THE PERIPARTURIENT INCREASE IN TRICHOSTRONGYLE EGG COUNTS IN YANKASA EWES IN ZARIA, NORTHERN GUINEA SAVANNAH ZONE OF NIGERIA

BY

NGOZI PAULINE CHIEZEY
(B.Sc, UNN 1978)
(M.Sc, ABU 1985)

THESIS SUBMITTED TO THE POST GRADUATE SCHOOL
AHMADU BELLO UNIVERSITY,
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DEPARTMENT OF PARASITOLOGY/ENTOMOLOGY
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AHMADU BELLO UNIVERSITY,
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NOVEMBER, 1998

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NOVEMBER 1998

DECLARATION

I declare that this work is solely my effort and has not been presented to an Institution or Body for any award or honours

Ngozi Pauline Chiezey

Date: 30-3-99

CERTIFICATION

This thesis entitled ***"The Periparturient increase in Trichostrongyle egg counts in Yankasa ewes in Zaria, Northern Guuinea Savannah zone of Nigeria"*** by Ngozi Pauline Chiezey meets the regulations governing the award of the degree of Doctor of Philosophy (PhD) of Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This thesis is dedicated to :

all women in the workplace who are also wives,

mothers, daughters and sisters,

but most of all to the Great Designer who keeps their cup full to overflowing.

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ABSTRACT

Studies were carried out in Zaria to investigate the occurrence of a pcriparturient increase in Trichostrongyle egg counts in naturally infected Yankasa ewes lambing at various times of the year, using serial egg counts. The first study using ewes in the last month of gestation and non-pregnant ewes as controls, showed that a significantly ($P < 0.05$) greater increase in egg counts occurred in the lambing ewes during the periparturient period when compared with the non-pregnant ewes sampled within the same period. A second study using ewes in their last month of gestation, grazing a separate pasture, confirmed the occurrence of a periparturient increase in egg counts in lambing ewes. Packed Cell Volume declined with increased egg counts but Total protein values did not show an appreciable change. In individual ewes, the periparturient rise in egg counts started about four weeks before parturition with a peak within the first four weeks of parturition/lactation. Larval culture showed that Haemonchus contortus was the main contributor to the rise.

Another experiment designed to evaluate egg counts in pregnant animals as well as evaluate the lambing performance of naturally infected ewes was carried out in a flock of 63 ewes which continuously ran with rams. Susceptibility of ewes at pregnancy was assessed by faecal helminth egg counts in ewes which were pregnant at periods of different pasture larval availability. Ewes lambing in October which had been pregnant in the months of June through September, months of high larval availability did not show significant differences ($P > 0.05$) in egg counts in early and mid pregnancy but there was a significant increase in egg counts in late pregnancy and this started about the 19th week of gestation. Similarly, ewes lambing in June which had gone through pregnancy through

the months of February to May when there was only a low level of larvae available on pasture did not show a significant difference in egg count between early and mid pregnancy but showed a significant increase in late pregnancy and this started about the 17th week of gestation. Ewes that lambed in June had significantly higher egg counts in late pregnancy than ewes lambing in October.

Ewes that lambed in the early dry season had significantly higher egg counts ($P < 0.05$) during the pre-lambing period than ewes lambing in the early wet or late wet seasons. There were no significant differences in the Packed cell volume (PCV) concentrations of ewes lambing in the different seasons but ewes that lambed in the late dry seasons had consistently lower PCV concentrations.

Lambs born in the early dry season had picked up low level helminth infections which were below 500 egg through the dry season months. In the rainy season the highest mean egg count in the lambs was 3,016 ± 1015 In contrast, lambs born in the early wet season became infected as early as seven weeks of age , showing egg counts of up to 10,000 egg, and high mortalities. Growth rate of lambs were however poor as they weighed an average of 19.2 ± 0.5 kg at 10 months of age.

Analysis of the overall lambing performance showed that mean birth weight of lambs was 2.7 kg, fecundity was 1.1 lambs, and there were significantly ($P < 0.05$) less lambs born in the late wet season, lambing percentages being 9.5%, 23.3%, 25.9% and 38% for late wet, late dry, early wet and early dry season lambings respectively. 17.4% of ewes had lambing intervals (L I) of 193 days and another 34.8% had LI of 220 days. the

remaining 47.8% had Lambing intervals greater than 220 days. Low mean egg counts (5025 ± 4441epg) were associated with long lambing intervals, and lambing intervals over 220 days were assoeiated with mean egg counts of 16,317 ± 2315 egg. Ewes with high egg counts ending pregnancy in low body condition neglected their lambs which subsequently died of hyponutrition. Progesterone profiles of the ewes evaluated by radioimmunoassay showed that long lambing intervals were due to delayed resumption of ovarian activity post partum.

In a study to show the effect of weaning on the occurrence of the periparturient rise in egg counts, three groups of 10 ewes each had their lambs weaned at week 0, week 1 and week 4 of lambing respectively. Weaning did not seem to have a significant effect on the occurrence of the periparturient rise in egg counts unless the lambs were separated from the dams immediately after birth (weaned week 0).

Treatment of ewes with levamisole hydrochloride at one week after lambing (OWAL), one week before lambing (OWBL), two weeks before lambing (TWBL) and week of lambing (WOL), showed that treatment immediately after lambing or not later than one week of lambing significantly eliminated the occurrence of the periparturient rise in egg counts.

This study is the first to comprehensively demonstrate the occurrence of a periparturient rise in helminth egg output occurs in Yankasa ewes. The study also demonstrated that the increase in egg output arises from the development of inhibited

populations of larvae in the ewes as well as from newly acquired infections. Larvae derived from the periparturient rise in faecal egg count was shown to be the major source of gastrointestinal nematode infection for young lambs.

Taken together, this study also indicates that helminlhosis while not directly interfering with reproduction, may decrease reproductive efficiency in the ewe by depreciating its body condition. The increase in helminth egg output at the periparturient period indicates that time of parturition can influence the general level of parasitism within a flock due to the breakdown in ewe defence mechanisms as a result of endocrine events associated with late pregnancy and early lactation. Therefore in breeding flocks, measures must be taken to control helminth infections at this time to avoid a build up of larvae on pasture and the subsequent morbidity and production losses associated with parasitism.

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LIST OF ABBREVIATIONS

CD	=	Cluster Designation
EPG	=	Eggs per Gram
FAO	=	Food and Agricultural Organisation
FEC	=	Faecal Egg Count
gm	=	gram
Hb	=	Haemoglobin
IAEA	=	International Atomic Energy Agency
IgA	=	Immunoglobin A
IgE	=	Immunoglobin E
IgG	=	Immunoglobin G
Kg	=	Kilogram
LI	=	Lambing Interval
ng/ml	=	nanogram per ml
°C	=	Degree Centigrade
Oct	=	October
PCV	=	Packed Cell Volume
PGE	=	Parasitic Gastroenteritis
	=	Progesterone
PPR	=	Peri Parturient Rise
SEM	=	Standard Error of the Mean
Spp	=	Species
Temp	=	Temperature
TP	=	Total Protein
Wk	=	Week

CHAPTER ONE

1.0 INTRODUCTION

Sheep occupy an important and strategic position in the socio economic life of the people of Nigeria. Apart from being an important source of the much needed protein in the diet, they feature predominantly in the socio-cultural festivities throughout the country, providing important trade between rural and urban areas and immediate sources of cash for small-holder farmers. They contribute about 11% of the nation's meat supply, and make up about 18% of the livestock population in Nigeria (Adu and Ngere, 1979). The Yankasa is the most numerous breed and also has the widest distribution, being found throughout the sub-humid and semi-arid zones (FDLPS 1991). It is estimated to constitute abut 60% of a National sheep population of 22.1 million (Osinowo 1992). It is a hardy, medium sized, short horned breed with predominantly white coat and patches of black colour around the eyes, muzzle and feet. Obvious advantages of sheep are that they are not in competition with man for feed, being able to convert poor quality fodder into high protein food, having a high fecundity, short generation interval and not requiring intensive capital for their production. (Ademosun, 1973). Despite these advantages they are traditionally viewed as slow growing scavengers, requiring little investment of capital and labour (Bayer, 1986), and large scale production of sheep has largely not been undertaken.

Major constraints to the development of the sheep industry have been identified as high rates of internal parasitism as well as poor management factors (ILCA, 1979b); these often lead to low productivity and high lamb mortality (Adu and Buvanendren, 1982; Agei

1993). Although parasites are widely prevalent, the clinical signs they cause in infected animals can be less obvious than signs of other livestock diseases. For this reason, infections with helminth parasites are among the most neglected areas of veterinary care in much of the developing world (Hansen and Perry, 1994). Allonby and Urqhart (1975) however point out that one of the most significant features of helminthosis (especially where haemonchosis is prevalent) is not necessarily the acute syndrome characteristically associated with the disease, but the fact that a few hundred worms persisting over a long period could produce chronic anaemia and ultimately loss of condition and death in sheep, especially if they are grazing low quality pasture. Some of these effects have been reported to include poor growth rates, low fertility of ewes and cows (Lewis, 1975); low birthweight of lambs, (Johnstone *et al.*, 1979) as well as a reduced percentage of ewes rearing lambs to weaning (Mackay, 1980). These insidious effects of chronic haemonchosis have important implications for the attainment of maximum productivity in adult sheep

The interaction of parasitism and reproductive activity in the ewe characterised by a periparturient rise (PPR) in faecal worm egg output in ewes has been well documented (reviewed by Connan, 1968, Michel, 1974). Various studies (O'Sullivan and Donald, 1970; 1973; Connan, 1968; Michel *et al.*, 1979; Donald *et al.*, 1982). have shown that the PPR results from a general reduction in immunological responses to worms at late gestation and lambing. Resistance to the establishment of in-coming larvae, constraints on the fecundity of female worms and the capacity to expel adult worms are all diminished The epidemiological importance of this phenomenon is that sheep are known to be more

susceptible to the ill effects of internal parasites than other domestic livestock (Pal and Quayum, 1992) and the relaxed immunity places the ewe at greater risk of parasitic and other diseases and to production losses, while larvae hatched from the numerous ova become an important source of nematode infection when the new lamb crop begins to graze (Salisbury and Arundel, 1970).

This has been recognised as a flock problem in sheep farming countries and is incorporated into routine health care of breeding flock . In Nigeria, there is very little information on the occurrence of a PPR and it's contribution to the epidemiology of gastroenteritis in the indigenous breeds. Van Geldorp and Schilhorn van Veen, (1976) described its occurrence in a few Uda sheep while Schilhorn van Veen and Ogunsusi, (1978) described it in seven Uda sheep lambing over a one-month period. With the increasing interest in intensive production systems with selection for accelerated twice-yearly lambing ewes, there is a need to study the contribution of the PPR to the epidemiology of gastro-intestinal helminths in the different ecological areas and adequate control strategies worked out.

This study was therefore designed to :

(i) investigate the occurrence of the periparturient rise in helminth egg output in naturally infected ewes lambing at different times of then year.

(ii) study the sequential development of helminth infections in lambs born to the ewes and any effects on lamb growth performance.

(iii) investigate the effect of lactation on the occurrence of the periparturient rise in helminth egg counts by weaning lambs at various periods relative to lambing.

(iv) study the sequential development of helminth infections in lambs born to the ewes and any effects on lamb growth performance.

(v) to design an appropriate strategic treatment regime to eliminate the occurrence of the peri parturient increase in egg counts.

CHAPTER TWO

REVIEW OF LITERATURE

2.1 Host -Parasite Relations

2.1. 2. Aetiology

The family Trichostrongylidae includes small, (5-30mm) thin bursate nematodes which occur in the abomasum and small intestine of ruminants and cause a syndrome called Parasitic gastroenteritis (PGE). They include nematodes of the genera: *Haemonchus, Ostertagia, Trichostrongylus, Cooperia* and *Nematodirus.*

In Nigeria, species of importance in small ruminants are *Haemonchus contortus* and *Trichostrongylus colubriformis* (Schilhorn van Veen, 1978; Eysker and Ogunsusi, 1980; Chiejina, 1987). In its typical form PGE is a disease of young animals, occurs mainly during the rainy season (Schilhorn van Veen and Brinckman, 1975; Eysker and Ogunsusi, 1980; Chiejina, 1987) and is characterised by anorexia, diarrhoea, loss of weight or reduced weight gain, hypoalbuminemia, protein-losing gastroentheropathy and sometimes anaemia (Soulsby, 1982).

2.1.3 Life Cycle and Transmission

The basic life cycle of the Trichostrongylid nematodes are the same. The life cycle is direct. Adult nematodes inhabit and mate in the gastrointestinal tract . Eggs produced by the female are passed out in the faeces. The eggs embryonate and hatch into first stage

larvae, (L1) which in turn moult into second stage larvae (L2), shedding their protective cuticle in the process. The L2 moult into third stage larvae (L3) but retain the cuticle from the previous moult. This double-cuticled L3 is the infective stage of these nematodes. The time required for the eggs to develop into infective larvae depends on temperature and relative humidity. The ideal temperature for larval development of many species is between 18° and 26°C. Some parasites will continue to develop at temperatures as low as 5°C but at a much lower rate. Development can also occur at higher temperatures but mortality is high at these temperatures. The ideal humidity for larval development is 100% and the minimum humidity required for development is about 85%. The survival of larvae in the environment depends upon adequate moisture and shade. Desiccation from lack of rainfall kills eggs and larvae.

Ruminants are infected by ingesting the L3. Most larvae are picked up during grazing and pass to the abomasum or intestine, ex-sheathing the extra cuticle in the process. The L3 of the Trichostrongyle group penetrate the mucous membrane (in the case of *Haemonchus* and *Trichostrongylus*) or enter the gastric glands (as in *Ostertagia*) During the next few days, the L3 moult to the fourth stage (L4), and remain in the mucous membrane or gastric gland for 10-14 days.

The period between the infection of an animal by ingestion of infective L3 and the first egg production by the adult female called the prepatent period, varies from 2-4 weeks, depending on the species. The development of infective larvae ingested by an animal during adverse environmental conditions may be temporarily arrested in the abomasal or intestinal mucosa. This suspension of development helps some nematode parasites survive

the dry seasons. The L4 then emerge and moult to a young adult stage, L5. Most Trichostrongyles mature and start egg production about 3 weeks after infection Different species of nematodes have different egg laying capacities. The individual female *Cooperia* for example produces many eggs but is not very pathogenic. Females of *Trichostrongylus* are quite pathogenic but produce few eggs. *Haemonchus* has the greatest fecundity, an adult female laying up to 5000 eggs per day. The number of eggs produced by an adult will also depend on the level of immunity to the intestinal parasite the host possesses.

2.2 Epidemiology of Trichostrongylid Parasites in Nigeria

In Nigeria, prevalence rates of gastrointestinal helminths in small ruminants are known to be 50- 100% all year round; (Fabiyi, 1973; Okon, 1975; Ogunsusi, 1978) and parasitic gastroenteritis of sheep is known to be caused by *Haemonchus contortus, Oesophagostomum,* and *Trichostrongylus* species (Kuil, 1970; Schilhorn van Veen, 1973; Anosa, 1973; Fabiyi, 1973) with *Haemonchus* being the most prevalent and most pathogenic. Chronic infections are common and adult animals are known to develop some immunity to the infections (Ogunsusi, 1978).

Availability and longevity of larvae on pasture and transmission of infection are determined primarily by climatic factors . In a survey of seasonal incidence of different species of gastrointestinal nematodes in dwarf goats from the Zaria area of northern Nigeria, Fabiyi (1973) observed that worm burdens were highest during the rainy season but with definite sequence in the succession and abundance of the species. *H. contortus* predominated during June and August with a peak in burden in July. *T. colubriformis* on the other hand was most numerous during August and November with a peak incidence in

September. Thus *Haemonchus* predominates during the early part of the rainy season while other species appeared during the second half of the rainy season or early dry season. Fabiyi (1973) attributed the differences in the order of succession and in the peak incidence largely to differences in the fecundity constant and generation intervals of the species. The relevance of this to the host population is that haemonchosis will be the overwhelming infection in the PGE of domestic animals. Also the ability of *Haemonchus* and *Cooperia* species to survive the dry season in domestic ruminants as arrested larvae or as adults enables them to appear early in a favourable season.

Domestic animals in the rain forest zone of southern Nigeria acquired nematode infections all the year round because there is adequate rainfall and temperature for the development and survival of the pre-parasitic stages throughout the year in that part of the country (Okon and Akinpelu, 1982). Similar observations were made by Fagbemi and Dipeolu (1982) from work carried out in the tropical rain-forest and derived savannah zones of south western Nigeria. In contrast, in northern Nigeria, rainfall is highly seasonal. The rainy season lasts from May to October and dry season occurs from November to April. In the extreme north, the rainy season may last for only three months. In the Guinea savannah zone of northern Nigeria, the development and survival of the pre-parasitic stages of strongyle nematodes and hence pasture contamination only occur during the wet season. and only negligible infections are acquired by animals during the dry season. Also in the northern guinea savannah zone due to unfavourable weather conditions arrested development of *H. contortus* and *Trichostrongylus* species in sheep (Ogunsusi and Eysker, 1979) occur. They observed an increasing percentage inhibition of *H. contortus* in sheep from August onwards with nearly 100% inhibition by the end of the rains in October.

The term hypobiosis which literally means a slowing down of life processes was first used by Gordon (1973), to describe a temporary but sometimes prolonged period of arrest of development of nematodes in their natural hosts This usually occurs when conditions are unfavourable for transmission of the free-living stages from one host to another. Hypobiosis includes such terms as arrested, retarded, inhibited or suppressed development when these are applied to worms that have not completed their development in the host within the commonly accepted interval for the species (Gordon, 1973). Initially hypobiosis was considered to be the result of host resistance to worm infections which served to regulate populations of adult worms against irregular intakes of larvae. Later work provided evidence that hypobiosis is mainly an adaptation of the life cycle, that serves to synchronise the development of the parasite with changes in its environment, thereby facilitating its persistence and transmission.

The epidemiological importance of arrested larval development is that it does not only ensure the survival of the nematode during periods of adversity but also the subsequent maturation of arrested larvae increases the contamination of the environment and can sometimes result in clinical disease in grazing animals. Fabiyi *et al.*, (1979) reported an outbreak of clinical parasitic gastro-enteritis during the late dry season and associated this with the synchronous resumption of development of large numbers of L4 of *H. placei, C. pectinata* and *C. punctata* which had been arrested in the host due to unfavourable weather conditions for their development. Studies done indicate that such larvae are the only important means of carry-over of *Haemonchus* species infections from one rainy season to the other in the north (Hart,1964; Eysker and Ogunsusi ,1980). In the south, particularly in the derived savannah areas, Chiejina *et al.*, (1988) and Fakae (1990) found very limited

arrested development of *H. contortus* in goats and no survival of extra- host stages of the parasite on pasture. Therefore neither hypobiosis nor dry season survival of the infective larval L3 on pasture are likely to be important in the initiation of infection in sheep and goats at the onset of the rainy season in these areas.

2.3 Hypobiosis and the Periparturient Rise in Sheep

Several factors have been proposed as inducers of hypobiosis. These include host effects, both immunologic and physiologic, environmental stimuli acting on the larvae, and parasite-related factors such as genetic predisposition and population interactions (Gibbs, 1982). Generally there are a number of factors that may be responsible for inducing this state even within a given species but most workers agree that there are basically two types of hypobiosis. Non-specific arrest occurs at any time and its causes could be either host-related or parasite-related factors which affect the immediate environment of the nematode, causing inhibition in development. Seasonally arrested development on the other hand represents an annual event during the same season and is dependent on nematodes being adapted to a particular environment and being responsive to external environmental stimuli acting upon the infective larvae which result in arrest at a later stage in development. In temperate climates, seasonal arrest seems to be related to the advent of cool temperatures, but in tropical and arid areas such conditions are not a factor. In these regions, arrest seems to be related to the onset of hot, dry conditions. These larvae remain inhibited in the abomasal glands during the dry season until the next rainy season or until the animal is calving or sick.

Both types of hypobiosis ultimately cumulate in the maturation of larvae; and this often occurs spontaneously and synchronously. This results in the typical "spring rise" in faecal egg counts seen with *H. contortus* and other parasites (Taylor,1935; Gibbs, 1964) Hypobiotic forms are generally unaffected by immune mechanisms and they are singularly resistant to anthelmintics. Generally hypobiotic larvae resume development in modest numbers to replace adult worms removed by anthelmintic treatment, or spontaneously lost, thereby maintaining a turnover of worms even in animals which have no access to fresh infection.

Factors responsible for the initiation of resumption of development of the arrested larvae are now known. The once commonly held view that resumption of development was due to loss of resistance in the host was excluded by the work of Blitz and Gibbs (1971) which showed that transfer of arrested L4 to non- immune pregnant ewes did not alter their time of emergence from arrest, from that seen in other sheep. Furthermore, attempts at stimulation of resumption of development in arrested larvae by immunosuppression of the host have been unsatisfactory. Gibbs (1967) using chlorambucil, an immunosuppressive alkylating agent was unable to stimulate resumption of development of arrested *H. contortus* in ewes in spite of a marked lymphocyte depression in these ewes that suggested that they were severely immunosuppressed. However, resumption of development was later observed in these ewes after treatment was discontinued, at the same time as a periparturient increase in egg counts was observed in other ewes. Similar results using adrenocorticotrophic hormone, another immunosuppressive agent was observed. Prichard *et al.*, (1974) were also unsuccessful in their attempts to induce maturation with immunosuppressive drugs. It therefore appears unlikely that a depression of immunity is

responsible for the resumption of development of arrested larvae. Only in one instance did removal of adult worms with anthelmintic cause larvae of *O. circumcincta* that were arrested to resume development (Dunsmore,1963).

There is a great deal of work demonstrating that resumption of development or activation of quiescent larvae can be influenced directly or indirectly by changes occurring in the host that are related to pregnancy or lactation. Although spring rises in egg counts have been observed in barren ewes and male sheep (Brunsdon, 1966; Gibbs 1967) and maximum expression of the rise has been associated with parturition and lactation (especially in the latter). Most workers believe that this reflects a fall in the immune status of the host associated with the endocrinologic changes of parturition and lactation a periparturient relaxation of immunity. (Connan, 1968; O'Sullivan and Donald, 1970) However in some ewes, the rise in faecal egg counts may begin prior to lactation (O'Sullivan and Donald, 1970).

It appears that when the immunosuppression of lactation coincides with the presence of larvae in the gut of sheep, the larvae are able to reach adulthood and remain for extended periods of time. With some specie of nematodes, this may also apply when there is intake of larvae from pastures during this period. This results in a post parturient rise in egg counts because of the number of adult worms that become established in these lactating ewes (O'Sullivan and Donald, 1970; O'Sullivan and Donald, 1973; Donald *et al.*, 1982).

The effect of lactation may best be interpreted in the light of its known ability to suppress the expulsion of worms through its hormonal interferences. It thus appears that

there is a two-phase phenomenon associated with the maturation of hypobiotic larvae (Gibbs, 1986). During the first phase, there is a seasonally-related maturation of the larvae that is either a result of an internal larval stimulus; or to some external stimulus acting on these larvae. In non-lactating animals, these larvae stimulate an immune reaction that results in their expulsion. Relatively few worms reach maturity and produce eggs, and even these do not persist. This is responsible for the transient low-level rise in egg counts that is seen in this class of sheep. In lactating animals on the other hand, because there is a *temporary suppression of immunity (the periparturient relaxation of immunity)*, large numbers of the developing larvae reach maturity and are thus responsible for the high and more persistent egg counts that are seen during the periparturient rise (Gibbs and Barger, 1986). In addition, should these sheep be exposed to populations of infective larvae of some species on pasture during this time, substantial populations will reach maturity in these ewes, resulting in a post parturient rise in egg counts or contributing to an already existing rise.

2.4 Pathophysiology of Gastrointestinal Nematode Infections

Gastrointestinal parasitism has a wide range of pathophysiological effects on the host and many of the pathological effects manifest as distinct morphological and functional derangements, either locally in the gastrointestinal tract or systematically (Chiejina, 1987).

2.4.1 Clinical Signs

Anemia is the most astounding symptom in acute infections in areas where *Haemonchus* species predominate, and lambs in particular may die of blood losses without warning. In more prolonged infections, anaemia is associated with oedamatous swellings

under the jaw and sometimes the ventral abdomen. The body fat is replaced by gelatinous tissue and so emaciation may not be apparent. In cases in which *Trichostrongylus* predominates, diarrhoea, with thin black coloured faeces may be noted in addition to anaemia and cacexia.

2.4.2 Morphological Changes in the gastrointestinal Tract

H. contortus infections, produce predominantly hemorrhagic lesions and anaemia. The gastric mucosa shows numerous petechial haemorrhages, representing areas of attachment of the blood-sucking worms. Frank or coagulated blood and fibrin clots, which may be mixed with mucus and desquamated epithelial cells, may be present in the abomasal content and on the mucosal surface in peracute and acute infections, when numerous young worms may also be present (Soulsby, 1982). In chronic infections, moderate degree of hyperplasia of the glandular epithelium may be present. *Trichostrongylus* infections on the other hand produce mainly proliferative gastropathy accompanied by catarrhal inflammation and epithelial ulceration.

In heavy infections with *T. axei*, numerous worms will be found inside dilated gastric glands or beneath the epithelium of the duodenum and proximal jejenum, where they cause displacement, disruption and increased shedding of the epithelial cells and loss of villi (Taylor and Pearson, 1979). In heavy intestinal trichostrongylosis, the activity of the parasites produce severe abnormalities in the villous architecture and mucosal surface pattern, ranging from mild or moderate to severe villous atrophy. The lamina propia in nematode-induced villous atrophy is oedematous, grossly thickened and infiltrated by eosinophils, lymphocytes, mast cells and plasma cells (Murray *et al.*, 1971). Other

morphological reactions to the parasites include pronounced crypt cell and goblet cell hyperplasia (Coop and Angus, 1975), increased mucus production and the replacement of normal intestinal absorption cells (enterocytes) with immature non-functional cells sequel to deranged epithelial cell dynamics (Symons, 1965). This leads to a reduction in the surface area available for absorption of water, electrolytes and nutrients. Affected animals may have diarrhoea, passing dark green soft faeces which fowl the wool of the breech. Lamb and yearly flocks are most seriously affected and a constant mortality begins, a few animals dying each day.

2.4.3 Structural changes outside the gut

Infections with abomasal and small intestinal nematode parasites in small ruminants produce osteoporosis and retardation of growth of long bones (Sykes *et al.*, 1977). This occurs even in subclinical infections and is the result of disorderd calcium and phosphorus absorption and metabolism, coupled with impaired protein utilisation. Since growth and development of bone influence muscle development, the poor mineralisation and stunting of bone also result in poor muscle development in parasitised animals (Coop and Angus, 1981). In infected animals, the percentage of water was higher and the deposition of fat, protein and skeletal calcium and phosphorus was lower in than in worm-free controls (Symons and Steel, 1978). Apart from this, energy utilisation is compromised (Sykes and Coop, 1977) as the host uses energy and protein to replenish the mucosa, and plasma proteins which have been depleted. This places demands on the liver and increases its nutrient utilisation. There is increased excretion of nitrogen as urea in urine, demonstrating that recycling of the nutrients is not completely efficient in maintaining nitrogen balance, even though considerable energy costs are incurred by the host for increased protein

synthesis (Parkins *et al.*, 1990) Steel, (1974; 1978) has reviewed the metabolic consequences of gastrointestinal parasitism with particular reference to sheep. He came to the conclusion that helminth disease causes a series of metabolic changes to occur in the animal, the primary impact of which is on protein metabolism. The effects however carry through to the metabolism of other nutrients. The result is to produce a syndrome analogous to undernutrition.

2.4.4 Haematological changes

Anaemia is the most important change of pathogenic and diagnostic significance in PGE. It occurs mainly in infections with *Haemonchus* and other blood sucking worms. Considerable damage is caused by the fourth-stage larvae (L4) of abomasal parasites (*Haemonchus* and *T. axei*). The L3 enter the mucus membrane within six hours of entering the host and will usually stay in the mucus membrane or the glands. Both L4 and adult *Haemonchus* are blood suckers and by passing large amounts of the host's blood through their alimentary tracts, cause loss of all blood components including erythrocytes and plasma proteins resulting in anaemia and hypoproteinemia (Kuttler and Marble, 1960; Leland, 1961). Hypoproteinemia is associated with leakage of albumin into the gastrointestinal tract. Nitrogen is also lost but a great deal of it is reabsorbed along the small intestine, although this is not so if the host has a concurrent intestinal worm burden (Steel, 1978, Steel *et al.*, 1982).

