Grazing Behaviour, Inappetence and Production Losses in Cattle with Sub-clinical Parasitic Gastroenteritis

Proefschrift ter verkrijging van de graad van doctor in de diergeneeskundige wetenschappen aan de faculteit diergeneeskunde Universiteit Gent, 2008

door Andrew Forbes

Promotor
Prof. dr J.Vercruysse

Vakgroep Virologie, Parasitologie en Immunologie
Faculteit Diergeneeskunde, Universiteit Gent
Salisburylaan 133, B-9820 Merelbeke

Acknowledgements

massive contribution to the work that comprises the bulk of this thesis: it would simply have been impossible without them and their knowledge, diligence and patience. Malcolm has further provided, unstintingly, valuable guidance and assistance during the preparation of this thesis as one of my supervisors.

The outcome of this work could have remained as a number of individual papers in the scientific literature, but, because of the unusual, if not unique, combination of elements of parasitology, agronomy, nutrition, grazing behaviour, animal performance and pathophysiology, a summation of the different studies was contemplated. It was about this time that another conversation took place, again somewhat serendipitously, with Professor Jozef Vercruysse, who was talking about another (mature!) colleague who had consolidated a number of studies into a thesis at the University of Ghent. A debt of gratitude is therefore owed to the staff and administration of the University of Ghent for accommodating such approaches to the achievement of a Ph.D. I am naturally delighted that Professor Vercruysse agreed to act as my Promoter for this thesis. His enormous breadth and depth of knowledge within parasitology in general and veterinary parasitology in particular has been invaluable in helping me to produce this thesis. It goes without saying that his legendary enthusiasm, stamina and hospitality have also provided me with the stimuli to keep my studying and writing on schedule.

My third supervisor is Dr Frank Jackson from the Moredun Institute in Edinburgh. Dr Jackson has worked and published extensively and diversely in the field of ovine gastrointestinal parasitism. The aspects of his research that have been particularly valuable to me in writing this thesis have been his work on nutrition-parasite interactions and the effects of parasitism on foraging behaviour in sheep. But more than that, Dr Jackson epitomises the bonhomie and camaraderie that – perhaps surprisingly to those outside this discipline – pervades veterinary parasitology: work can, and should be, fun as well as hard graft. Dr Jackson also brings a slightly tenuous (geographical) link with my *alma mater*, the Royal (Dick) School of Veterinary Studies, Edinburgh University.

Specific thanks are due to my company Merial and various bosses over the years, including Graham Davenport, Mel Brewer, David Biland and Jean-Luc Michel for their forbearance in fostering and supporting my research ideas and initiatives, however unconventional they may have seemed at the time. Without their confidence in my judgement and ability it would have been impossible to carry out the studies

Table of contents

List of abbreviations

AFRC	Agricultural and Food Research Council
AGRP	Agouti-related protein
BCS	Body condition score
BM	Bite mass
BR	Bite rate
C	Carbon
CCK	Cholecystokinin
cm	Centimetre
CNS	Central nervous system
CP	Crude protein
Cr_2O_3	Chromic oxide
CRH	Corticotrophin releasing hormone
CV	Coefficient of Variation
D	Diet digestibility
DCP	Digestible crude protein
DM	Dry Matter
DMI	Dry Matter Intake
ELISA	Enzyme-Linked ImmunoSorbent Assay
EPG/epg	Eggs per gram
ES	Excretory/secretory
FEC/fec	Faecal egg count
FGS	First Grazing Season
FM	Fresh matter
FMIR	Fresh matter intake rate
FO	Faecal output
GE	Gross energy
GH	Growth hormone
GHS	Growth hormone secretagogue
GHS-R	Growth hormone secretagogue receptor
GI	Gastrointestinal
GIN	Gastrointestinal nematodes
GJM	Grazing jaw movements
GLP	Glucagon-like peptide
GMT	Greenwich Mean Time
GRP	Gastrin-releasing peptide
ha	Hectare
HCl	Hydrochloric acid
HFRO	Hill Farming Research Organisation
HI	Herbage intake
IL	Interleukin
IR	Intake rate
IWL	Insensible weight loss
kg	Kilogram
L/l	Litres
L_1 L_2 L_3 L_4 L_5	First, second etc stage nematode larvae
LW	Live weight

M	Month
MAFF	Ministry of Agriculture, Fisheries & Food
mcg	Micrograms
MCH	Melanin concentrating hormone
ME	Metabolizable energy
ME_m	Metabolisable Energy for maintenance
ME_p	Metabolisable Energy for production
mg	milligram
MJ	Megajoules
ml/mL	Millilitre
MP	Metabolizable protein
mRNA	Messenger ribonucleic acid
MSH	Melanocortin stimulating hormone
MTD	Minimal total discomfort
N	Nitrogen
NDF	Neutral detergent fibre
NEFA	Non-esterified Fatty Acid(s)
ng	nanogram
NPY	Neuropeptide Y
NS	Not Significantly different
ODR	Optical Density Ratio
OM	Organic Matter
OMIR	Organic matter intake rate
pg	Picogram
PGE	Parasitic gastroenteritis
pH	Hydrogen ion concentration
pmol	picomole
PYY	Peptide YY
R^2	Regression coefficient
RIA	Radioimmunoassay
s.e.d	Standard Error of the Difference
SCM	Solids-corrected milk
SGS	Second Grazing Season
SSH	Sward surface height
TET	Total eating time
TGJM	Total grazing jaw movements
TGT	Total grazing time
TIT	Total idling time
TMR	Total Mixed Ration
TNF	Tumour necrosis factor
ToD	Time of Day
TRT	Total ruminating time
U	International Units
VFA	Volatile Fatty Acid(s)
W	Weight
$W^{0.75}$	Metabolic Weight

General Introduction: Bovine Parasitic Gastroenteritis and its Impact on Cattle Health and Production

_____

1. Introduction

Parasitic gastroenteritis (PGE) is common, if not ubiquitous, in cattle of all ages and is responsible for both clinical and sub-clinical disease with consequences that include poor welfare, ill health, poor production and financial losses. In order to understand when PGE occurs and why it has such an impact, it is necessary to describe pertinent aspects of its pathophysiology and epidemiology and to consider these in the context of commercial cattle farming. Armed with such knowledge, it should then be possible to develop a rationale for control of infections and to mitigate the consequences in a pragmatic and practical manner.

2. Gastrointestinal nematodes in the bovine

The parasitic nematode fauna of the bovine gastrointestinal (GI) tract comprise some twenty species, with representatives from around seven genera (Table 1.). Generally, species are site specific and are located either in the abomasum, the small or the large intestine. Equally, most species are host-specific and parasitize cattle alone, although cross-infection between sheep and other ruminants can occur, for example with *Nematodirus battus* and *Haemonchus* spp. The most notable exception to this generalisation is *Trichostrongylus axei*, which can parasitize a variety of ungulate hosts, including cattle, sheep, pigs and horses. Bovine gastrointestinal nematodes (GIN) are common in grazing animals and parasitism can be considered the norm. The pathogenicity of the various parasitic species and stages, the numbers of parasites present at any time, the level of innate or acquired immunity and the nutritional status of the host determine the expression of PGE in cattle, which can extend from frank clinical disease to apparent normality.

3. Species Abundance and Distribution

Amongst the species listed in Table 1, two are particularly important as they are both common and pathogenic: *Cooperia oncophora* and *Ostertagia ostertagi* (Parkins et al., 1990). *C. oncophora* is particularly common in young cattle during their first grazing season (FGS) and is the main contributor to faecal egg counts (FEC), certainly up to mid-summer (Hertzberg et al., 1992). Cattle appear to mount a rapid immune response to this parasite; consequently both intestinal burdens and faecal egg counts tend to decline towards the end of the first grazing season and remain low in subsequent years.

_____

Table 1. Common Gastrointestinal Parasitic Nematodes of Cattle in Europe

Site	Nematode genus/species
Abomasum	***Ostertagia ostertagi***
	Haemonchus spp.
	Trichostrongylus axei
Small Intestine	***Cooperia oncophora***
	Cooperia spp.
	Trichostrongylus spp.
	Nematodirus spp.
Large Intestine	*Oesophagostomum radiatum*
	Trichuris spp.

O. ostertagi infections can also be acquired from turnout, through ingestion of over-wintered larvae on pasture, but generally they do not predominate until the second half of the first grazing season. Since *O. ostertagi* is a relatively low egg producer and because egg output is density-dependent, i.e. the greater the worm burden, the lower the individual worm egg production, *O. ostertagi* abundance may be under-estimated from larval culture of faeces samples (Eysker and Ploeger, 2000). Immunity to *O. ostertagi* takes longer to develop than in the case of *Cooperia* and cattle are not normally considered to be functionally immune until they have experienced two complete grazing seasons (Armour, 1989). The immunity is not a sterile one and adult cattle frequently harbour burdens of *O. ostertagi*, which in some individuals can be high (in the hundreds of thousands) and which occasionally result in clinical disease (Orpin, 1994; Gross et al., 1999; Agneessens et al., 2000; Borgsteede et al., 2000). The focus of the remainder of this introduction and of Chapter I is PGE resulting from infections with *O. ostertagi* and/or *C. oncophora* in cattle.

4. Epidemiology

The epidemiology of PGE in cattle arises from complex interactions between the parasites - in both the parasitic and free-living stages – the pasture and the hosts.

4.1. Biology of the Parasitic Phase (see Figure 1)

The parasitic phase commences with exsheathment of the infective 3rd stage larvae in the rumen, stimulated by factors including low pH, digestive secretions and changes in temperature and carbon dioxide concentration. Exsheathment can be affected by diet: maximum percentage (>97%) of exsheathed *O. ostertagi* larvae was observed 120 minutes after exposure in the rumen in grass-fed cattle and after 360 minutes in

_____

grain-fed cattle (DeRosa et al., 2005). Correspondingly, 60% of larvae in the rumen of grass-fed cattle began to exsheath after 30 minutes exposure, compared to 120 minutes for grain-fed animals. The 3rd stage larvae then migrate to their predilection sites: in the case of *O. ostertagi* the gastric glands of the abomasum (Ritchie et al., 1966) and for *C. oncophora*, the crypts of the intestinal mucosa (Armour et al., 1987). There, after a few days, the worms moult to the 4th larval stage, which continue to develop to the 5th stage and subsequently the adult stages, which are found on the surface of the mucosa. In the early adult stage, the male and female worms mate and the females commence egg production. The minimum pre-patent period (ingestion of L$_3$ to egg production) is typically 15-18 days for *C. oncophora* and 18-21 days for *O. ostertagi*, but for practical purposes in the context of control programmes, three weeks is typically used as a standard.

A variation in the standard life cycle, particularly in *O. ostertagi,* can occur in the autumn and over winter. Some infective larvae acquired during the latter part of the grazing season, instead of proceeding through the normal moults and development to adults over the subsequent few weeks, enter a period of inhibition. This generally occurs within a few days of entry into the host at the early L$_4$ stage, when the larvae (~1 mm long) become hypoactive and fail to continue with their normal development. Resumption of development occurs after several months of inhibition, typically towards the end of the winter, although it can happen earlier (Michel et al., 1974). Synchronous emergence of large numbers of previously inhibited larvae in the late winter can result in severe clinical disease in some individuals – a condition known as Ostertagiosis Type II (Armour, 1970). Arrested development is probably a parasite adaptation to enhance over-winter survival and to ensure that pastures are seeded with worm eggs the following Spring, when susceptible hosts (young cattle) are grazing (Eysker, 1997).

4.2. Immunity and the Parasitic Phase

Following repeated exposure, cattle generate an acquired immunity to gastrointestinal nematodes. The response is to some degree genetically controlled and individual animals vary somewhat in the speed and extent to which immunity develops.

_____
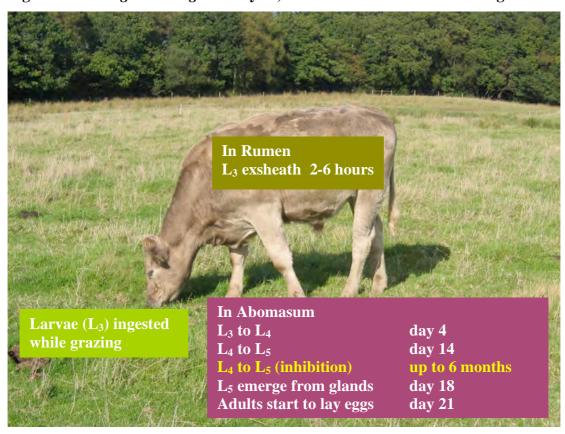
Figure 1. *Ostertagia ostertagi* **Life Cycle; Transmission and Parasitic stages**

Within a relatively closed population, using faecal egg counts as an indicator, it has been shown that ~25% of calves appear to be innately resistant to GINs, 50% generate an acquired immunity during the first grazing season and 25% have an inadequate response and fail to show a reduction in FEC consequent to exposure (Leighton et al., 1989). Thus, at the end of a grazing season, ~75% may be functionally immune, whereas ~25% may still carry relatively high nematode burdens. Evidence that aggregation is typical of ruminant nematode infections is provided by field observations that show that parasite population levels in individual animals within a herd have a skewed distribution with high levels of infection occurring in only a small proportion (~20%) of individual hosts (Barger, 1985).

