## UNIVERSITI MALAYSIA SABAH

В	ORANG PENGESAHAN STATUS TESIS
JUDUL: THE EFFICACY COCCIDIOSIS CATFLE WEANER	OF MONEINSIN SODIUM ON CONTROLLING AND AVERAGE PAILY GAIN IN FEEDLOT BEEF IS AND YEAR LINGS
UAZAH: BACHELOR OF	AGRICULTURE SCIENCE WITH HONOURS
SAYA: WONG YIEN SII	
Mengaku membenarkan tesis * (LPSM/s syarat-syarat kegunaan seperti berikut:-	Sarjana/Doktor-Falsafah) ini disimpan di Perpustakaan Universiti Malaysia Sabah dengan
	iti Malaysia Sabah. sia Sabah dibenarkan membuat salinan untuk tujuan pengajian sahaja. buat salinan tesis ini sebagai bahan pertukaran antara institusi pengajian tinggi.
SULIT	(Mengandungi maklumta yang berdarjah keselamatan atau kepentingan Malaysia seperti yang termaktub di AKTA RAHSIA RASMI 1972)
TERHAD	(Mengandungi maklumat TERHAD yang telah ditentukan oleh organisasi/badan di mana Penyelidikan dijalankan)
TIDAK TERHAD	JAMIUD MICHEAL LUKALIAN UNIVERSITI MALAYSIA SABAH
	Disahkan Oleh:
(TANDATANGAN PENULIS)	(TANDATANGAN PENYELIA)
Alamat Tetap: 202A, Taman (hin Jalan Sultan Iska 97000 Bintulu, Sarawak.	BO KIDON DE KANNAN
Tarikh: 26.4.2010	Tarikh: 26.4.2010
berkuasa/organisasi	berkenaan. JLIT atau TERHAD, sila lampirkan surat daripada pihak yang i berkenaan dengan menyatakan sebab dan tempoh tesis ini perlu SULIT atau TERHAD.

Tesis dimaksudkan sebagai tesis bagi Ijazah Doktor Falsafah dan Sarjana Secara penyelidikan atau disertasi bagi pengajian secara kerja kursus dan Laporan Projek Sarjana Muda (LPSM)



# THE EFFICACY OF MONENSIN SODIUM ON CONTROLLING COCCIDIOSIS AND AVERAGE DAILY GAIN IN FEEDLOT BEEF CATTLE WEANERS AND YEARLINGS

WONG YIEN SING

DISSERTATION SUBMITTED IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR THE DEGREE OF BACHELOR OF AGRICULTURE SCIENCE WITH HONOURS

> LIVESTOCK PRODUCTION PROGRAMME SCHOOL OF SUSTAINABLE AGRICULTURE UNIVERSITI MALAYSIA SABAH 2010



## DECLARATION

I hereby declare that this dissertation is based on my original work except for citations and quotations which have been duly acknowledged. I also declare that no part of this dissertation has been previously or concurrently submitted for a degree at this or any other university.

Sing .

Wong Yien Sing HP2006-6151 15<sup>th</sup> March 2010



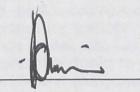
1. Dr. Kiron Deep Singh Kanwal SUPERVISOR

Dr. Punimin Abdullah

CO-SUPERVISOR

2.

DR. KIRON D.S KANWAL Pensyarah / Penasihat Akademik Sekolah Pertanian Lestari Universiti Malaysia Sabah



DR. PUNIMIN ABDULLAH PENOLONG PENGARAH

3. Madam Mary Magdaline Siambun EXAMINER

Mr. Januarius Gobilik 4. **FXAMINER** 

MARY SIAMBUN PENSYARAH Sekolah Pertanian Lestari Universiti Malaysia Sabah

JANUARIUS GOBILIK Lecturer / Academic Advisor School of Sustainable agriculture Universiti Malaysia Sabah

5. Prof. Dr. Ridzwan Abdul Rahman DEAN School Sustainable Agriculture

PROF. DR. RID ZWAN ABDUL RAHMA

V Dekan Sekolah Pertanian Lestari Universiti Malaysia Sabah



#### ACKNOWLEDGEMENT

Initially, this dissertation is prepared to fulfill part of the requirements required for us to obtain the degree. Yet along the process of writing this dissertation, the snag and challenges faced had provided me a new experiences and joy. Studying about the effect of monensin has always been an interesting subject for me. This has been the main reason I chose this title for my dissertation.

During the writing of this dissertation, I received great encouragement and support from many individuals to whom I would like to express my gratitude. First of all, I would like to thank my supervisor, Dr. Kiron Deep Singh Kanwal, who had guided me to finish my dissertation. Besides, I would like to express my thanks to my cosupervisor, Dr. Punimin Abdullah, who had given me this title and giving me lots of ideas to improve my dissertation. I also appreciate for the helpful in my dissertation from Encik Assis bin Kamu and Wee Sok Kok which help me a lot in ideal of SPSS and I would like to thank them for kindly spending their time discussed with me.

Finally, I would like to thank all School of Sustainable Agriculture lecturers and all my friends for all their guidance and efforts for this past four years. Thank you very much for being patients and tolerance for me.



#### ABSTRACT

Coccidiosis is an infectious disease of cattle particularly affecting confined animals. Intensive animal husbandry practices can increase the spread of coccidiosis. The efficacy of monensin sodium in controlling coccidiosis infection in beef weaners and yearlings from different breed of cattle and age groups kept under feedlot system at Livestock Breeding Centre, Tawau was examined. There were 67 heads (24 heads of Brahman, 23 heads of Droughtmaster, and 20 heads of Bali cattle) of male beef weaners and yearlings raised in the feedlot were used. The beef weaners and yearlings were fed daily with 20 kg of fresh signal or grass and 2 kg/head/day concentrates. Monensin sodium (Elancoban, Elanco®) was added into the mixture of the concentrates at the rate of 600 mg/head/day. The experiment with the feeding supplementation with monensin was started in August 2009 and ended in December 2009. Individual fecal samples were collected from the beef weaners and yearlings before and after treatment. The coccidia oocysts counts were determined by McMaster method. The beef weaners and yearlings were weighed in March, June, August, September and December. All the samples were tested positive for coccidia oocysts with the infection rate between one and three oocysts/g. Monensin showed an increase of mean average daily gain (ADG) of the Bali beef yearlings from 0.19 kg to 0.32 kg and in the 19-26 months age group beef yearlings from 0.25 kg to 0.29 kg. Monensin given in the diet was effective in controlling bovine coccidiosis infections and increase ADG of the beef yearlings. Thus, monensin was suitable to be use in the cattle industry especially in feedlot diets to control coccidiosis infections. Further study need to be done to examine the relationship between the dosage of the monensin and the average daily gain of beef weaners and yearlings.



