UNIVERSITI PUTRA MALAYSIA

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

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By

MOHAMED ALI BEN-GEHSHIR

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DEDICATION

To my parents, brothers, sisters, my wife and my children, Haneen and Isrra, 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

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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* L_3 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* L_3. 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* L_3 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

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Julai 2000

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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 antihelmintik pada minggu ke-8 selepas jangkitan dan dicabarkan dengan satu dos yang mengandungi 5,000 L_3 *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. Walaupaun 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 dicerai susu supaya status tindakbalasnya boleh ditetapkan. FEC, PCV, PP dan haiwan-haiwan berat badan ini selepascabaran 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.

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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, Foresty 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).