian ini bercadang bebiri boleh diasingkan kepada yang HR dan LR dengan menggunakan kriteria parasitologi yang mudah, disokongi oleh PCV dan PP dan ketahanan terhadap jangkitan *H. contortus* dapat diwurisi.

ACKNOWLEDGEMENTS

All praise to Almighty Allah, the Merciful and the Benevolent. Had it not been due to His will and favour, the completion of this study would not have been possible.

I would like to express my sincere gratitude and appreciation to my supervisor, Associate Professor Dr. Rehana Abdullah Sani; she has devoted a lot of her time for invaluable advice, guidance, support, and patience and kind encouragement throughout the duration of this study.

I wish to express my sincere gratitude to my co-supervisors Dr Daud Israf Ahmed and Dr Jothi Malar Panandam for their kind encouragement, guidance and sincere constructive comments. I also thank Dr. Mahmood Ameen Abdullah for his technical assistance in histology. Thanks are also due to Dato. Prof. Sheikh Omar Abdull Rahman, Dean of Faculty of Veterinary Medicine, Dr. Douglas Gray at the Livestock Research Division, Philippine Council for Agriculture, Forestry and Natural Resources and Development (PCARRD), Los Banos, Laguna and Dr. D. J. Weilgama from Department of Parasitology, Faculty of Medicine, University of Peradeniya, Sri Lanka for there advices, comments, support and friendly relationship. Special thanks goes to Dr. Jothi Malar Panandam for her assistance in the statistical analysis.



I am especially grateful to the Ministry of High Education and University of Omar Al-Mukhtar, Libya for providing the scholarship during the duration of my study

I would also like to thank the management and staff at the sheep farm, Pusat Pembiakan Bebiri, Gajah Mati, Kedah for their kind hospitality during the course of sample collection, particularly Puan Sharifah Norhaimi Mohd Salleh and Dr. Ibrahim Jalil

I also acknowledged the skilful assistance and untiring help rendered by the staff of the Parasitology Laboratory, Mr. Lee Chu Chong and Encik Bohari Yaacob, and other colleagues who had in one way or another helped to make the study a success and most memorable.

I would like to express my gratitude to Associated Professor Dr Rasedee Abdullah and members of Clinical Pathology Laboratory, who have provided the facilities of their laboratories during the course of this study. I wish to thank Mr. Karim from the Computer Centre for providing the SAS software for my statistical analysis

I have also been very fortunate in receiving assistance from a number of my colleagues and friends. Many of them went with me during the collection of samples or data. However, I would like in particular to thank Mr. Zaki, Mr. Khor Yew Lee and Mr. Vijay Kumar.



Last but not least, I am greatly indebted to my parents, my dear wife and beloved children, Haneen and Israa and all of my brothers and sisters, for their support and understanding and sacrifices when I have been unable to give them my attention.



TABLE OF CONTENTS

		Page
	DEDICATION.	ii
	ABSTRACT.	iii
	ABSTRAK	vi
	ACKNOWLEDGEMENTS.	ix
	APPROVAL SHEETS.	xii
	DECLARATION FORM.	xiv
	LIST OF TABLES.	xviii
	LIST OF FIGURES	xx
	LIST OF ABBREVIATIONS	xxii
CHAPTER		
I	INTRODUCTION	1
II	LITERATURE REVIEW	5
	General	5
	Life cycle and Transmission of Gastrointestinal Nematode	5
	Pathogenesis and Clinical Signs	6
	Immunology of Haemonchus Infections	7
	Diagnosis	8
	Differential Resistance of Sheep to G. I. Parasites	8
	Selection Criteria for Resistance	11
	Artificial and natural Infections	15
	Breed Differences	17
	Effect of Dietary Protein upon Developing Immunity	20
	Immunological mechanism of gastrointestinal parasites	25
	Cell Types and Cellular Response	26
	Eosinophil Response	26
	Mast Cells Response.....	28
	Plasma Cells Response.....	30
	Role of Mucus in Immunity	31
III	INFLUENCES OF DIETARY PROTEIN SUPPLEMENTATION ON REGULATION OF <i>HAEMONCHUS CONTORTUS</i> POPULATIONS OF DORSIMAL LAMBS.	33
	Introduction	33
	Materials and Methods	34
	Animals and Feed	34
	Experimental Design	35
	Infective Larvae	35
	Haematology	37
	Blood Collection	37
	Packed Cell Volume (PCV)	37
	Haemoglobin (Hb) Concentration	37