## KEMUJARAHAN MONENSIN SODIUM PADA JANGKITAN KOKSIDIOSIS DI KALANGAN ANAK SAPIH DAN HAIWAN SETAHUN LEMBU PEDAGING FIDLOT

#### ABSTRAK

Koksidiosis ialah sejenis penyakit berjangkit lembu terutamanya mempengaruhi ternakan kurung. Pengurusan ternakan intensif meningkatkan rebakan penyakit ini. Kemujaraban monensin dalam mengawal jangkitan koksidiosis di kalangan anak sapih dan haiwan setahun lembu pedaging fidlot yand berlainan spesies dan umur dikaji di Stesen Pembiakan Ternakan, Tawau. Terdapat 67 ekor (24 ekor Brahman, 23 ekor Droughtmaster, dan 20 ekor Bali) anak sapih dan haiwan setahun lembu jantan pedaging fidlot yang dikaji di kajian ini. Anak sapih dan haiwan setahun lembu tersebut diberi makan sebanyak 20 kg signal atau rumput segar dan 2 kg/ekor/hari konsentrat. Monensin sodium (Elancoban, Elanco®) ditambahkan ke dalam konsentrat pada tahap 600 mg/ekor/hari. Esperimen bermula pada Ogos 2009 and berakhir pada Disember 2009 dengan memberikan monensin sebagai tambahan. Sampel tahi individu dikumpul daripada anak sapih dan haiwan setahun lembu sebelum dan selepas rawatan. Pengiraan oosit koksidia ditentukan melalui McMaster. Anak sapih dan haiwan setahun lembu tersebut ditimbang pada bulan Mac, Jun, Ogos, September, dan Disember. Semua sampel oosit koksidia menunjukkan positif untuk rawatan sebelum dan selepas denagn kadar jangkitan antara satu dan tiga oosit/g. Monensin menunjukkan purata tambahan berat badan harian meningkat daripada 0.19 kg kepada 0.32 kg bagi haiwan setahun Bali dan daripada 0.25 kg kepada 0.29 kg bagi kumpulan umur 19-26 bulan haiwan setahun lembu pedaging. Monensin yang ditambahkan pada makanan berkesan untuk mengawal jangkitan koksidiosis dan meningkatkan purata tambahan berat badan harian anak sapih dan haiwan setahun lembu pedaging. Oleh itu, monensin sesuai digunakan dalam industri lembu untuk mengawal jangkitan koksidiosis terutamanya bagi lembu fidlot. Kajian lanjut perlu dilakukan untuk mengkaji hubungan antara dos monensin dan purata tambahan berat badan harian anak sapih dan haiwan setahun lembu pedaging.



## **TABLE OF CONTENTS**

DE VE AC AB AB TAI LIS LIS	Intent ICLARATION RIFICATION KNOWLEDGEMENT STRACT STRAK BLE OF CONTENTS T OF TABLES T OF FIGURES T OF FIGURES T OF SYMBOLS, UNITS AND ABBREVIATIONS	Page ii iv vv vi vii ix x xi
		<b>1</b> 1 2 2
<ul> <li>CHA</li> <li>2.1</li> <li>2.2</li> <li>2.3</li> <li>2.4</li> <li>2.5</li> </ul> 2.6 <ul> <li>2.7</li> <li>2.8</li> </ul> 2.9 <ul> <li>2.10</li> <li>2.11</li> <li>2.12</li> <li>2.13</li> </ul>	Coccidia Species in Cattle	<b>3</b> 3 4 5 5 6 6 6 7 7 7 8 8 9 9 10 10 10 10 10 10 10 11 11 11



CHAPTER 3MATERIALS AND METHODS3.1Location3.2Animals3.3Weighing of Animals3.4Parasitological and ADG3.4.1Sampling3.4.2Fecal Analyses3.4.3Equipments3.4.4McMaster Procedure3.4.5The Level of Infestation3.4.6ADG3.5Data Analysis	21 21 22 22 22 22 22 23 23 23 23 23 24 24
CHAPTER 4 RESULTS 4.1 Coccidia Oocysts 4.2 Live Body Weight 4.3 ADG	
CHAPTER 5 DISCUSSION	31
CHAPTER 6 CONCLUSION	34
REFERENCES	35
APPENDICES	40



## LIST OF TABLES

Table		Page
3.1	The definition for the level of infestation	23
4.1	The oocysts count before treatment for each breed of cattle and age groups	25
4.2	The oocysts count after treatment for each breed of cattle and age groups	26
4.3	The mean weight of the weaners and yearlings according to breeds before and after treatment	26
4.4	The mean weight of the weaners and yearlings according to ages before and after treatment	27
4.5	The average daily gain of weaners and yearlings according to breeds before and after treatment	28
4.6	The average daily gain of weaners and yearlings according to ages before and after treatment	29



## **LIST OF FIGURES**

Figure		Page
3.1	The weighing schedule	22
4.1	The mean average daily gain in Bali, Brahman and Droughtmaster cattle	28
4.2	The mean average daily gain in age groups cattle	30



## LIST OF SYMBOLS, UNITS AND ABBREVIATIONS

°C % R ANOVA ADG cm kg km kg/day kg/head/day mg/day mg/monensin/head/day ml mm No. sg

**Degree Celsius** Percentage Registered Analysis of variance Average daily gain Centimeter Kilogram Kilometer Kilogram per day Kilogram per head per day Milligram per day Milligram per monensin per head per day Milliliter Millimeter Number Specific gravity



### **CHAPTER 1**

#### INTRODUCTION

### 1.1 Introduction

Gastro-intestinal parasites are widespread and are a serious problem to the livestock in the tropics. Helminths and coccidian are the most common and significant gastro-intestinal parasites in ruminants (Kusiluka *et al.*, 1996). Coccidiosis is considered to be one of the economically most significant diseases in intensive production of ruminants in the world.