2.4.5 Changes in Feed Intake and Body Weight

Reduction in voluntary feed intake or inappetance is an important feature of gastrointestinal parasitism and widely recognised as a major feature in the pathogenesis of

such infections (Holmes, 1987) Change in body weight of the host is the most common criteria for judging the effects of gastro-intestinal parasitism. The onset is insidious and young animals start to loose weight or fail to grow. They are unthrifty and lack vitality. When observed closely, their feed intake can be seen to have reduced. The degree of inappetance can vary considerably and can be reduced by as much as 20% in moderate to heavy infections with trichostrongyles. The degree of inappetance has also been found to vary with the level of protein nutrition (Abbot *et al.*, 1986).

2.4.6 Gastrointestinal secretions

Gastrointestinal secretions have been shown to be altered in parasitised ruminants. Infections of the abomasum with *Ostertagia* spp and *H. contortus* are associated with elevations of abomasal pH and rises in blood pepsinogen concentrations. This is as a result of damage caused by the 4th stage larvae, which apart from being haematophagic, cause damage during penetration and emergence respectively from the gastric glands.

The basic lesion produced is a discrete raised nodule in the gastric mucosa produced by the parasite housed in and dilating the gastric gland. Functional acid-producing (parietal) and pepsinogen-producing (zymogenic) cells are damaged and replaced by poorly differentiated, non-functional cells especially the parietal HCl- producing cells. As a result, acid secretion is impaired and abomasal fluid pH is elevated (Ritchie *et al.*, 1966) and there is increased permiability of the bowel wall to macromolecules The most important consequences of these changes is a reduction in feed intake and utilisation associated with abdominal pain, elevated abomasal pH, gastrin and plasma pepsinogen levels. Pain may be due to local damage in the abomasum; and the elevation of the pH from 2 to7 or higher,

results in a failure of conversion of pepsinogen to pepsin and a failure of the primary digestion mechanism. This is because elevated pH limits abomasal protein digestion and the altered amino acid production could affect food intake since some amino acids are known appetite stimulants (Lang, 1981). In addition, there is an increase in the number of bacteria in the abomasum due to the loss of the bacteriocidal effect of an acid environment and this is associated with diarrhoea and anorexia resulting in reduced food and water intake at a time such are needed. Proteins are also lost into the gastrointestinal tract as a result of impaired cell junction integrity of mucosal epithelial cells (Murray, 1969).

Animals tend under these circumstances, to run down their pool of plasma proteins because production in the liver cannot keep pace with the loss, even though the synthesis rate is unusually high. Adjustments are made to other nitrogen-using processes of lower priority, notably synthesis of wool and muscle leading to poor carcass quality (Sykes, Coop and Angus, 1977), reduced wool (Symons *et al.*, 1981) and milk production (Bliss and Todd, 1976). The result again is the development of a malabsorption syndrome resulting in diarrhoea, malnutrition, and manifested by weight loss.

2.5 Factors Influencing Pathogenesis

The outcome of worm infections is influenced by the age of the animal, genetic make-up factors, acquired immunity, and nutritional status of the host.

2.5.1 Effect of Age

The susceptibility of grazing animals to nematode infections as well as the pathogenesis of the parasites are influenced by age. Age is most important in the first

grazing season or when young stock are turned out to pasture with older animals which are of higher egg output (Adejinmi and Harrison, 1996). Young animals are more susceptible than older animals principally because of their relative immunological incompetence. Immunological unresponsiveness of young lambs is associated both with host factors as well as parasite factors. In particular young lambs show an inability to develop protection against re-infection with most but not all gastrointestinal nematodes. For example, self cure of *H. contortus* has been seen in suitably sensitised lambs as young as 9 weeks of age (Valera-Diaz, 1970) Six to eight week old lambs infected with *N. battus* expelled the parasite within 3-4 weeks of infection and strong protection against re-infection ensued (Lee and Martin, 1976). Further, foetal and neonatal lambs and calves can respond to a wide range of bacterial and viral antigens (Silverstein *et al.*, 1963; Fahey and Morris 1978) and neonatal calves can be immunised orally against infection with *Escherichia coli* (Porter *et al.*, 1977). In contrast, lambs do not develop protection against re infection with *H. contortus* and *T. colubriformis*. Manton *et al.*, (1962); Urquhart *et al.*, (1966); Benitez-Usher *et al.*, (1977); Gregg *et al.*, (1978) and Smith and Angus (1980) all demonstrated the lack of responsiveness of young lambs to immunisation with normal or irradiated larvae in comparison with the high degree of resistance conferred using the same dosing regime in older animals. This unresponsiveness lasts until the animals are at least 3-6 months of age. A comparable situation is seen in the field where lambs showed high faecal egg counts which did not terminate until the lambs were about 6 months old (Crofton ,1955; Soulsby, 1966) and could be mimicked experimentally (Chiejina and Sewell, 1974; Gibson and Parfitt, 1972). Indeed Ross (1939) observed that a one year old lamb was still susceptible to infection even though it was previously infected in the field. Similarly, Lopez and Urquhart (1967) reported that Merino sheep developed immunological maturity only

sometime between 7 and 24 months of age. Smith *et al.*, (1985) compared the performance of four-and a half and 10-month-old primed lambs to challenge with *O. circumcincta* and found that the increased susceptibility of the younger animals was correlated with a much weaker immune response in the gastric lymph.

The mechanism of this immunological unresponsiveness is not fully understood and various hypothesis have been put forward as to the specific defect in lambs which prevents responsiveness to nematodes such as *H. contortus* and *T. colubriformis*. These include a "feedback" inhibition by maternally transferred antibodies, the colostral transfer of antigen or soluble suppressor factors, and the generation or the presence of natural suppressor cells in the neonate (Soulsby *et al.*, 1981). However more recent studies on the lymphoid system of sheep and cattle show that in contrast to humans and rodents, ruminant systems contain a large number of CD4 and CD8- T cells. CD4+and CD8+ positive cells have T- cell receptors composed of alpha and beta chains similar to immunoglobulins. However, in CD4- and CD8- T-cells a different type of receptor composed of gamma and delta chains are present. These are sometimes called gamma delta (γδ) T -cells and a substantial proportion are found in the epithelial and mucosal surfaces (Wyatt *et al.*, 1994). The γδ T-cells are present in higher concentrations (up to 30%) in peripheral blood of young ruminants relative to CD4+ and CD8+ cells. If CD4+ cells play an important role in gut immunity, unresponsiveness of young animals against intestinal parasites may be a reflection of inadequate numbers or function of the CD4+ sub-population compared with older animals. The relative distributions of these subsets change with age and may account for the changing pattern of immunity to parasites in sheep. Also in most sheep, the development of the ability to respond to nematodes occurs at about the same time as the

onset of sexual maturity (Coop *et al.*, 1990) Although steroid sex hormones and hormones associated with lactation are known to be capable of influencing the quantitative expression of an established immune response. Coop *et al.*, (1990) have suggested that the maturation of the immune system is controlled by brain neurohumoral stimuli in a manner similar to the neurohormonal stimulation of the onset of puberty (Jankovic, 1989). Blalock (1989), as well as Ader *et al.*, (1990) support this hypothesis. They also point to the fact that the neuroendocrine system and immune system share identical signal molecules in the hormones and cystokines that they use for communication and regulation.

2.5.2 Effect of Nutrition

There is a close relationship between nutrition and helminthiasis. Gastrointestinal nematodosis in particular is considerably influenced in severity by the level of host nutrition. Reduction in overall food intake, in quality such as deficiency in protein, vitamins and mineral content leads to a lowering of host resistance to infections (Gordon, 1964; Sykes, 1978). This can result in enhanced establishment of worm populations in the host or to the increased pathogenicity of an existing worm burden and hence to clinical disease (Schilhorn van Veen, 1974; Sykes, 1978). In northern Nigeria, the dry season brings with it severe shortage of feed and water for grazing animals as grasses and shrubs wither away with green fodder available only in the marshy areas (Pullan, 1980). Where no supplementary feeding is given, the grazing animals are each year subjected to a prolonged period of malnutrition and this has been reported to exacerbate parasitism in animals (Schilhorn van Veen, 1974). Likewise, improved nutrition can compensate for the detrimental effects of animal parasitism. Blackburn *et al.*, (1991) reported decreased pack

cell volume (PCV) in goats with increased worm infections, and these increased as nutrition was increased. Blackburn, *et al.*, (1992) also observed that animals with no parasites and on high planes of nutrition had more leucocytes in their blood than animals on low planes of nutrition when exposed to accidental infections. This was taken to imply that their immune defence mechanisms were better prepared to resist eventual challenges and that high levels of infection coupled with poor nutrition depressed the defensive capabilities of animals.

The protein content of the diet has also found to affect the rate of development of immunity to parasitic infections (Biesel, 1982; Gibson, 1983). Abbot *et al.*, (1988) found that weaned lambs fed 170 g crude protein/kg dry developed immunity to a trickle infection of H. contortus whereas lambs fed 80 g/kg dry matter showed no protection. Bown *et al.*, (1991) showed that three-and -a half-month-old lambs that received infusion of protein into the abomasum appeared to develop immunity to a trickle infection of *T. colubriformis* more rapidly than unsupplemented controls. Additional control groups indicated that the accelerated response was due to the nitrogen content of the protein supplement rather to its energy value Supplementation therefore with a protein-rich concentrate influences lymphocyte traffic in the intestinal lymph and so has a major impact on the development of acquired immunity to gastrointestinal nematodes in young sheep (Shaw *et al.*, 1995; Coop *et al.*, 1995). Coop *et al.*, (1995) attributed lower immune responses of lambs to competing demands for available nutrients between growth, the repair of gastrointestinal pathology and the immune response. They postulated that in the young lamb, there may be a bias towards the first two processes at the expense of the immune response.

Studies in laboratory animals have shown that protein- energy deficiency is an important cause of defective T-cell function (Beisel, 1982; Chandra, 1984), and T-cells ave been found to play a pivotal role in mediating acquired resistance to helminthoses in sheep (Gill *et al.*, 1993).

2.5.3 Effect of Acquired Immunity

A slow development of protective immunity characterise the majority of nematode infections in ruminants. This is in contrast with the situation in a majority of laboratory animals in which protective immunity, evidenced by early expulsion of the adults from a primary infection and by rapid expulsion of challenge infections occurs. In ruminants, adult parasites arising from a primary infection may persist for 2-6 months or more and experimentally several immunising infections may be required for expression of an effective immune response. However in the field, in the face of continual challenge, infection initially accumulates but subsequently an abrupt expulsion of the acquired nematodes and resistance to re-infection develops (Crofton, 1955; Chiejina, 1974; Chiejina and Sewell, 1974).

2.5.4 Effect of genetic make-up

The chronicity of infections with nematodes such as *H. contortus* and *T. colubriformis* in sheep is also influenced by the genetic make-up of the host. The rate of acquisition of immunity can vary widely between individuals (Barger, 1985). Various breeds of sheep and cattle differ in their susceptibility to infection with gastrointestinal nematodes and this may operate determining the level of infection and duration of a primary infection. Breed differences can be substantial. For example, Scottish Blackface sheep were

more resistant to *H. contortus* worm establishment from both primary and secondary infections than were Finn Dorset (Altif and Dargie, 1978). The resistance appeared to act first against establishment of the parasites and subsequently is reflected in an earlier expulsion of adult parasites (Preston and Allonby, 1978). This genetic resistance to infection is highly heritable (Whitlock and Madsen, 1958; Windon and Dineen, 1981). Whitlock (1955), termed the inheritance of resistance in sheep the "Violet effect", dubbed after the increased resistance of the progeny of the ram Violet. There is evidence however, that in sheep and calves genetically selected for resistance to nematodes such as *H. contortus* and *Cooperia oncophora*, respectively, productivity was affected adversely (Albers *et al.*, 1987; Riffkin and Dobson, 1979). Wagland *et al.*, (1984) however, found no evidence to suggest that production and resistance to *T. colubriformis* in lambs were genetically linked. Wagland *et al.*, (1984) suggested that the developing immune response competes with production for the resources of the host which would be important where energy is limited.

2.6 Immunity to Gastro-intestinal Nematodes

The immunological response to gastrointestinal nematodes is a complex interaction of humoral, cellular and parasite antigens (Miller, 1984). The contribution of individual components in this multifactorial immunity may vary without necessarily interfering with overall effectiveness of resistance and may be directed at different stages of the parasite life cycle. Knowledge of the immune response to gastro-intestinal tract nematodes is derived from mainly animal models. The work with rodents has helped to clarify the understanding of the immune response of the intestinal tract of ruminants to parasitic helminths.

2.6.1 The Cellular Immune Response

The most striking feature of a gastro-intestinal tract nematode infection is probably the cellular response and it involves an increase in the number of T lymphocytes, as well as accumulation of non-lymphoid cells (globule leukocytes, eosinophils, basophils) within the mucosa (Rothwel, 1989). Athymic animals genetically deficient in T-cells or made T-cell deficient by thymectomy, irradiation or bone marrow reconstitution, have illustrated the central role of T-cells in the immune response to helminth infections. These T-cell deficient animals cannot expel an intestinal nematode infection and show no intestinal mast cell hyperplasia. The small lymphocyte population of T-cells can be distinguished from B-cells by it's different cell surface proteins which act as markers or cluster designation (CD). This antigen recognition site on T-cells consists of either CD4 or CD8. CD4 is expressed by T-helper cells while CD8 is expressed by T-supressor or T- cytotoxic cells. Lymphocytes have cell surface receptors for many other molecules, eg cytokines. Cytokines are peptide hormones that act as signals between the cells of the immune system (Emery and Wagland, 1991). In sheep, the involvement of T-cell-mediated immunity in resistance to H. contortus was demonstrated by Gill et al., (1993) who abrogated immunity in genetically resistant sheep, by administration of anti- CD4+ (helper) monoclonal antibodies. The two subsets of helper T-cells designated Th1 and Th2 cells are defined on the basis of the cytokines they produce (Coffman *et al.*,1988; Madden *et al.*, 1991). Th1, produces gamma interferon (gINF) and IL-2 (interleukin 2) and is associated with activation of macrophages and Delayed Type hypersensitivity; Th2 produces IL4 which induces B cells to switch immunuglobulin production to IgE, and IL5 which with eosinophils is the basis of Immediate Type hypersensitivity. There is cross regulation: IL10 from B cells stimulates Th2 responses and down regulates Th1 while IL12 produced by

macrophages stimulates Th1 cells and the consequential production of gINF down regulates Th2 responses.

In rodent systems, IL-4 was found to regulate mucosal responses in immunity to gastro-intestinal nematodes. Immunity to *Heligmosomoides polygyrus*, a helminth which has similarities to *H. contortus* was found to be controlled by both CD4+ T lymphocytes and IL-4 dependent immune mechanisms (Urban *et al.*, 1991a,b). IL4-dependent immunity has also been implicated in immunity to *Trichuris muris* (Else *et al.*, 1994) and *N. brasilensis* (Urban *et al.*, 1995). It has been therefore established that Type II helper lymphocytes regulate immunity to several gastro-intestinal nematodes even though the involvement of Type 1 responses cannot be excluded in all cases. For example increased gamma interferon production was associated with more rapid expulsion of intestinal *Trichinella spiralis* infection (Pond *et al.*, 1989). Urban *et al.*, (1995) also pointed out that IL-4 complexed with anti-IL4 antibody caused significant elimination of *H. polygyrus* and *N. brasilensis* in mice with severe combined immuno-deficiency. These results suggest that immunity to gastro-intestinal tract nematodes is regulated by antigen-specific Type II T-helper lymphocytes and it is likely that they do this by regulating other immune components which mediate immunity. Studies in sheep showed that the survival of mucosal mast cells are highly T-cell dependent (Mayrhofer, 1979) and differentiation and growth of mast cells is promoted by lymphokines from primed T-cells (MacMenamin *et al.*, 1987).

Huntley *et al.*, (1984; 1986) have shown that mucosal mastocytosis including the presence of intra-epithelial globule leucocytes is associated with the development ofresistance to gastro-intestinal tract helminthiasis. The globule leucocyte (GL) has been

described as being derived from the mucosa and its accumulation has been regarded as indicative of resistance to nematodes in both sheep and cattle (O'Sullivan and Donald, 1973; Jarret *et al.*, 1967, Douch *et al.*, 1986). In sheep the detection and purification of a specific mast cell proteinase in mast cells termed sheep mast cell proteinase (SMCP), (Huntley *et al.*, 1986) has enabled the quantitative evaluation of mucosal mast cell ((MMC) proliferation in gastrointestinal tissues. It's concentration is correlated with the number of mucosal mast cells and globule leukocytes present in the tissues (Huntley *et al.*, 1984). Immune expulsion of nematodes is also associated with increased mucosal permeability and concomitant release of mast cell proteinase into mucus following challenge with nematode larvae. Severe hyperemia, intestinal fluid and mucus secretion accompanied release of the protease from the mucosal mast cells and so, an immediate- type hypersensitivity reaction with release of anaphylactic mediators of inflammation was known to mediate the reaction (Murray, 1972). These mast cell-derived mediators have been found to consist of pre-formed mediators such as histamine, 5HT, proteoglycan, serine proteases and chemotactic peptides as well as secondarily generated mediators such as prostaglandins and leucotrines (Rothwell *et al.*, 1974; Richards *et al.*, 1977). These factors have been suggested to be the agents directly responsible for the expulsion of nematodes by causing reversible smooth muscle contraction which may result in reduced motility or paralysis, thus expelling the nematode or preventing the larvae from establishing in the gastro-intestinal tract mucosal tissues (Rothwell *et al.*, 1974). These mediators also have permeability-inducing and chemotactic activities. Thus, they may also act by promoting both the translocation of plasma proteins/antibodies across the mucosal epithelium, and the recruitment of immuno-inflammatory cells such as neutrophils and

eosinophils as well as promote secretion by epithelial absorptive cells and mucus-secreting cells (Murray, 1972).

The importance of mucus in expulsion of nematodes was suggested by the fact that treatment of immune rats and sheep with corticosteroids prevented the parasite expulsion (King *et al.*, 1985, Miller *et al.*, 1986) as did the administration of intraduodenal mucolytic agents (Miller and Huntley, 1982). Mucus from the gastro-intestinal tract of sheep resistant to a nematode infection, inhibited larval migration by up to 93% in vitro whereas mucus from sheep reared helminth-free or from heavily infected sheep did not significantly inhibit larval migration (Douch *et al.*, 1983). The larval migratory inhibitory (LMI) activity of mucus from resistant sheep was associated with components having some properties of slow reacting substance of anaphylaxis (SRS-A) of which the major components were leucotrines. Lee and Oglive (1981) had also reported that mucus from resistant rats immobilised exsheathed larvae of *Trichinella spiralis* while Miller *et al.*, (1981) observed that *Nippostrongylus brasilensis* larvae are trapped in the mucus of primed rats during rapid expulsion phase and suggested that this was responsible for their elimination.

Eosinophilia has for many years been considered an important indicator of helminthiasis and are traditionally viewed as effector cells in parasitic infections. Soulsby (1963) observed adhesion and granulation of eosinophils on the surface of ascarid larvae previously sensitised with immune serum. He suggested that this might release pharmacologically active substances which affect the structure and function of the cuticle. McLaren *et al.*, (1972) also demonstrated adhesion of eosinophils to and their degranulation

upon parasite surfaces with the release of enzymes such as peroxidase. Binding of eosinophils to target is restricted to certain antibody isotypes: IgG and IgE. There is evidence that eosinophils can be associated with cell-mediated immune responses, immediate type hypersensitivity and the interaction of antigen- antibody complexes with complement (James and Coiley, 1976; Miller, 1984) but there is no evidence that eosinophils have a direct effector role in expulsion of nematodes from the gut. Neither lactating sheep nor guinea pigs were able to expel an infection with *T. colubriformis* even though they had sufficient numbers of eosinophils in their gut (O'Sullivan and Donald, 1973; O'Sullivan, 1974). Similarly, expulsion of *N. brasiliensis* did not occur in lactating rats even after their lamina propria had been infilterated with large numbers of eosinophils and mast cells (Kelly and Oglive, 1972) . The work of Gregg et al., (1978) showed that there was no correlation between lamina propria eosinophils and worm burdens. However, blood eosinophilia was inversely related to faecal egg counts and was under genetic control of the host (Dawkins *et al.*, 1989; Buddle, 1992).These authors concluded that selection of sheep for high eosinophil count alone might be enough to confer on sheep, an appreciable resistance to *T. colubriformis*. Gill (1991) did not observe any inverse relationship between FEC and eosinophils but lambs bred to be resistant to *H. contortus* had higher mast cell and eosinophil numbers than randomly bred lambs.

2.6.2 The Antibody Immune Response

Complete immunity to some parasites may be achieved by passive transfer of immune serum. The complement-fixing antibodies, particularly IgG2 are thought to mediate host protection (Lloyd and Soulsby, 1978). Windon and Dineen (1981) reportedan inverse relationship between complement-fixing antibody levels to larval antigens and

faecal egg counts (FEC) when sheep were vaccinated with irradiated larvae of *T. colubriformis* and challenged with normal larvae. The class of antibodies developing in Merino wethers was investigated by McClure *et al.*, (1992). They immunised wethers against *T. colubriformis* by administration of three doses of *T. colubriformis* larvae and collected serum and mucus from sheep killed at intervals during the development of the immune response. Their results showed that early rejection of the parasite occurred within the first day in immune sheep and was associated with increased concentrations of *T. colubriformis* specific IgG1 and IgG2 in intestinal mucus. The rejection of the remaining worms occurred between three and fourteen days after challenge and was associated with increased *T. colubriformis*- specific IgA and IgG2 in intestinal mucus. They concluded that immunity was associated with increased parasite-specific antibodies. In sheep exposed to multiple infections, Smith (1977) detected anti-larval antibody which was associated with IgA and IgG immunoglobulin subclasses. The response developed slowly and declined soon after re-infection ceased. No immunological response was observed when sheep were challenged later. Local production of specific IgA was suggested whereas specific IgG was considered to be plasma derived. The number of arrested larvae was also found to be positively associated with the activity of parasite-specific IgA and IgG1 (Stear *et al.*, 1995).

Anti- *H. contortus* IgA hemaglutinins have been reported in the sera of sheep infected with this parasite and high anti-parasite antibody levels were found in the IgA1 and IgA2 fractions of colostrum of sheep subjected to repeated infections up to the time of parturition (Valera Diaz, 1970). However, maternal transfer of such antibodies did notresult in protection against infection in lambs. IgA antibodies to *O. circumcincta* in

draining lymph from immune sheep were found to be increased (Smith *et al.*, 1983; 1984) but this was delayed in comparison to expulsion suggesting that IgA antibodies were not involved in protection. Bell and McGregor, (1979) postulated that the "mucus-like" stretch of IgA perhaps making it more miscible with mucus could be important in entrapping and expelling nematodes.

More recent studies (Jeffcoate *et al.*, 1992; Stear *et al.*, 1995) linked IgA with worm length and fecundity. They postulated that IgA may be the most important regulator of worm fecundity and attributed the ability of some sheep to regulate worm fecundity largely to strong IgA responses to a set of specific parasite molecules which are not recognised by all sheep.

Some helminth infections are marked by high levels of IgE antibody. This has encouraged the view that IgE represents an important line of defence. IgE is found in serum in minute amounts but is produced primarily in plasma cells in mucosal tissues and in the lymph nodes that drain them. IgE is found bound very firmly to the Fc receptors of mast cells and it is thought that the main physiological role is the protection of the external mucosal surfaces of the body by local recruitment of plasma factors and effector cells through triggering an acute inflammatory reaction (Miller, 1984). IgE is a powerful mediator of anaphylactic reactions and Murray (1977) postulated that amine release from mast cells, synchronously produced in response to parasitic infections by IgE antibody-antigen reactions, created pathway between the cells of the intestinal mucosa for the leakage of protective antibodies. They demonstrated a striking relationship between the increase in mast cell numbers, the onset of worm expulsion and macromoleculer leakage

into the gut. Ishziaka *et al.*, (1969) had earlier suggested that the possible role of IgE antibody-antigen reactions in the protection of the mucosal surface against infection was by changing permeability so that serum immunoglobulin may leak into the area and contribute to protection. Infectious agents penetrating the IgA defences would combine with specific IgE on the mast cell surface and trigger the release of vasoctive agents and factors chemotactic for granulocytes so, leading to an influx of plasma IgG, complement, polymorphs and eosinophils. The parasite becomes coated with specific IgG and C3b. Where the opsonised organism is too large for phagocytosis, they are killed by an extracelluler mechanism after attachment by their Fc receptors. This phenomenon is termed antibody-dependent cell mediated cytotoxicity (ADCC). This reaction is the mechanism for killing of schistosomulae and nematode larvae (Capron et al., 1980) but there is no evidence that such mechanisms operate against gut-dwelling nematodes. However, tissue invading larval stages of parasites such as *Oesophagostomum* spp and *N. dubius* which enter the sub-mucosa may be targets for antibody-dependent killing since massive accumulations of eosinophils surround dead and dying worms in animals (Elek and Durie, 1966). Thus, the precise role of IgE and other anaphylactic antibodies remains unidentified and can only be inferred from indirect observations of the hypersensitivity responses to infective larvae.

In summary, the hypothesis consistent with available evidence suggests that the mechanism of expulsion of nematodes is complex and involves both humoral and cell-mediated immunity. Antibodies cause biochemical and morphological damage to the parasites and lymphocytes enhance immunity by secreting cytokines which recruit haemopoietic precursor cells to the site of parasite infestation, stimulate their

differentiation and finally activate their inflammatory cell progeny i.e. macrophages, neutrophils, mast cells and eosinophils (Haig *et al.*, 1990). This results in expulsion of the damaged worms. Antibodies do not cause worm expulsion per se and lymphocytes do not cause expulsion of normal undamaged adult worms (Dineen and Kelly, 1973). This mechanism could account for the rapid rejection of adult worms, including immune exclusion of H. contortus and T. colubriformis L3 from immune sheep (Jackson *et al.*, 1988a; 1988b).

2.7 Effect Of Immune Responses On The Nematodes

The most dramatic and important response by the parasite to host immunity is that it fails to establish or that it is expelled. More subtle responses have also been observed particularly related to the morphology of the parasites. The immunological responses against the parasites may be expressed in different ways, and different mechanisms appear to be responsible.

2.7.1 The Self- Cure Reaction

The term "self-cure" reaction refers to a the rapid expulsion of an established burden of adult parasites in a suitably sensitised host following the ingestion of a challenge dose of infective larvae of the homologous specie (Stoll, 1929) The newly invading larvae mediates the abrupt expulsion of the existing established burden of adult nematodes although occasionally fecundity of the parasites may be affected without actual expulsion (Stewart, 1955). The invading larvae themselves are not necessarily affected and may reach maturity, sometimes in sufficient numbers to be fatal. Animals which experience this phenomenon are not necessarily immune subsequently and the mechanism may

therefore be different from those which induce protection against re-infection. (Stewart, 1950). The self-cure reaction occurs in graded intensity ranging from the classical self cure and protection characterised by rejection of adult worms plus obstruction to the development of the infective larvae, to reactions where there is no loss of the existing adult population but the prevention of establishment of the new infection.

Michel (1963) contended that the self-cure occurred when the population or biomass of parasites exceeds limits set by immune responses of the host. The reaction may occur in sheep carrying a primary infection but is more prevalent and explosive in sheep that have been sensitised by several large infections (Stewart, 1953). Several smaller superimposed infections may not provide such effective sensitisation (Dargie and Allonby, 1975) ·A latent period is required between the initial primary sensitising infection and the administration of larvae which induce the self cure reaction. The length of the latent period and efficacy of the self cure reaction may be age-dependent. Manton *et al.*, (1962) and Adams (1983a) observed self cure in adult sheep re-infected at 30-40 days after the primary infection, but 3-5 month old lambs similarly infected and challenged underwent either a partial self cure or no self cure at all. Chiejina and Sewell (1974) infected lambs from 3 weeks of age with exponentially increasing doses of *Trichostrongylus colubriformis* to mimic field infections and found no evidence for self cure in the 16-20 week experiment. In contrast, Valera-Diaz (1970) described effective self cure in lambs challenged when only 9 weeks old, but a minimum latent period of 42-49 days was required to induce the effect. Initiation of self cure is antigen-specific. The reaction requires local abomasal release of antigens during the moult of larvae from the third to the fourth stage. Dead or ectopically administered larvae do not induce self cure (Stewart, 1955; Soulsby and

Stewart, 1960). The effector mechanisms appear to be associated with an immediate-type hypersensitivity reaction. A transient rise in blood histamine and an intense oedema accompanied by eosinophilia occur in the abomasal mucosa with superficial aggregation of eosinophils. Antihistamines abrogated both the self cure and the rise in the blood histamine (Stewart 1953; 1955).