There are several tiers of expression of acquired immunity to gastrointestinal nematodes in cattle (Armour, 1989; Vercruysse and Claerebout, 1997):

1. Decrease in fecundity

2. Stunting of growth

3. Retardation and arrested development

4. Expulsion of adult worms

_____

5.   Failure of incoming infective larvae in establishing in the GI tract

These effects occur sequentially as the acquired immune response develops in the host. One of the consequences of the reduction in parasite fecundity is that faecal egg counts can be very low in 'immune' animals and hence do not necessarily reflect the adult worm burden.

4.3. Ecology of Free-living Phases (see Figure 2)

Worm eggs are excreted within the faeces and hatch and undergo further development within the faecal pat. The dynamics of the free-living stages of both *O. ostertagi* and *C. oncophora* are very similar and will be considered together (Rose, 1961, 1962, 1963).   Hatching and larval development are primarily temperature-dependent processes, although moisture is also required as these stages are susceptible to desiccation. First stage larvae (L_1) hatch from the eggs, feed on bacteria, and moult into second stage larvae (L_2), which also feed on bacteria. The L_2 in turn moult into third stage larvae (L_3), which are the infective stages.   The time taken for development from egg to L_3 under laboratory conditions for both *O. ostertagi* and *C. oncophora* is 3-7 and 3-9 days respectively at 22-23°C; 7-16 and 4-21 days at 14-16°C and 18-28 and 21-56 days at 10-11°C (Rose, 1961, 1963). On pasture, these figures equate to minimum development times <1 to 3 weeks over a typical grazing season starting in April and ending in October: development times during the winter months can range between 3 and 20 weeks (Rose, 1961, 1963). The L_3 retain the cuticle of the L_2 as a sheath that appears to confer some protection to the larvae as they are relatively resilient to environmental fluctuations: *O. ostertagi* and *C. oncophora* L_3 can be recovered from herbage ~2 years after deposition in the dung (Rose, 1961, 1963). There is some evidence that the soil may also act as a reservoir for infective larvae and facilitate their long term survival (Al Saqur et al., 1982). The L_3 are motile but appear to have limited capacity for active migration, in part due to the fact that they do not feed and therefore have a finite, non-renewable energy reserve. Nevertheless, there is some active movement within the dung as the majority of L_3 are eventually found in the top third of the pat (Rose, 1961), presumably in readiness for translocation on to the surrounding herbage.

Translocation from the pat to the surrounding herbage requires moisture to facilitate both active and passive movement. The ability of infective larvae to move actively over distances of more than a few centimetres appears to be limited.
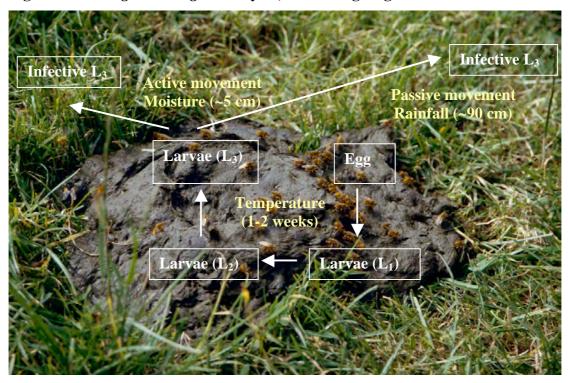
Figure 2. *Ostertagia ostertagi* **Life Cycle; Free living stages**

Studies have shown that most larvae move no more than ~5 cm horizontally from the pat of origin (Rose, 1961) and their propensity to travel vertically up the herbage appears to be equally limited, with the majority of larvae being found in the lower 5 cm of the sward (Silangwa and Todd, 1964; Williams and Bilkovich, 1973). However, because guideleines for the continuous stocking management of cattle grazing temperate ryegrass swards, currently recommend that the sward height should be maintained between 5 and 8 cm, there is ample opportunity for such stock to ingest infective larvae, particularly if they graze close to older dung pats. Nevertheless, ruminants normally avoid grazing on vegetation close to dung, except when grazing pressure is high (Cooper et al., 2000; Bosker et al., 2002; Scantlebury et al., 2004).

Passive mechanisms however can facilitate dispersal of infective larvae over greater distances from the faecal pat. Rainfall is known to be a very important agent in facilitating passive movement of larvae away from dung and onto pasture (Rose, 1962; Gronvold, 1984b, 1987). The mechanism involves an initial wetting and softening of the dry crust, which typically forms on pats after deposition, followed by infective larvae close to the surface of the pat being splashed out in droplets through the kinetic energy of the falling rain. Passive movement of larvae by this means can account for 90% of the translocation of larvae from the pat to the pasture and larvae

_____

can be found up to 90 cm from the pat. The trajectory of the droplets carrying infective larvae is normally at a height of 30 cm above ground, so, on landing on the herbage, they will initially be deposited on the upper leaves of the herbage and hence will be prone to ingestion by the grazing animal (Gronvold and Hogh-Schmidt, 1989). Spread of infective larvae beyond this range almost certainly takes place passively through water flow and through transport hosts, including invertebrates such as earthworms (Gronvold, 1979), and insects (Tod et al., 1971) and vertebrates such as birds (Gronvold, 1984a) and cattle themselves. Viable infective L_3 have been found in samples of encrusted faeces on the feet and limbs of grazing cattle (Hertzberg et al., 1992).

5. Management Systems

The two most obvious distinctions in terms of cattle farming systems and their effect on the epidemiology of parasitic gastroenteritis (PGE) in young cattle are between dairy farms, where calves are removed from their dams at or soon after birth, and beef breeding farms, where the calves typically remain with their dams until weaning, generally when calves are six to eight months of age. For practical reasons, young stock are typically considered as either first grazing season (FGS) or second grazing season (SGS) animals, but as can be seen in this section, this does not necessarily imply that they will have had the same level of exposure to parasites, which depends on the month when they are born and subsequent management. The calving pattern can affect parasite epidemiology: generally beef herds have a Spring or Autumn/Winter calving period, which for management reasons is normally reasonably short – ideally ~two months. Dairy herds can also calve seasonally (usually Spring or Autumn), although it is mainly Spring-calving herds producing milk from grass for direct supply to dairies for manufacture (e.g. in Ireland) that operate a strict calving season of two months. In many dairy herds, calving occurs all year round with only minor seasonal peaks and on such farms the epidemiology of nematode parasitism can be a mixture of the patterns described below.

5.1. Beef Farms
5.1.1 Spring-calving Herds
In spring-calving beef breeding herds the cows are typically immune and excrete low concentrations, 0-100 eggs per gram (epg), of worm eggs in their faeces (Forbes et al.,

_____

2002). Nevertheless, because of the fresh weight of dung (25-30 kg) produced by adult cattle, they can contribute significantly to pasture contamination, adding to over-wintered larval populations and the subsequent appearance of infective larvae on the herbage (Stromberg, 1997; Stromberg and Gasbarre, 2006; Yazwinski and Tucker, 2006). The young calf is parasite naïve and fully susceptible to parasitic nematodes at birth, but its diet comprises predominantly its mother's milk for the first few months of life. Studies in sheep indicate that milk in the diet has some inhibitory effects on infective larvae of *Telodorsagia (Ostertagia) circumcincta* and lower burdens were observed in milk-fed lambs compared to similar aged lambs that had been weaned (Zeng et al., 2001; Zeng et al., 2003): this could also be the case in calves. It is not until the calf is nearly six months old and close to weaning that herbage dry matter (DM) intake exceeds milk DM intake (Boggs et al., 1980). The consequence of this is that spring-born pre-weaned beef calves generally acquire quite modest burdens of gastrointestinal nematodes up to mid to late summer, as reflected in mean epgs in the range of 0-250 (Forbes et al., 2002), and clinical disease and marked production penalties are uncommon. Thereafter, higher worm burdens may be acquired, particularly post-weaning, the magnitude depending on the size of pasture larval populations and exposure; growth retardation can result (Forbes et al., 2002). Because of the relatively limited exposure to nematode infection in their first year of grazing, beef calves may fail to acquire protective immunity to *O. ostertagi* and in their second grazing season, high faecal egg counts, elevated plasma pepsinogen and reduced growth rates can result (Taylor et al., 1995).

5.1.2. Autumn-calving Herds

Autumn-born suckled beef calves, particularly if they have been housed, will have had little or no experience of worm infection by the Spring and by then will be consuming a high proportion of grass in their diet. They are therefore more prone than spring-born calves to acquiring significant infection earlier in the grazing season; their exposure is dependent on over-wintering survival of infective larvae on the pasture, which may be enhanced by additional contamination with worm eggs from their dams. These animals will then follow the classical sequence of events of acquiring infection while grazing, initially from over-wintered and maternally derived larvae and subsequently from auto-infection. Under the influence of typical seasonal temperature and rainfall patterns in northern Europe, this results in high concentrations of infective larvae on pasture from mid July onwards and,

_____

consequently, a high risk of both clinical disease and production losses (Urquhart et
al., 1987).

5.1.3. Non-breeding Farms

Some farmers will purchase animals destined for beef either from beef breeding herds
or dairy herds for fattening. Cattle may be just a few weeks old on arrival in the case
of dairy calves or be weaned beef calves 6-9 months old or more. The approach to
parasite control on these farms will vary according to several factors, including the
(grazing) history of the animals prior to purchase, their intended weight and age when
they are to be sold, the time of year, the availability of pasture and the type of farm –
i.e. mainly arable or mixed (other livestock species).

5.2. Dairy Farms

The fundamental difference between beef and dairy herds is of course that in the
former, the calves normally stay with their dams for several months before weaning;
in the latter, calves are removed from the cows at or soon after birth and thereafter are
raised independently of their dams and fed on milk substitutes and concentrates until
weaning at ~3 months of age. These differences in husbandry can affect parasite
epidemiology qualitatively and quantitatively. Additionally, many dairy farms will
sell their male calves for veal or beef production; hence their young stock comprises
mainly heifer replacements. Finally, because of the high nutritional and management
inputs required for high genetic merit dairy cows, some farmers adopt confined
systems in which lactating cows are fed total mixed rations (TMR), which are
designed to provide all the nutrient requirements for production. On such farms,
grazing may be restricted to young stock or dry cows and consequently it is mainly
these groups that are at risk of PGE.

5.2.1. Spring-Calving Herds

Spring-calving in dairy herds is characteristic of farms that aim to produce the bulk of
milk from grazed grass. Production can be aimed at fresh milk markets, but
commonly – for example in Ireland and New Zealand – milk is supplied directly to
dairies for further processing into products such as butter and cheese. Regions that
favour this style of production are those with a long growing season and calving
typically occurs in late winter/early Spring; February and March in Ireland, August
and September in New Zealand (Harris and Kolver, 2001). Hence, in northern
Europe, weaned calves may be turned out to grass as early as April at ~2 months of

age and nematode epidemiology then follows a similar pattern to that described above for autumn-born beef calves (but without any influence of the dams). If the calving season is more protracted, then farmers may keep the calves inside until after weaning (typically at around 3 months of age) and this may mean that calves are not pastured until mid-summer or later. If such calves are put on to pasture that has been grazed by cattle, particularly young animals, these calves could be exposed to high levels of larval challenge from the herbage and be at risk of clinical disease. On the other hand, if pasture is available that hasn't been grazed in the current year, levels of larval contamination could be low, particularly if the fields have been used for forage conservation, and the risk of disease would be correspondingly less.

5.2.2. Autumn-calving herds

Autumn-born dairy calves are in a similar situation to autumn-born beef calves in that they will be weaned and largely or totally dependent on grass as their feed following turnout to pasture in the Spring. Hence they will be fully susceptible to PGE and, unless appropriate control measures are in place, will be exposed to the risk of both clinical and subclinical PGE from mid-summer onwards (Shaw et al., 1998).