Coccidiosis particularly affects confined animals, and intensive animal husbandry practices increase the spread of coccidiosis. All infected animals showed dullness, anorexia, diarrhea, dehydration, and loss of body weight (Bangoura and Daugschies, 2007b). More importantly, coccidiosis can have a negative impact on performance and carcass quality of beef cattle because of reduction in weight gain and feed efficiency. This may cause economic loss to the farmers practicing feedlot system.

In one study, all calves kept under conventional system were infected by coccidiosis early in life (Lucas *et al.*, 2007). The clinical disease mostly affects calves between three weeks and six months, or soon after weaning. The highest prevalence of oocyst shedding and incidence of clinical disease occurs in calves less than one year of age (Lucas *et al.*, 2007). Management practices such as over crowding expose calves to high number of infective oocyst that result increase in infection and disease (Gauly *et al.*, 2004).

Humoral (Faber *et al.*, 2002) and cellular immune responses (Ghanem *et al.*, 2008) are produced by the animals infected with *Eimeria bovis*. Immune responses help to control coccidiosis infection. Beside immune responses to control coccidiosis infection, there are several anticoccidial drugs that are available and effective to

prevent and control coccidiosis in beef calves (Mundt *et al.*, 2005a). One of them is monensin sodium, also called rumensin. It is used for treatment and control of coccidiosis caused by *Eimeria bovis* and *Eimeria zuernii* species in growing cattle on pasture or in dry lot. Moreover, monensin sodium also helps in increasing the rate of weight gain in cattle.

Monensin has wide acceptance by the cattle industry since its use in feedlot diets. It is routinely added to feedlot diet to improve feed efficiency. Monensin improves dry matter digestibility by reducing methane (De and Singh, 2003). When fed in high energy diets, monensin lowers daily feed intake without reducing weight gain. Monensin also reduces lactic acid production (Goodrich *et al.*, 1984) and the likelihood of feedlot bloat, and it may reduce heat production and aid in control of coccidiosis. Nevertheless, the use of monensin as an anticoccidial in beef cattle weaners and yearlings has not been investigated in Malaysia.

### 1.2 Justification

The purpose of feedlot is to produce marketable beef in the shortest possible time. And, coccidiosis is a common disease in feedlot beef cattle especially young calves, and causes great economic losses worldwide (Ghanem *et al.*, 2008) which prolonged the rearing period. Monensin is an anticoccidial agent used to prevent coccidiosis in cattle, and as a feed additive it improves growth rate and carcass yield of beef cattle (Goodrich *et al.*, 1984) and thus help to shorten the rearing period.

## 1.3 Objective

The objective of this study is to determine the effect of feeding monensin sodium in controlling coccidiosis infection in beef weaners and yearlings of different breed and age kept under feedlot situation. In addition, the study will also examine the average daily gain of the animals. It was hypothesize that the use of monensin will control coccidiosis infection and in addition, will increase the average daily gain.



## **CHAPTER 2**

#### LITERATURE REVIEW

### 2.1 Introduction

Coccidiosis had been given various common names. These included scours, bloody scours, hemorrhagic enteritis, dysentery, and bloody diarrhea (Ernst and Benz, 1981). Nevertheless, since all coccidial organisms of cattle belong to the genus *Eimeria*, bovine eimeriosis was the most precise name but the common name that investigators preferred to use for this disease was bovine coccidiosis (Ernst and Benz, 1981).

Bovine coccidiosis affected cattle in most countries from the tropics to temperate zones. It was an infectious disease of calves all over the world especially in major cattle-raising countries and caused economic loses (Matjila and Penzhorn, 2002; Samson-Himmelstjerna *et al.*, 2006; Bangoura and Daugschies, 2007b) every year either to the beef or dairy industries (Ghanem *et al.*, 2008). The prevalence of the disease was significantly higher in weaners than in adults (Matjila and Penzhorn, 2002). Therefore, more weaners were excreted a high number of oocysts compared to adult (Fayer *et al.*, 2000).

#### 2.2 Coccidia Species in Cattle

*Eimeria* species represented important protozoan parasitosis in cattle coccidiosis, caused high impacted on animal health and profitability (Matjila and Penzhorn, 2002) of the cattle industry. *Eimeria bovis* and *Eimeria zuernii* were the most prevalent species infected the confined cattle (Matjila and Penzhorn, 2002; Ghanem *et al.*, 2008), which were often numerous in bovine fecal samples (Samson-Himmelstjerna *et al.*, 2006). In the other words, the two most pathogenic of the protozoa that caused disease in confined cattle were *Eimeria bovis* and *Eimeria Zuernii* (Radostits and Stockdale, 1980; Fayer *et al.*, 2000; Matjila and Penzhorn, 2002; Bangoura and

Daugschies, 2007b; Lucas *et al.*, 2007; Ghanem *et al.*, 2008). Other species frequently appeared in confined cattle were *Eimeria ellipsoidalis*, *Eimeria cylindrica*, *Eimeria auburnensis*, *Eimeria alabamensis*, *Eimeria subspherica*, *Eimeria pellita*, *Eimeria brasiliensis*, *Eimeria canadensis*, *Eimeria bukidnonensis*, *Eimeria illinoisensis*, and *Eimeria wyomingensis* (Cornelissen *et al.*, 1995).

*Eimeria alabamensis* was the predominant species in grazing cattle with clinical coccidiosis (Samson-Himmelstjerna *et al.*, 2006). *Eimeria bovis, Eimeria auburnensis, Eimeria zuernii, Eimeria ellipsoidalis,* and *Eimeria bukidnonensis* were other species frequently found in grazing cattle (Svensson, 1998). *Eimeria ellipsoidalis* appeared early in animals which was found in calves on their third week of life and had a marked predominance on the oocysts culture until 25 days of age (Sánchez *et al.*, 2008). *Eimeria brasiliensis* and *Eimeria wyomingensis* were the two species occurring rarely in the cows but were not found in calves (Faber *et al.*, 2002). A calf shed few oocysts of *Eimeria pellita* which was not observed in the cows (Faber *et al.*, 2002).

## 2.3 Transmission of Coccidia

Coccidiosis was transmitted by infected host through oral and it was a basic source for the disease transmission. Different coccidia were specific to their own hosts. Coccidial infections of bovine origin had not been found in other animals; none of the species of nonbovine coccidia were demonstrated in cattle (McAllister, 2006). Coccidia may infected healthy adult animals and produce great number of oocysts. The animals became carriers of coccidiosis and the source of infection to other animals (McAllister, 2006).