These effector mechanisms once initiated by specific antigenic stimulation act non-specifically. A challenge with *H. contortus* for example given to *H. contortus* sensitised and infected sheep, will induce self cure not only of the population of adult *H. contortus*, but also of other concurrent nematode infections (*T. colubriformis* and *Ostertagia circumcincta*) in the abomasum and small intestine. According to Stewart (1955) "this lack of specificity is dependent on the latter species being in the same location or posterior in the gastrointestinal tract to that of the parasite inducing the response. Stewart came to this conclusion when he found that self-cure initiated by *T. colubriformis* larvae and affecting *T. colubriformis* adults in the small intestine did not induce self cure of H. contortus adults in the abomasum. This may be associated with specific antigen or mediators of inflammation being carried down the gastrointestinal tract to affect sensitised cells or the intestinal mucosa or parasites respectively, in posterior locations. Absorption of antigen and its distribution via the circulation to react with sensitised cells in the other part of the gut cannot occur, since reactions in the small intestine do not initiate inflammation and nematode expulsion from the abomasum which is located anteriorly. The self cure is well known experimentally and is an important mechanism for terminating infection in the field as has been demonstrated in different breeds of sheep. Evidence suggests a relationship between bloodtype and resistance and the ability to mount a self-cure reaction

(Salisbury and Arundel, 1970) and that climatic or pastoral changes are sufficient stimuli for a self-cure. Strain or breed differences also occur (Gordon, 1948; Soulsby, 1957; Preston and Allonby, 1979). The practical importance of the self- cure other than a temporary remission is the misleading effect it may have on the assessment of control programs.

2.7.2 Rapid Expulsion

Protection against re-infection with gastro-intestinal tract nematodes is a well defined phenomenon and expulsion of the invading burden of parasites can occur in a reaction distinct from the self-cure reaction. In rapid immune exclusion in- coming larvae are excluded from entering the glandular tissues and are consequently rapidly lost from the host. This protective immune response which prevents establishment of a challenge infection, develops slowly in the field in adult sheep, cattle and goats (Crofton, 1949). The rapid expulsion of invading larvae from ruminants has been detected through the identification of exsheathed third stage larvae of *T. colubriformis* or *Cooperia punctata* in the faeces of sheep and cattle 24-48 hours after infection. (Tetley, 1949; Chiejina and Sewell, 1974).

Stimulation of the intestine is required for expression of some of the manifestations of rapid expulsion. Expression of mast cell and goblet cell hyperplasia in rats adoptively immunised with immune mesenteric lymph node cells from *N. brasilensis*-infected donors required the presence of *N. brasilensis* in the intestine of recipient rats (Miller and Nawa, 1979). Bell and McGregor (1979) demonstrated that this stimulation need not be

antigen-specific. Thus a specific systemic immunity plus an intestinal response which is probably non-specific is required for the expression of rapid expulsion.

Expulsion of the invading larvae in immune animals is rapid and the majority of larvae are expelled within 4-8 hours (Rothwell *et al.*, 1978; Miller *et al.*, 1981; Rothwell, 1989). Again an immediate-type hypersensitivity reaction and release of anaphylactic mediators of inflammation from local mucosal mast cells are likely candidates of mediation of the response. Mucus discharge may be triggered by the anaphylactic response with antigen or immune complexes. Collaboration between released mucus and a heat-stable antibody and a heat-labile serum factor, perhaps, complement, occurs to entrap and expel the nematodes, perhaps aided by intestinal peristalsis (Lee and Oglive, 1981). Entrapped in viscid strings or goblets of mucus, *H. contortus* larvae penetrated below the mucus layer but could not penetrate between the villi (Miller *et al.*, 1983). The entrapment with mucus prevented the worms from adhering to the to the mucosa. Thus 50% of *H. contortus* larvae had penetrated the glandular abomasal tissue 4 hours after infection whereas larvae were excluded from the glandular tissue in immune sheep. Also intestinal mucus inhibited larval migration. Specific worm antibody and a heat-labile, non-specific serum factor may be involved in the entrapment of the invading larvae in mucus (Miller and Huntley, 1982). Whichever mechanism is involved the parasites are expelled rapidly but are not permanently damaged since larvae expelled from the intestine of immune rats were infective for naive animals.

2.7.3 Direct Anti-Nematode Effects

Direct anti-worm effects as a result of host immune responses have been observed. These can range from inhibition of larval establishment through retarded growth and development, reduced fecundity of the invading worms (Oglive and Jones, 1973) to morphological changes in the parasites (Michel, 1967). The anti-nematode effects have been shown to be a separate expression of immunological responsiveness and not simply a consequence of expulsion of the adult parasites. For example, even though expulsion of adult Nippostrongylus brasiliensis arising from a trickle infection given to immune rats was prevented, immunity was demonstrated by stunting of the worms and a reduced egg production compared with normal primary infection worms (Oglive *et al.*, 1969) .

2.8 Suppressed Immunological Reactivity to Helminth Parasites

2.8.1 Increased Susceptibility

The protective immune response against infection with gastro-intestinal nematodes in ruminants is suppressed during the periparturient period. Sheep, cattle and goats show increased faecal egg counts which begin in late pregnancy and peak in early lactation (Connan, 1976; Lloyd, 1983). The time of peak output of the eggs varies, depending on the species present (Borgsteede, 1978). The increased faecal egg counts reflect an increased susceptibility to infection associated with an inability of the periparturient animal to prevent establishment of newly acquired infection, to suppress egg production by the female worms and to expel developing worms which have arisen from maturation of hypobiotic larvae during the spring rise phenomenon. For example there is a marked increase in the susceptibility of sheep to newly acquired infection of *H. contortus* and *T. colubriformis*

after either a trickle infection or a single large dose of larvae (O'Sullivan and Donald, 1973) A similar increase in susceptibility to *O. ostertagi* infection is seen in cattle in late pregnancy and lactation (Michel *et al.*, 1979).

O'Sullivan and Donald (1973) and Shubber *et al.*, (1981) demonstrated that ewes in late pregnancy behaved similarly to lactating ewes. These ewes when infected with *H. contortus*, showed a reduced ability to resist newly acquired infection, to suppress egg production and to eliminate the parasites present.

Connan (1968; 1974) as well as O'Sullivan and Donald (1970; 1973) demonstrated that the periparturient rise in faecal egg counts was also contributed to by an increased fecundity of parasites present. Furthermore, parturition and lactation amplified the effects of an on-going spring rise phenomenon, but this effect was maximal only if lactation was superimposed on the on-going spring rise. Lambing before or after this optimum period in the absence of newly acquired infection was not associated with increased faecal egg counts (Morgan *et al.*, 1951; Brunsdon, 1967) Resistance returned rapidly if lactation was terminated and sheep expelled the parasites they had acquired in late pregnancy when their lactation was terminated immediately after parturition (Connan, 1968; Shubber *et al.*, 1981). Also faecal egg counts fall in the latter half of lactation. According to these authors, this probably reflects spontaneous expulsion or self cure of the adult parasites.

The putative immunological defect during late pregnancy and lactation remains unclear. In general, studies which have examined immunosuppression during pregnancy in human and laboratory animals in relation to survival of the foetus and infection with

bacteria and viruses have suggested that cell-mediated immune responses and not humoral immunity responses, are affected (Lloyd, 1983). Similar conclusions have been drawn from rats infected with *N. brasiliensis* and mice infected with *T. muris* since protective levels of serum antibodies were produced whether or not the animals were infected during lactation (Kelly and Dineen, 1973; Connan, 1973). Further evidence for the efficacy of serum antibodies in protection against infection with gastrointestinal nematodes is lacking. An increase in antibody titres to an extract of *T. vitulorum* larvae about three months before parturition in buffaloes was reported by Amerasinghe et al., (1994). However, the titres declined at about the time of parturition and remained low for up to three months into lactation.

Suppressed reactivity in responsiveness of lymphocytes to polyclonal mitogens and to antigens of *H. contortus* and *T. colubriformis* has been observed in nematode-infected sheep in late pregnancy and lactation (Chen and Soulsby, 1976; Shubber et al., 1981) as well as in buffaloes infected with *T. vitulorum*.(Amerasinghe *et al.*, 1994). Not only did the pattern of suppressed lymphocyte responsiveness follow lactation, but rapid increase in lymphocyte reactivity was observed when lactation was terminated immediately after parturition.

In general, lymphocyte responsiveness is impaired in lactating animals both in the induction of responsive lymphocytes and in expression of expulsion even though potentially reactive lymphocytes were present. Dineen and Kelly (1972) had suggested that a bone marrow, myeloid component of the effector arm of the immune response was impaired during lactation. Evidence for the impairment of mast cell and eosinophil function during

lactation has been presented. Thus lactation suppressed or delayed the degree of intestinal mast cell hyperplasia and eosinophil numbers in the intestine of parasitised, lactating rats, guinea pigs, mice and sheep (Kelly and Oglive, 1972; O'Sullivan and Donald 1973; 1974).

An involvement of endocrinological changes in the periparturient immuno-suppression has been postulated. High concentrations of a variety of steroid hormones of reproduction are capable of influencing both humoral and cell mediated immune responses (Connan, 1976; Lloyd, 1982) . In considering which of the hormones associated with reproduction may possibly be involved, one may consider them under two effects: those of pregnancy whose high concentration or fall in concentration, allows the PPR to occur (for example progesterone) and those of late pregnancy and lactation(such as prolactin) which may bring it about directly.

2.8.2 The Role of Progesterone and other Hormones of Pregnancy

An important role for progesterone in immunological unresponsiveness during pregnancy is suggested by a number of experiments conducted both in vitro and in vivo. (Szekeres *et al.*, 1981; Dobson, 1966). In vivo studies demonstrated that the administration of exogenous or synthetic progestins prolonged the survival of skin and renal allografts in rabbits and dogs respectively (Hulka *et al.*, 1965; Truycotte *et al.*, 1968) Monkeys treated with progesterone had an increased susceptibility to Rous Chicken Sarcoma virus (Munroe and Windle, 1967).

The relevance of this progesterone- immunosuppression to the periparturient rise in helminth egg counts cannot be satisfactorily explained. Cell- mediated immunity is depressed in pregnant animals but normal levels of immunity against nematode parasites are maintained through pregnancy until the PPR begins a week or two before parturition (Grossman 1989; Schuurs and Verheul, 1990). Progesterone is essential for the implantation of the ovum on the uterus and maintains pregnancy after implantation. Following ovulation, the rising progesterone levels inhibits LH release from the anterior pituitary (Cumming *et al.*, 1971) This in turn causes the corpus luteum to stop secreting progesterone. This decreasing progesterone level has a negative feed- back effect on the hypothalamus and this starts to produce FSH and LH again. If fertilization takes place, the egg becomes implanted in the uterine wall and pregnancy results. Under this circumstance, the corpus luteum instead of degenerating, persists and continues to secrete progesterone. During the course of pregnancy, plasma progesterone concentrations are similar to that found during the luteal phase of the estrus cycle up to about day 60 of gestation and then increases between day 60-140 due to placental progesterone production. This coupled with the small secretion of oestrogen from the ovary maintains the continued development of the uterus. The progesterone concentration starts to decline at about 135 days of gestation and then falls rapidly over the last 3-5 days due to decreased placental secretion (Basset *et al.*, 1969).

Connan (1967) reported that the structure of *Ostertagia* species populations were similar in pregnant and barren ewes and in castrated male sheep with respect both to dormant and developing larvae. Furthermore, administration of exogenous progesterone and dexamethasone had no effect on faecal egg output (Coop *et al.*, 1990; Gibbs, 1967).

The authors concluded that this appeared to preclude the effect of the hormones of pregnancy and parturition and pointed out that endocrine changes that occur during lactation may be important.

With regards to oestrogen, Gibbs (1967) showed that diethylstilbesterol when injected into sheep in corn oil, brought about a rise in faecal egg output but he concludedthat this may have been a pharmacological action since at term and in early lactation in the ewe, oestrogens are still at relatively low levels .

2.8.3 Role of Prolactin and other Hormones of Lactation

Of the protein hormones, the principal lactogenic hormone, prolactin was originally suspected as being of primary importance in the loss of immunity to nematodes in periparturient ewes because of the synchronous appearance of the PPR and elevated prolactin levels (Dunsmore, 1965) It is known that during pregnancy, prolactin remain at basal levels, increasing five to ten fold during the week before parturition, and high during lactation (Kann and Denamur, 1974).

During the last month of gestation, there are many endocrine changes that culminate in parturition. The most dramatic change is that seen in unconjugated oestrogens that rise sharply within the last two days of gestation from below 20 pg/ml to around 200 to 1000 pg/ml (Challis, 1971). This estrogen surge is derived from the conceptus and so there is a sharp fall to low levels within a day after birth. In contrast, pituitary prolactin rises in the last 48 hours of gestation and remains elevated after delivery of the lamb (Basset *et al.*, 1967). Prostaglandin F is also detectable in maternal uteroovarian venous plasma on the

day before parturition and tends to remain at plateau concentrations of about 10 ng/ml until labour is advanced when there is a rapid increase (Battye *et al.*, 1988). Postpartum, this declines quickly but considerable quantities continue to be secreted for several days presumably associated with uterine involution. Progesterone and oestrogen are also responsible for the growth of the mammary glands during pregnancy, in readiness for milk production. After parturition the flow of milk is initiated by prolactin secreted from the anterior lobe. Surgical removal of the pituitary during lactation stops the flow of milk immediately. Before birth, prolactin secretion is inhibited by oestrogen and progesterone. The sudden drop in these two hormones at the end of pregnancy permits the onset of lactation.

In the rat, the high secretion was related closely to the suckling stimulus and fell rapidly at the withdrawal of the litter and was also related to the size of the litter. In the ewe, it has often been noted that the rise in faecal egg count may begin as early as five weeks prior to parturition and that even worm numbers may be increased at this time. This was also consistent with results of experiments where dry ewes were treated daily with diethylstilbesterol (Gibbs, 1967) or acepromazine (Connan, 1974), and exhibited a PPR which did not appear in untreated ewes. Both treatments are known to induce increased levels of plasma prolactin. Fleming and Conrad (1989), reported that treatment of ovariectomised barren ewes with exogenous prolactin increased egg counts resulting from a single *H. contortus* infection, in comparison with egg counts in similar ewes treated with progesterone plus prolactin or saline. Egg counts and worm burdens in all groups were so low in relation to the challenge dose of 10,000 larvae that immunity did not seem to be diminished in any treatment.

Another indirect study on prolactin was based on the observation (Connan, 1976) that a number of drugs induce the release of prolactin. A group of four 18-month-old virgin ewes was injected with 25mg acepromazine maleate acetylpromazine twice daily for 28 days. During the first three weeks the ewes were daily dosed with infective larvae of *Ostertagia* specie. Another group of four ewes received larvae but no injections. Plasma prolactin on day 28 was very significantly elevated in all four injected ewes. The faecal egg counts increased almost immediately after injections began, and rose steeply after one week. At post mortem on day 28, there was a 10-fold difference in the number of *Ostertagia* present in the two groups

In yet another model, the secretion of prolactin was modified by varying the suckling pattern of the ewes. Three groups of lactating ewes were allowed to suckle twin lambs, single lambs or single lambs whose access to the ewe was restricted to two half-hour periods per day. The pulse release of prolactin at suckling was confirmed in a series of plasma samples collected during the second and fourth weeks (McNeilly, 1971) and hopefully therefore confirming differing exposure of the ewes to high prolactin levels. Although there was a numerical difference in the number of worms present in each group, 6 weeks post partum the differences were not significant owing to the large within-group variation . Lactating mice infected with *Trichinella spiralis* failed to develop protective immunity to re-infection (Ngwenya, 1977) and lactation was accompanied by a failure to expel intestinal worms (by the self-cure reaction) in rats infected with *Nippostrongylus brasilensis*, and guinea pigs infected with *Trichostrongylus colubriformis* (Connan, 1970, O'Sullivan, 1974) A periparturient rise in faecal egg counts was seen also in cattle naturally infected with gastrointestinal helminths; lactation but not pregnancy, in cattle has

been found to reduce the number of *Ostertagia ostertagi* larvae undergoing arrest and permitted development of larvae; and Type 11 ostergiasis may be precipitated in calving heifers (Michel *et al.*, 1979; Hammerberg and Lamm, 1980).

There is thus some indirect evidence to suggest a part played by prolactin, whose secretion pattern corresponds closely to the characteristics of the PPR but there are some anomalies. In rats, it is known that circulating prolactin reaches a high peak on the evening of proestrus with a secondary peak on the following day. Therefore in adult female rats elevated prolactin levels comparable to those in lactating rats are present for a significant period. Connan (1974) drew attention to the lack of effect of elevated prolactin levels around oestrus on expulsion of worms in rats or on egg count of ewes. Failure of elevated prolactin during the oestrus cycle to influence the expulsion mechanism in the rat or the faecal egg count in ewes may be due to several factors. The character of the prolactin secretion may be important, the height attained at the peak perhaps being less important than the elevated basal level.

More recent studies cast further doubts on the primary role of prolactin in the PPR Jeffcoate *et al.*, (1990) administered the prolactin antagonist bromocryptine to immune ewes for two weeks following parturition. These ewes and untreated immune ewes were challenged with *O. circumcincta* larvae for two weeks over the periparturient period. Bromocryptine treatment reduced plasma prolactin concentrations to extremely low levels, but this had no detectable effect on the extent and timing of the PPR, or on immune responses to horse red blood cells or BCG vaccine. Coop *et al.*, (1990) compared the dynamics of the PPR in pregnant and dry ewes with that in dry ewes induced to lactate by

exogenous treatment with oestradiol-17b and progesterone for 30 days, followed by oestradiol-17b , dexamethasone and progesterone for 7 days. All ewes were exposed to a trickle infection *O. circumcincta*. The hormone treatment induced what appeared to be a normal lactation in the treated ewes, including a similar elevation in plasma prolactin to that observed in the naturally lactating ewes but, only the naturally lactating ewes exhibited a PPR. It seems therefore to have been tacitly assumed that prolactin exerts an immunosuppressive effect, as the mere coincidence of elevated prolactin and the PPR is hardly a sufficient basis on which to erect an hypothesis. Reviews by Grossman (1989) and Ader *et al.*, (1990) indicate that this assumption may be incorrect. Far from suppressing immune responses, prolactin appears to be an important regulatory hormone that enhances immunity. It can restore both humoral and cell-mediated immune responses in hypophysectomised or dopamine receptor agonist-treated mice, reconstitute immunity in nude mice, increase mitogenic responses of lymphocytes, and reverse the immuno-supressive activity of glucocorticoids and cyclosporin. Another puzzling aspect of the PPR is the maintenance by ewes of normal adult levels of immunity against nematode parasites through pregnancy until the PPR begins a few weeks before parturition, when it is known that cell mediated immunity is depressed in pregnant animals (Schuurs and Verheul, 1990).

CHAPTER THREE

THE EPIDEMIOLOGY OF THE PERIPARTURIENT RISE IN HELMINTH EGG COUNTS IN YANKASA EWES

3.1 Summary

Studies were carried out in Shika, Zaria, on the occurrence of a periparturient increase in Trichostrongyle egg counts in naturally infected Yankasa ewe, using serial egg counts. The first study using ewes in the last month of gestation and non-pregnant ewes as controls, showed that a significantly ($p<0.05$) greater increase in egg counts occurred in the lambing ewes when compared with the non-pregnant ewes sampled within the same period. A second study using ewes grazing a separate pasture confirmed the occurrence of a periparturient increase in egg counts in lambing ewes. Packed cell volume values in these ewes declined with increased egg counts but Total protein values did not show an appreciable change. Larval culture showed that *Haemonchus contortus* was the main contributor to the rise in helminth egg counts.

In another study to examine helminth egg counts in pregnant animals, this showed that there was no significant difference in helminth egg counts in early and mid pregnancy but egg counts rose significantly in late pregnancy.

Ewes that lambed in the early dry season had significantly higher egg counts ($P<0.05$) during the periparturient period than ewes lambing in the early wet or late wet

seasons. There were no significant differences in the Packed cell volume (PCV) concentrations of ewes lambing in the different seasons but ewes that lambed in the late dry seasons had consistently lower PCV concentrations.

Lambs born in the early dry season had picked up low level helminth infections which were below 500epg through the dry season months. In the rainy season the highest mean helminth egg count in these lambs was 3016 $\pm$ 1015 In contrast, lambs born in the early wet season became infected as early as seven weeks of age, showing egg counts of up to 10000 and high mortalities.

The periparturient rise in helminth egg counts is an important epizootiologic consideration in gastrointestinal helminthiasis since it results not only in the development of infections in the lamb but may lead to severe and fatal infection in the ewe.

3. 2 Introduction

Helminths have been recorded as the most prevalent cause of disease in small ruminants in Nigeria. (Fabiyi, 1968; Okon, 1975; Akerejola, 1980; Schilhorn van Veen, 1974 and Trimnell *et al.*, 1985) and a major cause of lamb mortality (de Leeuw; 1972; Anosa, 1977,; Akerejola *et al.*, 1979). The prevalence of infection is high and ranges from 80-90% (Fabiyi, 1970; Schilhorn van Veen and Ogunsusi, 1978; Schilhorn van Veen and Brinckman, 1975). Animals are known to be infected throughout the year but with an increase in infection at the end of the dry season. The flush of young grass that accompanies the early rains, although highly nutritious, comes with concurrent rise in levels of both internal and external parasites. (Adeoye, 1985) Animals are infected as early

as six weeks of age and their state of health and productivity is determined by the amount of infection acquired. Production losses in the form of severe morbidity and high mortalities therefore occur in sheep in Nigeria in spite of the availability of highly efficacious anthelmintics.

The periparturient increase in egg count in ewes is one aspect of importance that has been neglected in the study of the epidemiology of helminth infection in Nigeria. Van Geldorp and Schilhorn van Veen (1976), and Shilhorn van Veen and Ogunsusi, (1978) have demonstrated the occurrence of the periparturient rise in a few Udah and Yankasa ewes respectively, and Okon (1980) reported its occurence in goats, but the increase in egg counts as a flock phenomenon under field conditions has not been studied. With the growing importance attached to commercial value of sheep in Nigeria, sophisticated production systems are being developed, with pen feeding of lambs for slaughter being increasingly practised. With intensive production comes increased parasitic infections.

Given the importance consequences of the periparturient rise in ewes, both for the epidemiology of nematodosis and the economic potential of sheep, it is an aspect that deserves further investigation. This work was therefore designed to:

(i) Investigate the occurence of the periparturient rise in helminth egg output in naturally infected ewes lambing in different times of the year.

(ii) Study the susceptibility of the ewe to helminth infection at the different trimesters of pregnancy

(iii) Study the sequential developments of helminth infections in lambs born to the ewes and any effects on lamb growth performance.

3.3 MATERIALS AND METHODS

3.3.1 Study Site

The National Animal Production Research Institute, (NAPRI) Shika, Zaria where these studies were carried out, is located on latitude 11o 12' N and longitude 7o 37' N with an altitude of 610m and annual rainfall of 1107mm. . The climate of the area is subhumid while the vegetative zone is Northern Guinea savannah. Rainfall is distributed in such a way that the year can be divided into four seasons namely late dry (January -March); early wet (April - June); late wet (July - Sept) and early dry (Oct -December) The seasonal distribution of rainfall is about 0.1% during the late dry season; 25.8 % in the early wet season; 69.6 % in the late wet season; and 4.5 % in the early dry season . The first significant rains come in late April or May and precipitation reaches a peak in July or August and ends abruptly in October but the pasture is sometimes still wet up to November. The average temperature and humidity during the wet season are 24.8o C and 72% respectively The early dry season commences with a period of dry cool weather known as the harmattan lasting from mid October to January followed by dry hot weather February to April. The dry season is characterised by considerable diurnal variation in temperature with daily temperatures ranging from 14- 30°C with relative humidity of 10-20%.

3. 3. 2 Animals and Management

The study was done using three different flocks of sheep, each flock comprising a separate experiment. The sheep in experiments one and two were

animals from the NAPRI sheep breeding Project. The sheep breeding project of the Institute covers 50 hectares of grazing land divided into paddocks with improved pasture. All sheep were managed under a semi-intensive system involving grazing on improved pasture 6-8 hours daily plus 0.3-0.5 kg/day of a 15% crude protein supplement depending on physiological status i.e. pregnant, nursing, dry. The concentrate was made up of 62.6% maize, 33.7% cottonseed cake, 2.5% bonemeal, and 0.25% vitamin mineral premix. Water and mineral salt licks were supplied ad libitum. Animals were housed in well ventilated pens with concrete floors which were cleaned daily. The animals received routine veterinary care including vaccination against pest de petit ruminant and dipping against ectoparasites at the appropriate time.

Breeding of ewes was by flock or pen mating, to rams of the same breed. Following parturition, ewes and their lambs were separated into a nursing pen and kept under observation for one week after lambing. While indoors the ewes were given supplementary feed as well as chopped sun- dried green fodder during the rainy season or hay during the dry season.

All animals used for the experiment were naturally infected from pasture and did not receive any experimental infections

3.3.3 *Experimental design*

3.3.3.1 Experiment 1

Consisted of 36, 2-3 year old ewes, which were pregnant and were in the last late of pregnancy. 20 non- pregnant maiden ewes served as controls and grazed the same pasture with the pregnant ewes. The pregnant ewes lambed between the months of August and September and had received a routine monthly anthelmintic treatment in July, a month before lambing was due to begin. Weekly rectal faecal samples were collected from each animal from four weeks pre to six weeks post lambing. The purpose of this experiment was to see if there was any difference in the helminth egg counts of ewes at lambing and that of non-pregnant ewes grazing the same pasture.

3.3.3.2 Experiment 2

These were also animals of the NAPRI sheep breeding project but these were housed differently and grazed a separate pasture from ewes in experiment 1. They consisted of 35 , 2-3 year old pregnant ewes in late pregnancy and they lambed between the months of February and April . Weekly faecal samples were collected from these animals from 2 weeks before to 6 weeks after lambing. Weekly blood samples were also taken for Packed cell volume (PCV) Haemoglobin concentration (Hb) and Total protein (TP) determinations during the same period.

3.3.3.3 Experiment 3

Consisted of a flock of 63 ewes, 2-3 years old, which had been bought from the open market. They were housed and grazed separately from experiments I and II animals but feeding and management were the same.

Four rams of the same breed selected from the NAPRI sheep project were joined to the ewes. The rams were fitted with aprons bearing coloured chalk so as to be able to identify any ewe that had come on heat and had been and bred by the ram. The date on which any ewe was marked was taken and the ewe subsequently monitored until it lambed. The ewes in this flock were continuously exposed to rams except for the immediate week following parturition. A total of 116 lambings were monitored and the births grouped according to season. To study the helminth egg output of the ewe during pregnancy, nine ewes that lambed in June and therefore were pregnant during the dry months of February -May, were used. Similarly, five ewes that lambed in October were used to evaluate the helminth egg output of the pregnant ewe during the wet months of June - September. The gestational period was divided into three periods of eight weeks each, constituting early, mid and late gestation. The periparturient period here was taken to include the period, four weeks before parturition and the immediate six weeks post parturition.

3.3.3.4 Experiment 4

Ten lambs born in the early dry season and another 10 born in the early wet season were monitored weekly for helminth eggs in their faeces, as from 12 weeks of age for a 23 week sampling period, to find out when they would acquire

their first helminth infection. At the end of the first 10 weeks of sampling, which coincided with the end of the dry season, the lambs were dewormed with an anthelmintic and the pattern of infection was again followed for the rainy season for the rest of the 14 week period. These lambs were also weighed monthly for 10 months to monitor growth rates. The same pattern of study was to have been carried out in another group of 10 lambs born in the wet season but due to an early and acute helminthosis, these set of lambs were only monitored twice for egg counts before death of 6 of the lambs, warranted an anthelmintic treatment of the rest .

3. 3.4 *Faecal Egg Counts (FEC)*

Rectal faecal samples were collected at one week intervals and egg counts were carried out using a modified McMaster technique (Whitlock 1948). The results were expressed as eggs per gram (epg) faeces.

3. 3.5 Larval Culture

Faecal cultures for recovery of infective larvae were prepared by incubating 4-10g faeces from pooled batches of faeces from the animals in lightly capped jars at 23-25°C in a humid atmosphere for 7 days. About 200 infective larvae from each culture were differentiated at the generic level with the aid of standard methods (Anon, 1971).

3. 3.6 *Blood Collection*

About 5mls of blood were collected by jugular venipuncture weekly into labelled vacutainer tubes with Ethylene Diamine Tetracetic Acid (EDTA) as anticoagulant for haematological and hormonal studies. The vacutainers were placed on ice in a cooler and transported to the laboratory. A portion of the blood was used for haematological studies, the balance provided plasma which was preserved at -20°C until needed for hormonal assay.

3. 3.7 Haematological Indices

The haematological indices monitored were haemoglobin (Hb), Packed Cell Volume (PCV) and Total Protein (TP). Haemoglobin was estimated as cyanmethaemoglobin using a Coulter Haemoglobinometer (Coulter Electronics Ltd, England). Percentage PCV was determined using a Hawksley microhaematocrit centrifuge and reader, the tubes being centrifuged for five minutes at 1200g. Total protein was read using a Goldberg Refractometer.