5.2.3. All-year-round calving herds

Dairy herds that calve all year round do so either because they aim to provide a steady supply of milk in every month or because they are unable to maintain a seasonal calving pattern because of poor reproductive performance, such that a 365-day calving interval is precluded, or possibly a combination of both. In such systems, calves can be born in any month and hence their first grazing experience could last for anything from a few months (in the autumn) to the complete duration of a grazing season. Calves born late in the summer or autumn may not graze till the following year, so that they enter their second year of life in a state of relative parasite naivety. To achieve the normal target of calving at 2 years of age, dairy heifers need to be served by 15 months of age at 85% of their expected adult live weight (Losinger and Heinrichs, 1997), so it is not surprising that parasite control is crucial, at least up to this age, to ensure good growth rates - 0.7 to 0.8 kg/day (Van Amburgh et al., 1998) - at pasture. Clearly, a sound understanding of dairy husbandry and parasite epidemiology is required to ensure that herd production and nematode control are in harmony.

_____

6. Production losses associated with PGE in cattle

On welfare grounds alone, there is a clear need for clinical parasitic gastroenteritis to be adequately controlled, particularly in the most vulnerable animals in their first grazing season. However, in many cases, PGE is present sub-clinically and it is consequent production losses that assume greater importance. The impact of sub-clinical nematode infections has been demonstrated both through classical experiments comparing the performance of uninfected control and artificially infected animals and through numerous field studies in which naturally infected ruminants of all ages, treated with anthelmintics, have shown production responses consequent to effective parasite control (Hawkins, 1993; Shaw et al., 1998; Gross et al., 1999; Vercruysse and Claerebout, 2001). Performance penalties from sub-clinical gastrointestinal parasitism have been observed in essentially every class of cattle from the suckled calf to the adult dairy cow (Table 2.).

Table 2. Loss of Production resulting from Sub-clinical Parasitic Gastroenteritis

Class of Stock	Effect on Production
First Grazing Season weaned calves	Reduced Growth Rate
Confined cattle	Inferior Feed Conversion Efficiency
Second Grazing Season cattle	Reduced Growth Rate
	Delay in Puberty & Conception in Heifers
	Reduced Pregnancy rate in Heifers
	Loss of Carcass Yield and Quality
First Calf Dairy Heifers	Decreased Milk Yield
	Longer Calving to Conception Interval
1st Calf Beef Heifers	Decreased Pregnancy Rates
Dairy Cows	Decreased Milk Yield
	Longer Calving to Conception Interval
Beef Cows	Lower Weaning Weight of their Calves
	Decreased Pregnancy Rates

The magnitude of these losses can vary considerably, but, for example in a review of 85 studies on GIN infections in Western Europe in FGS calves, either untreated (control) or subject to anthelmintic chemoprophylaxis, clear patterns emerged (Shaw et al., 1998). In studies in which the control group experienced clinical PGE, the

difference in daily liveweight gain was 62% greater in the treated calves compared to the controls (600 versus 370 g/day respectively). In studies in which no clinical disease was observed in the control group, i.e. infections were subclinical, there was still a difference in daily liveweight gain of 28% between the treated calves and the controls (690 versus 540 g/day respectively). In dairy cows, strong negative associations have been found between the concentrations of antibodies to *O.ostertagi*, measured by ELISA and expressed as Optical Density Ratios (ODR) in the bulk tank milk, and the average herd milk production (Guitian et al., 2000; Charlier et al., 2005). Statistical analyses of data from several thousand herds show that the difference in average daily milk yield in cows in herds at the 25th percentile of ODR values was typically ~1 kg/day greater than that of cows in herds at the 75th percentile. Both these examples illustrate quantitatively the potential of subclinical (and clinical PGE) to adversely affect production, with consequent economic losses.

7. Pathology and Pathophysiology

The underlying mechanisms for these production losses can be better understood from knowledge of some of the pathological and pathophysiological changes that occur in PGE. Furthermore, the pathophysiological changes can provide diagnostic markers (Eysker and Ploeger, 2000) and also indicators of the biochemical and neuroendocrine pathways that are involved (Fox et al., 1989b). These subjects are dealt with in more depth in Chapter 1 insofar as they affect inappetence, but the general effects are summarised in this section.

7.1 Ostertagiosis

The pathogenesis of ostertagiosis has been well documented (Ross and Todd, 1965; Ritchie et al., 1966; Murray et al., 1970); the principal biochemical, histopathological and clinical signs are observed shortly after the emergence of the adult worms from the gastric glands of the abomasum. At this time there is hyperplasia and loss of cellular differentiation, particularly of the hydrochloric acid-producing parietal cells; this occurs in adjacent, unparasitised glands as well. These cellular changes have several consequences:

- Elevation of the pH of abomasal contents from 2 to 7, leading to
  - Failure to convert pepsinogen into pepsin

_____

- o Loss of ability to initiate protein breakdown
- o Hypergastrinaemia
- o Loss of bacteriostatic effect/change in bacterial flora
- Greater permeability of the abomasal wall to macromolecules, leading to
  - o Elevated plasma pepsinogen levels
  - o Hypoalbuminaemia

The hypergastrinaemia is strongly associated with a consistent feature of both subclinical and clinical ostertagiosis, namely depressed appetite (Fox et al., 2002). Inappetence has been observed in several studies and the consequent reduction in Dry Matter Intake (DMI) can account for much of the observed production losses (Fox et al., 1989a).

In heavy infections, the cellular changes can be observed as gross pathology with affected glands appearing swollen and pale and the mucosal surface of the abomasum taking on an appearance described as 'Morocco leather' (Armour, 1970). These pathological changes in the abomasal wall can lead to a marked increase in the weight of the organ and associated lymph nodes. For example, in one study, seven weeks after infection, the infected-untreated control calves weighed 131 kg and the infected-treated calves 135 kg. The weight of the abomasal wall in the infected-untreated control was 958 g, compared to infected-treated calves in which it was 532 g; the corresponding weights of the associated lymph nodes were 2.78 g and 0.78 g respectively (Yang et al., 1993).

7.2. Cooperiosis

C. oncophora parasitizes the small intestine but generally causes less structural damage than that seen in parasitic gastritis. Nevertheless, a mucoid enteritis results from infection and a loss of villus architecture can be observed in infected regions of the alimentary tract. Consequent to such changes are inappetence, slow growth, lowered nitrogen retention and loss of plasma proteins into the gut (Armour et al., 1987).

7.3. Mixed *Cooperia* and *Ostertagia* Infections

Mono-specific infections with either parasite do not represent the likely field occurrence of parasitic gastroenteritis, particularly in young stock: co-infection is

more typical. Experimental mixed infections with *O. ostertagi* and *C. oncophora* have shown that severe disruption of normal gut function can result, manifest as diarrhoea, inappetence, hypoalbuminaemia and weight loss (Parkins et al., 1990). Dual infections appeared to cause greater effects than comparable mono-specific infections and may reflect a reduction in the ability of the host to compensate for dysfunction at various locations in the gastrointestinal tract.

8. Conclusion

The broad and profound adverse effects that can be associated with PGE underline the fact that its control should be one of the cornerstones of any cattle enterprise and a primary focus for the veterinarian. However, because PGE in cattle, especially older animals, is commonly expressed in its sub-clinical form, it may be given less attention than more 'dramatic' clinical diseases. Because reduced feed intake is a common feature of PGE and because feed intake is also fundamentally important to successful livestock husbandry, it is something to which most farmers, veterinarians and advisors can easily relate. Growth, the production of milk and meat and the efficient functioning of biological processes, including the immune and reproductive systems, all ultimately depend on nutrient supply, nutrient intake, nutrient partitioning and nutrient utilisation. Hence, the fact that inappetence alone can be responsible for many of the losses associated with PGE, helps bring it into sharp focus. Since most of the research on the effect of parasitic nematodes on feed intake in cattle has been conducted under housed conditions in young animals, it is important to be able to demonstrate inappetence in parasitized animals under field conditions that closely resemble commercial farming and with cattle of different ages and physiological states. Such data, presented in a readily assimilated form, could make a compelling case for rational parasite control in cattle.

_____

9. References

Agneessens, J., Claerebout, E., Dorny, P., Borgsteede, F.H., Vercruysse, J., 2000, Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 90, 83-92.

Al Saqur, I., Bairden, K., Armour, J., Gettinby, G., 1982, Population study of bovine *Ostertagia* spp infective larvae on herbage and soil. Res Vet Sci 32, 332-337.

Armour, J., 1970, Bovine ostertagiasis: a review. Vet Rec 86, 184-190.

Armour, J., 1989, The influence of host immunity on the epidemiology of trichostrongyle infections in cattle. Vet Parasitol 32, 5-19.

Armour, J., Bairden, K., Holmes, P.H., Parkins, J.J., Ploeger, H., Salman, S.K., McWilliam, P.N., 1987, Pathophysiological and parasitological studies on *Cooperia oncophora* infections in calves. Res Vet Sci 42, 373-381.

Barger, I.A., 1985, The statistical distribution of trichostrongylid nematodes in grazing lambs. Int J Parasitol 15, 645-649.

Boggs, D.L., Smith, E.F., Schalles, R.R., Brent, B.E., Corah, L.R., Pruitt, R.J., 1980, Effects of Milk and Forage Intake on Calf Performance. J Anim Sci 51, 550-553.

Borgsteede, F.H., Tibben, J., Cornelissen, J.B., Agneessens, J., Gaasenbeek, C.P., 2000, Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 89, 287-296.

Bosker, T., Hoekstra, N.J., Lantinga, E.A., 2002, The influence of feeding strategy on growth and rejection of herbage around dung pats and their decomposition. J Agric Sci 139, 213-221.

Charlier, J., Claerebout, E., Duchateau, L., Vercruysse, J., 2005, A survey to determine relationships between bulk tank milk antibodies against *Ostertagia ostertagi* and milk production parameters. Vet Parasitol 129, 67-75.

Cooper, J., Gordon, I.J., Pike, A.W., 2000, Strategies for the avoidance of faeces by grazing sheep. Appl Anim Behav Sci 69, 15-33.

DeRosa, A.A., Chirgwin, S.R., Fletcher, J., Williams, J.C., Klei, T.R., 2005, Exsheathment of *Ostertagia ostertagi* infective larvae following exposure to bovine rumen contents derived from low and high roughage diets. Vet Parasitol 129, 77-81.

Eysker, M., 1997, Some aspects of inhibited development of trichostrongylids in ruminants. Vet Parasitol 72, 265-272; discussion 272-283.

Eysker, M., Ploeger, H.W., 2000, Value of present diagnostic methods for gastrointestinal nematode infections in ruminants. Parasitology 120 Suppl, S109-119.

Forbes, A.B., Cutler, K.L., Rice, B.J., 2002, Sub-clinical parasitism in spring-born, beef suckler calves: epidemiology and impact on growth performance during the first grazing season. Vet Parasitol 104, 339-344.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Gale, D.L., 1989a, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on

_____

appetite, digestibility, rate of passage of digesta and liveweight gain. Res Vet Sci 47, 294-298.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Simmonds, A.D., 1989b, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on the hormonal control of digestive and metabolic function. Res Vet Sci 47, 299-304.

Fox, M.T., Uche, U.E., Vaillant, C., Ganabadi, S., Calam, J., 2002, Effects of *Ostertagia ostertagi* and omeprazole treatment on feed intake and gastrin-related responses in the calf. Vet Parasitol 105, 285-301.

Gronvold, J., 1979, On the possible role of earthworms in the transmission of *Ostertagia ostertagi* third-stage larvae from feces to soil. J Parasitol 65, 831-832.

Gronvold, J., 1984a, On the possible role of birds as transport hosts of 3rd-stage trichostrongyle larvae. Preliminary results. Acta Vet Scand 25, 143-144.

Gronvold, J., 1984b, Rain splash dispersal of third-stage larvae of *Cooperia* spp. (Trichostrongylidae). J Parasitol 70, 924-926.

Gronvold, J., 1987, A field experiment on rain splash dispersal of infective larvae of *Ostertagia ostertagi* (Trichostrongylidae) from cow pats to surrounding grass. Acta Vet Scand 28, 459-461.

Gronvold, J., Hogh-Schmidt, K., 1989, Factors influencing rain splash dispersal of infective larvae of *Ostertagia ostertagi* (Trichostrongylidae) from cow pats to the surroundings. Vet Parasitol 31, 57-70.

Gross, S.J., Ryan, W.G., Ploeger, H.W., 1999, Anthelmintic treatment of dairy cows and its effect on milk production. Vet Rec 144, 581-587.

Guitian, F.J., Dohoo, I.R., Markham, R.J., Conboy, G., Keefe, G.P., 2000, Relationships between bulk-tank antibodies to *Ostertagia ostertagi* and herd-management practices and measures of milk production in Nova Scotia dairy herds. Prev Vet Med 47, 79-89.

Harris, B.L., Kolver, E.S., 2001, Review of Holsteinization on Intensive Pastoral Dairy Farming in New Zealand. J Dairy Sci 84, E56-61.