The other source of the transmission that resulted in coccidiosis was the ingestion of sporulated oocysts. The infection was acquired from feed or water or by licking contaminated hair coat (Ernst and Benz, 1981). Once ingested, the oocysts must undergo sporulation to become infective. The oocysts need two to six days to undergo sporulation and need oxygen and moisture condition. The oocysts sporulated at 15°C to 30°C (Ernst and Benz, 1981). Temperatures above 30°C were killed the oocysts quickly while temperatures below freezing killed the oocysts slowly. Cold temperatures above freezing only avoided sporulation to occur (Ernst and Benz, 1981; McAllister, 2006).



## 2.4 Epidemiology of Coccidiosis

Coccidiosis was caused by a parasite that lived inside the cells of the infected cattle's intestinal tract. The coccidian mainly infected the large intestine such as cecum and colon (Bangoura and Daugschies, 2007b). Prepatent periods of *Eimeria bovis* and *Eimeria zuernii* were about 17 days (Mundt *et al.*, 2005b; Faber *et al.*, 2002; Bangoura *et al.*, 2007b). There were two asexual stages for both organisms (Radostits and Stockdale, 1980). For first-generation, *Eimeria bovis* were found in endothelial cells lined the lacteals of villi while *Eimeria zuernii* were found in the lamina propria usually closed to the muscularis mucosa (Ernst and Benz, 1981). Microscopically, *Eimeria bovis* schizonts of *Eimeria zuernii* were often surrounded by a thin multicellular enveloped outside the host cell and schizonts of *Eimeria zuernii* were often surrounded by one or more layers of Imphocytes (Ernst and Benz, 1981).

For second-generation, *Eimeria bovis* and *Eimeria zuernii* had little or no pathologic effect until mature (Ernst and Benz, 1981). Microscopically, the infected host cell enlarged and the host-cell nucleus broke into irregular shape because the mass of the schizont. There was little inflammation reaction occurred. However, the lamina propria had increased numbers of neutrophils, lymphocytes, and macrophages (Ernst and Benz, 1981).

## 2.5 Clinical Signs of Coccidiosis

### 2.5.1 Diarrhea

Bovine coccidiosis infected animals at any age, led to inflamed intestine, slowed weight gain, and economic lose (Matjila and Penzhorn, 2002; Bangoura and Daugschies, 2007; Ghanem *et al.*, 2008). The common clinical sign of coccidiosis in calves was diarrhea. Coccidium was one of the enteropathogen that caused diarrhea in calves. The diarrheic feces contained mucous and blood (Ernst and Benz, 1981; McAllister, 2006). The prevalence of coccidiosis and median oocyst per gram of feces in diarrheic animals was significantly higher than in non-diarrheic animals (Chibunda *et al.*, 1997). Thus, *Eimeria* species may caused diarrhea, which may also caused by other microorganisms (Chibunda *et al.*, 1997; McAllister, 2006).



## 2.5.2 Mild and Acute Coccidiosis

In mild coccidiosis cases, the animals showed diarrhea, weakness, and lose of appetite or anorexia as clinical signs (Ernst and Benz, 1981; Ghanem *et al.*, 2008). The feces may bloody. In acute coccidiosis cases, the animals had signs that included emaciated, dehydrated, weak, and listless. The feces were very fluid, bloody, and often have an extremely smelly odor. Besides, they may have rough coats, dropping ear, and sunken eye (Ernst and Benz, 1981). Clinical bovine coccidiosis was usually deceptive. Signs were often apparent at three to eight weeks after initial infection (Matjila and Penzhorn, 2002). However, *Eimeria bovis* and *Eimeria zuernii* had shown the first clinical signs at approximately about two to three weeks post-infection (Matjila and Penzhorn, 2002).

### 2.5.3 Nervous

According to McAllister (2006), the nervous form of coccidiosis was another clinical sign. Acute dysentery with nervous signs showed muscular tremor, convulsions, and occasional blindness. The mortality rate was about 50%. Animals with nervous involvement may die during a convulsion on the first day of illness or may survive for three to five days then died (Ernst and Benz, 1981). Nevertheless, animals infected by terminal convulsion may recover within one week (Ernst and Benz, 1981; McAllister, 2006).

### 2.5.4 Weight Gain

Many cattle were infected subclinically and this resulted in economic lose (Ghanem *et al.*, 2008), mainly due to lower weight gain. Subclinically infected animals suffered from decreased feed intake, feed conversion, and growth performance although appeared normal outwardly (Ernst and Benz, 1981). The animals reduced feed efficiency, slower weight gain made it susceptible to other diseases. The severity of the infection was related to the number of ingested oocysts (Ernst and Benz, 1981; Cornelissen *et al.*, 1995; Fayer *et al.*, 2000; McAllister, 2006).



### 2.6 Type of Animals Infected by Coccidiosis

#### 2.6.1 Confined Animals

The problem of coccidiosis can occur particularly in confined animals reared under intensive animal husbandry practiced. Coccidiosis was most frequently found in young calves, six to nine months of age, and reared in feedlot (Goodrich *et al.*, 1984). *Eimeria zuernii* was a paramount causal agent of clinical coccidiosis in dry lot calves. The consequence of the disease was related to massive diarrhea and decreased weight gain (Mundt *et al.*, 2005b; Bangoura and Daugschies, 2007b). Coccidiosis due to *Eimeria zuernii* caused significant pathological changed in the intestine. There was little inflammation of the small intestine, while lesions in large intestine with lose of epithelium and presence of fibrin in which cellular debris and bacterial colonies were incorporated (Mundt *et al.*, 2005b).

#### 2.6.2 Grazing Animals

Certain pasture which recently fertilized with fresh manure or pasture rotation practices may be the sources of coccidiosis (McAllister, 2006). In grazing animals, diarrhea may be caused by protozoan pathogens of *Eimeria* species (Samson-Himmelstjerna *et al.,* 2006). There were several *Eimeria* species that were capable of established clinical disease related to watery to bloody diarrhea, high morbidity and high mortality will be depended on the dominant species of the *Eimeria* involved (Waggoner *et al.,* 1994; Farkas *et al.,* 2007). Polyinfections were commonly found in the field, but monoinfections were detected in some cases (Samson-Himmelstjerna *et al.,* 2006).