3.3.8 Statistical Analysis

The weekly strongyle egg count for each animal was transformed, replacing the actual count, x, by log (x +1). The means of the transformed counts for the different groups in a given period were analysed on Minitab Version 10.1, by using one- way analysis of variance and the student's t test. The major effect of the transformation was to stabilise the variance and compensate for the zero egg counts (Snedecor and Cochran, 1980) .

3. 4 Results

3. 4. 1 Meterological Data

Meteorological data on conditions prevailing from the commencement of the study in 1990 through 1991 and 1994 as collected at the meteorological station, Institute for Agricultural Research, Ahmadu Bello University, Zaria, are shown in Figure 3.1.

3. 4. 2 Experiment 1: Helminth egg counts in lambing and non-lambing ewes

In experiment 1, pregnant ewes produced significantly ($P < 0.05$) more eggs during the periparturient period than did non-pregnant control ewes (Figure 3.2) Faecal egg counts increased from a mean of 19.4 ± 11 pre-lambing to 357.1± 94 epg 4weeks post lambing while in non-pregnant ewes equivalent egg counts at this time were 14.0 ± 12 and 190.0 ± 162 respectively. Individual peak counts ranged from 600-2400. From 6 weeks post lambing there were no appreciable differences in helminth egg counts of lambing and non-lambing ewes.

3.4.3 Experiment 2: Egg counts and haematological indices of lambing ewes

The mean values of PCV, Hb and Total protein of ewes of this experiment together with their concurrent mean faecal egg counts are shown in Figure 3.3. A post parturient increase in egg output that had a peak 2 weeks post lambing occurred in the ewes on lambing. At the peak of egg output at 2 weeks post lambing egg counts had increased from 187 ±124 two weeks pre-lambing to 1589 ± 429 epg, a more than 8-fold increase over pre-lambing epg. Egg counts were significantly higher ($p < 0.05$) than pre-lambing values between the week 0 of lambing and 3

weeks post lambing. PCV decreased with increasing egg counts, being lowest four weeks after lambing. Haemoglobin values followed the same trend, but Total protein values remained fairly stable Larval cultures showed that *Haemonchus* spp constituted 90% of the worms causing the PPR, *Trichostrongylus* and *Oesophagostomum* constituting 9 and 1% respectively.

3.4.4 Helminth egg counts and haematology of ewes lambing in the different seasons

The mean periparturient egg counts of ewes lambing in the different seasons are shown in Figure 3.4. Ewes that lambed in the early and late wet seasons as well as those lambing in the early dry season showed a rise in faecal egg counts post lambing while ewes lambing in the early dry season did not show a post lambing rise in egg counts but had significantly higher ($P<0.05$) egg counts pre-lambing, than ewes lambing at other seasons. The egg output pattern and magnitude varied in the various seasons and was minimal in ewes lambing during the late dry season. In ewes that lambed in the early dry season, egg counts progressively increased to a peak of 2860 $\pm$ 834 epg on the week before lambing and subsequently showed decreases. In ewes lambing in the early wet season egg counts progressively increased week 1 after lambing to a peak of 3838 $\pm$ 598 on week 5 post lambing. Ewes that lambed in the late dry and late wet seasons also showed progressive increases in helminth egg counts after lambing but attained peaks of 1800 ± 765 at week 5 and 4201 ± 1813 at week 4 post lambing respectively.

Post lambing, ewes that lambed in the early dry season had PCV that were consistently higher ($P<0.05$) than PCV of ewes lambing in the early wet and late

dry seasons. (Figure 3.5). Ewes that lambed in the late dry seasons had consistently lower PCV than ewes that lambed at any other season. PCV of individual ewes ranged from 16-32 gm/dl and anaemic ewes showed pallor of the mucus membrane as well as emaciation.

3.4.5 Helminth egg counts during pregnancy

There was no significant difference between the egg counts of ewes that were pregnant between February-May (lambed in June) and those pregnant May-Sept (lambed in October), in early, and mid pregnancy. However ewes that lambed in June had significant higher ($P<0.05$) helminth egg counts than those that lambed in October, during late pregnancy. For both groups egg counts in the late pregnancy were significantly higher($P<0.05$) than the values in early and mid pregnancy respectively (Figure 3.6).

3.4.6 Lamb helminth egg counts and weights

Mean weekly helminth egg counts of the lambs from when they were 12 weeks of age (sampling week 1) up to a subsequent 23 week sampling period is shown in Figure 3.7 while monthly weights are shown in Table 3.1. The lambs did not show any appreciable levels of infection from birth in the month of November until they were three months old in February. Mean helminth egg counts of the lambs remained below 500 epg all through the dry season months and peak increses in helminth egg counts occurred between the 16th -19th week of sampling, by which time the lambs were about eight months, and the rainy season had set in. At 10 months of age, which was when the experiment was terminated,

the lambs weighed an average of 19.2 kg and had gained a mean of 11.75 kg (Table 3.1). In contrast, lambs born in the wet season months of June - September, were overwhelmed by helminth infections before they were weaned, with epg in excess of 20,000 epg. Six out of the 10 lambs died and Table 3.2 summarises their helminth egg counts and survival from infection.

Table 3.1 Mean monthly weights (±sem) and average daily weight gains of some lambs born in the dry season, from birth to 10 months of age

Age (months)	Weight(kg) (±sem)	Average daily gain (gm)
0	2.7 ±0.14	-
1	5.1 ±0.18	80.0
2	6.2 ±0.29	36.7
3	7.6 ±0.50	46.6
4	10.1± 0.38	83.3
5	10.8± 0.41	23.3
6	10.9 ±038	3.3
7	12.8 ±0.62	63.3
8	14.8 ±0.83	66.7
9	17.3 ±0.68	83.3
10	19.2 ±0.47	63.3
Mean	11.75 ±0.54	54.98

Table 3. 2 Helminth egg counts and ages at infection of lambs born during the wet season

Lamb Number	*EPG at sampling	Age at sampling	Probable age infected	Age at which lamb died
0988	27,16	5 week	3 weeks	11 weeks
0971	9,540	5 weeks	3 weeks	Survived
097	31,740	7 weeks	5 weeks	8 weeks
0974	36,080	8 weeks	6 weeks	8 weeks
0962	20,200	9 weeks	7 weeks	9 weeks
0967	28,100	2 weeks	10 weeks	12 weeks

*EPG: Eggs per gram of faeces

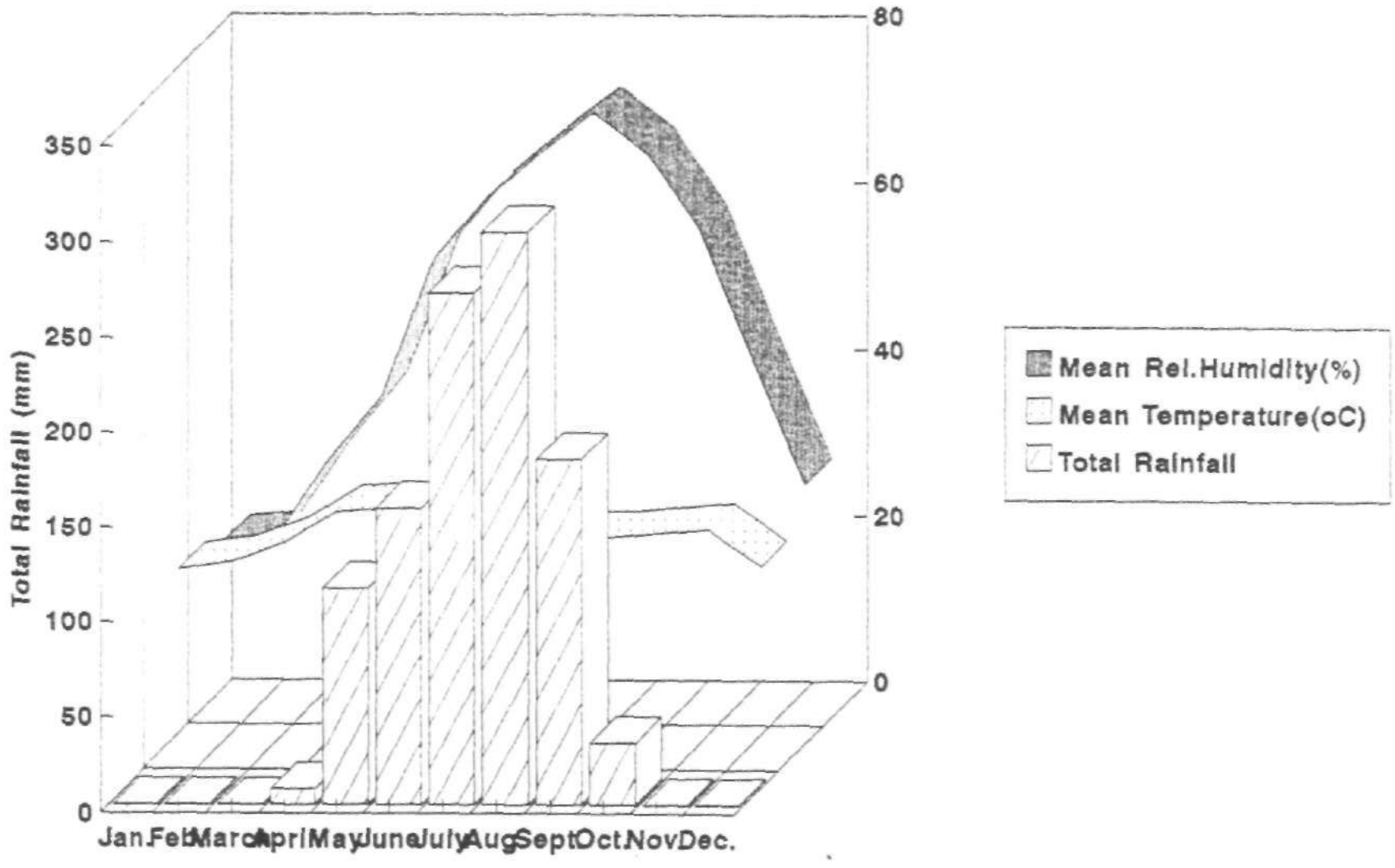


Figure 3.1:Long-term mean monthly meterological data for the experimental area.

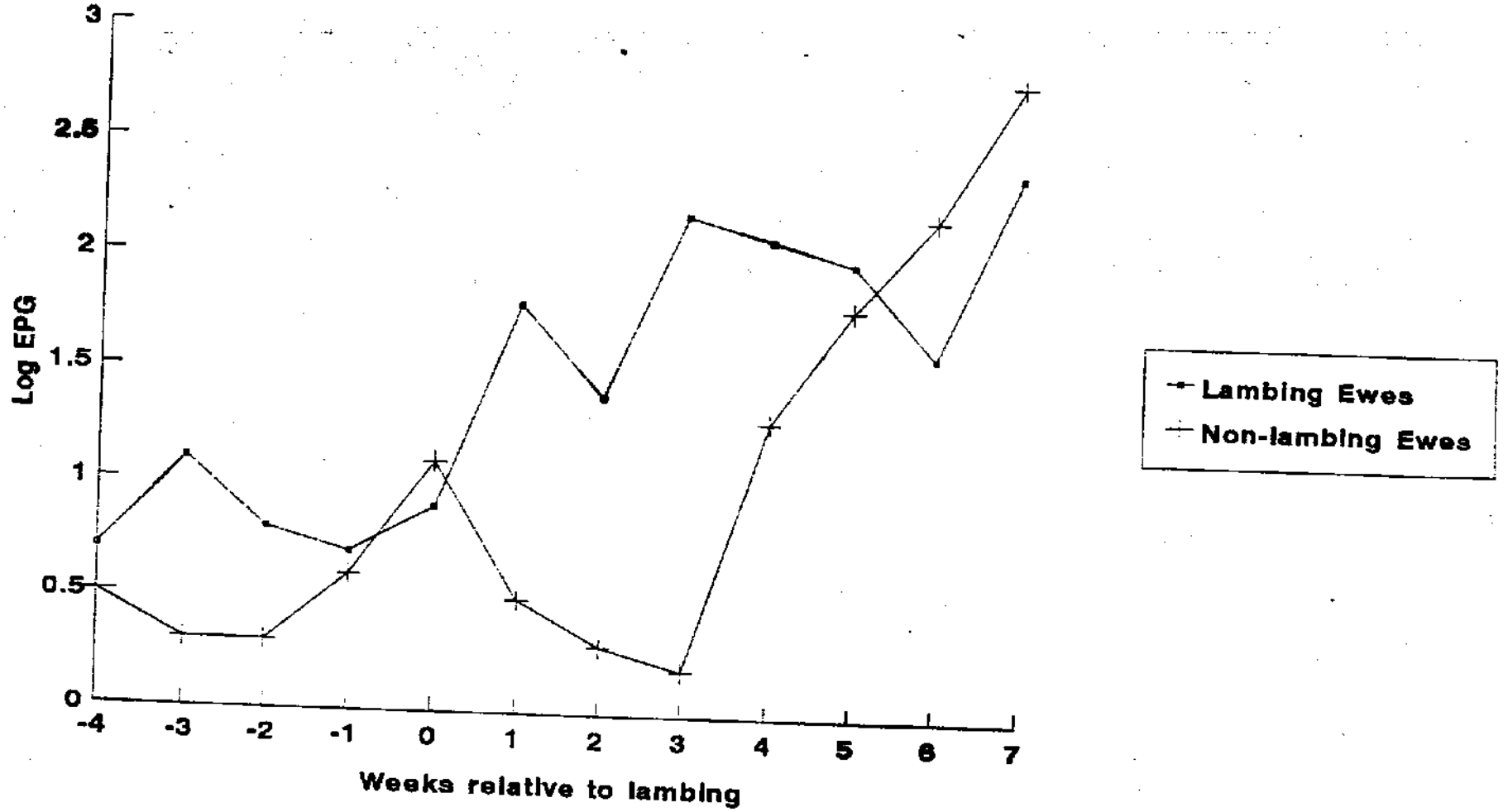


Figure 3.2: Mean weekly helminth egg counts of parturient and non-parturient ewes from four weeks before to seven weeks after lambing

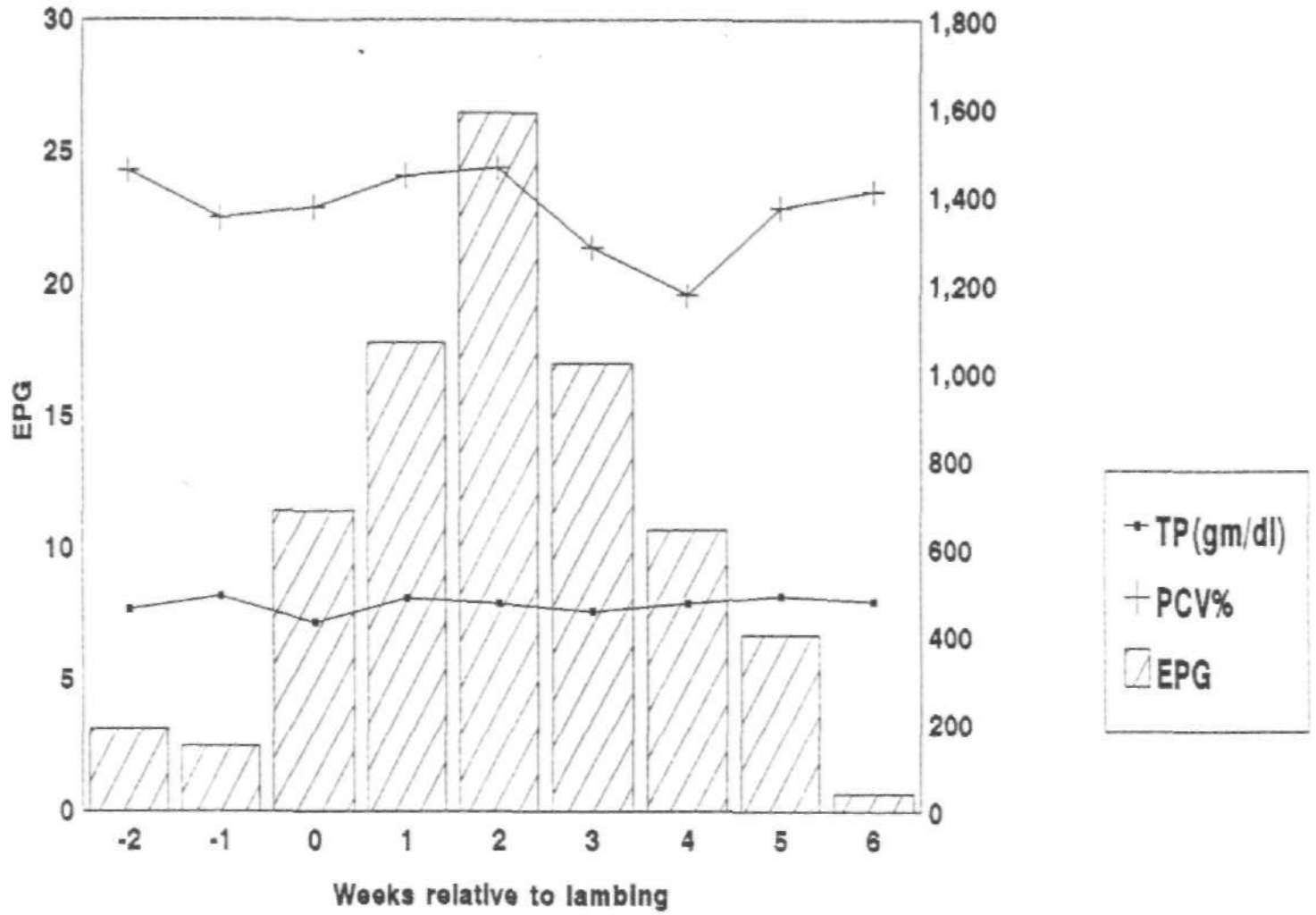


Figure 3.3: Mean helminth egg counts, Packed cell volume and Total protein of ewes from two weeks before to six week after lambing

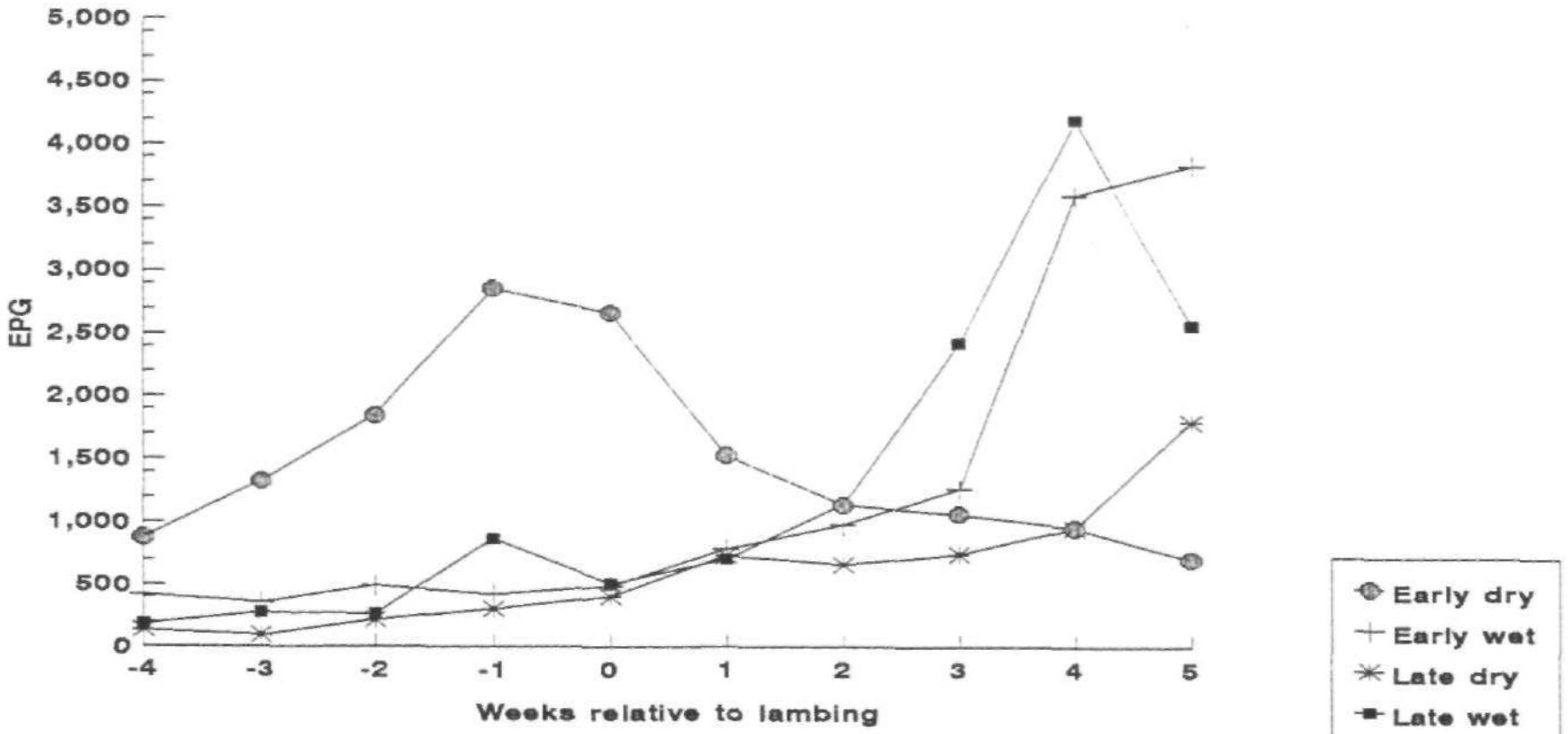


Figure 3.4: Mean weekly helminth egg count during the periparturient period of ewes lambing during the the different seasons

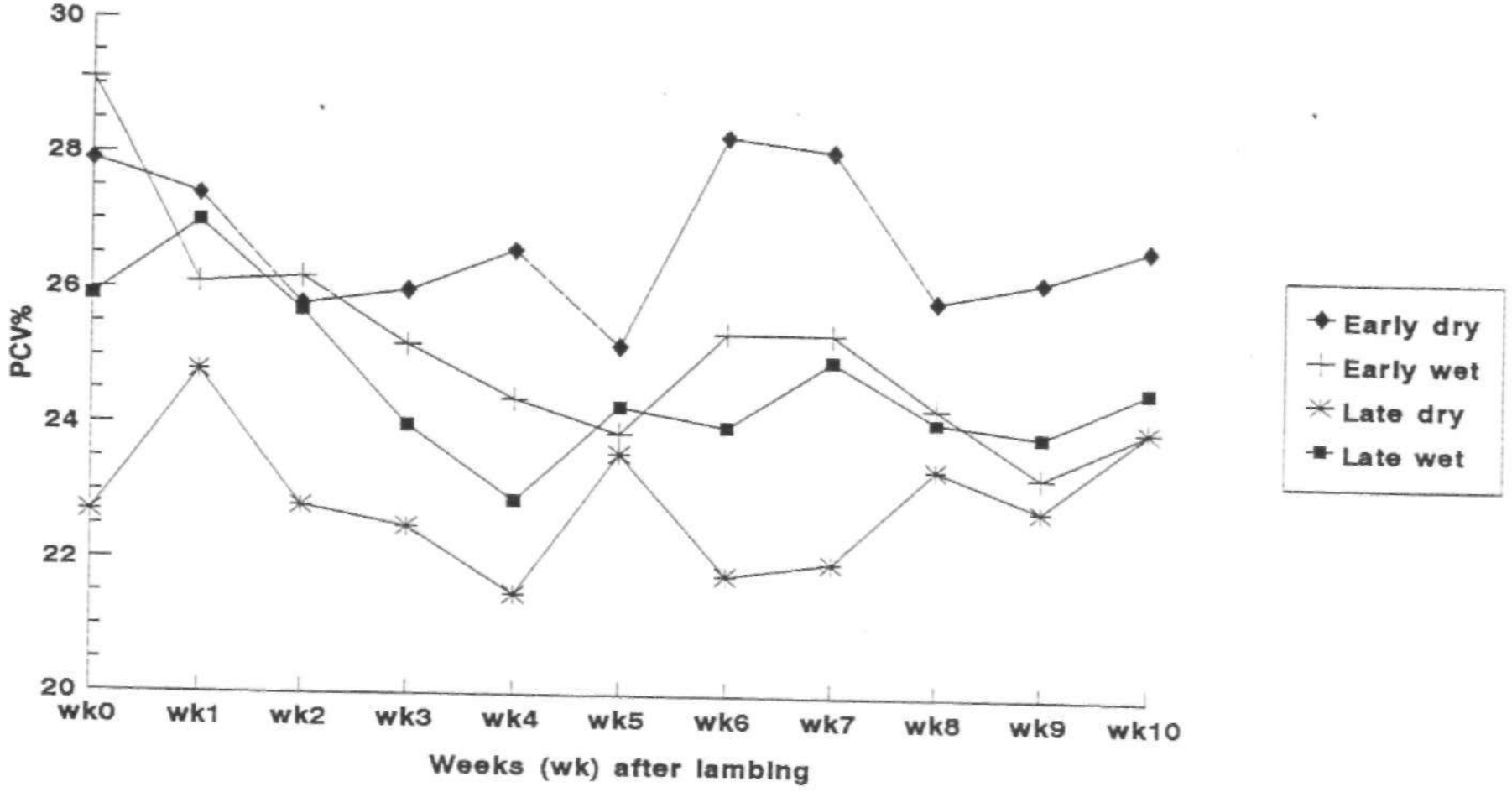


Figure 3.5: Mean weekly Packed cell voume values (%) post lambing, for ewes that lambed in different seasons of the year

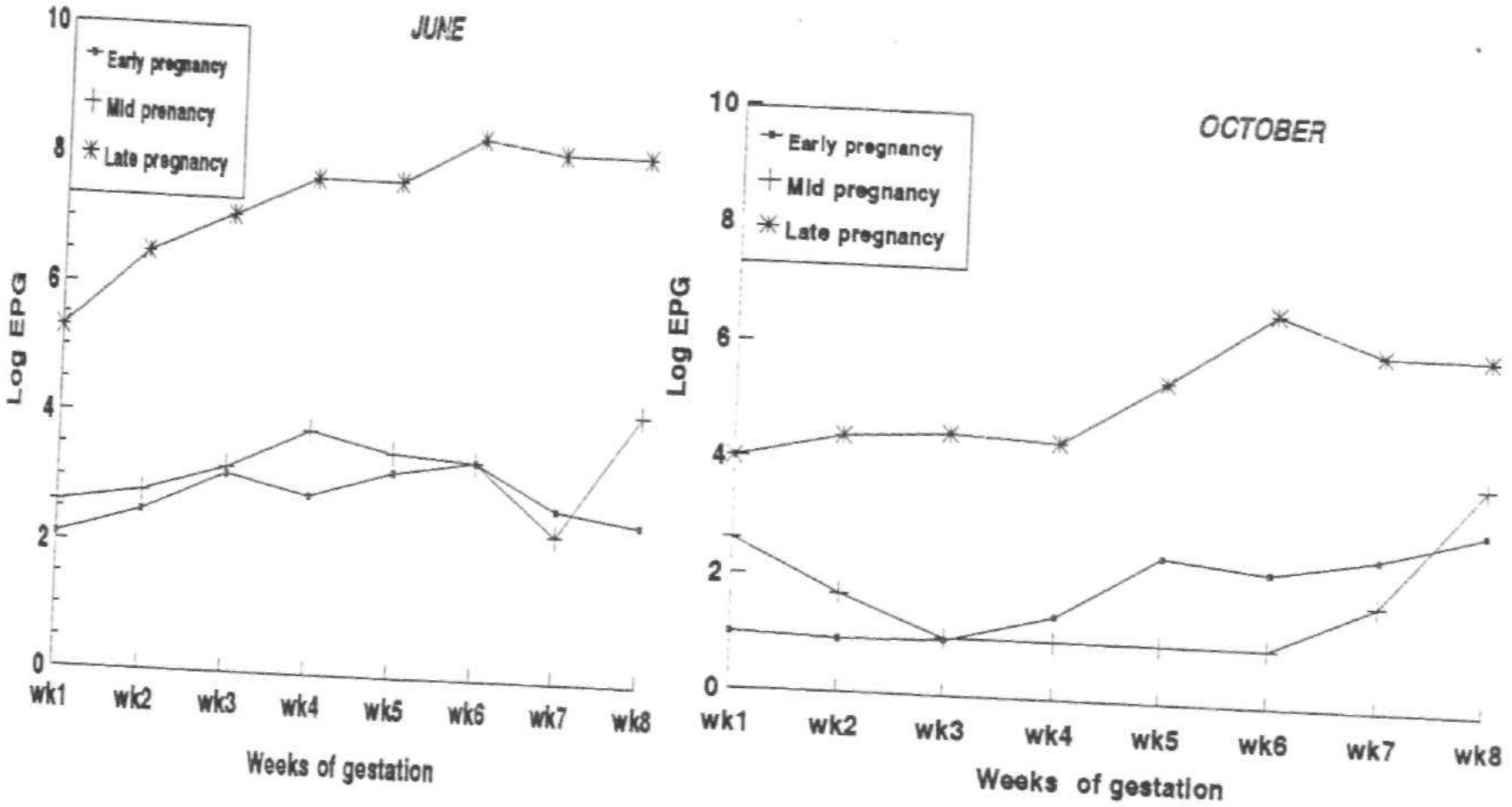


Figure 3.6: Mean weekly helminth egg counts per gram (EPG) for early, mid and late pregnancy of ewes that lambed in the months of June and October respectively