Hawkins, J.A., 1993, Economic benefits of parasite control in cattle. Vet Parasitol 46, 159-173.

Hertzberg, H., Schnieder, T., Lopmeier, F.J., Stoye, M., 1992, The influence of weather and egg contamination on the development of third-stage larvae of *Cooperia oncophora* on pasture. Int J Parasitol 22, 719-730.

Leighton, E.A., Murrell, K.D., Gasbarre, L.C., 1989, Evidence for genetic control of nematode egg-shedding rates in calves. J Parasitol 75, 498-504.

Losinger, W.C., Heinrichs, A.J., 1997, An analysis of age and body weight at first calving for Holsteins in the United States. Prev Vet Med 32, 193-205.

Michel, J.F., Lancaster, M.B., Hong, C., 1974, Studies on arrested development of *Ostertagia ostertagi* and *Cooperia oncophora*. J Comp Pathol 84, 539-554.

_____

Murray, M., Jennings, F.W., Armour, J., 1970, Bovine ostertagiasis: structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. Res Vet Sci 11, 417-427.

Orpin, P.G., 1994, Ostertagiasis in suckler cows. Vet Rec 135, 584.

Parkins, J.J., Taylor, L.M., Holmes, P.H., Bairden, K., Salman, S.K., Armour, J., 1990, Pathophysiological and parasitological studies on a concurrent infection of *Ostertagia ostertagi* and *Cooperia oncophora* in calves. Res Vet Sci 48, 201-208.

Ritchie, J.D., Anderson, N., Armour, J., Jarrett, W.F., Jennings, F.W., Urquhart, G.M., 1966, Experimental *Ostertagia ostertagi* infections in calves: parasitology and pathogenesis of a single infection. Am J Vet Res 27, 659-667.

Rose, J.H., 1961, Some observations on the free-living stages of *Ostertagia ostertagi*, a stomach worm of cattle. Parasitology 51, 295-307.

Rose, J.H., 1962, Further observations on the free-living stages of *Ostertagia ostertagi* in cattle. J Comp Pathol 72, 11-18.

Rose, J.H., 1963, Ecological Observations and Laboratory Experiments on the Free-Living Stages of *Cooperia oncophora*. J Comp Pathol 73, 285-296.

Ross, J.G., Todd, J.R., 1965, Biochemical, Serological and Haematological Changes Associated with Infections of Calves with the Nematode Parasite, *Ostertagia ostertagi*. Br Vet J 121, 55-64.

Scantlebury, M., Hutchings, M.R., Allcroft, D.J., Harris, S., 2004, Risk of disease from wildlife reservoirs: badgers, cattle, and bovine tuberculosis. J Dairy Sci 87, 330-339.

Shaw, D.J., Vercruysse, J., Claerebout, E., Dorny, P., 1998, Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. Vet Parasitol 75, 115-131.

Silangwa, S.M., Todd, A.C., 1964, Vertical migration of Trichostrongylid larvae on grasses. J Parasitol 50, 278-285.

Stromberg, B.E., 1997, Environmental factors influencing transmission. Vet Parasitol 72, 247-256; discussion 257-264.

Stromberg, B.E., Gasbarre, L.C., 2006, Gastrointestinal Nematode Control Programs with an Emphasis on Cattle. Veterinary Clinics of North America: Food Animal Practice 22, 543-565.

Taylor, S.M., McMullin, P.F., Mallon, T.R., Kelly, A., Grimshaw, W.T., 1995, Effects of treatment with topical ivermectin three and eight weeks after turnout on nematode control and the performance of second-season beef suckler cattle. Vet Rec 136, 558-561.

Tod, M.E., Jacobs, D.E., Dunn, A.M., 1971, Mechanisms for the dispersal of parasitic nematode larvae. 1. Psychodid flies as transport hosts. J Helminthol 45, 133-137.

_____

Urquhart, G.M., Armour, J., Duncan, J.L., Dunn, A.M., Jennings, F.W., 1987, Veterinary Parasitology, First Edition. Longman Scientific & Technical, 286 p.

Van Amburgh, M.E., Galton, D.M., Bauman, D.E., Everett, R.W., Fox, D.G., Chase, L.E., Erb, H.N., 1998, Effects of Three Prepubertal Body Growth Rates on Performance of Holstein Heifers During First Lactation. J Dairy Sci 81, 527-538.

Vercruysse, J., Claerebout, E., 1997, Immunity development against *Ostertagia ostertagi* and other gastrointestinal nematodes in cattle. Vet Parasitol 72, 309-316; discussion 316-326.

Vercruysse, J., Claerebout, E., 2001, Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Vet Parasitol 98, 195-214.

Williams, J.C., Bilkovich, F.R., 1973, Distribution of *Ostertagia ostertagi* infective larvae on pasture herbage. Am J Vet Res 34, 1337-1344.

Yang, C., Gibbs, H.C., Xiao, L., Wallace, C.R., 1993, Prevention of pathophysiologic and immunomodulatory effects of gastrointestinal nematodiasis in calves by use of strategic anthelmintic treatments. Am J Vet Res 54, 2048-2055.

Yazwinski, T.A., Tucker, C.A., 2006, A Sampling of Factors Relative to the Epidemiology of Gastrointestinal Nematode Parasites of Cattle in the United States. Veterinary Clinics of North America: Food Animal Practice 22, 501-527.

Zeng, S., Brown, S., Przemeck, S.M.C., Simpson, H.V., 2003, Milk and milk components reduce the motility of *Ostertagia circumcincta* larvae *in vitro*. N Z Vet J 51, 174-178.

Zeng, S., Lawton, D.E.B., Przemeck, S.M.C., Simcock, D.C., Simpson, H.V., 2001, Reduced *Ostertagia circumcincta* burdens in milk-fed lambs. N Z Vet J 49, 2-7.

Chapter 1: Grazing Behaviour and Feed Intake in Cattle with Parasitic Gastroenteritis

A Review of the Published Literature.

_____

1.1 Introduction

A reduction in, or loss of, appetite – inappetence or anorexia - is commonly observed in many disease states, particularly those associated with infectious agents (Hart, 1990). Inappetence is a common element of parasite infections in ruminants and has been described consequent to infection with several common helminths, including abomasal nematodes *Ostertagia ostertagi* (Fox et al., 1989a), *Teladorsagia circumcincta* (Coop et al., 1977), intestinal nematodes *Trichostrongylus colubriformis* (Kyriazakis et al., 1994) and the liver fluke *Fasciola hepatica* (Cawdery and Conway, 1971; Dargie et al., 1979). Most of this research has been conducted in young animals and there appears to be relatively little information on inappetence in adult ruminants with helminth infections (Leyva et al., 1982; Greer et al., 2005b). The importance of inappetence as a component of production losses resulting from parasitic gastroenteritis (PGE) in young animals has been demonstrated in studies that have shown that between 60-73% of the reduced growth rate in lambs and calves can result directly from a reduction in feed intake (Sykes and Coop, 1977; Fox et al., 1989a).

There is only one paper on PGE in cattle in which any attempt was made to estimate feed intake in animals at pasture (Bell et al., 1988) and none in which grazing behaviour, as it relates to appetite, was quantified.

1.2. Objectives

The objectives of this chapter are firstly to review the normal grazing behaviour of cattle in order that subsequent observations can be seen in perspective, then to describe the methodologies that can be used to measure feed intake and grazing behaviour, with emphasis on those techniques that can be used under field conditions. Because appetite is under the influence of several extrinsic and intrinsic factors and is controlled ultimately by the central nervous system (CNS), the subsequent section summarises the orchestration of appetite and its neuro-endocrine control in the ('healthy') ruminant. This then forms the background against which the biochemical and pathophysiological changes that have been observed in cattle with ostertagiosis and/or cooperiosis can be assessed in order to determine possible mechanisms that might account for parasite-induced inappetence. Finally there is a summary of the scientific literature in which inappetence, feed intake and PGE have been studied in cattle in order that qualitative and quantitative aspects can be reviewed.

_____

1.3. Grazing Behaviour

Introduction

The innate value of domestic ruminants to the livestock farmer is that they are able to harvest readily available nutrients in grass and other forages and convert them, with the help of a well adapted ruminal flora and fauna, into energy and protein that can be used by man in the form of draught power, meat, milk and fibre (van Soest, 1994). Furthermore, under many types of husbandry and for much of the year, ruminants do not need to be 'artificially' fed, but can satisfy all/most of their nutrient requirements during the process of grazing. Intake of grass is achieved through manipulation of the sward by means of the incisor teeth, the dental pad and the tongue, which, notwithstanding sward factors, constrains a single bite size in cattle to <1 gram Dry Matter (DM) (Hodgson, 1990). As an adult dairy cow may consume 15-20 kg DM herbage per day when grazing, it can readily be appreciated that a large number of bites and correspondingly long period of time must be spent each day eating in order to achieve such intakes. In addition to this, cattle must devote some time each day ruminating in order to digest the herbage and, of course, there are other important activities that must be incorporated into each 24-hour period, such as walking, resting, social interaction and, in cows, suckling and being milked. Hence the daily budget of activities in ruminants plays an important role in their biological functioning and efficiency.

Components of Grazing Behaviour

The daily consumption of herbage (Herbage Intake, HI) depends on the daily intake rate and the amount of time spent eating, hence the equation:

HI (kg DM) = Intake Rate (kg DM/hour) x Eating Time (hours)

Intake rate can be broken down into Bite Rate, generally expressed as bites/minute, and Bite Mass, usually expressed in g DM, hence the equation becomes:

HI (kg DM) = Bite Mass (g DM) x Bite Rate x Eating Time (min)/1000

Bite mass varies according to the anatomical dimensions of the muzzle (Gordon and Illius, 1988; Woodward, 1998) and sward characteristics, of which one of the most important is sward height (Gibb et al., 1997). The interrelationships between some these components can be illustrated with examples using some typical ranges of values for dairy cows:

_____

Bite mass (g DM)	0.35-0.75
Bite rate (bites/min)	50-60
Eating time (hours/day)	8-10
Daily intake (kg DM)	8.4-27

The main behavioural constraints appear to be bite rate and the amount of time spent grazing per day, hence bite mass (strongly influenced by sward height and bulk density) tends to be the most important determinant of intake. An intake of 19.0 kg herbage DM per day can be taken as a reasonable average figure for a lactating dairy cow (Kolver and Muller, 1998; Gibb et al., 1999).

Through the use of modern jaw-movement recorders, components of grazing behaviour can be further broken down into constituent parts (Gibb, 1998). For example, bite rate can be calculated as the product of the total number of grazing jaw movements (GJM) and the proportion of GJM that are bites (as opposed to other manipulative and masticative jaw movements preparatory or subsequent to biting herbage during grazing).

Furthermore, actual eating time comprises the total grazing time minus intra-meal intervals, which are defined as periods of jaw inactivity >3 seconds and <5 minutes, when the animal is walking or searching, but no food is ingested (Gibb et al., 1997). Eating time can be sub-divided into meals or bouts, which comprise continuous periods of grazing separated by breaks in activity of >5 minutes, thus daily eating time can be determined from the number of meals and their duration (Rook and Huckle, 1997).

Duration of Daily Grazing

In a review of some of the early literature it was reported that grazing time in cattle ranged between 4 and 9 hours per day; the same duration as the time spent ruminating daily (Hafez et al., 1969). A subsequent review published in 1993 provided a summary of 27 studies in which grazing time had been recorded and included, the variables grazing management and supplementation regimen. Although the animals involved were mainly beef calves, steers, heifers and cows, three of the studies involved dairy cows (Krysl and Hess, 1993). The overall range in daily grazing time was 359-771 minutes and a simple mean value of all 56 data sets was 531 minutes (~9 hours) per day. The longest total daily grazing time quoted of 771 minutes per day seems to be an artefact insofar as two methods of calculating grazing time were used: for Charolais x Angus cows in the summer, the values for the two methods were 14.8

_____

and 9.0 hours per day and the latter figure seems more realistic (Stricklin et al., 1976). In fact more recent studies, even in high producing dairy cows, suggest an upper limit for daily grazing time in cattle of 12-13 hours (Rook et al., 1994).