Infection of *Eimeria alabamensis* at turnout was either during the first few days of grazed or before turnout which led to low oocysts excretion. *Eimeria alabamensis* infections had shown the clinical signs of diarrhea within the first week post turnout. The diarrhea usually started four to six days after turnout. Symptoms of infected calves were watery diarrhea, poor appetite, depression, abdominal pain, and decreased growth rate (Samson-Himmelstjerna *et al.*, 2006). *Eimeria alabamensis*-dominated infections had low morbidity (Samson-Himmelstjerna *et al.*, 2006; Farkas *et al.*, 2007). However, it reduced weight gained during the first months of the grazing period. Affected cattle may lose weight depended on the severity of infection. Within two to three weeks of turnout, the calves may lose up 15% of body weight (Svensson, 1998). The consequence was fatal if no treatment given. Reduction in weight gain was also

7

found in natural infections. Mortality of *Eimeria alabamensis*-infected cattle was very low as below 1% although infections with more than one species were present (Samson-Himmelstjerna *et al.*, 2006).

*Eimeria alabamensis* oocysts can be transmitted by fed contaminated hay even though it had been stored for eight months (Samson-Himmelstjerna *et al.*, 2006). Animals that higher intake of the grass had ingested more oocysts. Other animals showed only mild signs that included poor appetite and softened feces (Bangoura and Daugschies, 2007b). The prepatent period of *Eimeria alabamensis* was between the ranges of six and ten days (Samson-Himmelstjerna *et al.*, 2006). It became prevalent at approximately seven to nine days post turnout of contaminated pasture. It can be diagnosed on eighth to tenth day of grazed due to the presence of the large numbers of oocysts (Samson-Himmelstjerna *et al.*, 2006).

## 2.7 Causing Factors of Coccidiosis Infection

The development of clinical coccidiosis in cattle was mainly dependant on the *Eimeria* species involved (Ernst and Benz, 1981; Sánchez *et al.*, 2008), the age of the infected animals (Cornelissen *et al.*, 1995; Fayer et al., 2000; Faber *et al.*, 2002), the number of oocycts ingested (Ernst and Benz, 1981; Samson-Himmelstjerna *et al.*, 2006; Bangoura and Daugschies, 2007b), the presence of a concurrent microbial infection (Matjila and Penzhorn, 2002), the production system (Sánchez *et al.*, 2008) and management practices used by the farmer (Cornelissen *et al.*, 1995; Gauly *et al.*, 2004; Lucas *et al.*, 2007).

## 2.7.1 Species of Coccidia

*Eimeria bovis* and *Eimeria zuernii* were important agents caused coccidiosis in confined cattle, while *Eimeria alabamensis* was infected to grazing cattle. *Eimeria bovis* and *Eimeria zuernii* were two pathogenic species caused gross lesions in the large intestine of cattle, which can result in severe bloody diarrhea and more seriously death (Ernst and Benz, 1981; Cornelissen *et al.*, 1995). *Eimeria alabamensis* was less pathogenic and caused less mortality (Samson-Himmelstjerna *et al.*, 2006; Farkas *et al.*, 2007).



### 2.7.2 Age of Infected Animals

*Eimeria* infections occurred frequently in all age classes. Nevertheless, coccidiosis was primarily a disease of young animals, which were one or two months to one year of age (Fayer *et al.*, 2000). Thus, most of the young cattle were infected by coccidia during first year of life. *Eimeria bovis* was infected more in cattle that less than one year of age than those over a year of age (Ernst and Benz, 1981; Sánchez *et al.*, 2008). Therefore, the prevalence coccidiosis in calves was more than in yearlings. This was because new-born calves had incomplete specific antibodies and cells (Rauprich *et al.*, 2000; Faber *et al.*, 2002; Sánchez *et al.*, 2008).

The prevalences of coccidiosis were low in the higher age classes. Thus, the oocysts number in yearlings were semi-quantitative due to coccidiosis was not commonly infected to yearlings. In other words, yearlings infected by mild coccidiosis compared to calves which was severe coccidiosis (Cornelissen *et al.*, 1995). Multiple infections of coccidiosis also occurred. The maximum number of species per sample ranged from five to seven in calves and yearlings respectively (Cornelissen *et al.*, 1995).

#### 2.7.3 Management of Farms

Management practices were the most significant cause of the development of coccidiosis infections. In general, calves were individually housed during the first few weeks and subsequently kept in small group in relative large pens (Cornelissen *et al.*, 1995). This was to provide enough space to animals to avoid overcrowded (Chibunda *et al.*, 1997). More importantly, general hygienic levels were high and manure was frequently removed (Ernst and Benz, 1981; Fayer *et al.*, 2000). Feeds should be placed in elevated feeders to reduce fecal-oral transmission (Ernst and Benz, 1981; McAllister, 2006). All these practices help in decreased the intake of high numbers of infective occysts by calves.

Management of farm that exposed high numbers of infective oocysts increased the infection and caused clinical signs (McAllister, 2006). High stocking densities and restrictive indoor living environments caused coccidiosis infections and it was rapid spread once infected (Gauly *et al.*, 2004). After protozoa being passed in feces, coccidial oocysts typically took two to four days to sporulate and became infectious (McAllister, 2006). Intensively reared animals were likely to become infected with large numbers of oocysts though fecal matter (Sánchez *et al.*, 2008). The oocysts were extremely resilient and resistance to disinfectants so it is hardly to overcome environmental contamination due to oocysts (Ernst and Benz, 1981; Ovington *et al.*, 1995; Gauly *et al.*, 2004; Sánchez, *et al.*, 2008).

## 2.7.4 Stress

When non-immune animals exposed to contaminated environments, coccidiosis infection occurred easily (Ernst and Benz, 1981; Faber *et al.*, 2002). This was because the animals had low immunity and under stress. Stress reduced immune response and animals were more prone to coccidiosis infections (Sánchez *et al.*, 2008). Animals can be exposed to stressful conditions such as malnutrition, bad hygiene, parasitism, and pollution. Moreover, transport, allocation to new group, and other infectious diseases also imposed stress in calves (Daugschies *et al.*, 2007; Sánchez *et al.*, 2008).

## 2.8 Effects of Coccidiosis on Animal Productivity

## 2.8.1 Animal Productivity

*Eimeria* infections occur frequently in cattle and can be a significant factor limited animal productivity, especially in calves and yearlings (Ernst and Benz, 1981; Lucas *et al.*, 2007). The subclinical infections of coccidiosis caused negative influence on animal productivity, as mild infections with *Eimeria bovis* resulted in a decreased feed consumption and lose of body weight (Cornelissen *et al.*, 1995; Matjila and PenzhoHaern, 2002; Bangoura and Daugschies, 2007b).