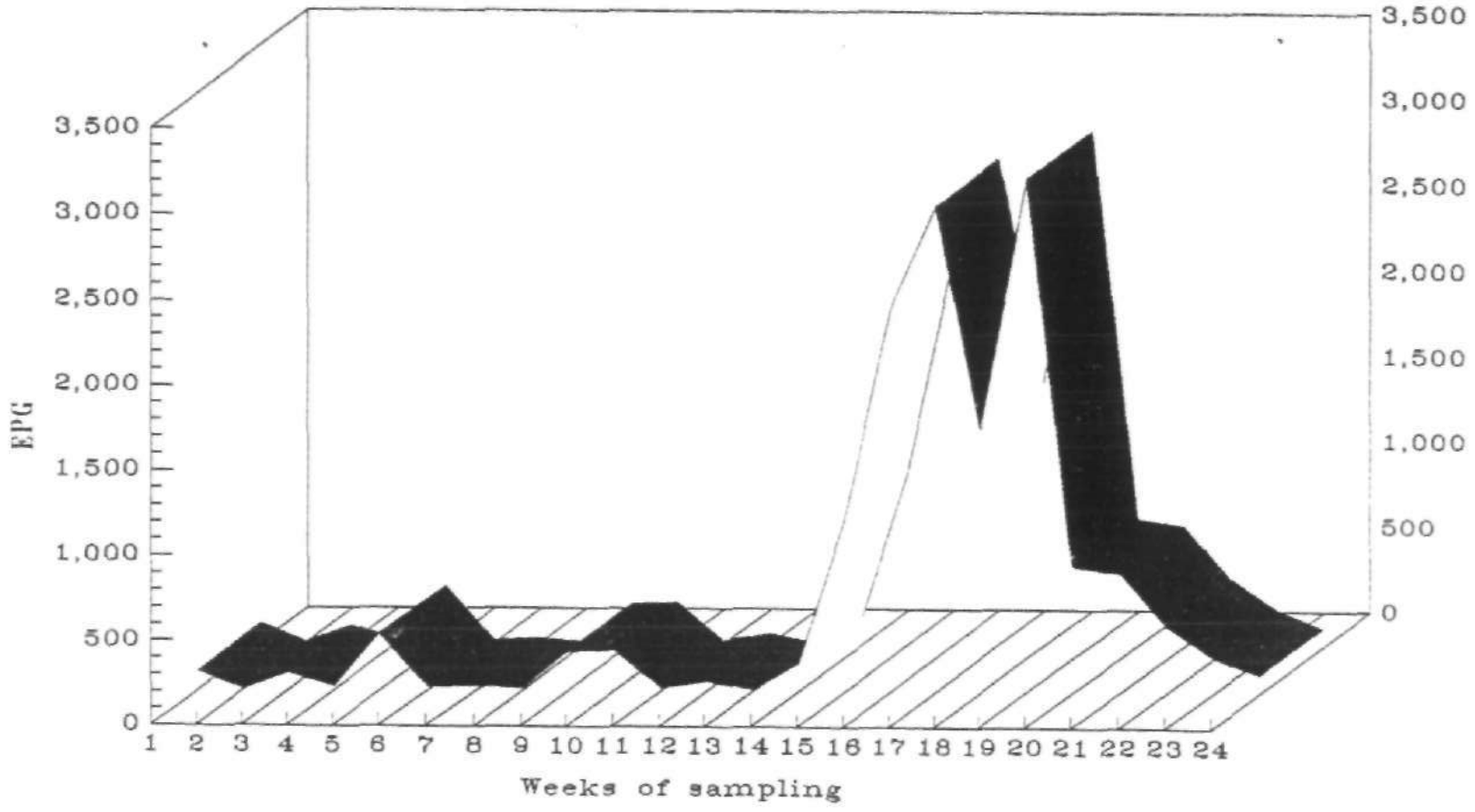


Figure 3.7: Mean weekly helminth egg counts per gram (EPG) of 12- week -old lambs sampled for a 24 week period.

3.5 Discussion

A periparturient increase in helminth egg counts occurred in lambing ewes in the three experiments. In contrast, no appreciable increase in egg counts occurred in non-lambing ewes, thus demonstrating that the increase in faecal egg counts was associated with parturition. Moreover, there were no significant differences in helminth egg counts in the ewes in early and mid pregnancy until a few weeks before lambing, suggesting that the increased egg counts were associated with endocrine events at the periparturient period.

In the present study, the peak of the periparturient rise (PPR) in egg counts occurred between 2 and 4 weeks after lambing. An early peak in egg counts is associated with helminths with short generation intervals such as Haemonchus. The time of peak output of eggs varies with the species present (Borgsteede, 1978) and so the early peak of two weeks in the faecal egg counts post lambing, reflects the short generation interval of 14-18 days for *H. contortus* which was the predominant specie present in the ewes. The decline in egg counts observed after the peak values may be a self-cure reaction. Such decrease in egg counts are known to be as a result of host immune reaction against infection (Adams *et al.*, 1990; Adams 1993), and can be taken as criteria for a return of immunity after the periparturient immunosupression.

The same pattern was seen in wet and dry season lambing ewes though the magnitude of the rise differed in the seasons, with ewes lambing in the rainy seasons showing greater intensity of infection, and more animals manifesting poor clinical

condition as a result of infection. These findings are similar to that observed in other breeds of sheep (Vercruysse, 1983; Jacquiet *et al.*, 1992). Although the importance of rainfall in the epidemiology of the pre-parasitic stages of helminths in tropical climates is well known. Although pasture larval counts were not done in this study, an earlier report by Ogunsusi (1978) in the same northern Guinea savannah area indicated that infective larvae were not available on pasture in the months of November to May.

A seasonal arrest of development of *H. contortus* of sheep in the Zaria area is known to occur as the dry season sets in, being greatest among larvae ingested from pasture in the late dry season (Ogunsusi, 1978). These larvae remain arrested until May/June when they mature asynchronously. The PPR in ewes that lambed in the late dry season, when there is little larvae on pasture, are likely to be related to the maturation of some larvae previously arrested in the ewes. Maturation of arrested larvae during the dry season has been reported by Fabiyi (1973) in the same area and has been associated with outbreaks of dry season clinical parasitic gastro-enteritis (Fabiyi *et al.*, 1979; Trimnell *et al.*, 1985). Trimnell *et al.*, (1985) attributed this peak partly to the nutritional stress of animals during the dry season which lead to a re-activation of arrested larvae in the immunocompromised animals.

However, the authors failed to mention if the ewes had lambed prior to the onset of the gastro-enteritis. Even though these reasons may not be strictly applied to the animals in this study, as they received protein supplement, the

lowered immune state of the periparturient ewes, might have stimulated the re-activation of some of the arrested larvae as well as allowed an increase in the fecundity of the residual adult population that had low egg productivity during pregnancy. Michel, (1976) has summarised the various studies on this phenomenon and concluded that there is a clear relationship between the physiological status of the ewe and the "activation" of arrested larvae acquired the previous grazing season. He proposed that parasite populations are controlled by a biorhythm of parasite origin, in that arrested larvae in sheep of all descriptions mature at the same time, while in parturient and lactating animals, parasites are retained longer than in barren animals. This explanation is consistent with the parasitological findings of this study. Connan (1976) has reviewed the evidence that endocrine changes in sheep associated with parturition are associated with suppression of the immune response against gastro-intestinal nematodes. Hence, the arrival of new, fecund parasites from the reservoir of arrested larvae occurs at a time when host factors have minimal impact on the parasite population.

The finding of low levels of helminth egg counts during pregnancy in this study is in agreement with the results of Gibbs (1967), Connan (1974), Fleming and Conrad (1989) and indeed Jeffcoate and Holmes (1988) who found zero egg counts in infected pregnant ewes and concluded that pregnant ewes are resistant to the development of infection to patency. The maintenance of normal levels of immunity against nematode parasites during pregnancy, until the PPR begins a few weeks before parturition, seems rather puzzling. In pregnancy in general, the immune response of the female is known to be decreased in order to enhance the

acceptance of the foetus (Andresen and Munroe, 1962). In humans, monoclonal antibodies used to detect B cells and various sub sets of T cells in patients examined during pregnancy, during labour, and after birth revealed that there was a decline in overall numbers of T cells throughout pregnancy, numbers returning to normal 3-5 months after birth. No changes occurred in the number of B cells during pregnancy (Svidane *et al.*, 1982). T cells are important in immune responses to helminths and are known to be depressed during the periparturient period. This depression of T cells does not seem to operate during the early and middle stages of pregnancy in sheep and probably other domestic animals. In *Toxascaris* spp infection in dogs and cattle for example, normal levels of immunity are maintained in the non-pregnant animal and in pregnancy and the parasites remain quiescent in the adult host. However, a resumption in activity of the parasites have been associated with a depression of immunity near parturition with the result that adult parasites appear again in the intestines of the female, and a trans-mammary infection to the young occurs. Normal immunity is restored a few weeks after birth (Lloyd *et al.*, 1983). These responses are dependent on T lymphocytes and are indicative of the important influence of the physiological and endocrinological events associated with parturition.

Lambs born in the early dry season did not acquire infection with Trichostrongyles until 3-4 months after birth even though adult worms were present in their dams. The absence of strongyle eggs in the faeces of these lambs may be due to the absence on pasture of infective larvae because of dry environmental conditions usually prevailing between November and February as earlier report by

Ogunsusi (1978) in the same area. He reported that pasture infectivity resumes as from June to October, and this may be responsible for the acute infection in lambs born in the wet season. *H. contortus* was the predominant parasite in the faecal cultures of these lambs but *Trichostrongylus* species may have played a role n the helminth infections since diarrhoea was present in some of the lambs and ewes. *Trichostrongylus colubriformis* is known to cause black scours and contribute to parasitic gastro-enteritis in lambs. Helminthosis in lambs in this experiment was also associated with low haematocrit values, (observed in individual sick lambs), ill thrift and low weight gains. Similar situations of clinical haemonchosis has been described by Schilhorn van Veen (1978) and Maclure *et al.*, (1976).

The growth rate of the lambs in our study was low when compared to those reported for sheep of same breed (Anon 1978; Adu and Buvanendran, 1982). This reduced growth rate will mean lateness in attainment of slaughter weights and delay in first ovulation and conception in females. The liveweight of 19.2 kg at 10 months of age and mean daily average as obtained in study is unsatisfactory but cannot be directly related to worm burdens since egg counts were not high. However, some of the earliest work on the effects of parasitism on the performance of livestock (Franklin *et al.*, 1946; Gordon, 1950) established that even small intakes of nematode larvae reduced growth rate in young growing lambs. Similarly in production studies with grazing animals on fertilised pasture, McClure *et al.*, (1978) reported that daily weight gains of helminth-infected lambs decreased sharply after the first 6-7 weeks in spite of favourable pasture quality. Since the season of birth has such an effect on the magnitude of helminth infection acquired

by lambs, the adjustment of breeding date for dry season lambing may be a useful adjunct to parasite control programmes in intensive sheep production systems. In order to avoid the negative effects of the periparturient rise in helminth egg counts, control measures should be undertaken by treating ewes and lambs even before weaning.

CHAPTER FOUR

EFFECTS OF NEMATODE WORM BURDENS ON REPRODUCTIVE PERFORMANCE OF YANKASA EWES

4.1 Summary

The reproductive performance of a flock of 63 Yankasa ewes carrying varying levels of Trichostrongyle burdens were examined. They were continuously bred by being run with rams and the interval from one lambing to the next conception were monitored by progesterone assay.

Analysis of the overall lambing performance showed that mean birth weight of lambs was 2.7 kg, litter size was 1.1 lambs, and up to 50% of ewes lambed two or more times within an 18 month period. There were significantly ($P<0.05$) less lambs born in the late wet season, than any other season, lambing percentages being 9.5%, 23.3%, 25.9% and 38% for late wet, late dry, early wet and early dry season lambings respectively. Seventeen percent of ewes had mean lambing intervals (L I) of 193 days and another 34.8% had mean LI of 220 days. The remaining 47.8% had lambing intervals greater than 220 days. Low mean egg counts (5025 $\pm$ 441 epg) were associated with long lambing intervals, and lambing intervals over 220 days were associated with mean egg counts of 16,317 $\pm$ 2315 epg. Progesterone profiles of the ewes showed that long lambing intervals were due to delayed resumption of ovarian activity post partum.

Ewes with high egg counts ending pregnancy in low body condition and anaemia, neglected their lambs which subsequently died of hyponutrition. High levels of helminth worm burdens may have an effect on expression of the full reproductive potential of the ewe. The mechanics were not explored but this may operate through an effect on live weight and condition and/or through indirect disruptions of the reproductive cycle.

4.2 Introduction

Tropical breeds of sheep are not seasonal breeders (Devendra and Burns, 1983) like breeds in the temperate regions. This is an obvious advantage in the study of the periparturient rise in egg counts. Any seasonal lambing peaks in tropical sheep have been attributed primarily to nutrition linked to local variation in rainfall, vegetation, growth and availability of forage (Amaoh and Gelaye, 1990). Ovarian activity is also established early in lactation and with good management and adequate nutrition, it is possible to obtain two lambings per year from indigenous breeds provided no other factor is limiting (Osinowo and Ekpe, 1985). However the humid and sub-humid environments also enhance the reproductive cycle of parasites and surveys indicate that about 95% of the small ruminant population in sub-saharan Africa is infected by parasites.

The consequent high mortality and poor productivity negates the high reproductive performance of small ruminant breeds (Ademosun, 1985). Lambing interval is an important production trait in domestic animals because of its effect on efficiency of production and reproduction. This interval may be prolonged by

conception failure, stillbirth and or abortion, delayed resumption of ovarian cyclical activity and disease among other factors.

Helminthosis has been found to decrease reproductive efficiency in sheep and other ruminants, though no pathological lesions have been reported in any of the regulating organs of reproduction. Anoestrus (Hayat *et al.*, 1983); reduced fertility of ewes and cows (Lewis, 1975; Hope-Cawdery, 1976; Oakley *et al.*, 1977; Mackay, 1980) as well as reduced birth weight of lambs born to infected ewes (Crossland *et al.*, 1977; Johnstone *et al.*, 1979) are some of the reported effects of helminths on reproduction. The mechanics have not been fully explored, but seem to operate through an effect on live weight and condition and/or through indirect disruptions of the reproductive cycle.

The development of radioisotopic techniques for hormone analysis has become an important tool for studying reproductive functions in domestic animals. In this regard progesterone assay has been found to be of great practical importance in assessing the reproductive status of the female (Perera and Abeyrante, 1979). Progesterone levels in the blood normally follow a cyclical pattern, and reflects corpus luteum function and therefore serves as a good indicator of the oestrus cycle stage and in diagnosis of pregnancy. It is also an important diagnostic tool in the elucidation of clinical syndromes in the post partum period either when it is too low at a time when the levels should be high or when they are higher than expected (Basset et al., 1967).

Information on the effects of helminth parasites on reproductive efficiency in Yankasa sheep is not available. The important physiological characteristics of the breed have been studied Litter size and twining rate are 1.25 and 12% respectively (Ferguson, 1964; Adu *et al.*, 1979). Birth weight is 3.2 kg (Adu, 1978); liveweights of about 18 kg at 4 months of age and 35 kg has been given as mature weight of females (Adu and Ngere, 1979). The aim of the present study was to monitor ovarian activity through progesterone profiles, determine parturition intervals and any effects on reproductive performance of Yankasa ewes naturally infected with helminth parasites.

4.3 MATERIALS AND METHODS

4.3.1 Animal feeding and management

All sheep were managed under a semi- intensive system involving grazing on improved pasture 6-8 hours daily plus 0.3-0.5 kg/day of a 15% crude protein concentrate supplement depending on physiological status i.e. pregnant, nursing or dry. The concentrate was made up of 62.6% maize, 33.7% cottonseed cake, 2.5% bonemeal, and 0.25% vitamin minral premix. Water and mineral salt licks were supplied ad libitum. Animals were housed in well ventilated pens with concrete floors which were cleaned daily. The animals received routine veterinary care including vaccination against PPR and dipping against ectoparasites at the appropriate time.

Breeding of ewes was by flock or pen mating, to rams of the same breed. Following parturition, ewes and their lambs were separated into a nursing pen and

kept under observation for one week after lambing. While indoors the ewes were given supplementary feed as well as chopped sun- dried green fodder during the rainy season or hay during the dry season.

All animals used for the experiment were naturally infected from pasture and did not receive any experimental infections

4.3.2 Progestrerone assay

Plasma obtained from centrifuging blood samples to which EDTA had been added, were deep frozen at -20^{0}C until assayed. Plasma progesterone (P 4) was measured using a solid phase radioimmunoassay (RIA) kit validated for use in sheep (Oyedipe *et al.*, 1986) and supplied by IAEA, Vienna. The assay procedure was as follows: to each antibody-coated tubes, 100ul of standard (0-6.3 nmol/L), quality control or sample was added. 1.0 ml of buffered 1125- labelled progesterone was then added to every tube and vortexed for 30 seconds. The contents were then incubated for 3 hours at room temperature, after which the liquid phase was decanted vigorously, and the tubes tapped on paper tissue to absorb all residual droplets. The bound radioactivity of the antibody-coated tubes was then counted for one minute in a gamma counter. The gamma counter plotted a standard curve from which the sample progesterone concentrations were extrapolated.

4.3.3 Reproductive indices

Reproductive indices assessed were: lambing interval, lamb birthweight, litter size, abortions/stillbirths as well as single/multiple births. Lambing interval

was calculated from day of one lambing to the day prior to the day of the next lambing. A total of 116 lambings were analysed to determine the mean and distribution of lambing intervals while 45 ewes whose progesterone profiles were monitored from one lambing to when they became pregnant again were used to determine parturition to conception intervals. Sheep were assumed to have resumed ovarian function after lambing if progesterone concentrations in two consecutive samples was above 0.2 ng/l with values at or near baseline in the subsequent sample. The occurrence of acyclicity was assumed when P_4 concentrations were below 0.2 ng/ml in at least two or more consecutive samples. Pregnancy was assumed in the ewes if P_4 concentrations were in excess of 1.0ng/l for four consecutive weeks and confirmed by eventual lambing of the ewe.

4.3.4 Faecal egg counts (FEC)

Rectal faecal samples were collected at one week intervals and egg counts were carried out using the modified McMaster technique (Whitlock 1948). The results were expressed as eggs per gram (epg) faeces.

4.4 Results

4.4.1 Reproductive indices

Mean lamb birth weight was 2.7± 0.14 kg, while mean litter size and abortion/stillbirth rates were 1.04 and 4.5% respectively. About 50% of the ewes lambed two times within the 18 month period and the lambing performance is summarised in Figures 4.1 and 4.2.

Lambing was fairly evenly distributed between the late dry, early wet, and early dry seasons but with there were significantly ($P<0.05$) less lambs born in the late wet season, lambing percentages being 9.5%, 23.3%, 25.9% and 38% for late wet, late dry, early wet and early dry season lambings respectively (Figure 4.3). January the month when the peak in lambing was observed, corresponded with the high conception rate in September.

A summary of lambing intervals is given in Table 4. 1. The overall mean lambing interval was 241 ± 45.5 days. About 17% 0f ewes had lambing intervals (LI) of less than 200 days with a mean of 192 ± 7.5 days, while 35% had an average lambing interval of less than 240 days (mean 220.4 ±10.4 days), A further 39% had average lambing intervals of 259.3 ±17.3 days and 8.7% had mean lambing intervals of 349 ± 46.9 days.

Mean helminth egg counts (5025± 4441) were significantly ($P < 0.05$) lower in ewes with mean lambing intervals of 192 days but there was no significant difference in egg counts between ewes that had mean lambing intervals of 220, 259 or 349 days respectively (Table 4.1). There were wide individual variations but generally the ewes with longer lambing intervals had higher mean helminth egg counts. Clinical signs observed in the flock in association with high egg counts (5000 epg) included anaemia, rough hair coat, progressive loss of condition as well as diarrhoea in some animals (Plates 1-VI).

4.4.2 Progesterone profiles

Parturition to conception intervals as shown by Progesterone profiles exhibited by sheep in this study are shown in Figures 4.4 to 4.6. and fall into three broad categories. Each figure is a prototype of an animal in each category.

The first category (Figure 4.4) was seen in ewe numbers 0651 and 0670 and represent ewes which resume ovarian activity as early as 3-4 weeks post partum. These exhibited P_4 profiles with basal values between peaks which then became consecutively elevated; coinciding with periods of oestrus, conception and pregnancy respectively in the animals.

The second category consisted of ewes that resumed ovarian activity between 5-9 weeks after lambing and was characterised by 5 weeks or more of basal P_4 level following parturition. This was recognised clinically as temporary anoestrus (Figure 4.5).

The third category of ewes were those which after one lambing, exhibited basal P_4 levels and were not pregnant up to the end of the study, and these were recognised as being anoestrus altogether (Figure 4.6).

Table 4.1 Mean lambing intervals and helminth egg counts of ewes with natural helminth infections

% of Animals	Mean lambing intervals (Days)	Mean eggs per gram (epg)
17.4	192 ± 7.5	5025 ± 4441
34.8	220 ± 10.4	13,011 ± 6959
39.1	259 ± 17.3	16,317 ± 2315
8.7	349 ± 46.9	12,555 ± 3914

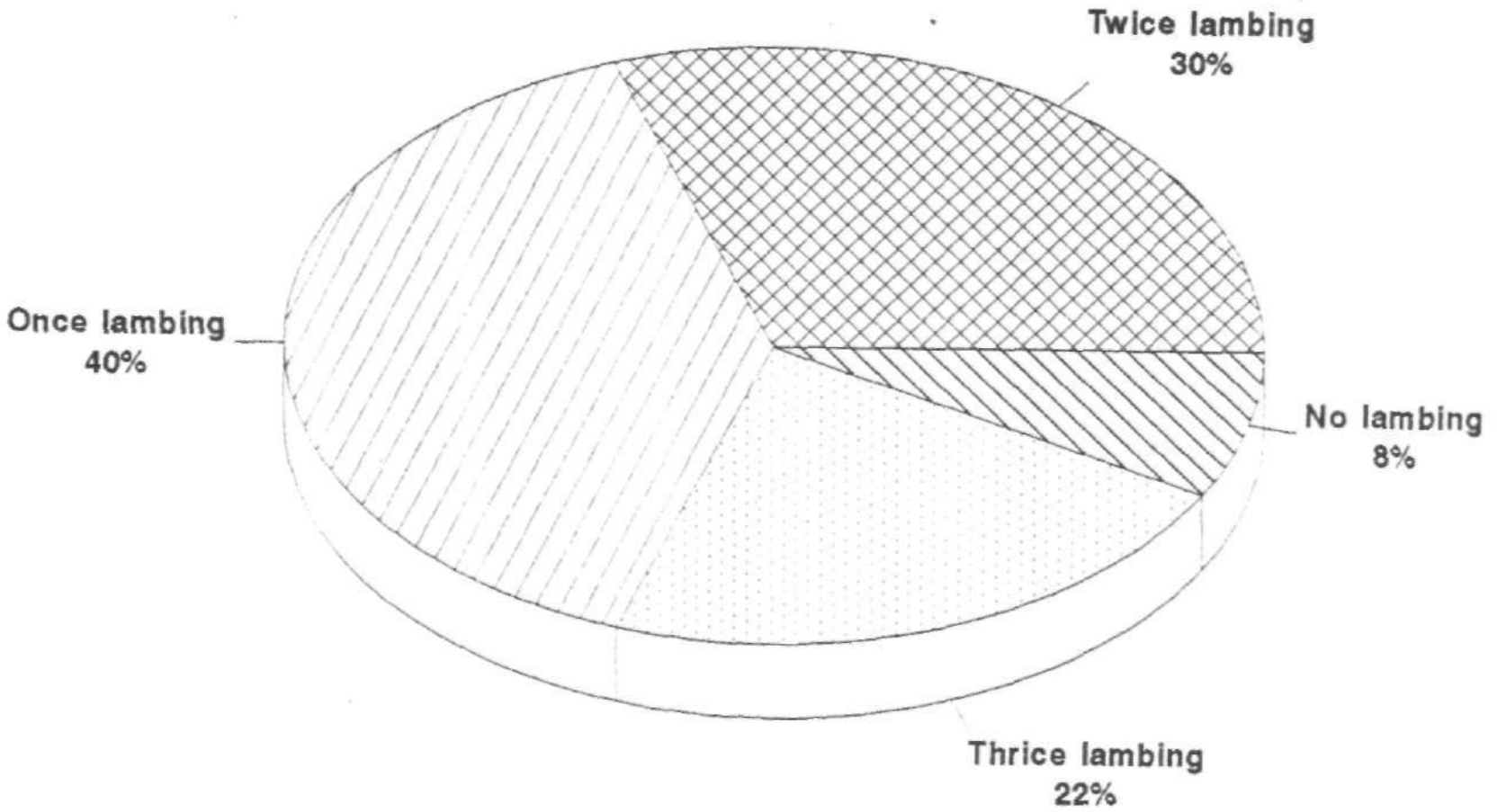


Figure 4.1: Lambing performance of ewes with varying levels of natural helminth infections over an 18 month period

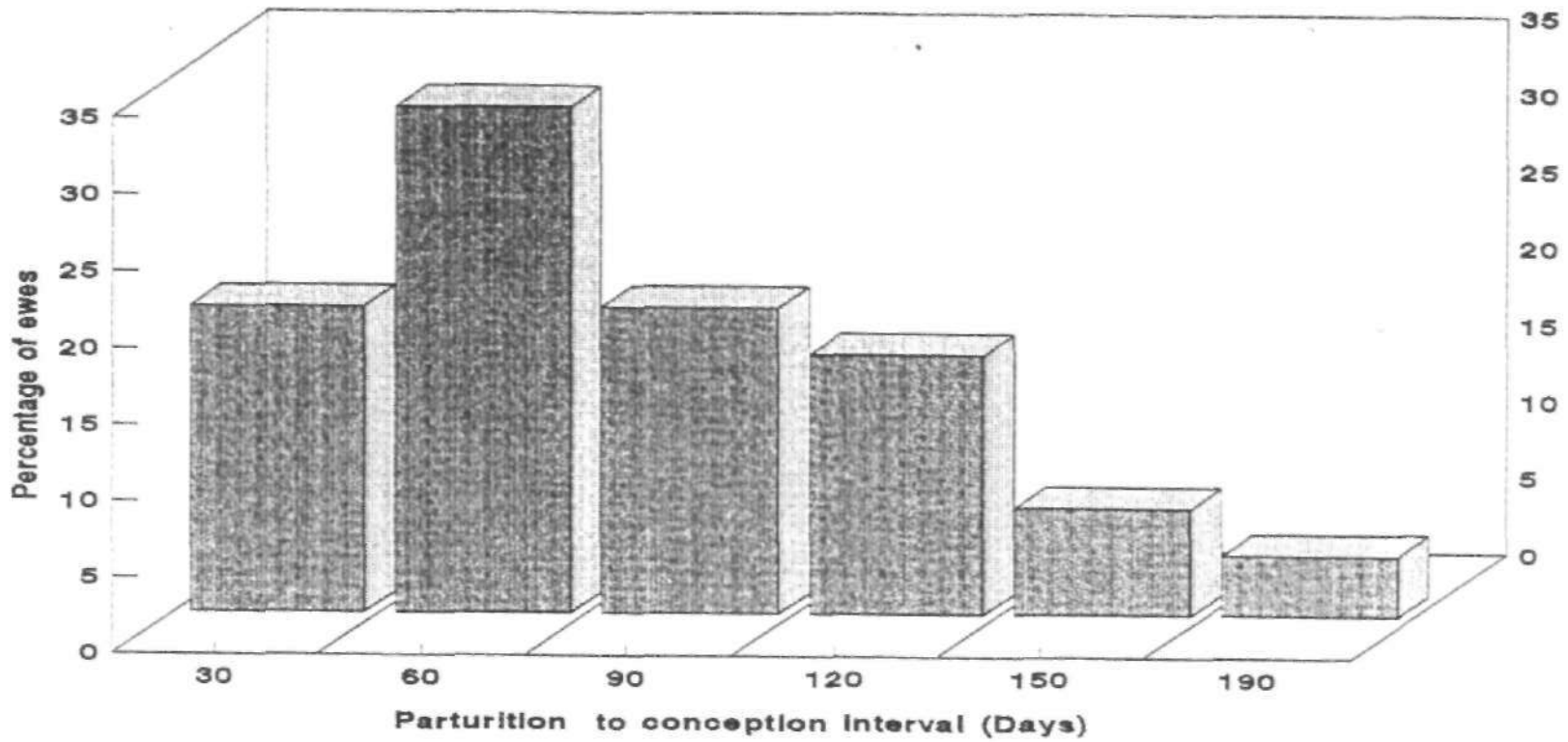


Figure 4.2: Frequency distribution of percentage of ewes that conceived between one to six months after lambing