_____

Table 1.1. Grazing behaviour studies in temperate regions 1994-2004: time spent grazing, eating and ruminating

Animal	Pasture/Management	Variables	Grazing (min)	Eating (min)	Ruminating (min)	Reference
Lactating dairy cows	Grass/clover Continuous	Sward height Supplementation Month	537-765		250-382	(Rook et al., 1994)
Lactating dairy cows	Grass Continuous	Sward height	581-628		335-439	(Gibb et al., 1997)
Lactating dairy cows	Grass Continuous	Time of day	632		434	(Gibb et al., 1998)
Lactating dairy cows	Grass/clover Continuous	Clover content Supplementation	590-622		313-331	(Rook and Huckle, 1997)
Dairy cows, dry or lactating	Grass Continuous	Sward height , Physiological state	452-624	419-607	361-495	(Gibb et al., 1999)
Lactating dairy cows	Grass Rotational	a.m. vs. p.m.		461-462	433-454	(Orr et al., 2001)
Lactating dairy cows	Grass Continuous	Level of Supplementation Month	554-629	531-599	332-493	(Gibb et al., 2002a)
Lactating dairy cows	Grass Continuous	Type of Supplement		402-507	369-432	(Gibb et al., 2002b)
Yearling dairy heifers	Grass or Clover Continuous	Grass vs. Clover	536-436* *Clover		526-267* *Clover	(Rutter et al., 2002)
Lactating dairy cows	Grass	Pasture allowance Supplementation	522-626		116-263	(Bargo et al., 2002a)
Lactating dairy cows	Grass	Partial supplementation using a Total Mixed Ration (TMR) or concentrate ration	252†-572 †TMR			(Bargo et al., 2002b)
Lactating dairy cows	Grass	Continuous or Rotational	535-563		365-393	(Pulido and Leaver, 2003)
Yearling dairy heifers	Grass or Clover Rotational	Grass vs. Clover, Month		672-382* *Clover	624-323* *Clover	(Orr et al., 2004)

_____

Summary information on twelve studies that have been conducted since 1994 is presented in Table 1.1. With the exception of one (Pulido and Leaver, 2003), they have all been conducted using similar methodology, with automatic recording and the definitions and terminology are similar, so the data should be broadly comparable. However, in some of the studies, it is not possible to differentiate grazing time and eating time, which are not synonymous. The data summarised in Table 1.1. can be compared to those in a review that summarised eight publications that focussed on the effects of supplementation of dairy cows while grazing on pasture (Bargo et al., 2003), which included three of the studies cited in Table 1.1. (Rook et al., 1994; Bargo et al., 2002a; Gibb et al., 2002a). In the review by Bargo et al. (2003), the range of daily grazing times was from 398 to 765 minutes and a simple mean value of all 43 data sets was 541 minutes (nine hours) per day.

It can be instructive to look at the conditions under which the 'extreme' results were generated. For example, the upper limit for daily grazing time from Table 1.1. was 765 minutes per day (12 hours 45 minutes) and this was observed in a group of cows that were grazing on paddocks with a sward height of only 4 cm and which were not given any supplement (Rook et al., 1994). The large increase in grazing time compared to the other treatment groups (the next highest was 660 minutes, 11 hours) appears to be a compensatory mechanism resulting from the very short height of the sward; indeed, because of an unreasonable loss of body weight, the group on 4 cm swards with no supplementation did not complete the study. In contrast, the appetites of heifers grazing pure clover swards were apparently satisfied within 452 minutes per day, (~7.5 hours), and the high digestibility of this diet also allowed them to have a short time for rumination – 267 minutes, (~4.5 hours) (Rutter et al., 2002). All of the variables listed in Table 1.1 affected the total time spent grazing each day or the temporal pattern of grazing. Thus sward height, supplementation (type and amount), month, clover content of the sward, sward monoculture type (grass or clover), physiological state (lactating or dry) of the cows and continuous or rotational grazing can all affect grazing behaviour. Another factor known to affect grazing time is fasting and in a study in the USA it was found that steers that had been deprived of food for 36 hours spent 44% more time grazing when returned to pasture compared to animals that had been removed for only 30-60 minutes (Greenwood and Demment, 1988).

_____

Table 1.2. Grazing behaviour studies in temperate regions 1994-2004: components.

Animal	Pasture/ Management	Bite mass (mg)	Total Number. of GJM/day	GJM Rate/min	Bite Rate/min	No. of meals/day	Duration of each meal (min)	Reference
Lactating dairy cows	Grass/clover Continuous	280-580 (DM?)		72-94	45-65	7-12	63-103	(Rook et al., 1994)
Lactating dairy cows	Grass Continuous	230-330 (OM)	48,400-61,800	80-97	74-79			(Gibb et al., 1997)
Lactating dairy cows	Grass Continuous	302-438 (OM)		77-81	47-59			(Gibb et al., 1998)
Lactating dairy cows	Grass/clover Continuous					8-11	66-79	(Rook and Huckle, 1997)
Dairy cows, dry and lactating	Grass Continuous	292-439 (OM)	31,300*-49,300[†] *dry; [†]lactating	74-81	58-62	4-7	72-142	(Gibb et al., 1999)
Lactating dairy cows	Grass Rotational	276-684 (DM)		76-86	70-80			(Orr et al., 2001)
Lactating dairy cows	Grass Continuous	231-346 (OM)	42,400-48,100	72-83	51-65	3-5	114-182	(Gibb et al., 2002a)
Lactating dairy cows	Grass Continuous	321-488 (OM)	30,800-37,700	74-76	57-60	7-12		(Gibb et al., 2002b)
Yearling dairy heifers	Grass/Clover Continuous	197-238* (DM) *Clover		65-79	53-70			(Rutter et al., 2002)
Lactating dairy cows	Grass	550-600	28,500-35,200		54-56			(Bargo et al., 2002a)
Lactating dairy cows	Grass	480-550	14,300-31,500		54-57			(Bargo et al., 2002b)
Lactating dairy cows	Grass							(Pulido and Leaver, 2003)
Yearling dairy heifers	Grass/Clover Rotational	74-371 (DM)		81-99	61-77			(Orr et al., 2004)

Table 1.2 lists some additional components of grazing behaviour that were measured in some of the studies. It is not in every case clear whether it is 'bites' or 'Grazing Jaw Movements' (GJM) that are being described and also some of the rates were measured over a 24-hour period, others were over a short period (~1 hour), when intake rates were recorded, nevertheless, the figures represent a range of typical values. These results can again be compared with those cited in two of the three reviews referred to earlier in this section. Hafez quotes figures of 24,000 bites per day with a rate during grazing of 50-80 bites/minute (Hafez et al., 1969); comparable figures quoted by (Bargo et al., 2003) are 16,000-47,000 bites per day and 45-78 bites per minute. An interesting calculation in one paper was that in pastured dairy cattle receiving a concentrate supplement there was a significant positive relationship ($R^2 = 0.74$) between the number of bites per day and milk production, such that there was an increase of 5 kg/day in milk for every 10,000 additional bites (Bargo et al., 2002b).
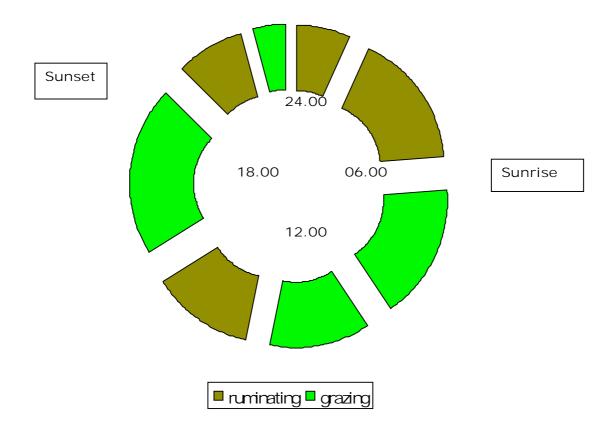
Circadian patterns of Grazing in Cattle

The total amount of time spent grazing each day is naturally not continuous and is divided into a number of bouts or meals, which from Table 1.2 can be seen to range in number from 3 to 12 per day and in duration from 63-182 minutes (~1-3 hours). It should be noted though that all these figures were measured in lactating dairy cows, which have an additional management constraint on grazing behaviour insofar as they are typically milked twice daily away from the grazing area, generally at set times. What is evident from the literature is that the majority of grazing takes place during daylight hours, when animals are at pasture for 24-hours per day. For example, between 65 and 100% of grazing took place in daylight hours in the studies cited in two reviews (Hancock, 1953; Krysl and Hess, 1993); Rook et al reported 88% in one study (Rook et al., 1994) and similar trends can be deduced from the activity profiles shown in several other papers (Gibb et al., 1998; Orr et al., 2001; Gibb et al., 2002a). It is clear therefore that there will be some seasonal changes in grazing behaviour consequent to the time of sunrise and sunset.

The general pattern of grazing in young cattle is to have three main periods of grazing activity per day (Figure 1.1.): the first starts soon after dawn, the second is during late morning and the third from late afternoon to dusk (Hodgson, 1990). Interspersed with these main meals is rumination, idling, exercise, drinking etc and additional short bouts of grazing. In dairy cows, this general pattern holds true and the imposition of milking generally means that the main period of grazing starts when the cows are

_____

returned to their paddocks after afternoon milking, and continues until dusk (Gibb et al., 2002a). There may be benefits of grazing late in the day as, through photosynthetic activity, the nutrient quality and density in grass increases in the afternoon (Delagarde et al., 2000; Orr et al., 2001).

Figure 1.1. Circadian pattern of Grazing & Ruminating in young Cattle

1.4. Methodology for determining Feed Intake

Housed Animals

Studies in housed animals can provide high levels of precision and replication and also allow for ease of sampling and measurement and are therefore popular. In order to measure voluntary feed intake, it is important that the ration is offered in excess of the expected nutrient requirements so that the animals can express feeding behaviour without constraint. This is typically achieved by offering a weighed amount of feed *ad libitum* (i.e. 10 to 20 per cent in excess of intake) and then weighing the amount that has not been consumed ('orts'), ideally on at least a daily basis.

It is preferable that intake is measured on an individual basis and this can be achieved by penning or tethering animals separately or through the use of automated feeding systems with transponder-driven access to feeders and electronic recording of

_____

amounts of feed consumed. Intake is normally converted from 'fresh weight' to Dry Matter (DM) or Organic Matter (OM) in order to standardise results and for comparative purposes. These can also be expressed in absolute terms or relative to live weight (LW) expressed in kilograms, or metabolic weight: $LW\,kg^{0.75}$.

In all but one of the papers cited in Table 1.7, feed intake was measured in housed cattle in order to evaluate possible effects of PGE on appetite. However, the conditions in such studies are clearly far removed from typical nematode epidemiology and normal cattle husbandry at pasture. Additionally, any changes in grazing/feeding behaviour and interactions with the sward that might be associated with PGE-associated inappetence can only be investigated on very reduced spatial and temporal scales, for example through the use of artificial sward boards (Hutchings et al., 1998). Results from behavioural experiments using turf/sod boards should be extrapolated with caution to field scenarios because of their unnatural setting and the short time frames over which measurements are typically made. (WallisDeVries et al., 1998).

Grazing Animals

Estimation of feed intake in grazing animals requires the use of indirect methods as it is virtually impossible to measure herbage intake directly (Penning, 2004b). Furthermore the swards themselves are dynamic and can change over time both as a result of normal plant biology – affected by factors such as topography, nutrient supply, temperature, solar radiation and rainfall - and as a result of defoliation, defaecation and trampling through animal grazing.

Grazing behaviour under field conditions can be monitored in a number of ways including classical human observation, video recording and the use of various remote sensors attached to the limbs, neck or head. The last 25 years has seen some significant advances in the study of grazing behaviour, notably:

- Agreed terminology and definitions for the detailed study of grazing behaviour (Gibb, 1996; Rook and Huckle, 1997; Gibb, 1998).
- Jaw movement sensors that can provide accurate, non-invasive tools with which to explore many facets of grazing behaviour without interference under natural free-range conditions (Penning, 1983; Penning et al., 1984; Rutter et al., 1997).
- Customised software programmes that allow for rapid analyses of behavioural patterns over a 24-hour period (Rutter, 2000).

_____

The currently available techniques for measuring feed intake in grazing ruminants have been extensively described, critiqued and reviewed (Penning, 2004b) and include the following:

- Sward-based estimates in which measurements of height, mass and composition are made before and after grazing from which intake can be calculated.

- Direct precision weighing of grazing animals to estimate intakes over short periods of time.

- Estimates of herbage intake using markers based on natural plant constituents or synthetic markers.

- Estimates based on observations of grazing behaviour; in particular the time spent grazing.

- Calculations based on nutrient requirements and performance of grazing animals predicted from feeding standards.

In grazing studies it is not uncommon for more than one of these methods to be included in the same study to provide complementary data.

Estimates based on measurements of the sward

The principle of this technique is in fact the same as in indoor systems, i.e. it is based on offering a known initial mass of herbage and then measuring the residual herbage mass when grazing has finished.