## 2.8.2 Haemoglobin and Leukocytes

Calves infected by coccidiosis had reduced weight gain because lose of fluid, decreased food intake, and malabsorption (Bangoura and Daugschies, 2007b). The higher was the level of infection in calves, the lowest of the weight gained. Although animals infected were not anorectic, but bad performance was observed. There was decreased in haemoglobin in *Eimeria zuernii* infected calves due to loss of blood through intestine (Bangoura and Daugschies, 2007b). *Eimeria zuernii* infections also caused lose of leukocytes due to acute inflammation of intestine and hemorrhagic diarrhea (Daugschies *et al.*, 1997; Mundt *et al.*, 2005b; Bangoura and Daugschies, 2007b).



## 2.8.3 Cholesterol and Creatine Kinase

There was low cholesterol concentration in coccidiosis infected calves and caused fat mobilization. This was caused by impaired intestinal absorption of fatty acids which led to decrease esterification in the liver (Bangoura and Daugschies, 2007a). All this decreased weight gain in infected calves. Creatine kinase levels were particularly high in infected animals. This was associated with muscular damaged (Bangoura *et al.*, 2007) and muscular dystrophy because of increased protein mobilization followed by malnutrition. Thus, weight loses occurred. Coccidiosis infections caused nitrogen loses due to intestinal bleeding. The disease also reduced nutrient digestibility and caused weight lose due to anorexia and lose of organic compounds which induced mucosal lesions (Daugschies *et al.*, 1998).

## 2.8.4 Free Fatty Acid, Total Protein, Albumin, Bilirubin, and Urea

The serum level of coccidiosis infected animals was low than healthy calves. Free fatty acid concentration increased in the periods of lipolysis because malabsorption through the lesion in the intestine. Protein concentration was low in the severely infected animals because of decreased in albumin concentration. The decreased in albumin decreased the serum antibody levels too (Bangoura *et al.*, 2007). *Eimeria alabamensis* infections increased bilirubin levels due to decreased food intake (Bangoura *et al.*, 2007). Urea levels were high in infected calves. Azotaemia appeared during endogenous protein catabolism because of lesion tissue, intestinal hemorrhage, dehydration, and electrolytes imbalance (Bangoura and Daugschies, 2007b).

#### 2.8.5 Electrolytes

The importance of imbalance in the metabolism of water and electrolytes in diseases related to diarrhea had been showed (Daugschies *et al.*, 1997; Bangoura and Daugschies, 2007a). The pathogenesis of *Eimeria bovis* which caused mild to hemorrhagic diarrhea in calves was due to alterations of the water and mineral balances (Daugschies *et al.*, 1997). Calves that suffered from severe diarrhea which disturbances of the absorptive capacity of the altered mucosa of the large intestine had low apparent digestibility and became anorectic (Daugschies *et al.*, 1997). The *Eimeria bovis* coccidiosis caused lose of fluid through the intestine. This was because low absorption of water from the infected large intestine and lose of blood into the gut lumen (Daugschies *et al.*, 1997).



#### 2.8.6 Bicarbonate

When coccidiosis infections occurred, the intestinal lose of buffer ions so excess of acids emerges. Thus, alterations of acid-base homoeostasis occurred (Daugschies *et al.*, 1997). Bicarbonate was decline in infected calves (Bangoura and Daugschies, 2007a). However, the bicarbonate decreased only of moderate extent in mild infected calves. There was severe diarrhea in infected calves that highly declined in pH. The levels of carbon dioxide also declined in infected calves (Bangoura and Daugschies, 2007a).

## 2.9 Coccidiosis Control and Treatment in Cattle

Management practices were important to control coccidiosis in cattle (Fayer *et al.*, 2000; Matjila and Penzhorn, 2002). Crowded (Chibunda *et al.*, 1997; Gauly *et al.*, 2004) and commingled in areas where hygiene was deficient can serve to increase both the level of infection and incidence of clinical disease due to expose calves to high numbers of infective oocysts (Ernst and Benz, 1981; Chibunda *et al.*, 1997; Lucas *et al.*, 2007). Disinfection of farm should be done to ensure unfavorable conditions for oocysts survived in the environment (Farkas *et al.*, 2007). Strong solutions such as lye or bichloride of mercury were effective for disinfection. The solutions can be applied to walls and floors by used stiff brush (Ernst and Benz, 1981). Furthermore, manure and wet should remove daily to ensure hygiene (Fayer *et al.*, 2000; McAllister, 2006).

Interestingly, outbreaks of coccidiosis for clinical disease increased with the infection pressure in the environment (Ovington *et al.*, 1995; Gauly *et al.*, 2004) and may further increased under conditions that imposed stress on the calves. This was because of transport, allocation to new groups, inadequate feeding, and other infectious diseases (Sánchez *et al.*, 2008). Age-segregation of calves was a coccidiosis prevention method. Calves can be placed in separate pens for different age groups (Ernst and Benz, 1981) such as calves under three weeks old of group, calves three to six weeks old of group, calves six weeks to three months old of group, and calves over three months old of group (McAllister, 2006). The two youngest groups need special take care and attention because they were easy infected by coccidiosis due to low immunity (Faber *et al.*, 2002; Farkas *et al.*, 2007).

Several anticoccidial drugs were effective in treatment and control of coccidiosis in beef calves (Mundt *et al.*, 2005b). These drugs were toltrazurit (Mundt *et al.*, 2005b);