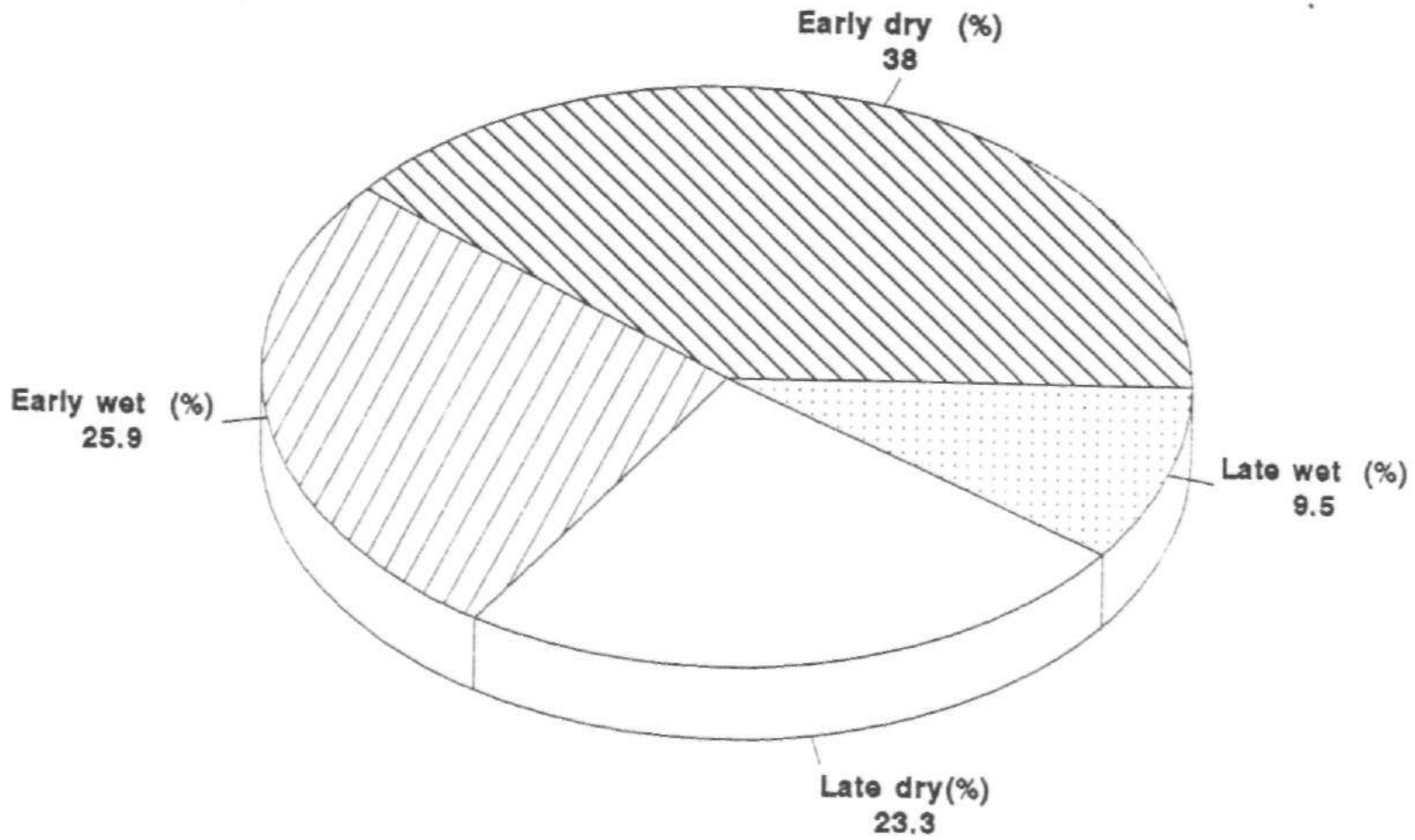


Figure 4.3: The distribution of lambings according to season

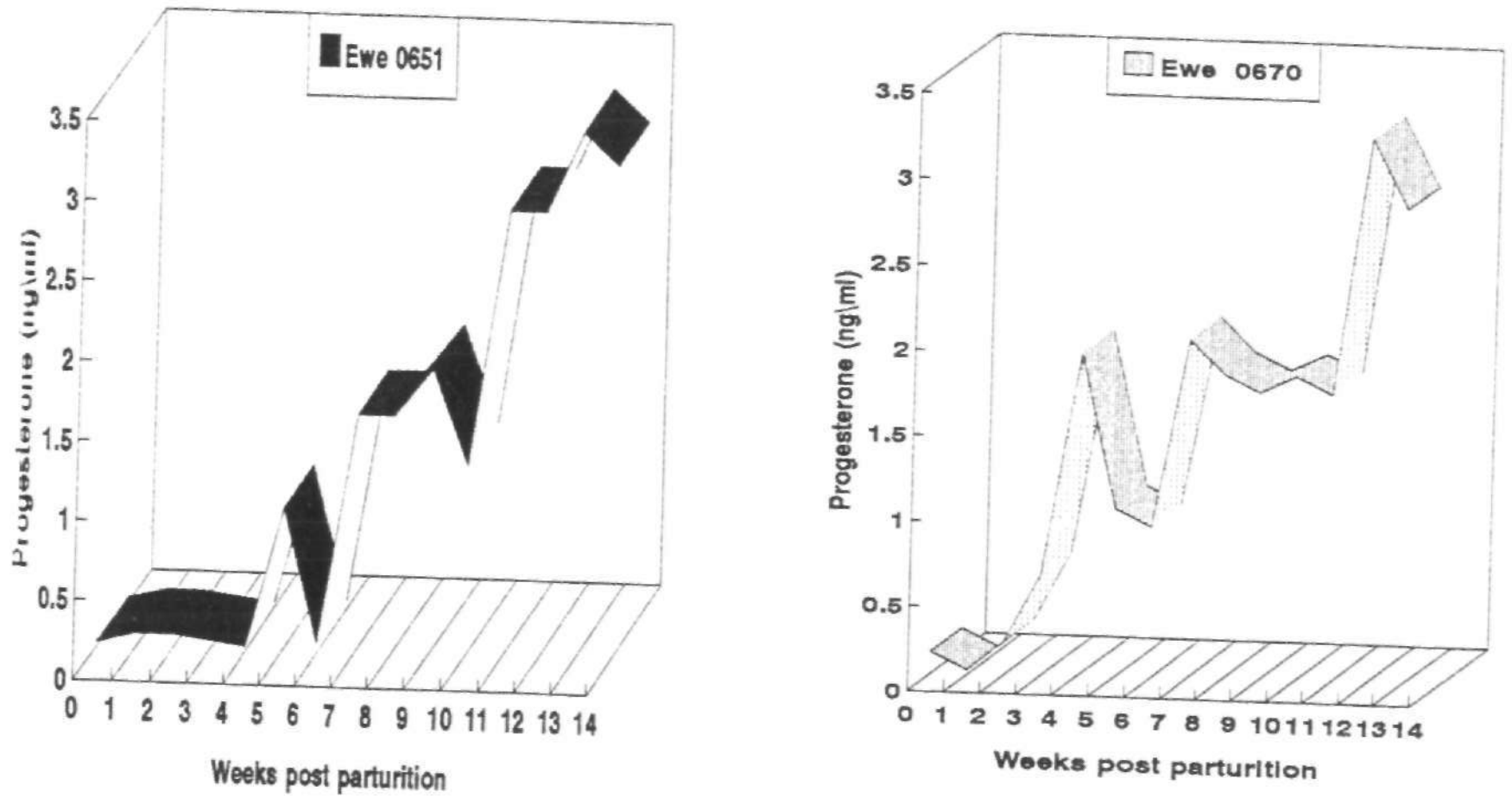
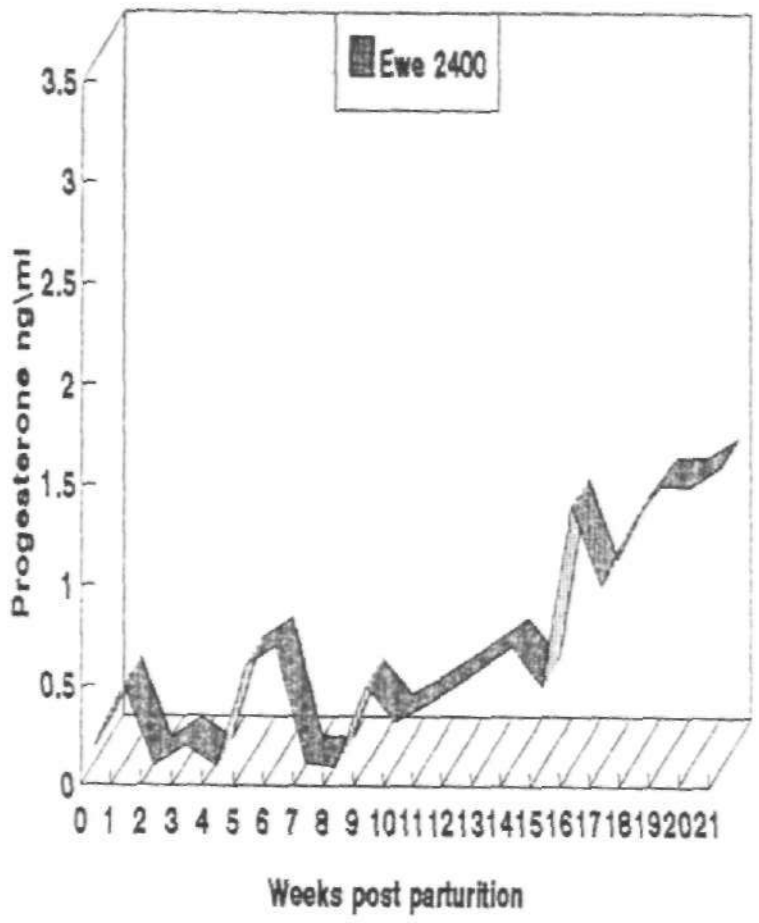


Figure 4.4: Progesterone profiles of ewes (0651 and 0670) that resumed ovarian activity between 3-5 weeks after lambing.



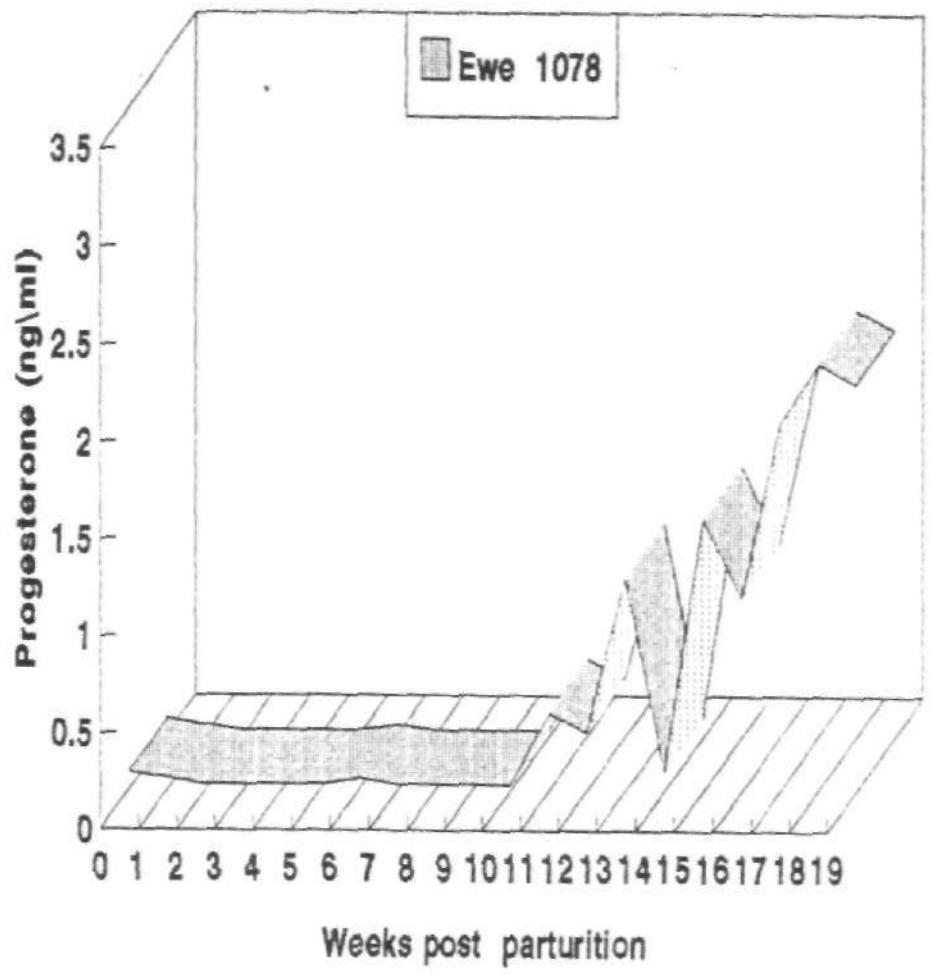


Figure 4.5: Progesterone profiles of ewes (2400 and 1078) that resumed ovarian activity between 5-9 weeks after lambing.

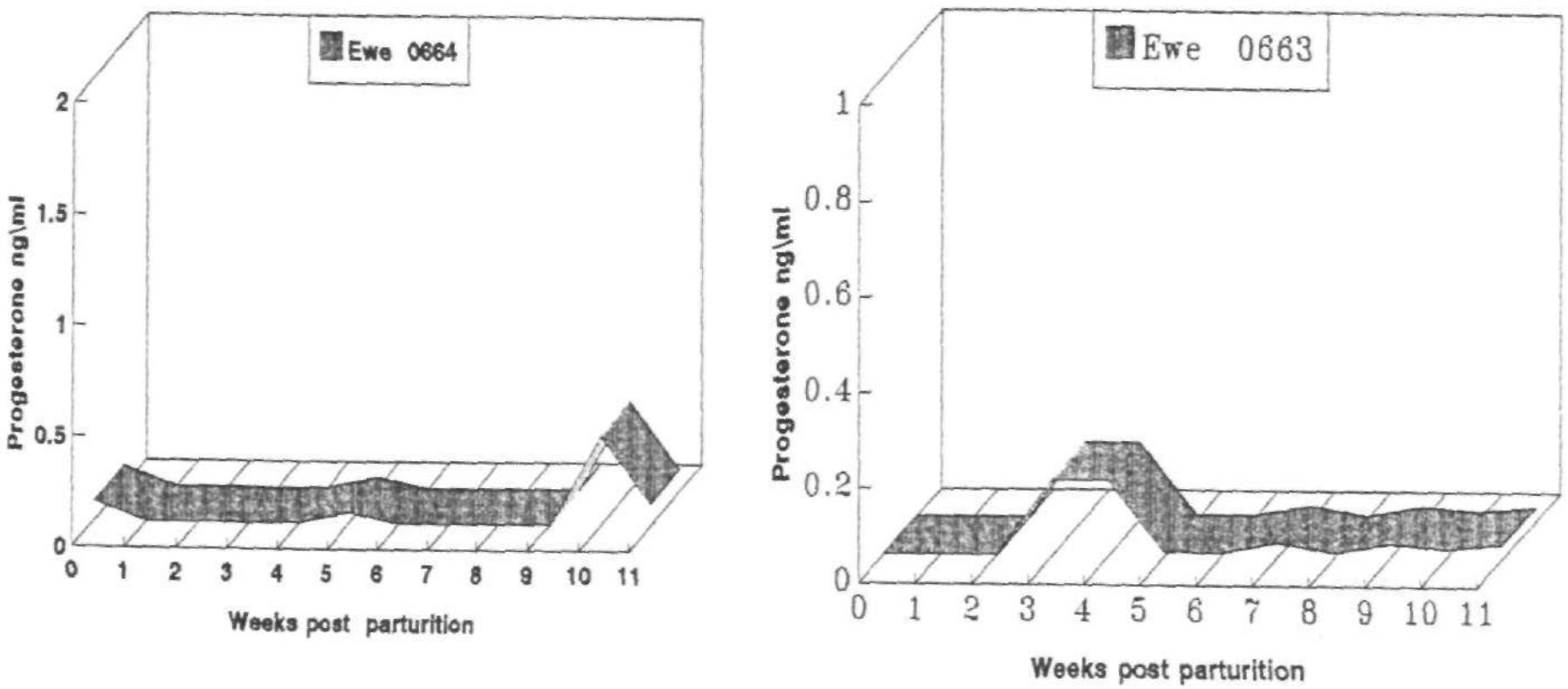


Figure 4.6: Progesterone profiles of ewes (0664 and 0663) that did not resume ovarian activity and did not lamb again till the end of the experimental period.



PLATE I The pregnant Yankasa flock carrying untreated natural Trichostrongyle infections

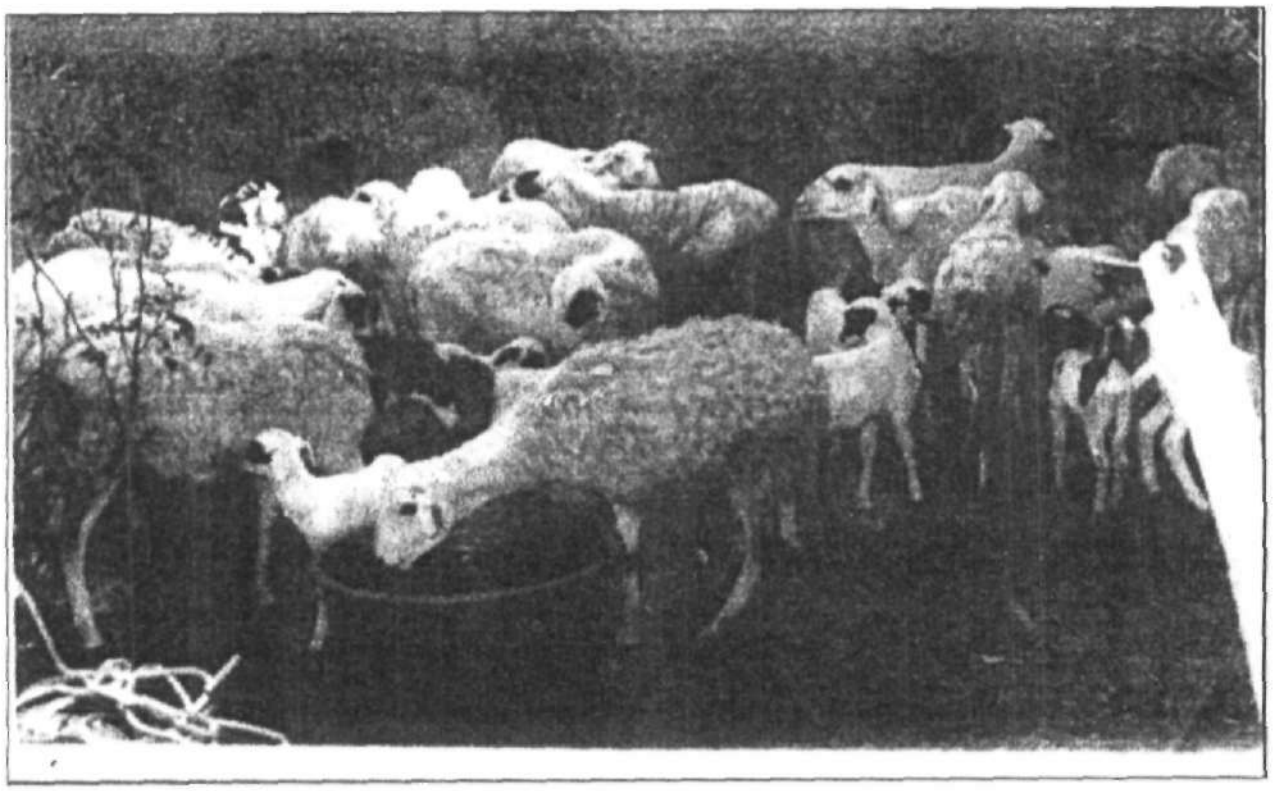


PLATE II The nursing flock of Yankasa ewes with their lambs, showing rough hair coat and diarrhoea in some animals

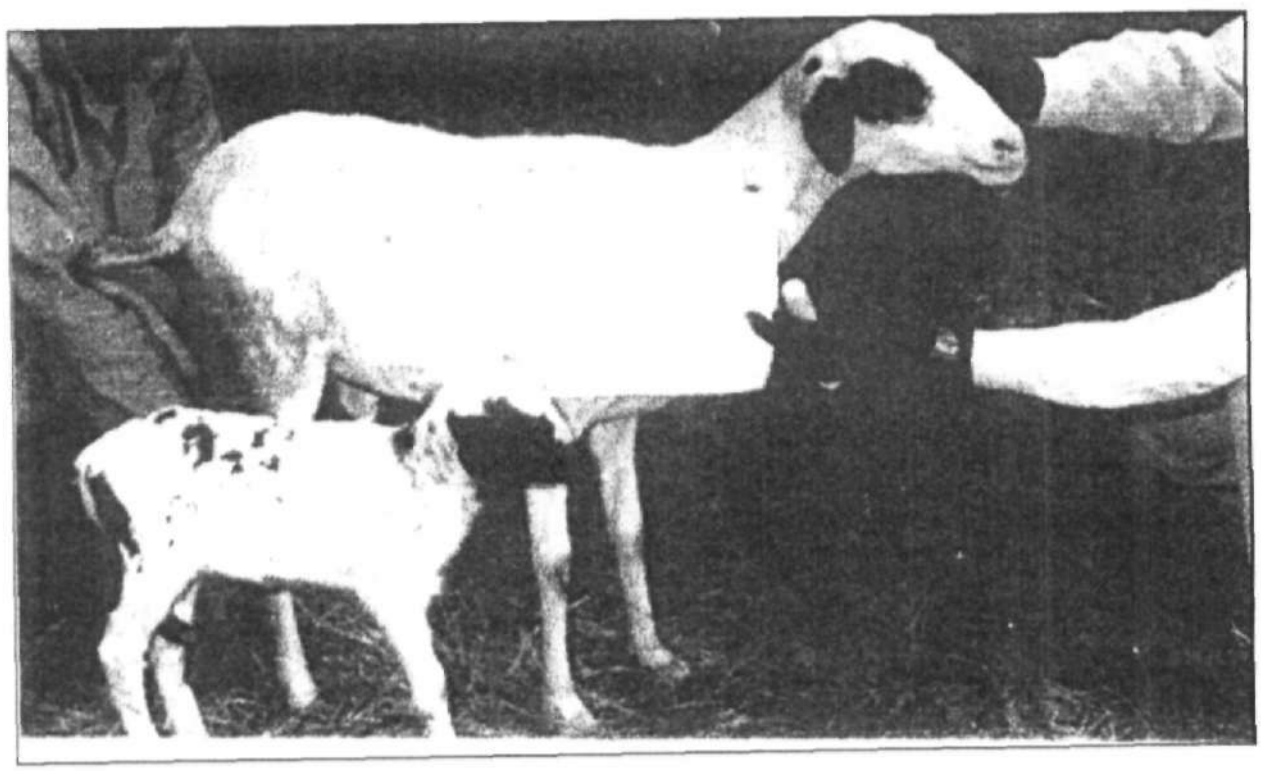


PLATE III Ewe number 0674 with its lamb, ewe had helminth egg counts of 9,820 per gram of faeces, was anaemic and emaciated

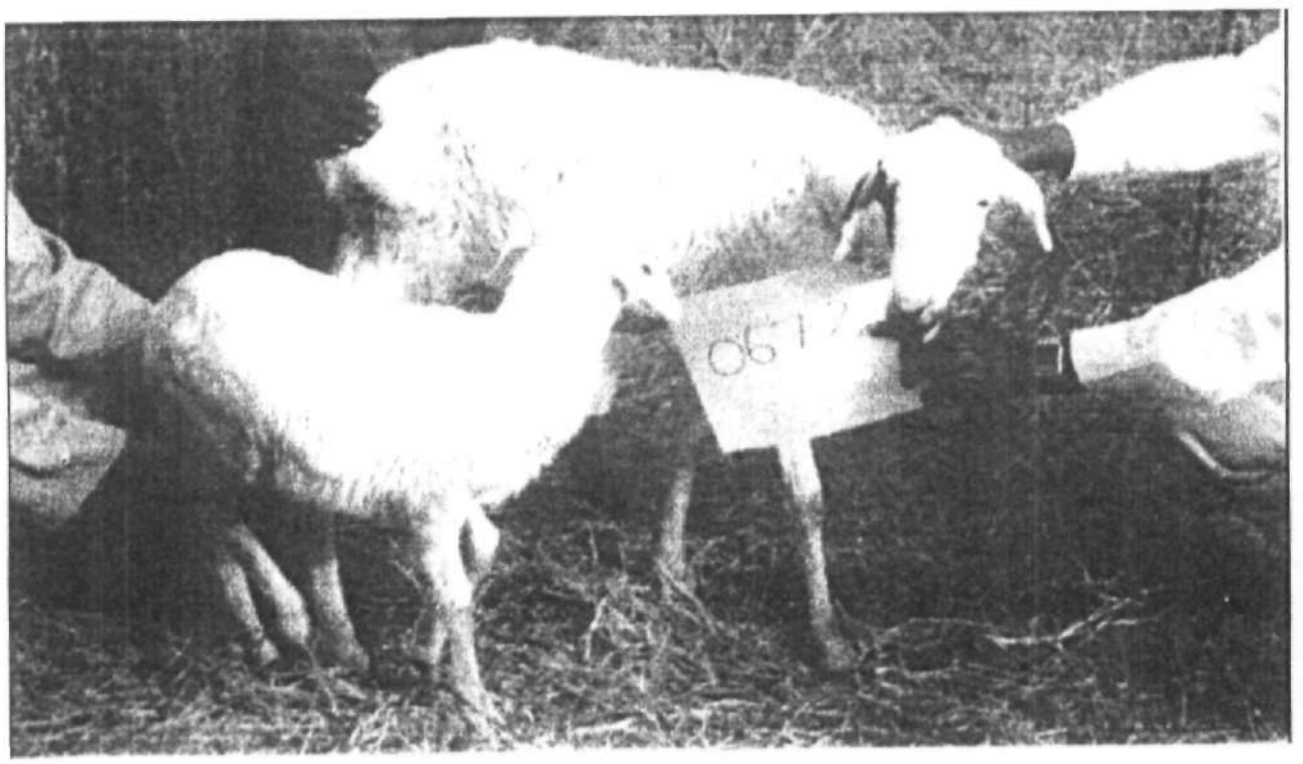


PLATE IV Ewe number 0672 with its lamb, ewe had helminth egg counts of 11,960 per gram of faeces, with rough, dull hair coat and in poor body condition

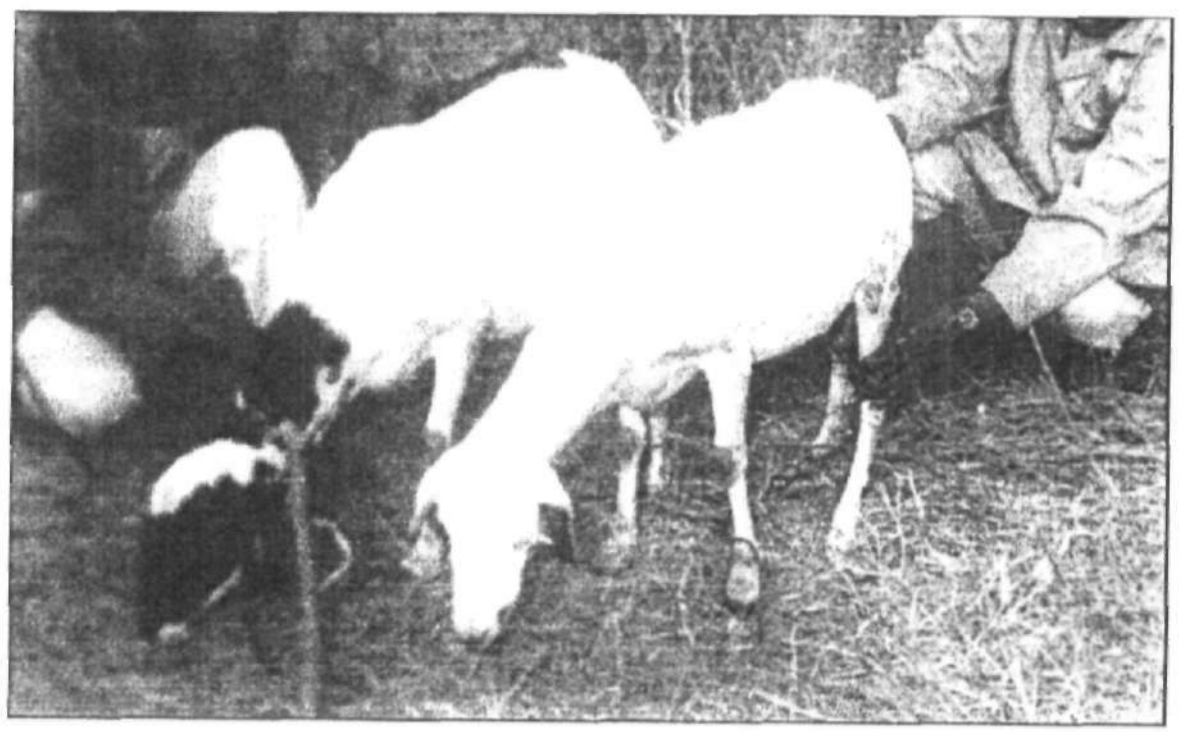


PLATE V Pregnant Yankasa ewes each with low helminth egg counts (< 500epg), having smooth hair coat and in good body condition