HI (kg DM) = Initial herbage mass (kg DM) - Post-grazing herbage mass (kg DM)

Because swards are dynamic and are generally growing during the grazing period, adjustments have to be made to the calculations to take this into account (Lantinga et al., 2004). For this reason, it is preferable that such studies are conducted over a relatively short time frame so that the proportion of re-growth in the residual herbage mass is relatively small compared to the mass lost through animal intake, hence limiting the size of potential errors.

Herbage mass (per unit of area) can be estimated using a number of techniques, including measurement of sward height in cm with a sward stick and/or measuring herbage mass with a rising-plate meter. In both systems, regression equations must be developed to allow an estimate of herbage mass in kg DM/hectare to be derived from the measured height readings. Additionally, representative samples should be collected from the pastures in order to determine dry matter, nutrient and species composition.

_____

Calculations based on weighing cattle before and after grazing

The principle of this method is deceptively straightforward. In essence it involves weighing cattle on accurate weigh scales prior to being released on to pasture and then repeating the procedure when they return – generally after a relatively short time, e.g. one hour. In order to account for all weight changes, animals must be fitted with faeces (F) and urine (U) collection bags in order that losses through these routes can be accounted for in the calculations. Additionally, water intake (L) must be measured or prevented and Insensitive Weight Loss (IWL) must be calculated by holding animals without access to food or water for a further period of time, typically ~1 hour. IWL in grazing animals comprises losses through evaporative/respiratory cooling, sweating and the loss of gases during respiration and digestion (Penning, 2004a).

Thus the rate of herbage intake (kg OM/hour) during the time spent at pasture is calculated from:

HI = (Weight after grazing + IWL + F +U) – Weight before grazing – L

However, in practice F and U are not weighed separately from the animal. The animal is weighed along with its collection harness and bag, together with any faeces/urine contained in it, before and after grazing and before and after measuring IWL. Furthermore, they are provided with water before being weighed and released onto the pasture, so L does no need to be determined. Thus the equation can be simplified to :

HI= Weight after grazing – Weight before grazing + IWL

The pasture must also be sampled in order to be able to calculate herbage DM content and composition, as required.

This technique is quite demanding in terms of equipment – accurate weigh scales, faeces/urine collection bags - manpower for fitting the bags, handling and moving the cattle, so is not appropriate for some studies and some locations. It can be used in conjunction with other measures of intake and grazing behaviour.

Estimates based on the use of inert markers

The equation underlying methods using this approach is:

HI kg DM/day = <u>Faecal Output (FO) kg DM/day</u>

(1 – Diet Digestibility, D)

Thus, if the daily faecal output (DM) is 4.0 kg (Marsh and Campling, 1970), the digestibility of the diet is 0.70 (Hodgson, 1990) , then intake is 4/0.3=13.3 kgDM.

_____

Total faecal output can be measured through the use of dung collection bags, but as stated previously, this is clearly very demanding in terms of equipment, logistics and manpower. An alternative is to estimate total faecal output through the use of external, usually inert, markers. Markers should be chemically discrete, easily recoverable, easily analysed and indigestible in the digestive tract (Dove and Mayes, 1991). In this technique, faecal output is normally calculated as Organic Matter (OM) rather than DM (Penning, 2004a) and the (simplified) equation for calculating Faecal OM becomes:

Faecal OM Output/day = <u>Weight of Marker given/day</u>
Mean concentration of marker in faeces OM

If the weight of the marker given daily is, say, 8 grams (g) and the concentration found in the faeces is 2 mg/g OM, then the faecal output is (8000/2)/1000 = 4 kg OM. The technique in practice essentially involves administering the marker - for example chromic oxide (CR_2O_3) – in a suitable carrier either on a daily basis or in a slow-release device for ~7 days for equilibration, followed by a 5-day measurement period. During the measurement period, faeces are collected either per rectum or from freshly deposited pats twice daily. This method was used in the only study in Table 1.7 in which intake was measured in pastured cattle with PGE (Bell et al., 1988).

Diet Digestibility is estimated from samples representative of the herbage that the animals are grazing, using one of several standard techniques, which are dealt with exhaustively in (Penning, 2004a), as are the various approaches to herbage sampling.

An extension to the use of inert markers for intake estimates in grazing animals is the use of n-alkanes, which are naturally occurring components of plant cuticular waxes that are largely indigestible. In most common pasture species of grass and clover, the carbon chain lengths of the main alkanes detected are in the range C_{25} – C_{35}, but within this range, the odd-numbered chains are present in much greater concentrations than the even-number alkanes. Thus by dosing animals with known quantities of appropriate even-chained alkanes, using a similar methodology to the chromic oxide technique above, faecal output can be calculated from the equation (Dove and Mayes, 1991):

FO = <u>Dosed + intake of even chained alkanes from herbage</u>
Faecal concentration of even-chained alkanes

Re-arranging the equation in various steps, the final equation for intake is (Penning, 2004a):

$$\text{HI kg DM/day} = \frac{D_{32}}{\dfrac{F_{32}* H_i - H_{32}}{F_i}}$$

Where: D_{32} = weight of dosed alkane C_{32} dosed (mg/day)

F_i = conc. of alkane of chain length i in faeces (mg/kg DM)

F_{32} = conc. of alkane of chain length 32 in faeces (mg/kg DM)

H_i = conc. of alkane of chain length i in herbage (mg/kg DM)

H_{32}= conc. of alkane of chain length 32 in herbage (mg/kg DM)

A further advantage of this method is that additional information on diet composition and digestibility of the diet can be determined from the same data (Lewis et al., 2003).

Estimates based on observations of grazing behaviour

 Measurements of grazing behaviour can be used not only to monitor behavioural changes, but also to calculate herbage intake. This is based on the equation:

Herbage Intake = Intake Rate x Grazing Time

Intake Rate can be broken down further into Bite Rate x Bite Mass; likewise, Grazing Time = Meal Duration x Number of Meals per day (Penning and Rutter, 2004).

In its simplest form, recording of grazing behaviour in animals at pasture involves detailed recording of individual activity at frequent intervals by observers positioned so as not to interfere with the animals' normal behaviour. Needless to say, this is labour-intensive, laborious, prone to operator error and can be inaccurate insofar as it may be difficult to accurately differentiate behaviours, if for example the animal is facing away from the observer. Video recording can counter some of these drawbacks, but still requires labour intensive assessment and analyses.

Significant advances in behaviour recording have been made in the last 25 years, principally because of developments using automatic recording systems and remote sensors. Specifically in terms of grazing and ruminating behaviour, the introduction of jaw-movement recorders was a major break through (Penning, 1983). Since then, a number of modifications have been made, both with the recording equipment and analytical software, so that now it is possible to record and analyse grazing behaviour with a high degree of accuracy and with a much reduced labour requirement (Rutter et

_____

al., 1997; Rutter, 2000). Additionally acoustic recordings via a microphone attached to the animal's forehead have been found to be useful and have the potential of allowing differentiation between various types of grazing jaw movement (Laca and WallisDeVries, 2000). Their value may, however, be constrained by the possibility of the microphone picking up extraneous sounds, including neighbouring grazing animals, the requirement for high levels of supervision, their suitability for only short term recordings and a lack of automation (Ungar and Rutter, 2006).

The recording equipment used at the Institute of Grassland and Environmental Research, North Wyke, Okehampton, EW20 2SB, UK is illustrated in Figure 1.2.

Figure 1.2. IGER Jaw Movement Recorder

Jaw movements recorded for 24 hours on a battery-powered microcomputer

Modified halter with noseband comprising a silicone tube packed with carbon granules

The electrical resistance of the sensor changes as the animal's jaw opens and closes

IGER Behaviour Recorder

The design is based on a conventional halter, but the noseband has been modified so that it comprises a silicone tube packed with carbon granules (graphite). When the animal's jaw opens and closes, the electrical resistance of the sensor changes and analogue signal of these changes are digitized and recorded over a 24-hour period on a battery powered microcomputer attached to the halter (Rutter et al., 1997). The total weight of the system for cattle is 1.5 kg. The data files are transferred from the memory cards in the microcomputer to a personal computer on which the customised programme 'Graze' facilitates categorisation and analysis of the recorded jaw-movements (Rutter, 2000). This programme displays a trace of the amplitude of the signal from the noseband over time and identifies periods of grazing, eating and ruminating. In addition, experienced operators are able to identify other activities such as drinking, eating supplements and grooming.
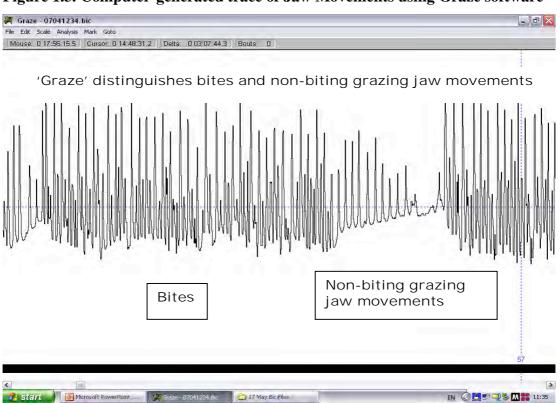
Figure 1.3. Computer-generated trace of Jaw Movements using Graze software

Jaw movements are initially analysed according to the height of the peaks above a set minimum amplitude and the lowest trough associated with that peak. Certain criteria then need to be satisfied in order for that peak to be defined as a jaw movement.

_____

Thereafter, 12 rules are used in order to discriminate between grazing and ruminating jaw movements (Rutter, 2000). Finally, grazing jaw movements (GJM) are subdivided into those associated with eating (bites) and those associated with other facets of manipulating the herbage during grazing: non-biting GJM.

A field validation trial using sheep to compare the results from automatic recording with those from a number (11) of manual observers showed an overall concordance of 91%, with values of 95.3% for eating, 92.9% for ruminating and 84.1 for others. The authors concluded that the automatic system was at least as accurate as manual observation and in fact the differences were more likely caused by errors in manual observation rather than the automated system (Rutter et al., 1997).

Calculations based on standard values for nutrient requirements.

This approach does not involve any actual measurement of the amount of herbage consumed nor of any aspects of grazing behaviour. Daily herbage intake (HI) in kg DM is calculated from theoretical values for the metabolisable energy (ME) in mega-joules (MJ) required per day for maintenance and production (MEm + MEp) of the test animals, based on the energy density (ME/kg DM) of the herbage that is being grazed (Baker, 2004).

$$\text{HI kg DM/day} = \frac{\text{MEm} + \text{MEp}}{\text{ME/kg DM}}$$

Thus a very simple example for a 600 kg Dairy Cow producing 30 litres of milk per day and grazing herbage with an ME of 10.0 MJ/kg, the calculation is:

Maintenance ME for 600 kg cow @ 0.1 MJ/kg LW =		60 MJ/day
ME for 30 kg milk @ 4.5 MJ/litre	=	135 MJ/day
ME density of herbage	=	10 MJ/kg DM
HI	=	$\frac{60 + 135}{10}$
	=	19.5 kg DM/day

Equations can be much more complex and sophisticated than this and may include allowances for components such as liveweight change, pregnancy, exercise, protein requirements, milk composition etc. but fundamentally, the calculations are based on the same principles.

_____

This approach was used in two studies on the epidemiology of PGE in dairy cows (Fox and Jacobs, 1980; Fox et al., 2007) and the calculation for intake used in those studies was that described in (Vadiveloo and Holmes, 1979).

Whilst this method has some attractions in terms of its simplicity (limited only by the complexity of the nutrient requirement calculations) and it may have value for comparative purposes, it is limited by the fact that there are no specific measurements of herbage intake nor of any variations between individuals in the quality of the diet selected or their digestive efficiency. Nevertheless, under some conditions it may be the only option and also it can be used to back-calculate from actual intake data generated by other methodologies to determine how close the results are to theoretical calculations.

1.5. Feed Intake and its Regulation

Introduction

The amount and type of feed that cattle eat is governed by numerous interacting factors that for clarity can be broadly subdivided into animal, nutritional and environmental components. Such a categorisation should not be interpreted as signifying separate processes because many of the elements are not mutually exclusive, indeed the whole process can be considered as being highly orchestrated and integrated. Table 1.3. includes examples from the general literature of factors that can affect and regulate feed intake in healthy ruminants in general, and grazing cattle in particular (Church, 1988; Phillips, 1993; van Soest, 1994; Forbes, 1995; Freer and Dove, 2002; McDonald et al., 2002).