#### REFERENCES

- Aziz, D.M. and Enbergs, H. 2005. Stimulation of Bovine Sperm Mitochondrial Activity by Homeopathic Dilutions of Monensin. *Homeopathy* **94**: 229-232
- Baile, C.A., McLaughlin, C.L. and Chalupa, W.V. 1982. Effects of Monensin Fed to Replacement Dairy Heifers during the Growing and Gestation Period upon Growth, Reproduction, and Subsequent Lactation. *Journal Dairy Science* **65**: 1941-1944
- Bangoura, B. and Daugschies, A. 2007a. Influence of Experimental *Eimeria zuernii* Infection in Calves on Electrolyte Concentrations, Acid-Base Balance and Blood Gases. *Parasitology Research* **101**: 1637-1645
- Bangoura, B. and Daugschies, A. 2007b. Parasitological and Clinical Parameters of Experimental *Eimeria zuernii* Infection in Calves and Influence on Weight Gain and Haemogram. *Parasitology Research* **100(6)**: 1331-1340
- Bangoura, B., Daugschies, A. and Fuerll, M. 2007. Influence of Experimental *Eimeria zuernii* Infections on Clinical Blood Chemistry in Calves. *Veterinary Parasitology* **150**: 46-53
- Boling, J.A., Bradley, N.W. and Campbell, L.D. 1977. Monensin Levels for Growing and Finishing Steers. *Journal Animal Science* **44**: 867-871
- Burrin, D.G. and Britton, R.A. 1986. Response of Monensin in Cattle during Subacute Acidiosis. *Journal Animal Science* 63: 888-893
- Bushmich, S.L., Randel, R.D., McCartor, M. and Carroll, L.H. 1980. Effect of Dietary Monensin upon Ovarian Response Following Gonadotropin Treatment in Pre-Puberal Heifers. *Journal Animal Science* **51**: 692-697
- Calhoun, M.C., Carroll, L.H., Livingston, C.W. and Shelton, M. 1979. Effect of Dietary Monensin on Coccidial Oocyst Numbers, Feedlot Performance and Carcass Characteristics of Lambs. *Journal Animal Science* **49**: 10-19
- Castillo, C., Benedito, J.L., Mendez, J., Pereira, V., Lopez-Alonso, M., Miranda, M. and Hernandez, J. 2004. Organic Acids as a Substitute for Monensin in Diets for Beef Cattle. *Animal Feed Science and Technology* **115**: 101-116
- Chibuda, R.T., Muhairwa, A.P., Kambarage, D.M., Mtambo, M.M.A., Kusiluka, L.J.M. and Kazwala, R.R. 1997. Eimeriosis in Dairy Cattle Farms in Morogoro Municipality of Tanzania. *Preventive Veterinary Medicine* **31**: 191-197
- Clanton, D.C., England, M.E. and Parrott, J.C. 1981. Effect of Monensin on Efficiency of Production in Beef Cows. *Journal Animal Science* **53**: 873-880
- Coleman, S.W. and Evans, B.C. 1986. Effect of Nutrition, Age and Size on Compensatory Growth in Two Breeds of Steers. *Journal Animal Science* 63: 1968-1982



- Cornelissen, A.W.C.A., Verstegen, R., Brand, H. Perie, N.M., Eysker, M., Lam, T.J.G.M. and Pijpers, A. An Observational Study of *Eimeria* species in Housed Cattle on Dutch Dairy Farms. *Veterinary Parasitology* **56**: 7-16
- Daugschies, A., Agneessens, J., Goossens, L., Mengel, H. and Veys, P. 2007. The Effect of a Metaphylactic Treatment with Diclazuril (Vecoxan) on the Oocysts Excretion and Growth Performance of Calves Exposed to a Natural *Eimeria* Infection. *Veterinary Parasitology* **149**: 199-206
- Daugschies, A., Burger, H.J. and Akimaru, M. 1997. Effects of Experimental Infection with *Eimeria bovis* on the Balance of Sodium, Potassium and Water in Calves. *Parasitological International* **46**: 159-169
- Daugshies, A., Burger, H.J. and Akimaru, M. 1998. Apparent Digestibility of Nutrients and Nitrogen Balance during Experimental Infection of Calves with *Eimeria bovis. Veterinary Parasitology* **77**: 93-102
- De, D. and Singh, G.P. 2003. Effect of Cold Process Monensin Enriched Urea Molasses Mineral Blocks on of Crossbred Calves Fed a Wheat Straw Based Diet. *Animal Feed Science and Technology* **103**: 51-61
- Ernst, J.V., and Benz, G.W. 1981. Coccodiosis. Diseases of Cattle in The Tropics: Economic & Zoonotic Relevance **31(6)**: 377 – 392
- Faber, J., Kollmann, D., Heise, A., Bauer, C., Failing, K., Burger, H.J. and Zahner, H. 2002. *Eimeria* Infections in Cows in the Periparturient Phase and Their Calves: Oocyst Excretion and Level of Specific Serum and Colostrum Antibodies. *Veterinarian Parasitology* **104 (1)**: 1–17
- Farkas, R., Szeidemann, Z. and Majoros, G. 2007. Studies on Coccidiosis of Calves in Hungarian Dairy Farms. *Parasitology Research* **101**: 113-120
- Fayer, R., Trout, J.M., Graczyk, T.K. and Lewis, E.J. 2000. Prevalence of *Cryptosporidium, Giardia* and *Eimeria* infections in Post-weaned and Adult Cattle on Three Maryland Farms. *Veterinary Parasitology* **93**: 103-112

Fisher, A. 1995. The UN-Cattle of Bali. International Development Research Centre.

- Gauly, M., Reeg, J., Bauer, C. and Erhardt, G. 2004. Influence of Production Systems in Lambs on the *Eimeria* Oocyst Output and Weight Gain. *Small Ruminant Research* **55**: 159-167
- Ghanem, M.M., Radwaan, M.E., Moustafa, A.M.M. and Ebeid, M.H. 2008. Comparative Therapeutic Effect of Toltrazuril, Sulphadimidine and Amprolium on *Eimeria bovis* and *Eimeria zuernii* Given at Different Times Following Infection in Buffalo Calves (*Bubalus bubalis*). *Preventive Veterinary Medicine* **84**: 161-170
  - Goodrich, R.D., Garrett, J.E., Gast, D.R., Krick, M.A., Larson, D.A. and Meiske, J.C. 1984. Influence of Monensin on the Performance of Cattle. *Journal Animal Science* **58**: 1484