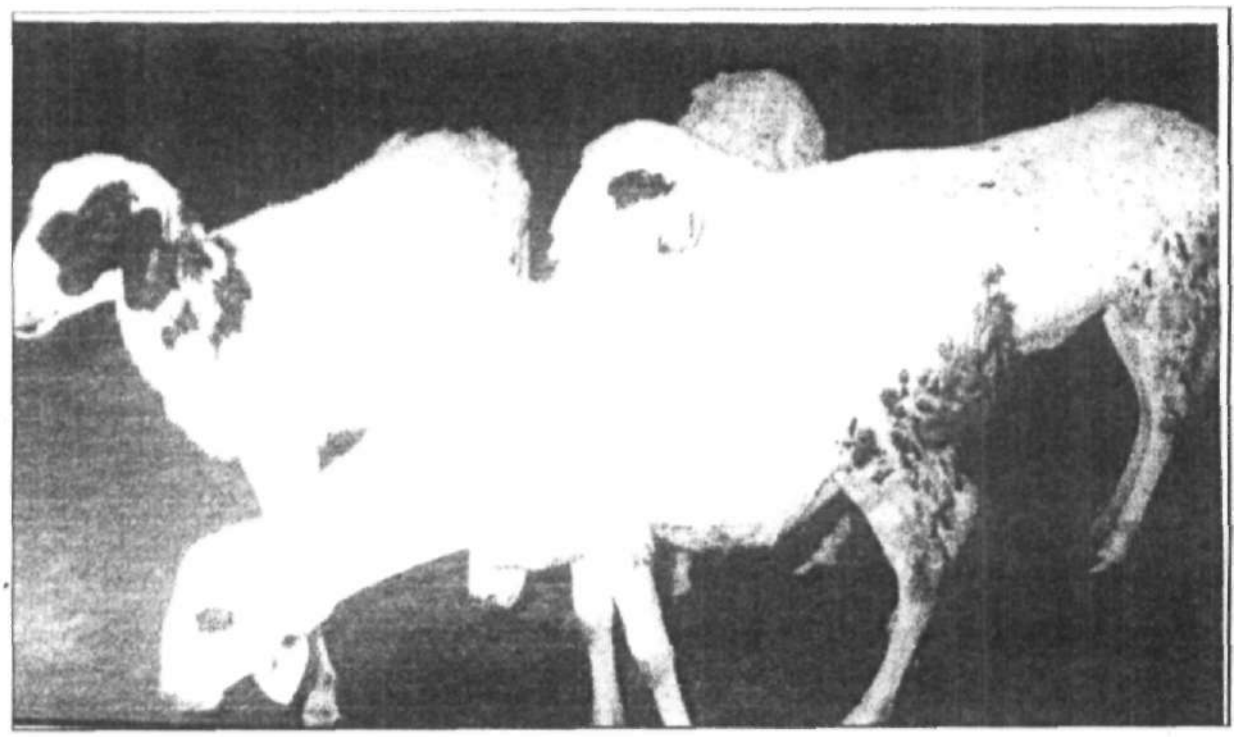


PLATE VI Pregnant Yankasa ewes each with helminth egg counts above 2000 epg looking dull but in fair body condition

4.5 Discussion

These results confirm that Yankasa like other tropical sheep breeds exhibit ovarian activity throughout the year (Devendra and Burns, 1983). Litter size, lamb birthweights as well as twining rates were much lower than what has been reported for this breed under similar management conditions . Lambing was distributed fairly evenly in the different seasons except for the late wet season when only 13% of the lambings took place. To lamb in the late wet season (July- September) animals must conceive in March - May which is the late dry season, a period associated with scarcity and low quality of forage (Amoah and Gelaye, 1990) and this, coupled with worm burdens may have been the cause of poor conception rates at this period. Quinivian (cited by Holmden *et al.*, 1973) in a study of ovulation rates in ewes given a pre-mating anthelmintic treatment, reported that ovulation rate appeared to increase more rapidly in treated ewes compared with untreated ones and attributed the increase to gains in body weight. Lewis (1975) also demonstrated a positive fertility response correlated with live-weight increase in ewes with egg counts above 100epg, when they were treated with anthelmintics.

Lambing interval defined as the period between 2 consecutive lambings is composed of the service period (period from lambing to conception) and the gestation period. The overall mean parturition interval of 241 days with a range of 182-418 showed that parturition intervals were short in a small number of animals but extended in some ewes. This means that under an accelerated twice-yearly lambing programme for improved sheep production, only the 17% of ewes with low worm burdens and with parturition intervals of 200 days or less qualify for

selection . The fact that a number of ewes became pregnant after a short number of days supports an earlier report by Osinowo and Ekpe (1985) that lactation is of only limited significance as a delaying factor in the rebreeding intervals in Yankasa sheep. Suckling/weaning did not seem to have any relevance on resumption of ovarian activity as even ewes which lost their lambs at birth exhibited long post partum anoestrus. P_4 profiles confirmed that long anoestrus periods and conception intervals in a number of ewes were due to late resumption of ovarian activity. However, the fact that some ewes in this experiment lambed two or three times does not seem to show any effect of worm burdens on conception or establishment of the foetus as reported by Hope-Cowdery (1976) and Hayat *et al.*, (1983). About 8% of ewes in this experiment did not lamb at all up to the end of this study but this could not be associated with egg counts that were significantly higher than that of other ewes.

However, it is possible that heavy parasitic burdens with concomitant inappetance, poor feed utilisation and anaemia may reduce the body condition of breeding ewes, reduce birth weights of lambs, and affect reproductive rate in a manner similar to undernutrition. Because the effects of parasitism and undernutrition are expressed in the same terms, it is often difficult to separate them (Sykes, 1978). This may lead to an underestimation of the effects of parasitism particularly in more resistant older animals where reduced growth rate may not be apparent. Deas et al., (1979) advocated that prevention and treatment of anoestrus in ewes basically depended on maintaining weight with an adequate diet and eliminating disease which causes loss of weight. They further elucidated that the

effect of undernutrition on the ovaries is not direct but mediated via the pituitary; *the response of the ovaries being consonant with their limited ability to respond to* reduction in normal stimuli. Pell and Bergman (1983) had earlier observed that glucose is an essential requirement for sheep brain and in the rat it has been implicated as an essential nutrient for Gn-RH - induced release and oocyte maturation (Sen *et al.*, 1979; Tsafrifri *et al.*, 1976). In ewes, cessation of oestrus has been known to occur as a result of a negative energy balance resulting from decreased energy intake (Allden, 1970; Lammond *et al*, 1972). Barger (1982) speculated that the effects of nematode parasitism on reproductive characters were probably manifestations of reduced liveweights. Alterations in nutritional status, body condition, liveweight and stress arising from infections are known to be important factors for endocrine dysfunction with respect to reproduction (Jones and Gillian, 1988) The activity of the hypothalamic- pituitary axis (HPA) is sensitive to stimuli such as trauma, fever hypoglycemia and other stress situations and reacts by increased production of glucocorticoids. Increased glucocorticoids initiate shifts in carbohydrate metabolism, increasing circulating energy substrates at the cost of energy stores. In the short term, glucocorticoids also limit immune and inflammatory reactions to infection. This seems to tie up with Osburn and Kennedy's (1981) observation that emaciation and the consequent hypoglycemia affects ovulation rate by affecting energy needed for the release of gonadotrophic-induced LH release.

Apart from having a low reproductive rate, heavily infected ewes are also more likely to neglect their lambs which would then die of hyponutrition. This may

account for the high mortality of lambs in the first week of life, observed in this experiment. Thomson and Thomson (1949), observed that malnourished ewes showed evidence of weakness after parturition, had little milk and that their maternal instinct was frequently impaired or absent . The findings of Thomas and Ali (1983) seem to support this theory: They observed significant losses in body weight and a 25% reduction in milk production in H. contortus-infected ewes during the periparturient period. Apart from such infected ewes rejecting their lambs, they also observed that surviving lambs from the ewes were infected early in life as they were forced to supplement their milk at an early age with contaminated herbage.

The abortion/stillbirth rates recorded in this experiment represent only foetuses seen inside the sheep pens. The possibility of foetal re-absorption or of aborted foetuses being missed cannot be ruled out. This is supported by the fact that P_4 profiles of some animals were consecutively high, reaching up to 7.3 ng/ml for several weeks only to come down abruptly without any recorded parturition or abortion. In the case of a hypophagic parasite like Haemonchus, severe maternal anaemia can cause foetal hypoxia which may precipitate abortion or foetal death.

Although there is no direct physiological explanation, this study as well as the studies of Lewis (1975), Oakley *et al.*, (1979), Mackey (1980) support the growing body of evidence that adequate parasite control may improve reproductive efficiency of sheep in tropical environments.

CHAPTER FIVE

EFFECTS OF WEANING ON THE PERIPARTURIENT RISE IN HELMINTH EGG COUNTS

5.1 Summary

For studies on the effect of weaning on the occurrence of a periparturient rise in helminth egg counts, three groups of 10 ewes each had their lambs weaned at week 0, week 1 and week 4 of lambing respectively. Ewes whose lambs were removed at birth (week 0), had significantly less ($P<0.05$) helminth eggs per gram of faeces than ewes whose lambs were weaned at one and 4 weeks after lambing. Mean helminth egg counts for the respective groups being 592 $\pm$127, 2278 $\pm$ 415 and 1318 $\pm$ 293.

Endocrine changes that take place in the last weeks of gestation that culminate in parturition and lactation could be responsible for diminished maternal immune responses that allows the periparturient rise in helminth egg counts to occur.

5.2.1 Introduction

Among the factors that can influence the general level of parasitism within a flock are season of the year, stocking density and time of parturition. It appears that hormonal changes associated with late pregnancy and lactation suppress immunity, allowing an abrupt increase in faecal egg output (Connan, 1968). Due to this temporary loss of immunity, all parasitological manifestations of acquired immunity (resistance to establishment, arrested development, reduced worm

fecundity, expulsion of established worms) appear to be compromised (Connan 1968; O'Sullivan and Donald, 1970; 1973; Michel et al., 1979; Donald et al., 1982) Increased susceptibility at the periparturient period places the ewe at greater risk of parasitic disease and production loss, while larvae hatched from the numerous ova become an important source of nematode infection when the new lamb crop begins to graze.

The phenomenon is associated with lactation rather than pregnancy although it may begin in late pregnancy. O'Sullivan and Donald, (1973) as well as Connan, (1967) demonstrated that the PPR can be abolished by removal of the suckling young, and this seemed to confirm the hypothesis of Dunsmore (1965); Brunsdon and Vlassof (1971); Fleming and Conrad, (1989); Blitz and Gibbs (1972) as well as Connan (1974), which stated that elevated prolactin levels at lactation was the cause of the PPR. Later studies (Jeffcoate *et al.*, 1990; Coop *et al.*, 1990 and Ader *et al.*, 1990) cast doubts on the primary role of prolactin in the PPR They argued that far from suppressing immune responses, prolactin appears to be an important regulatory hormone that enhances immunity. There is therefore yet no satisfactory explanation for the cause of the PPR and the role of the lactogenic hormone prolactin. As part of the study of the PPR it was considered desirable to investigate the effect of lactation on the occurrence of the PPR by following the course of natural Trichostrongyle infections in ewes from late pregnancy and different stages of lactation.

5.3 Materials and Methods

5.31 Management of animals

The animals were kept under a semi-intensive system of management, involving grazing on improved pastures for 6-8 hours daily, plus a 0.3 kg/day of a 15% crude protein supplement. Water and salt lick were provided *ad lib*. At night the animals were housed in concrete floor pens which were cleaned daily. The animals received routine herd health managemment carer including a monthly dipping against ectoparasites.

5.3.2 Experimental design

30 pregnant ewes which were in the last mmonth of gestation, carrying natural helminth infectiuons were uused for this study. They had been bred to rams of same breed. They were divided into 3 groups of 10 animals each:

Group 1 consisted of ewes whose lambs were removed at birth

Group 2 was made up of ewes whose lambs were weaned one week after birth

Group 3 consisted of eewes whose lambs were weaned after 4 weeks of birth

All lambs weaned prematurely were isolated from their dams and fostered to some other ewes as well as fed goat milk obtained from dairy goats. Faecal egg coounts (FEC) were monitored in animals from 4 weeks before to 8 weeks post lambing.

5.4 Results

Figure 5.1. shows the faecal egg counts (FEC) of the different groups of ewes. All groups showed a definite PPR with the exception of Group 1 animals that did not suckle their lambs at all. This group had mean helminth egg output significantly less ($P<0.05$) than ewes whose lambs were weaned at one week after lambing as well as those whose lambs were weaned four weeks after lambing. Overall mean egg counts for the groups being 492 ± 127, 2278 ± 4155 and 1290 ± 291 respectively . For Group 1 (lambs weaned immediately after lambing), FEC showed increases after lambing but mean egg counts never went beyond 2000epg after lambing. In group 2 (weaned week 1) egg counts increased from a mean value of 480epg to a peak of 5364epg three weeks after lambing. In group 3 (weaned week 4), egg counts increased from a mean of 240 epg before lambing to 3708epg one week after lambing. The rise in egg counts in Groups 3 and 4 animals also decreased with weaning of the lambs.

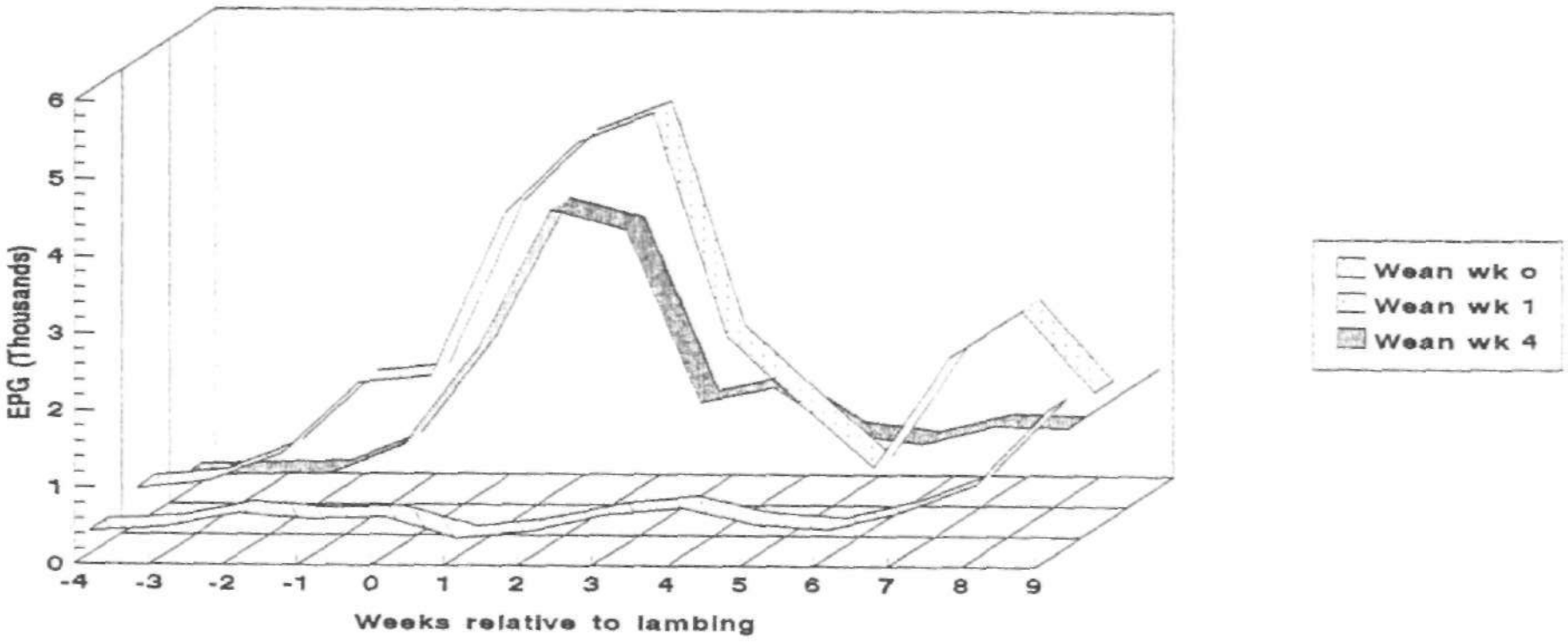


Figure 5.1: Mean helminth egg counts of ewes whose lambs were weaned at various periods relative to lambing

5.5 Discussion

The results of this experiment, particularly the absence of a PPR in ewes which did not suckle their lambs at all indicate that the rise in faecal egg counts are associated with lactation and the rise will occur once lactation is initiated. However, if lactation is not established, the magnitude of the PPR is less. This indicates that the PPR is triggered by lactation but wanes if lactation is not maintained by suckling. This is similar to the earlier findings of Arundel and Ford (1969) and Salisbury and Arundel (1970) who reported an abrupt termination of the post parturient rise following removal of the lambs; although the PPR in this experiment did not terminate abruptly. It however supports the evidence given by Connan, (1967) and Jacobs and Dunn, (1968) which stated that the post parturient rise reaches its full expression only when lactation is allowed to continue for some weeks.

Lactogenesis (initiation of lactation) and galactopoiesis (the maintenance of an established lactation) are 2-phase events which require a combination of hormones which act synergesically. These hormones typically consist of prolactin and/or growth hormone as well as corticosteroids (Mepham, 1976). In lactogenesis, some signal of the impending act of parturition must be conveyed to the mammary gland so that the biochemical apparatus involved in synthesising and secreting milk may rapidly be activated. In sheep, prolactin increases of 5-10 fold have been observed during the week before parturition (Kann and Denamur, 1974) and milk secretion may begin earlier than 4 weeks pre-partum. Galactopoiesis on the other

hand seems to be controlled by signals generated by the off-spring and increased secretion of corticosteroids have been observed at galactogenesis (Mepham, 1976). In essence, prolactin and adrenal corticosteroid levels are very low during pregnancy and rise abruptly just prior to parturition. High concentration of a variety of steroid hormones particularly cortisone and its derivatives not only prevent the development of resistance to helminths, but can also suppress the effects of acquired resistance including a suppression of the inflammatory response and depression of circulating eosinophils and lymphocytes (Robinson and Morris, 1960; Saba., 1965).

The occurrence of the PPR in this experiment parallels the features of the endocrine events at late parturition and lactation. In Yankasa ewes milk yield is known to increase from parturition to reach a peak on the 3rd week of lactation, gradually declining to very low levels at the 12th week of lactation (Afolayan et al., 1991) The transient PPR in ewes which suckled their lambs for as little as 1 week suggests that the initiation of lactation (with subsequent increase in immunosuppressive corticosteroids) is enough to cause a PPR though it may not be maintained as corticosteroid levels start to fall especially after the first two weeks of lactation. Artificial elevation of prolactin levels for example by the injection of diethylstilbestrol resulted in a rise in faecal egg output in sheep. Similarly, repeated injection of acepromazine maleate elevated prolactin levels and faecal egg count in ewes (Connan, 1973).

These reports and the observation of the occurrence of a PPR in ewes whose lambs were not removed at birth, suggest a relationship between the secretion of

hormones of lactation (probably prolactin acting synerstically with coricosteroids) and the changes in helminth worm burdens in animals. Studies of the rat-*Nippostrongylus brasilensis* model in which worm expulsion is suppressed during lactation have demonstrated that antibody production is unimpaired during lactation and that adult worms continue to be damaged by antibody at this time. However, damaged worms are not expelled and it has been suggested that prolactin-mediated suppression may compromise either the specific (antibody and lymphocyte) or the non-specific (the bone marrow derived myleoid elements and biogenic amines), component of the rejection mechanism (Kelly and Dineen, 1973). Connan (1976) suggested that the defect is the failure of sensitised lymphocytes to different to terminal effector cells. Dineen and Kelly (1972) have shown in the rat-*Nippostrongylus* system potentially reactive cells were present in lactating donors and that the immunological defect in lactating rats could be repaired by transfer of synegic cells from immune barren rats. The exact mechanism by which the hormones of lactation temporarily interfere with the immune response to nematodes is not fully understood but is definitely tuned in favour of the parasite.

CHAPTER SIX

THE EFFICACY OF STRATEGICALLY TIMED ANTHELMINTIC TREATMENTS IN ELIMINATION OF THE PERIPARTURIENT RISE IN HELMINTH EGG COUNTS

6.1 . Summary

Following information obtained on the epidemiology of the periparturient rise in helminth egg counts (PPR), treatment schedules for the strategic control of parasites at the periparturient period were evaluated. Four groups of 10 animals each, were treated with levamisole hydrochloride at two weeks before lambing (2WBL); one week before lambing (1WBL); week of lambing (WOL), and one week after lambing (1WAL). The treatments showed that treatment immediately after lambing or not later than one week of lambing significantly eliminated the occurrence of the periparturient rise in egg counts. Anthelmintic treatment immediately after lambing and a repeat treatment about 3-4 weeks after lambing is recommended.

6.2 Introduction

Since all the evidence indicates that the periparturient rise in egg count is brought about by the suppression of immunity, attempts to control by immunisation of the ewe are unlikely to be successful . The logical step is therefore 'to prevent the occurrence of the rise by use of anthelmintics. By treating animals with highly efficient anthelmintics at strategic periods in the epidemiology of the parasite, it should in theory be possible to prevent the periparturient rise in egg counts (PPR).When used prior to lambing, the drug must be safe and larvicidal.

Broad spectrum larvicidal drugs like Thiabendazole, Haloxon and Tetramisole have been used successfully prior to lambing where re-infection has been prevented (Nunns *et al.*, 1965; Helle, 1966; Connan, 1967) but has been unsuccessful under conditions where re-infection of the animals took place (Dunsmore, 1965). Connan (1973) advocated that where re-infection is inevitable, treatment should be delayed as late as possible or repeated at intervals. Craig *et al.*, (1982) reported the value of treatment with anthelmintic at or near the time of lambing.

Levamisole (Imidothazole group of compounds) has good activity against adult, trichostrongylids, developing larval stages and arrested larvae (Herd, 1984) though it is not ovicidal. However, in contrast to the Benzimidazoles, Levamisoles act rapidly as they can cross the nematode cuticle by diffusion rather than entering the worms by ingestion (Briscoe and Coles, 1980). It can also be used in pregnant ewes, with peak blood levels occurring soon after dosing. Withdrawal periods are also comparatively short.

The objective of this study was to determine optimum treatment strategies to be used in breeding ewes to eliminate the PPR, using a safe anthelmintic, under conditions of re-infection of the animals.

6.3 Materials and Methods

6.3.1 Management of animals

Forty pregnant ewes selected from the Experimental Unit flock were used for this experiment. They had been bred by flock mating to rams of same breed that were harnessed with coloured markers The animals had acquired natural helminth infections from grazing contaminated pasture. They were kept under a semi-intensive system of management, involving grazing on improved pastures for 6-8 hours daily, plus a 0.3 kg/day of a 15% crude protein supplement. Water and salt lick were provided *ad lib.* At night the animals were housed in concrete floor pens which were cleaned daily. The animals received routine herd health management care including a monthly dipping against ectoparasites

6.3.2 Experimental design

Each animal was weighed and allotted to one of 4 treatment groups of 10 animals each. A single strategically timed oral treatment with Levamisole hydrochloride (Ciba -Geigy Limited Switzerland) at a dose rate of 7.5 mg per kilogram body weight was administered. Faecal egg counts were determined before treatment and weekly thereafter up to 9 weeks post treatment. The animals in the groups were treated according to the following schedule:

Group 1 Two weeks before lambing (2WBL)

Group 2 One week before lambing (1WBL)

Group 3 Week of lambing (WOL)

Group 4 One week after lambing (1WAL)

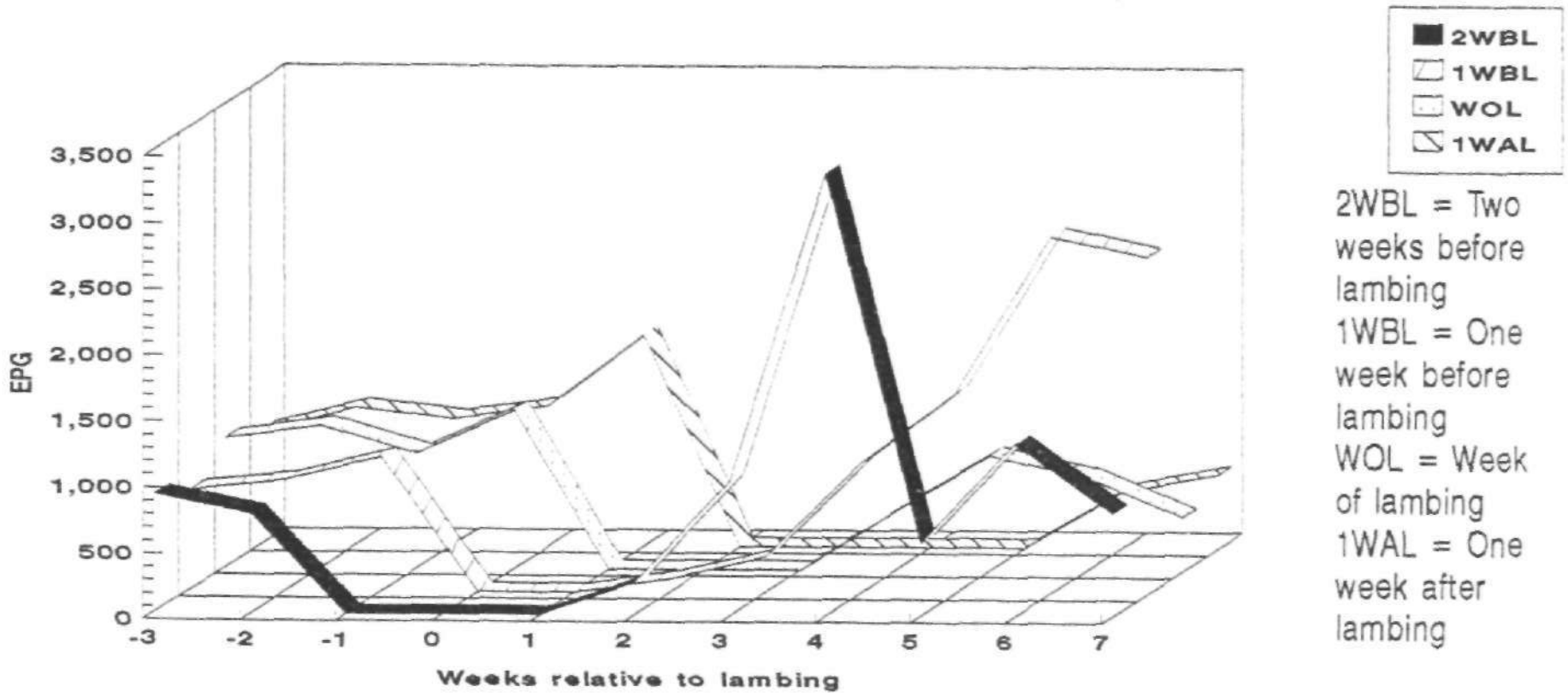
6.4 Results

The mean faecal egg counts of the ewes of the different groups are shown in Figure 6.1. After the treatments, faecal egg counts were negative in all groups for varying periods. Treatment 2 weeks before lambing (2WBL) resulted in egg counts being negative in the ewes only up to one week after lambing, and shifted the occurrence of the peak of the PPR to 4 weeks post lambing during which mean faecal egg counts reached values of 3340 ±215 epg at 4 weeks post lambing.

Anthelmintic treatment one week before lambing (1WBL) suppressed egg counts in the ewes only up to one week after lambing. Thereafter, epg progressively increased reaching a peak of 2700 ± 271 six weeks after lambing.