The animal based factors that govern feed intake have been condensed into a unifying model, based on a concept known as 'Minimal Total Discomfort' (MTD) (Forbes, 1999). Its principle is that animals will endeavour, through modifications in feeding/grazing behaviour, to ingest an optimal quantity and balance of nutrients, whilst avoiding excesses or deficiencies. Afferent signals from various receptors, for example those for rumen distension and those for various products of digestion or metabolism, are integrated by the central nervous system (CNS) in an additive manner and suitable efferent pathways are invoked to solicit the appropriate feeding behaviours. This approach has been developed in a model for grazing sheep: components include the metabolisable energy (ME) requirement of the animal, energy

_____

and neutral detergent fibre (NDF) content of the herbage, rate of feed intake (grams of DM per minute) and maximum hours grazed per day.

Table 1.3. Factors that can affect feed intake in cattle.

Animal	Nutrition	Environment
Metabolic Weight $W^{0.75}$ (W=Liveweight)	Herbage availability Sward height & density	Housing/shelter/outside Access to feed
Physiological/reproductive /productive status	Herbage composition Neutral detergent fibre Lignin, tannin	Temperature/wind
Fat/energy reserves	Stocking management	Rain/snow/frost
Genetics	Grazing pressure	Stocking density
Experience/acclimatisation	Digestibility	Faecal contamination
Social status/interactions	Heterogeneity Preference	Topography/exercise
Water and/or Mineral balance	Supplementation	Photoperiod/season

The relative discomfort associated with dietary components, within the grazing behaviour constraints defined in the models, enables a plot to be constructed from which, through addition, the daily feed intake associated with MTD can be calculated (Forbes, 2001). Limitations in the accuracy of such models are however acknowledged through examples of variations in individual feed intake by cows fed uniformly under the same conditions.

Central Control of Appetite

Appetite is ultimately under the control of the CNS and the hypothalamus appears to be fundamental to the intrinsic regulation of feed intake (Kalra et al., 1999; Mutch and Clément, 2006; Meister, 2007). The neural and neuroendocrine pathways that link changes in the gastrointestinal tract and other sites of metabolism to the CNS are complex (see Figure 1.4). The vagus and splanchnic nerve systems enervate the gastrointestinal tract and transmit signals to the hind brain and thence to the hypothalamus (Murphy and Bloom, 2006). Some of the endocrine responses require an intact enervation of the GI tract, while others appear to interact directly with the

_____

hypothalamus via specific receptors located in the arcuate nucleus (Fox et al., 2006; Fukumoto et al., 2008).

Figure 1.4. Afferent and Efferent Endocrine Pathways in the Control of Appetite

There has been a strong research focus into the neuroendocrinology of appetite control over the last decade, much of it conducted in rodents and humans, with the purpose of understanding and controlling obesity in the human population; several comprehensive reviews have resulted, including (Moran, 2004; Small and Bloom, 2004; Chaudhri et al., 2006; Murphy and Bloom, 2006; Näslund and Hellström, 2007). Much of this effort has been directed towards identifying and characterising gut peptides, ultimately with the aim of discovering novel therapies for obesity (Smith, 2000; Mendieta-Zerón et al., 2008). Whilst it is quite possible that observations at the neuroendocrine level in laboratory animals and man are equally applicable to ruminants, extrapolations should be cautiously applied. The presence of the rumen at the proximal end of the GI tract directly affects the flow of nutrients into the abomasum and the rumen itself is subject to both physical (distension) and chemical changes (accumulation of fermentation products, e.g. volatile fatty acids - VFAs), which can also mediate changes in appetite.

_____

The Hypothalamus

At the level of the hypothalamus, the arcuate nucleus is considered to be a key site for receiving neuroendocrine signals for the regulation of feed intake and energy balance and, through links with the other sites, such as the paraventricular nucleus, it plays a prominent role in controlling appetite (Smith and Grove, 2002). The arcuate nucleus is thought to have an incomplete blood-brain barrier and this allows some circulating hormones to have access to receptors in this part of the CNS (Meister, 2007). The arcuate nucleus contains distinct, but over-lapping, populations of neurons that mediate either orexigenic (increase appetite) or anorexigenic (decrease appetite) responses. Gene expression studies on various peptides and receptors in murine brain have resulted in detailed maps of this central circuitry (Olszewski et al., 2008). Some of the known hypothalamic modulators of feed intake are listed below (Friedman and Halaas, 1998; Smith, 2000; Murphy and Bloom, 2006), but it should be remembered that neurotransmitters generally have diverse properties and their activities can vary according to different physiological states.

Table 1.4. Examples of orexigenic and anorexigenic molecules found in the hypothalamus appetite centres.

Orexigenic	Anorexigenic
Agouti-related protein (AGRP)	Cholecystokinin (CCK)
Cortisol	Corticotrophin releasing hormone (CRH)
Galanin	Glucagon
Ghrelin	Insulin
Neuropeptide Y (NPY)	Leptin
Orexin	Melanocortin

Afferent Signals for Appetite from Peripheral Tissues

The role of numerous endocrine messengers, including gut peptides, in appetite control has been studied extensively (Moran, 2004). Interestingly, the majority have been shown to be appetite inhibitors (indicators of satiety) (Stephens et al., 2007) and those that are located in the GI tract are generally in the intestines, whilst a peptide that can strongly stimulate appetite is ghrelin, which is located primarily in the oxyntic (hydrochloric acid – HCl – producing) glands of the stomach.

_____

Table 1.5. Examples of peripheral signallers for satiety or hunger.

Signal Satiety	Signal Hunger
Leptin	Ghrelin
Insulin	
Amylin	
Cholecystokinin (CCK)	
Bombesin family e.g. gastrin-releasing peptide (GRP)	
Glucagon	
Enterostatin	
Apolipoprotein	
Somatostatin	
Peptide YY (PYY)	
Glucagon-like peptide 1 (GLP)	

Two of these molecules - ghrelin and leptin - will be described in more detail as, in addition to the work done in laboratory animals and man, both have been subject to some research in ruminants.

Ghrelin

Ghrelin is a 27/28-amino acid peptide that acts as a Growth Hormone Secretagogue (GHS) and is produced mainly in the oxyntic (parietal) glands of cow and sheep abomasa (Grouselle et al., 2008), but it is also found in other organs, including the rumen, intestine, pancreas and immune system (Hayashida et al., 2001; Gentry et al., 2003; Sugino et al., 2004). Ghrelin receptors in the oxyntic cells can be associated with gastrin receptors and there is evidence that gastrin may directly stimulate ghrelin release from the stomach and that both may increase gastric acid synergistically (Fukumoto et al., 2008). Ghrelin can cross the blood-brain barrier (Banks et al., 2002) and bind to receptors in the brain; GHS (ghrelin) receptors (GHS-R) are expressed mainly in the arcuate nucleus of the hypothalamus where they are associated locally with NPY expression (Olszewski et al., 2008). In general terms, concentrations of ghrelin in the circulation increase prior to scheduled meals and in response to fasting and sub-optimal levels of nutrition, while feeding generally suppresses ghrelin secretion (Wertz-Lutz et al., 2006; Bradford and Allen, 2008; Wertz-Lutz et al., 2008). In housed sheep fed once daily there was a surge in plasma ghrelin just before feeding, which declined within 1 hour of feeding; thereafter the ghrelin levels gradually increased until the surge before the next meal (Sugino et al., 2004). However, the relationships differed according to feeding regime: there was a ghrelin surge prior to feeding, when the ration was offered in discrete programmed meals, but

_____

if the ration was fed *ad libitum*, ghrelin concentrations in the blood fluctuated much less.

In adult dairy cows, plasma ghrelin levels were reduced within one hour of feeding and then returned to pre-feeding levels within 4 hours (Hayashida et al., 2001; Miura et al., 2004), though this pattern may also vary with stage of lactation (Bradford and Allen, 2008). In contrast, it was shown that in 3-month old calves there were no significant changes in ghrelin concentrations associated with feeding time (Miura et al., 2004). The relationship between ghrelin and feed intake was supported by the observation that selection for high genetic merit in dairy cows is associated with higher plasma ghrelin levels and increased DMI (Roche et al., 2006). The same authors measured ghrelin in dairy cows at 07.30 before milking, when they were fed concentrates, and at 10.00 when at pasture: supplementation was associated with a linear decline in post-prandial ghrelin concentrations and a decline in subsequent pasture intake (Roche et al., 2007).

Leptin

Leptin appears to play a central role in signalling and regulating energy homeostasis (appetite, nutrient partitioning, body composition), but it also is involved in diverse other functions, including reproduction and immunity (Garcia et al., 2002; Smith and Grove, 2002; Liefers et al., 2003; Chilliard et al., 2005; Kulcsár et al., 2005; Zieba et al., 2005). Leptin is a protein that is synthesised primarily in white adipose tissue and in adipocytes in other tissues (Bartha et al., 2005), but in ruminants, leptin gene expression has also been demonstrated in other organs including the rumen, abomasum and duodenum (Yonekura et al., 2002). Studies in calves have shown that mRNA expression of leptin can only be detected in the rumen and abomasum of pre-weaned (milk only diet) calves (Yonekura et al., 2002). In weaned 13–week-old calves and adult cattle, no leptin could be detected in the fore-stomachs, though it was still present in the duodenum. Its lipostatic role can be explained simply as a feedback mechanism such that when adipose tissue is abundant, high levels of leptin are synthesised and secreted into the circulation (Sansinanea et al., 2001; Delavaud et al., 2002). Circulating leptin reaches the hypothalamus, where the anorexigenic hormones, such as MSH, are stimulated, and the orexigenic hormones, such as NPY are depressed, thus reducing appetite and fat deposition (Hossner, 1998; Ingvartsen and Boisclair, 2001). In domestic animals and man, emphasis is typically put on its anorexigenic role in preventing excess fat deposition, but in wild animals or livestock

in harsh environments, it may be crucial in adapting to periods of undernutrition (Friedman and Halaas, 1998). Although the mediation of leptin effects on appetite via fat storage suggests a role in long-term nutritional control, in fact leptin expression can occur in the mid-term (days at a certain feeding level) and short term (minutes/hours after a meal) (Chilliard et al., 2005).

1.6. Anorexia: Regulation of Appetite in Disease

Anorexia is a universally common clinical sign in both infectious and non-infectious disease and there has been much discussion and debate in infectious disease as to whether the anorectic response is of functional benefit to the host, the pathogen, both or neither (Symons, 1985; Poulin, 1995; Exton, 1997; Kyriazakis et al., 1998). Attempts have been made to model feed intake responses in young animals, consequent to pathogen exposure and to try and establish thresholds for anorexia in order to account for effects seen at the subclinical level (Sandberg et al., 2006; Vagenas et al., 2007a, b). The mechanisms and pathways for inappetence in disease include alterations in the concentrations of peptides, proteins and other molecules that are known to regulate appetite in normal hosts, as described in the previous section. Other candidates for neuroendocrine roles in appetite regulation, particularly in infectious diseases, include pro-inflammatory cytokines, for example interleukin (IL) 1, IL6 and tumour necrosis factor (TNF) (Exton, 1997; Johnson, 1998, 2002; Konsman et al., 2002), which are associated with inflammatory and immune responses. Such mediators may also be invoked in helminth infections and therefore immune responses and immunopathology in gastrointestinal parasitism could also contribute to anorexia (Meeusen, 1999).

Biochemical changes in ruminants parasitized with gastrointestinal nematodes

The principal pathological, physiological, biochemical and endocrinological changes that have been observed in parasitized cattle are summarised in Table 1.6. The citations listed are not exhaustive, particularly for the commoner parameters. The specific pathophysiology of haematophagous nematodes, such as *Haemonchus* spp., has not been included. In only some of the studies cited were pair-fed controls included, i.e. a matched group of animals fed the same intake as that exhibited by the infected animals, hence some of the observations could at least partially be attributed to a reduction in feed intake rather than parasites alone.

_____

Table 1.6. Pathological, physiological, biochemical and endocrinological changes associated with PGE in cattle in temperate regions.