Plasma Protein (PP) Concentration	38
Peripheral Eosinophil counts	38
Removal and Preparation of Abomasum for Worm	
Counts	38
Worm Counts	39
Abomasal Tissue Sections for Histology	39
Mucosal Cell Counts	40
Statistical Analysis	40
Results	42
Faecal egg counts	42
Packed Cell Volume	44
Haemoglobin Concentration	44
Plasma Protein Concentration	48
Peripheral Eosinophil Counts	52
Worm Burdens	52
Tissue Cell counts	56
Correlation Analysis Between Worm Counts and	
Tissue Cell Counts	57
Discussion an Conclusion.....	61

IV	SCREENING AND SELECTION OF SANTA INES	
	SHEEP FOR RESISTANCE TO HAEMONCHUS	
	CONTORTUS	67
Introduction		67
Materials and Methods		68
Site of Study and Management		68
Experimental Animals		69
Breeding Program		69
Selection of Animals		72
Faecal Egg Counts		73
Infective Larvae		74
Counting of Larvae		75
Challenge Infection		75
Body Weight		76
Haematology		76
Blood Collection		76
Packed Cell Volume		76
Plasma Protein concentration		77
Statistical Analysis		77
Results		79
Classification of Responder Status		79
Faecal Egg Counts		79
Packed Cell Volume		89
Plasma Protein		95
Body Weight		102
Correlation Analysis of FEC between Field and		
Experimental Infections in Flock I and II		102
Faecal egg count of Family		103



	Correlation Analysis between Variables FEC, PCV, and PP of HR and LR Animals in Flock I, II and Lambs	111
	Discussion and Conclusion.....	115
V	GENERAL DISCUSSION AND CONCLUSION	123
	BIBLIOGRAPHY	130
	APPENDICES	150
	VITA	168



LIST OF TABLES

Table		Page
1	Experimental design	36
2	Least square means (\pm S E) for packed cell volume of all groups by week	46
3	Least square means (\pm S E) for haemoglobin of all groups by week	49
4	Least square means (\pm S E) for plasma protein of all groups by week	51
5	Least square means (\pm S E) for peripheral eosinophil counts of all groups by week	54
6	Least square means (\pm S E) for worm burdens in the four groups of Dorsimal lambs	55
7	Least square means (\pm S E) of tissue cell counts for all groups	58
8	Simple correlation coefficient between worm burdens and tissue cell counts for groups (IS and IUS)	59
9	Simple correlation coefficient between worm burdens and tissue cell counts for groups (UIS and UTUS)	60
10	Foundation flock size and selected animals ..	73
11	Lsmean (\pm S E) faecal egg counts of the 3rd and 4th sample following the natural infection	83
12	Least square means (back-transformed) faecal egg counts of HR and LR animals by week	87
13	Least square means for faecal egg counts of HR and LR animals by responder status and Sex	88
14	Least square means (\pm S E) for PCV of HR and LR animals by responder status and Week	90
15	Least square means (\pm S E) of PCV by responder status and Sex	97
16	Least square means (\pm S E) of plasma protein (PP) of HR and LR animals by week	99
17	Least square means (\pm S E) of plasma protein (PP) by responder status and sex	104
18	Least square means (\pm S E) for the body weight of HR and LR lambs by responder status and week	106



19	Simple correlation coefficient between field and experimental infection in Flock I and II	107
20	Simple correlation coefficient between field and experimental infection in Flock I	108
21	Simple correlation coefficient between field and experimental infection in Flock II	109
22	Mean FECs of pedigree following the artificial challenge	110
23	Correlation between parents and their offspring FECs	110
24	Correlation analysis between variables FEC, PCV, and PP related to the responder status and the duration in weeks	113
25	Correlation analysis between variables FEC, PCV, and PP related to the responder status	114