In the group treated on the week of lambing (WOL) , helminth eggs did not appear in the faeces until 4 weeks after lambing. At no time did egg counts get up to 1000 epg.

In the group treated one week after lambing (1WAL) the PPR was eliminated and egg counts were negative up to six weeks post treatment reaching only 564 ±73 at seven weeks post lambing.



2WBL = Two weeks before lambing
1WBL = One week before lambing
WOL = Week of lambing
1WAL = One week after lambing

Figure 6.1: Mean helminth egg counts of ewes treated with anthelmintic at various periods relative to lambing

6.5 Discussion

Anthelmintic treatment of ewes with Levamisole hydrochloride at about the time of lambing suppressed the occurrence of the PPR, except in the case of treatment two weeks before lambing where a significant PPR still occurred 5 weeks post treatment This was probably because treating two weeks before lambing eliminated the adult worm population but gave sufficient interval for the adult worm population to be replaced from the pool of inhibited late 4th stage larvae which must have been present in the ewes even after treatment. These new adults barely require 10-11 days to complete their life cycles and would produce maximum egg outputs at about 5 weeks post treatment. Helminth control in animals continuously grazing contaminated pasture is more difficult than when animals are treated and moved unto clean pasture. When left on the same pasture after treatment, the ewes become re-infected rapidly and the effect of dosing on the PPR is only transitory. Furthermore, the lambs born to the ewes become infected and start to pass eggs at an early age and thus also contribute to pasture contamination at the same time as the ewes. Chiejina, (1987) observed that anthelmintics were less effective when used as the only method of prophylaxis of parasitic gastroenteritis under conditions of high pasture infectivity and rapid re-infection such as exists in the rainy season in the savannah zones. Dunsmore (1965) advocated that where re-infection is inevitable, treatment should be delayed or the treatment repeated soon after lambing. He also noted that two treatments with phenothiazine during late pregnancy could not prevent the parturient egg count

rise while thiabendazole considerably reduced the magnitude of the rise but did not eliminate it.

The treatment schedules on the week of lambing and 1 week after lambing appeared to be the most successful in suppressing the PPR . This is in agreement with the work of Reid and Armour (1975) and Craig *et al.*, (1982) who also reported the immediate post-lambing period as the most appropriate time for treatment of breeding ewes. At this time, the ewes are carrying large numbers of adult nematodes and any inhibited worms are coming out of inhibition and therefore susceptible to anthelmintic treatment. Treatment during the immediate post lambing period would also eliminate speculation where breeding dates are not known with certainty. From these results, treatment during this period and a repeat treatment about 3-4 weeks post lambing is recommended. Levamisole appears not to have been very effective in removing these inhibited stages in this experiment. An ideal anthelmintic treatment for grazing animals should however, combine proper pasture management with strategic timing of the treatment based on knowledge of the local epidemiology of the helminths involved in that region.

CHAPTER SEVEN

GENERAL DISCUSSION AND CONCLUSIONS

7.1 General discussion

Reproduction can alter the course of ovine helminthiasis; the suppression of maternal immune responses to helminths in late pregnancy and lactation is manifested as an increase in helminth egg output at this period. Factors which have been incriminated as contributing to maternal immunosuppression during the periparturient period, include the hormones of pregnancy (Szekeres *et al.*,1981), lactational hormones (Dunsmore, 1965; Connan, 1974), as well as the maternal transfer of immunoglobulins via colostrum to the neonate (Brandon *et al.*, 1981). Based on the results of the investigation reported here, increased progesterone levels may not be responsible for immunosuppression in ewes. There was no significant increase in egg counts in mid pregnancy when P_4 levels were at their peak. Moreover, concentration of P_4 is known to decrease sharply at term before parturition (Basset *et a*l., 1969).

Earlier studies were in favour of prolactin as being the suppresser of maternal immune responses but the later studies (Jeffcoate *et al.*, 1990; Ader *et al.*, 1990) cast doubts on the role of prolactin in immunosuppression during the periparturient period. Based on the trend of findings in this work it is postulated along with Connan (1974) that adrenal corticoids may be involved along with prolactin in causing the PPR. During the last weeks of gestation, many other

endocrine changes that culminate in parturition and lactation take place. Increased levels of cortisol, oestrogens and prostaglandins associated with parturition could also be responsible for depressing lymphocyte function (Lloyd *et al.*, 1981, Rothwell *et al.*, 1982). A number of studies on the influence of corticosteroids on infection and immunity have shown that steroid therapy tends to depress the defence mechanism of the animal not only to helminth infections, but also to viruses, bacteria and fungal infections. Studies on the influence of cortisone generally indicate that cortisone-treated animals tend to have more larvae and more adult worms for a longer period of time, failed to develop an immune response, show a slower rise in antibodies, fail to develop resistance to re-infection and show more of an activation of latent infections than non-cortisone treated controls. Jackson *et al.*, (1988) reported that treatment of immune sheep with steroids abrogated mucosal mastocytosis and the concomitant loss of resistance to larval challenge. It was postulated that survival of sheep mucosal mast cells was highly T-cell-dependent. Mayrhofer (1979) postulated that the effect of steroids may be to wipe out sub-populations of small lymphocytes which produce lymphokines. Since lymphokines support mast cell growth, hyperplasia of mucosal mast cells is prevented.

The increased susceptibility to infection has practical implications for productivity in sheep, especially when it is considered that in breeding flocks the ewe spends a quarter of each reproducing year in this state of reduced responsiveness. Increased susceptibility at late gestation as seen in this study could be sufficient to result in clinical or subclinical disease. Field *et al.*, (1960), as well

as Gibbs (1964) reported acute and fatal haemonchosis in ewes that were lactating. Even sub-clinical infections may have economic effects on production (Tekeleye, 1992). Thomas and Ali (1983) observed that significant losses in body weight and milk production occurred in ewes infected with H. contortus over the periparturient period. The majority of weight loss occurred during lactation and was associated with a periparturient rise in egg counts. In addition, there was a 25% overall lowering of milk production in the infected ewes. Similar findings have been reported by Leyva *et al.*, (1982) in O. ostertagi infections. Infections reduced feed intake by 16% during lactation while milk production was decreased during the 4-6 weeks of lactation.

Reduction in milk production has important implications for the survival of the lambs, which if they survive will be forced to supplement their milk intake at an early age with contaminated herbage. The lamb crop is of course the pivot on which the profitability of the sheep industry revolves (Agei, 1993) and any condition or disease that would affect the lamb crop must be eliminated. The infection of lambs as early as four weeks of age in the rainy season as well as their poor growth rates reported in this experiment supports the carry -over effect of infection of the dam to her lamb. Losses of up to 40% in lambs up to 12 months old due to internal parasites have been reported (De Leauw 1972; Reynolds, 1991). Surviving animals had reduced daily weight gains and reproductive rates. In a separate study infected lambs treated with anthelmintics had weight gains which were superior to those given supplementation alone. The treatment also had a persistent effect on growth rates that neither nutrition, genotype nor season had.

The finding in this study that Yankasa sheep breed throughout the year simply supports the report of other studies in African breeds (Hunter, 1968). This removes one obstacle to increased productivity encountered in breeds with short breeding seasons (Hunter, 1968), provided no other factor is limiting. High levels of helminth infection observed in this study in ewes with long lambing intervals may represent more than a casual relationship that may require further investigation. Most of the evidence for reduced fertility response of infected ewes have been obtained indirectly using anthelmintic treatments. Holmden (1973) reported that pre-mating anthelmintic treatment of breeding ewes seemed to increase ovulation and body weight in treated ewes. Similarly, Lewis (1975) observed liveweight gains which were positively correlated with fertility response in anthelmintic treated ewes

The mechanism by which gastrointestinal parasitism affects reproduction is not clear but it is postulated that the effect on reproduction is not direct but in a manner analogous to undernutrition. This is sequel to reduced feed intake, inefficient food utilisation and redistribution of endogenous proteins for the purposes of homeostasis and tissue repair in infected animals (Topps, 1977; Anderson and Barger, 1987). This means that in heavy helminth infections, both energy and protein are diverted from vital processes such as meat and milk in order to repair the damaged gut and to maintain the level of protein in the circulating blood. The carcasses of parasitised sheep have been found to contain less protein per unit weight than worm-free controls (Sykes and Coop, 1971). In adult ewes, ovarian function is known to be conditioned by body weight and nutritional plane, heavier ewes having more multiple births; thinner ewes being more frequently barren or do

not conceive at first service (Degs *et al.*, 1979). Therefore infected ewes coming to the end of pregnancy with depressed feed intake, low maternal weights, and anaemia may take longer to return to cyclical ovarian activity post partum. This is thought to be the result of a negative energy balance and hyponutrition and emaciation in the ewe (Allden, 1970; Castillo, 1976). The negative energy balance prevents GnIH release and secretion from the anterior pituitary. GnIH is necessary for follicle development and ovulation. This can be contrasted with what happens with increasing feed intake just before the breeding season (flushing). Flushing appears to raise the level of ovarian function and increase the incidence of multiple births. Flushing is thought to act by decreasing atresia of follicles in late folliculogenesis. The number of follicles recruited for development during an oestrus cycle is greater than the expected ovulation rate (Goodman and Hodgen, 1983). Following recruitment, only a limited number of follicles are again selected for growth and maturation, the others stop their growth and become atretic. Flushing therefore acts by decreasing atresia during the selection phase of follicular development. The effects of gastrointestinal parasites on reproductive characters may therefore be just manifestations of reduced liveweights.

The insidious effect of chronic haemonchosis on body condition of the ewe has important implications for the attainment of maximum productivity in adult sheep. Further studies are required to clarify the aspects of reproduction affected by heavy infections with helminths. In this regard, it is suggested that a minimum body condition score be established for breeding sheep, since loss of body condition is a primary factor for endocrine dysfunction with respect to reproduction. The

increasing tendency towards intensive sheep production systems and use of synchronising agents to achieve twice -yearly lambing apart from being expensive, would give rise to a high failure rate if basic requirements like strategic pre-mating and post lambing deworming to get animals to appropriate body weights and body condition are not done. Heavily infected animals would not only have long lambing intervals but would also have high lamb mortality and these would add to the overall cost of production. To ensure the full expression of the ewe's potential fertility, gastrointestinal helminths must be controlled. Treatment regimes that do little but remove adult worms without controlling pasture contamination are not effective since production losses continue as a result of immediate re-infection in the interval between treatments. Any form of preventive control to avoid the occurrence of disease outbreaks and decreased productivity associated with it must take into account the periparturient rise in egg counts. Control of the periparturient rise in egg counts centres on the use of anthelmintics. Any drug used prior to lambing must be safe and also larvicidal in areas where hypobiosis of larvae plays a role in the epidemiology of parasites in that area. The occurrence of a periparturient increase in egg counts in ewes that lambed in the early dry and late dry seasons indicate that hypobiotic larvae play a role in the periparturient rise.

According to Ogunsusi (1978) under the conditions that exist in the Northern Guinea Savannah, the 6-month long dry season which starts in October ends in April. When the rains stop in October, pasture infectivity starts to decline from November and by January the presence of helminth larvae is nil on pasture due to very low humidity conditioned by lack of rainfall. All grass and shrubs on pasture

dry up and the alternation of the dry atmosphere with cold harmattan at this period, makes it impossible for helminth larvae to survive on pasture. Any increased infection at this period must have come from the maturation of larvae arrested previously in the host. Brunsdon (1964) and Gibbs (1967) reported that the timing of the periparturient rise in egg counts was more synchronous than would be the case if the rise was related only to lambing. Gibbs (1967) suggested that two factors may be of importance in initiating the periparturient rise in egg counts. The first he said, is a hypothalamic-pituitary mediated stimulus on the quiescent larvae of all sheep dependent upon the time of the year but independent of sex. The second is the stimulus due to hormonal changes associated with parturition and lactation. If this two-phase theory is correct, it would appear that ewes lambing in the wet season lambed when both the hypothalamic-mediated as well as the hormonal -mediated stimulus on hypobiotic larvae were operating and this contributed to a maximum expression of the periparturient rise in egg counts.

Treatment with a larvicidal drug will prevent the maturation of the hypobiotic larvae to adults to participate in the periparturient rise. In this regard, a pre-mating anthelmintic treatment is recommended just before the time of flushing of animals. This treatment will ensure that ewes obtain maximum benefit from flushing. Animals that are pregnant in the early to late wet season may also benefit from a mid-pregnancy treatment. as the season of lambing has an effect on the magnitude of the periparturient rise in egg counts, and upon the degree of pasture contamination to which lambs are subjected, the adjustment of breeding dates could

be valuable adjunct to a control programme aimed at minimising the periparturient rise in helminth egg counts.

7.2 Conclusions

These findings emphasise the major contribution of the breeding ewe as the source of helminth infection for lambs and other animals by pasture contamination during the periparturient rise in egg counts. The Trichostrongyle nematodes showed a seasonal pattern with pathogenic levels of infection occurring during the mid rainy season. Severe lamb mortality, clinical disease and production losses in the form of stunted growth, loss of weight, anaemia and long parturition intervals are some of the problems that are encountered in breeding sheep if nematode parasitism is not adequately controlled. Low risk of infection is maintained if the ewes are treated with highly efficacious anthelmintics within 0-2 weeks of lambing. Any form of preventive control to avoid the occurrence of disease outbreaks and decreased productivity associated with it must take into account the periparturient rise in egg counts.

In this regard, a pre-mating anthelmintic treatment is recommended just before the time of flushing of breeding animals. This treatment will ensure that ewes obtain maximum benefit from flushing. Animals that are pregnant in the wet season may also benefit from a mid-pregnancy treatment. As the season of lambing has an effect on the magnitude of the periparturient rise in egg counts, and upon the degree of pasture contamination to which lambs are subjected, the adjustment of

breeding dates could be a valuable adjunct to control programmes aimed at minimising the periparturient rise in helminth egg counts.

The young sheep and the breeding ewe particularly when pregnant and lactating are generally most susceptible to helminthosis. An effective parasite control programme should therefore be an important component of any successful sheep breeding programme. Treatment with anthelmintics to remove nematodes before mating is recommended, to improve ewe fertility. Monitoring of faecal nematode egg counts from breeding flocks is advisable, and if the mean egg counts of 5-10% of the flock is above 500 eggs per gram, it is recommended that the whole flock should be dewormed even if they do not show signs of helminthosis. It is also recommended that unweaned lambs be included in deworming programmes especially during the rainy season. Ewes and their lambs should be kept penned for up to 6 weeks post lambing before being allowed to graze since the ewe in early lactation appears more susceptible to helminth infections.

For further research, it is recommended that predictive markers of resistance to helminths be looked into in Yankasa sheep. This should go along with the study of the immune response of lambs of various age groups to endoparasites . This would be with a view of developing vaccination strategies and also breed consciously for resistance and incorporate selection for resistance to endoparasites into the genetic improvement of indigenous sheep in Nigeria.

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Appendix 1.A: Analysis of variance computations for weekly helminth egg counts of parturient and non-parturient ewes from four weeks before to six weeks after lambing

Source of Variation	DF	SS	MS	F
Between Groups	1	167.51	15.23	3.72*
Error	228	9322.28	4.09	
Total	229	1099.79		

Appendix 1.B: Mean weekly helminth egg counts(±sem) of parturient and non-parturient ewes from four weeks before to six weeks after lambing

Reproductive Status	Weeks of Lambing										
	-4	-3	-2	-1	0	1	2	3	4	5	6
Pregnant	19.4± 11.1	81.7± 41.1	31.1± 16.9	110.6±[a] 31.4	123.9±[a] 42.4	216.0± 96.8	201.1±[a] 56.6	212.1± 68.1	357.1±[a] 94.3	199.0±[a] 125.0	158.9± 83.8
Non-Pregnant	14.0± 12.1	10.1± 10.0	8.0± 8.0	44.0±[b] 26.9	49.0±[b] 28.7	46.0± 23.2	61.0±[b] 36.6	192.0± 141.0	190.0±[b] 162.1	112.0±[b] 62.1	142.0± 67.2

* significant at .05 level of probability

Appendix 2A: Analysis of variance data for mean helminth egg-count values of ewes from two weeks before, to six weeks after lambing

Source of Variation	DF	SS	MS	F
Between Weeks	7	166.71	23.82	21.02*
Error	272	308.24	1.13	
Total	279	474.95		

Appendix 2B: Mean helminth egg counts, Packed cell volume, Haemoglobin and Total Protein values (±sem) of ewes from two weeks before, to six weeks after lambing

	Weeks of Lambing								
Parameter	-2	-1	0	1	2	3	4	5	6
EPG	187± 124	149± 44	686±255	1086± 262	1589± 429	1021± 270	641± 142	403± 142	40.2± 180
PVC	24.3± 0.44	22.5±0.65	22.9± 0.45	24.1± 0.32	24.4± 0.31	21.4± 0.33	19.6± 0.42	22.9± 0.44	23.5± 0.17
HB	6.4± 0.18	6.4± 0.11	6.6± 0.10	6.7± 0.12	7.6± 0.14	7.8± 0.13	6.0± 0.17	7.8± 0.17	7.9± 011
TP	7.7± 0.31	8.2± 0.49	7.2± 0.28	8.1± 0.16	7.9± 0.40	7.6± 0.28	7.9± 0.28	8.2± 0.59	8.0± 0.13

* Significant at 0.05

Appendix 3A: Analysis of variance data for **Mean weekly helminth egg counts of ewes lambing at various seasons of the year**

Source of Variation	DF	SS	MS	F
Between Groups	3	183.7	61.2	19.3*
Error	47	149	3.2	
Total	50			

Appendix 3B: Mean weekly helminth egg counts (±sem) of ewes lambing at various seasons of the year

	Weeks of Lambing									
	-4	-3	-2	-1	0	1	2	3	4	5
Season										
Early dry	875±252^{a}	1325±437^{a}	1847±613^{a}	2860±834^{a}	2666±718^{a}	1531±225^{a}	1138±155^{a}	1065±157^{a}	957±128	708±129
Early wet	417±172^{b}	359±101^{b}	494±154^{ab}	426±123^{c}	489±168^{b}	788±371^{b}	979±378^{b}	1266±429^{b}	3600±1515	3838±1598
Late dry	134±30^{b}	96±23^{b}	218±64^{b}	306±101^{c}	405±139^{b}	736±275^{b}	662±198^{ab}	748±342^{b}	956±372	1800±765
Late wet	186±116^{a}	279±135^{ab}	266±124^{a}	868±501^{b}	507±224^{ab}	708±263^{ab}	1144±396^{ab}	2431±1081	4201±1813	2572±900

* Significant at 0.05

abc Means with different superscript (s) within each column differ ($P < 0.05$)

Appendix 4A.: Analysis of variance data for mean weekly **Packed** cell volume values post lambing, for ewes that lambed in different seasons of the year

Source of Variation	DF	SS	MS	F
Between Groups	3	211.1	70.4	3.04
Error	43	994.9	23.1	
Total	46	1206.0		

Appendix 4B: Mean weekly Packed cell volume (±sem) values post lambing, for ewes that lambed in different seasons of the year

	Weeks afer Lambing									
	0	1	2	3	4	5	6	7	8	9	10
Season											
Early dry	27.9±0.7^{a}	27.4±1.0	25.8±1.1^{a}	26.0±1.7	26.6±1.5^{a}	25.3±1.3	28.3±1.3^{a}	28.1±1.5^{a}	25.9±1.0	26.2±0.6^{a}	26.7±0.9
Early wet	29.1±1.6^{a}	26.1±1.2	26.2±1.1^{a}	25.2±0.9	24.4±0.9^{b}	23.9±0.8	25.4±0.8^{b}	25.4±0.6	24.3±0.7	23.3±0.6^{ab}	24.0±0.8
Late wet	25.9±1.1^{ab}	27.0±1.1	25.7±0.9^{a}	24.0±1.0	22.9±1.0^{b}	24.3±0.9	24.0±1.2^{b}	25±1.2^{b}	24.1±1.2	23.9±1.1^{ab}	24.6±1.4
Late dry	22.7±1.2^{c}	24.8±1.2	22.8±1.1^{b}	22.5±1.0	21.5±1.2^{b}	23.6±0.9	21.8±1.4^{b}	22±1.7^{b}	23.4±2.3	22.8±2.2^{b}	24.81.6±

^{abc} Means with different superscript(s) within each column differ ($P < 0.05$)

Appendix 5A: Analysis of variance data for mean weekly helminth egg counts for early, mid and late pregnancy of ewes that lambed in the month of June

Source of Variation	DF	SS	MS	F
Between Weeks	23	1060.67	46.12	7.41*
Error	192	1194.23	6.22	
Total	215	2254.89		

Appendix 5B: Mean weekly helminth egg counts (±sem) for early, mid and late pregnancy of ewes that lambed in the month of June

	Weeks of gestation							
Stage of Pregnancy	wk1	wk2	wk3	wk4	wk5	wk6	wk7	wk8
Early	51±32[b]	80±46	96±40[b]	122±58[b]	224±89[b]	404±175[b]	212±101[b]	184±93[b]
Mid	260±132[b]	373±211[b]	340±168[b]	540±318[b]	236±127[b]	236±127[b]	51±32[b]	21±86[b]
Late	318±106[a]	1089±426[a]	1939±641[a]	3551±809[a]	3550±608[a]	10269±3754[a]	10606±4014[a]	8895±2693[a]

* Significant at 0.05

[ab] Means with different superscript(s) within each column differ (P<0.05)

Appendix 6A: Analysis of variance data for mean weekly helminth egg counts for early, mid and late pregnancy, of ewes that lambed in the month of October

Source of Variation	DF	SS	MS	F
Between Weeks	23	501.18	21.79	5. 8*
Error	96	360.78	3.76	
Total	119	861.98		

Appendix 6B: Mean weekly helminth egg counts (±sem) for early, mid and late pregnancy of ewes that lambed in the month of October

	Weeks of gestation							
Stage of Pregnancy	wk1	wk2	wk3	wk4	wk5	wk6	wk7	wk8
Early	28±28	20±20	24±40	16±12	68±49^{b}	136±97^{b}	724±685^{b}	116±57
Mid	60±35	56±51	20±10	40±20	20±15^{b}	20±5^{b}	40±28^{b}	72±33
Late	90±55	124±69	132±56	146±72	360±156^{a}	2144±1231^{a}	844±361^{a}	888±348

* Significant at 0.05

ab Means with different superscript(s) within each column differ ($P<0.05$)

Appendix 7A: Analysis of variance data for mean helminth egg counts of ewes in late pregnancy that lambed in the months of June or October respectively.

Source of Variation	DF	SS	MS	F
Between Groups	1	47.49	47.49	11.29*
Error	12	50.45	4.20	
Total	13	97.94		

Appendix 7B: Mean weekly helminth egg counts (±sem) for late pregnancy of ewes that lambed in the months of June and October respectively

	Weeks of gestation							
Month of Lambing	wk1	wk2	wk3	wk4	wk5	wk6	wk7	wk8
June lambing	318±106[a]	1089±426[a]	1939±641[a]	3551±809[a]	3550±809[a]	10269±3754[a]	10606±4014[a]	8895±2693[a]
Oct lambing	90±55[b]	124±69[b]	132±56[b]	146±72[b]	360±156[b]	2144±1231[b]	844±361[b]	888±348[b]

* Significant at 0.05

ab Means with different superscript(s) within each column differ (P<0.05)

Appendix 8: Mean weekly helminth egg counts (±sem) of 12 week old lambs sampled for a 24 week period

Weeks of sampling	Mean weekly EPG
1	112±39
2	6±6
3	102±37
4	23±5
5	334±295
6	24±11
7	31±20
8	17±11
9	237±231
10	246±165
11	20±114
12	60±35
13	11±9
14	171±101
15	1011±449
16	2249±1481
17	2854±1813
18	1551±893
19	3016±1015
20	746±155
21	709±189
22	403±94
23	217±70
24	112±39

Appendix 9: Progesterone values(ng/mL) of individual ewes used as prototypes of their group from parturition to the next conception.

Ewe Tag No	←Weeks post parturition → 0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21
0651	0.03	0.09	0.09	0.06	0.03	0.9	0.06	1.5	1.5	1.8	1.2	2.8	2.8	3.3								
2347	0.009	0.5	0.25	0.9	0.06	1.5	1.5	1.6	1.5	1.2	1.8	1.9	2.1	3.2	2.9	2.4						
1077	0.06	0.003	0.003	0.006	000	0.8	0.5	0.005	0.9	1.1	0.8	1.1	1.1	0.8	1.2	1.9						
0670	0.13	0.03	0.2	0.6	1.9	1.0	0.9	2.0	1.8	1.7	1.8	1.7	3.2	2.8								
2400	0.09	0.6	0.0	0.1	0.0	0.2	0.0	0.01	0.0	0.5	0.22	0.3	0.0	0.0	0.0	0.4	1.3	0.9	1.2	1.4	1.4	1.5
1078	0.09	0.06	0.03	0.03	0.03	0.03	0.06	0.03	0.03	0.03	0.03	0.4	0.3	1.1	0.1	1.4	0.06	1.9	2.2	2.1		
2333	0.07	0.17	0.13	0.2	0.1	0.1	0.1	0.35	0.13	0.06	1.5	1.6	1.3	1.6	2.4							
1067	0.05	0.06	0.0	0.03	0.9	0.03	0.8	1.1	1.3	1.4	2.9	1.9										
0663	0.003	0.003	0.003	0.16	0.16	0.01	0.009															
0664	0.09	0.003	0.003	0.0	0.003	0.05	0.0	0.0	0.0	0.0	0.4	0.1										
2396	0.03	0.99	0.0	0.7	0.09	0.0	0.4	0.0	0.13	1.5	1.2	1.9	2.0	1.3	2.6	4.8	4.9	5.9				
2342	0.1	0.1	0.0	0.3	0.1	0.9	1.9	1.6	1.7	3.4	2.1	2.8	1.9	3.7	7.2	6.8						

Appendix 10: Actual lambing intervals and mean helminth egg counts (EPG) of some of the ewes (n = 45) during the experimental period.

Ewe Number	Lambing Interval (Days)	Mean Helminth Egg Count (EPG)
2347	181	1460
0687	182	2500
0687	187	1500
1067	195	1720
2347	195	2060
1010	198	2860
1075	199	2300
2377	199	1660
1082	206	11640
0683	208	6840
1082	209	5264
127	210	20220
0648	210	7120
1012	214	23220
0641	216	4020
1081	219	16900
1069	220	15240
0642	227	12440
1012	227	8460
1077	229	5800
1062	230	3140
1063	230	21980
0656	231	11680
1075	234	20300
1077	240	5800
1062	241	3140
1067	244	1720
2395	246	4000
0651	247	23760
1087	248	8920
0657	251	7200
0683	252	6840
0664	254	7720
0674	255	8980
1087	256	8920
0670	258	35760
1063	270	21980
1069	272	15240
2579	277	1160
1078	277	5820
2400	287	10280

Appendix 11A: Analysis of variance data for mean helminth egg counts of ewes whose lambs were weaned at various weeks relative to lambing

Source of variation	DF	SS	MS	F
Groups	2	2002096	10011048	7.90*
Error	39	494120008	1266975	
Total	41	694334104		

Appendix 11B: Mean helminth egg counts of ewes (±sem) whose lambs were weaned at various weeks relative to lambing

	Helminth egg counts per gram		
	GROUP		
Weeks relative to lambing	Weaned week 0	Weaned week 1	Weaned week 4
-4	318±23	480±40	240±46
-3	373±38	580±21	272±31
-2	561±54	960±83	286±47
-1	482±37	1857±56	670±64
0	510±77	1954±134	1916±148
1	238±75	4055±690	3708±354
2	340±21	4982±623	3458±421
3	548±17	5364±340	1218±195
4	644±7	2474±259	1418±83
5	426±51	1610±160	817±211
6	362±17	784±210	693±103
7	596±131	2200±140	925±96
8	962±76	2820±220	904±109
9	1932±114	1774±179	1533±122
Mean	592±144[b]	2278±415[a]	1318±293[b]

* Significant at 0.05

[ab] Means with different superscripts differ (P<0.05)

Appendix 12: Mean helminth egg counts (±sem) of ewes treated at various periods realtive to lambing

Group	Weeks relative to lambing										
	-3	-2	-1	0	1	2	3	4	5	6	7
TWBL	900±82	760±62	0	0	0	248±37	1020±108	3340±	1557±213	1306±121	788±95
OWBL	760±80	840±94	1020±270	0	0	102±23	270±60	982±82	1518±127	2700±271	2560±222
ZWOL	980±120	1080±200	871±80	1200±140	0	0	0	449±104	871±67	705±51	413±82
OWAL	860±120	1040±85	960±68	1060±160	1600±180	0	0	0	0	426±85	564±73

TWBL = Two Weeks Before Lambing
OWBL = One Week Before Lambing
ZWOL = Zero Week of Lambing
OWAL = One Week after Lambing