- Herald, F., Knapp, F.W., Brown, S. and Bradley, N.W. 1982. Efficacy of Monensin as a Cattle Feed Additive against the Face Fly and Horn Fly. *Journal Animal Science* 54: 1128
- Kusiluka, L.J.M., Kambarage, D.M., Matthewman, R.W., Harrison, L.J.S., and Daborn C.J. 1996. Coccidiosis of Small Ruminants in Tanzania. *Small Ruminant Research* **21**: 127-131
- Larsson, A., Dimander, S.O., Uggla, A., Waller, P. and Hoglund, J. 2006. Effects of Single or Concurrent Infections with *Eimeria alabamensis* and Gastrointestinal Nematodes on the Performance of Calves on Pasture. *Parasitology Research* 99: 84-89
- Lucas, A.S., Swecker, W.S., Lindsay, D.S., Scaglia, G., Elvinger, F.C. and Zajac, A.M. 2007. The Effect of Weaning Method on Coccidial Infections in Beef Calves. *Veterinary Parasitology* **145**: 228-233
- Matjila, P.T. and Penzhorn, B.L. 2002. Occurrence and Diversity of Bovine Coccidian at Three Localities in South Africa. *Veterinary Parasitology* **104**: 93-102
- McAllister, M.M. 2006. Protozoosis of the Calf: *Giardia, Cryptosporidium, Eimeria, Sarcocystis, Neospora.* World Buiatrics Congress, Nice, France
- McGuffey, R.D., Richardson, L.F. and Wilkinson, J.I.D., 2001. Ionophores for Dairy Cattle: Current Status and Future Outlook. *Journal Dairy Science* 84: 194-203
- Moseley, W.M., McCartor, M. and Randel, R.D. 1977. Effects of Monensin on Growthand Reproductive Performance of Beef Heifers. *Journal Animal Science* **45**: 961-968
- Mundt, H.C., Bangoura, B., Mengel, H., Keidel, J. and Daugschies, A., 2005a. Control of Clinical Coccidiosis of Calves due to *Eimeria bovis* and *Eimeria zuernii* with Toltrazuril under Feld Conditions. *Parasitology Research* **97(1)**: S134–S142
- Mundt, H.C., Bangoura, B., Rinke, M., Rosenbruch, M. and Daugschies, A. 2005b. Pathology and Treatment of *Eimeria zuernii* Coccidiosis in Calves: Investigations in an Infection Model. *Parasitology International* **54**: 223-230
- Nillo, L. 1970a. Bovine Coccidiosis in Canada. Canadian Veterinary Journal 11: 91-98
- Nillo, L. 1970b. Experimental Winter Coccidiosis in Sheltered and Unsheltered Calves. Can. J. Comp. Med. 34: 20-25
- Oliver, W.M. 1975. Effect of Monensin on Gains of Steers Grazed on Coastal Bermudagrass. *Journal of Animal Science* **41**: 999-1001
- Ovington, K.S., Alleva, L.M. and Kerr, E.A. 1995. Cytokines and Immunological Control of *Eimeria* spp. *International Journal for Parasitology* **25(11)**: 1331-1351
- Perry, T.W., Beeson, W.M. and Mohler, M.T. 1976. Effect of Monensin on Beer Cattle Performance. Journal Animal Science 42: 761

- Potter, E.L., VanDuyn, R.L. and Cooley, C.O. 1984. Monensin Toxicity in Cattle. Journal Animal Science 58: 1499-1511
- Potter, E.L., Wray, M.I., Muller, R.D., Grueter, H.P., McAskill, J. and Young, D.C. 1985. Effect of Monensin and Tylosin on Average Daily Gain, Feed Efficiency and Liver Abscess in Feedlot Cattle. *Journal Animal Science* **61**: 1058-1065
- Radostits, O.M. and Stockdale, P.H.G. 1980. A Brief Review of Bovine Coccidiosis in Western Canada. *Journal of Veterinary Canada* **21**: 227-230
- Raun, A.P., Cooley, C.O., Potter, E.L., Rathmacher, R.P. and Richardson L.F. 1976. Effect of Monensin on Feed Efficiency of Feedlot Cattle. *Journal Animal Science* 43: 670
- Rauprich, A.B., Hammon, H.M. and Blum, J.W. 2000. Influence of Feeding Different Amount of First Colostrum on Metabolic, Endocrine and Health Status and on Growth Performance in Neonatal Calves. *Journal Animal Science* **78**: 896-908
- Reed, B.K. and Whisnant, C.S. 2001. Effects of Monensin and Forage: Concentrate Ratio In Feed Intake, Endocrine, and Ovarian Function in Beef Heifers. *Animal Reproduction Science* 67: 171-180
- Richardson, L.F., Raun, A.P., Potter, E.L., Cooley, C.O. and Rathmacher, R.P. 1976. Effect of Monensin on Rumen Fermentation In Vitro and In Vivo. *Journal Animal Science* **43**: 657
- Rouquette, F.M., Griffin, J.L., Randel, R.D. and Carroll, L.H. 1980. Effect of Monensin on Gain and Forage Utilization by Calves Grazing Bermudagrass. *Journal of Animal Science* **51**: 521-525
- Salles, M.S.V., Zanetti, M.A., Titto, E.A.L. and Conti, R.M.C. 2008. Effect of Monensin on Performance in Growing Ruminants Reared under Different Environmental Temperatures. *Animal Feed Science and Technology* **147**: 279-291
- Samson-Himmelstjerna, G., Epe, C., Wirtherle, N., Heyden, V., Welz, C., Radeloff, I., Beening, J., Carr, D., Hellmann, K., Schnieder, T. and Krieger, K. 2006. Clinical and Epidemiological Characteristics of *Eimeria* Infections in First-Year Grazing Cattle. *Veterinary Parasitology* **136**: 215-221
- Sánchez, R.O., Romero, J.R. and Founroge, R.D. 2008. Dynamics of *Eimeria* Oocyst Excretion in Dairy Calves in the Province of Buenos Aires (Argentina), during Their First Two Months of Age. *Veterinary Parasitology* **151**: 133-138
- Svensson, C. 1998. Prevention of *Eimeria alabamensis* Coccidiosis by a Long-Acting Baquiloprim/ Sulphadimidine Bolus. *Veterinary Parasitology* **74**: 143-152
- Turner, H.A., R.J. Raleigh and D.C. Young 1977. Effect of Monensin on Feed Efficiency for Maintaining Gestating Mature Cows Wintered on Meadow Hay. Journal Animal Science 44: 338



- Virkel, G., Lifschitz, A., Sallovitz, J., Inza, G. and Lanusse, C. 2004. Effect of the Ionophore Antibiotic Monensin on the Ruminal Biotransformation of Benzimidazole Anthelmintics. *Veterinary Journal* **167**: 265-271
- Waggoner, J.K., Cecava, M.J. and Kazacos, K.R. 1994. Efficacy of Lasalocid and Decoquinate against Coccidiosis in Naturally Infected Dairy Calves. *Journal Dairy Science* 77: 349-353

Yolande, B. 1998. The Veterinary Formulary. Fourth Edition. Pharmaceutical Press.