LIST OF FIGURES

Figure		Page
1	Mean FEC (\pm S D) throughout the trickle infection period in Dorsimal lamb infected with <i>H. contortus</i>	43
2	Least square means of packed cell volume for all animals groups infected with <i>H. contortus</i>	45
3	Least square means of haemoglobin concentration for all animals groups infected with <i>H. contortus</i>	47
4	Least square means of plasma protein for all animals groups infected with <i>H. contortus</i>	50
5	Least square means of peripheral eosinophil counts for all animals groups infected with <i>H. contortus</i>	53
6	Photograph, abomasum, sheep, infected-supplemented group sub-mucosal infiltration by mast cell, eosinophil, plasma cell in response to <i>H. contortus</i> (McNamara's Giemsa \times 1000)	57
7	Breeding program	70
8	Means (\pm S E) FECs for HR and LR animals following natural pasture infection in Flock I	81
9	Means (\pm S.E) FECs for HR and LR animals following natural pasture infection in Flock II	82
10	Least square means faecal egg counts of HR and LR animals in Flock I infected with <i>H. contortus</i>	84
11	Least square means faecal egg counts of HR and LR animals in Flock II infected with <i>H. contortus</i>	85
12	Least square means faecal egg counts of HR and LR animals in Lambs infected with <i>H. contortus</i>	86
13	Least square means (\pm S E) packed cell volume of HR and LR animals in Flock I infected with <i>H. contortus</i>	91
14	Least square means (\pm S E) packed cell volume of HR and LR animals in Flock II infected with <i>H. contortus</i>	92
15	Least square means (\pm S E) packed cell volume of HR and LR animals in Lambs infected with <i>H. contortus</i>	94
16	Least square means (\pm S E) plasma protein of HR and LR animals in Flock I infected with <i>H. contortus</i>	98



17	Least square means (\pm S E) plasma protein of HR and LR animals in Flock II infected with <i>H. contortus</i>	100
18	Least square means (\pm S E) plasma protein of HR and LR Lambs infected with <i>H. contortus</i>	101
19	Least square means (\pm S E) body weight of HR and LR Lambs infected with <i>H. contortus</i>	105

LIST OF ABBREVIATIONS

μg	micro-gram
μl	micro-liter
μm	micrometer
B. wt	body weight
CSIRO	Commonwealth Scientific and Industrial Research Organization
d.f	degree of freedom
DM	dry matter
EDTA	ethylene-diamine tetra-acetic acid
EOS	eosinophil
epg	egg per gram
FEC	faecal egg count
GL	globule leukocyte
Hb	haemoglobin
HR	high responder
IHb	initial haemoglobin value
IPCV	initial packed cell volume value
IPP	initial plasma protein concentration value
LR	low responder
Lsmean	least square mean
MAFF	Ministry of Agriculture, Fisheries and Food (London)
MMC	mucosal Mast cell
MS	mean square
PBS	phosphate buffered saline



PCARRD	Philipine Council for Agriculture, Forestry and Natural Resources and Development
PCV	packed cell volume
PP	plasma protein concentration
rpm	revolution per minute
S.G	specific gravity
UPM	Universiti Putra Malaysia



CHAPTER I

INTRODUCTION

Sheep population in Malaysia is growing rapidly to meet the increasing demand for meat and the need to increase self-sufficiency has led to a major shift towards commercial production (Rajion *et al.*, 1993). However, one of the major problems faced by sheep farmers is gastrointestinal parasitism.

Gastrointestinal nematode, in particular *H. contortus*, is the major source of parasitic gastro-enteritis in tropical countries and was associated with large economic losses (Over *et al.*, 1992). Haemonchosis is one of the major disease problems affecting sheep production in Malaysia (Sani *et al.*, 1995). *Haemonchus contortus* and *Trichostrongylus spp.* are the most important strongyles in sheep and goats in Malaysia (Sani *et al.*, 1985; 1986).

The aim of any helminth control is to reduce parasitism to levels that have little impact on animal production. There are various ways to control helminthiasis in sheep. Anthelmintic prophylaxis is a very common control measure world wide, including Malaysia. Rotational grazing management alone or in combination with anthelmintic prophylactics and improved nutrition is another common control measure. The grazing management program is often impractical in a country like Malaysia, which has limited or shortage pastureland and the climate is favourable for parasites development and survival (Dorny *et al.*, 1994).