Parasite	Change (parasitized versus control)	Reference
O. ostertagi	Increase in abomasal pH from 2.9 to 6.6	(Purewal et al., 1997)
	Increase in plasma pepsinogen	(Fox et al., 1989b)
	Increase in plasma gastrin	(Fox et al., 1989b)
	Fundic (+96%) and pyloric (+31%) mucosa increase in mass	(Purewal et al., 1997)
	Increase in gastrin mRNA in pyloric mucosa	(Purewal et al., 1997)
	40x increase in the number of viable aerobic bacteria in abomasum	(Jennings et al., 1966; Symons, 1985)
	Reduction in nitrogen digestibility	(Fox et al., 1989a)
	Reduction in rate of passage of digesta	(Fox et al., 1989a)
	Increase in plasma non-esterified fatty acids (NEFA)	(Fox et al., 1989b)
	Hypoalbuminaemia	(Fox et al., 1989b; Yang et al., 1993)
	Urea levels increased	(Fox et al., 1989b)
	Glucose, cholesterol and calcium reduced	(Fox et al., 1989b)
	Reduction in plasma insulin	(Fox et al., 1989b)
	Increase in plasma growth hormone	(Fox et al., 1989b)
	No effect on blood CCK	(Fox et al., 2002)
C. oncophora	Reduced Nitrogen (N) retention	(Armour et al., 1987)
	Increased leakage of plasma protein N into alimentary tract	(Armour et al., 1987)
	Reduced digestibility of dietary fractions	(Armour et al., 1987)
O. ostertagi & C. oncophora	Elevated plasma pepsinogen	(Parkins et al., 1990)
	Hypoalbuminaemia & hypoproteinaemia	(Parkins et al., 1990)
	Reduced digestibility of dietary fractions	(Parkins et al., 1990)
	Reduced Nitrogen (N) retention	(Parkins et al., 1990)

Abomasal parasitism

The fundamental changes that occur in the abomasum following parasitism with *O. ostertagi* have been well described (Armour et al., 1973). Figure 1.5. illustrates some of the main features of the pathophysiology. Following a primary infection, coincident with the emergence of 5th stage larvae from the gastric glands at around 18 days, the pH in the abomasum increases rapidly. This is a consequence primarily of damage to the parietal cells in the gastric glands by the parasite, which results in a reduction in the secretion of hydrochloric acid (HCl). The loss of acidity in the abomasal contents has a number of consequences, including a failure of pepsinogen to convert to the active proteolytic enzyme, pepsin, hypergastrinaemia (Fox et al., 1989b) and a loss of bacteriostatic activity, manifest as increased numbers of aerobic (Jennings et al.,

_____

1966) and anaerobic bacteria (Simcock et al., 1999) in the lumen. Under experimental conditions in calves trickle infected with *O. ostertagi*, there appears to be a strong temporal association between increased concentrations of pepsinogen and gastrin in the blood and depression in appetite and feed intake (Fox et al., 1989a; Fox et al., 1989b). Additional work in non-parasitised calves treated with the proton pump inhibitor, omeprazole, showed that the resultant hypergastrinaemia was associated with a reduction in appetite, indicating that gastrin is a strong candidate for mediating inappetence in abomasal parasitism (Fox et al., 1989c).

Figure 1.5. The pathophysiological cascade in Ostertagiosis

Gastrin is secreted from the G cells in the abomasum and duodenum and binds to receptors on the parietal and enterochromaffin-like cells in the gastric mucosa, where it stimulates gastric acid (HCl) secretion. Through a negative feed-back mechanism, its release can be triggered by low acid concentrations (high pH) in the gastric lumen – as happens in abomasitis caused by *O. ostertagi* (Purewal et al., 1997) . At least two peptides of gastrin have been described, comprising 17 or 34 amino acids and they all share biologically active five C-terminal amino acids, known as pentagastrin. The five C-terminal amino acids of gastrin and cholecystokinin are identical and both

_____

compounds can bind to the same receptors, hence some of their activities may overlap (Kopin et al., 1992).

Intestinal parasitism

There have been many fewer pathophysiological studies in cattle infected with *C. oncophora* (Armour et al., 1987), but a number have been conducted with intestinal trichostrongylosis in sheep (Coop and Field, 1983; Kimambo et al., 1988; Dynes et al., 1990; Kyriazakis et al., 1994; Kyriazakis et al., 1996; Greer et al., 2005b). However, with the exception of the studies by Dynes and by Greer, discussed below, these have produced few indications of specific metabolic or endocrine changes that could be related to feed intake.

Pathophysiology and Inappetence

It is not inconceivable that any of the pathophysiological changes listed in Table 1.6, individually or collectively, could stimulate afferent pathways to the appetite/satiety centres in the CNS and cause a reduction in feed intake. Equally, some changes may be a consequence of a reduction in feed intake, hence resulting from efferent pathways, rather than be a cause of inappetence. There is some indirect evidence, certainly for nematodes that are found in the abomasum, that the depression of appetite is associated with the presence of (adult) worms in/on the mucosa and not with any associated pathology. For example, transplantation of *T. circumcincta* or *Haemonchus contortus* into the abomasa of uninfected sheep resulted in an immediate elevation of pH, serum gastrin and pepsinogen, before any significant pathological changes could have taken place (Simpson, 2000). Similar observations were made with *O. ostertagi* transplants in cattle (McKellar et al., 1986; McKellar et al., 1987). When infected cattle or sheep with depressed appetite are treated with an anthelmintic, improvement in appetite takes place rapidly (Fox et al., 1989a; Dynes et al., 1990), before the gut would have had time (2-3 weeks) to fully recover from nematode-associated pathology (Angus et al., 1979).

Additional evidence for a pathophysiological, rather than a pathological, basis for parasite-induced inappetence comes from studies in immunosuppressed sheep. It has been found that sheep infected with either *T. colubriformis* or *T. circumcincta* and treated with corticosteroids do not exhibit the depression of feed intake shown by untreated, infected sheep (Greer et al., 2005a; Greer et al., 2005b). Worm burdens at the end of the study with *T. colubriformis*, 11 weeks after infection was initiated, were

224 in the infected lambs and 22,387 in the immunosuppressed, infected lambs (Greer et al., 2005b). Thus appetite depression in the infected lambs was associated with a worm population ~1% the size of that in the infected immunosuppressed sheep. These observations lend further support to the proposed 'nutritional' cost of immunity, which has been recognised as being of importance in several biological systems (Sheldon and Verhultst, 1996; Lochmiller and Deerenberg, 2000; Viney et al., 2005) and may be of particular relevance in production animals, where economically important production penalties could result from the preferential partitioning of nutrients towards immunity (Houdijk et al., 2001; Colditz, 2002).

In a study with sheep infected with *O. (Teladorsagia) circumcincta*, an increase in leptin was associated with a decline in feed intake, whereas, when appetite increased, serum leptin levels declined (Fox et al., 2006). In the same study it was shown that afferent signals via the vagal or splanchnic nerves were not required to mediate the loss of appetite, lending further support to the importance of the role of the endocrine system in appetite regulation in (parasitized) ruminants. In another study with *T. circumcincta*, the effects of parasitism on feed intake and circulating leptin levels in two different breeds of sheep of differing susceptibility to GI nematodes was examined (Zaralis et al., 2008a). The main findings were that anorexia was only observed in the more susceptible breed and this occurred both during primary infections and during re-exposure; plasma leptin concentrations were higher in infected than in non-infected lambs at similar levels of feed intake. This apparently contradictory effect was explained by the fact that leptin responses in the infected lambs may have been subject to conflicting influences of reduced appetite (leptin decrease) and infection (leptin increase), the latter has been demonstrated in several studies in infectious diseases (Fantuzzi and Faggioni, 2000), including one in intestinal parasitism in rats (Roberts et al., 2000). In contrast, no interaction between anorexia, parasitism and leptin was observed in a study in peri-parturient ewes infected with *T. circumcincta* (Zaralis et al., 2008b). In a field study in which the effects of natural infections with GI nematodes were compared in two groups of young heifers, one of which was treated regularly with ivermectin to minimise worm burdens, there was a marked difference in growth rate. The age of puberty in the treated heifers was 29 weeks compared to 40 weeks in the untreated controls (P<0.001); there was a significant positive association between leptin concentration and body weight up to 150 kg, but no significant association between leptin and

_____

parasitism. This perhaps could also be explained by the conflicting roles of leptin in the regulation of feed intake/energy balance and immunity.

Conclusion

The evidence from the literature indicates that the appetite suppression, observed in ruminants parasitized with gastrointestinal nematodes, results from the presence of worms in the lumen or mucosa of the alimentary tract. It seems that nematode-associated pathology/histopathology is not a prerequisite for this response and that the mere presence of parasites is sufficient to provoke a loss in appetite. The putative mechanisms involve parasite excretory/secretory (ES) products (Simpson, 2000) that provoke host inflammatory/immune/endocrine responses in a neuroendocrine cascade of events that leads to the release of appetite inhibitors and/or the suppression of appetite enhancers in the hypothalamus.

1.7. Inappetence and Bovine Parasitic Gastroenteritis

Introduction

Whilst the phenomenon of anorexia in bovine PGE is well recognised, there are surprisingly few references in the scientific literature to studies in which feed intake has been measured quantitatively and many of the papers lack specific details on aspects such as the methodology used. In many cases, feed intake was one of a number of parameters measured in studies that were designed to investigate broader aspects of pathology, pathophysiology, digestion, metabolism and performance, resulting from bovine PGE in general or ostertagiosis in particular. Table 1.7 provides a summary of useable publications, in which sufficient detail is provided to enable the level of parasitism and the magnitude of any reductions in nutrient intake to be estimated.

Table 1.7 Descriptive Information and Summary of Effects of PGE on Feed Intake in Cattle

Animals Total & Age	Infection			Husbandry	Parasitism Score	Effect on Intake	Reference
	Natural	Treatment	Induced				
39 M <1year			1x 411,600 L$_3$ *O. ostertagi* & 176,400 *C. oncophora**	Feedlot	1	-11% NS**	(Ames et al., 1969)
18 M twins <1 year	27% *Ostertagia* 60% *Cooperia*	Cambendazole every 2 weeks		Pasture then housed	2	-10%	(van Adrichem and Shaw, 1977a)
48 F twins <1 year	19% *Ostertagia* 70% *Cooperia*	Cambendazole every 2 weeks		Pasture then housed	2	-9%	(van Adrichem and Shaw, 1977b)
12 M <1 year			1x 60,000 L$_3$ *O. ostertagi* & *C. oncophora*	Housed	1	-24%	(Randall and Gibbs, 1981)
			1x 600,000 L$_3$ *O. ostertagi* & *C. oncophora*	Housed	3	-58%	
10 dairy cows	*Ostertagia*	Thiabendazole x2 after housing		Pasture then housed	1	NS	(Fox et al., 1985)
39 M <1 year	*Ostertagia* *Cooperia*	Fenbendazole every 2 weeks		Pasture then housed	1	NS	(Entrocasso et al., 1986)
	Ostertagia *Cooperia*	Morantel bolus at turnout		Pasture then housed	1	NS	
40 M <1 year	*Ostertagia* *Cooperia*	Morantel bolus at turnout		Pasture (then housed)	3	-18%	(Bell et al., 1988)

_____

12 M <1 year			45x 10,000 L$_3$ *O. ostertagi*/day	Housed	2	-77%	(Fox et al., 1989a)
25 M <1 year			42x 2000 L$_3$ *O. ostertagi* /day	Housed	2/3	NS	(Taylor et al., 1989)
		Morantel bolus at start	42x 2000 L$_3$ *O. ostertagi* /day	Housed	2	NS	
20 M 1-2 years	*Ostertagia Cooperia*	Morantel bolus at turnout		(Pasture) then housed	1	NS	(Bell et al., 1990)
25 M <1 year			42x 2,000 L$_3$ *O. ostertagi*/day 42x 10,000 L$_3$ *C. oncophora*/day	Housed	3	-4% overall -10% days 35-42	(Parkins et al., 1990)
		Morantel bolus at start	42x 2,000 L$_3$ *O. ostertagi*/day 42x 10,000 L$_3$ *C. oncophora*/day	Housed	2	NS	
15 M <1 year			98x 1,428 L$_3$ *O. ostertagi*/day	Housed	1	NS	(Xiao and Gibbs, 1992)
			98x 14,285 L$_3$ *O. ostertagi*/day	Housed	3	-50%	
16 F <1 year			1x 200,000 L$_3$ *O. ostertagi*	Housed	2	-38%	(Fox et al., 2002)

*1x, 42x, 45x & 98x refer to the number of days over which the infections were given.
**NS over whole 189 days on feed, but for first 70 days, large differences reported and illustrated in graph, but no interim statistics provided.

The effects on feed intake are expressed as a percentage difference between infected and uninfected controls or between untreated controls and anthelmintic-treated groups. The units (where stated) in which Intake was measured include Fresh Weight, Dry Matter (DM), Organic Matter (OM); Gross Energy (GE) and Nitrogen (N) intake. Given the uniform diets offered in the studies in housed animals, it is assumed that all intakes, regardless of the units, are proportional to Dry Matter Intake (DMI). In some studies, intake was measured for the duration of the experiment, in others, particularly those focussing on digestibility, it was measured on one or more occasions over a short time at various intervals (in metabolism crates) during the period of infection. Parasitism is scored according to the following scale, based on the authors' descriptions or data:

    0   Uninfected

    1   Sub-clinical infection

    2   Mild/intermittent clinical

    3   Clinical

Additional data, where available, on the size of worm burdens at necropsy (normally at the end of the experiment) are provided in Table 1.8.

Overview

In all the studies cited, whether infections were acquired naturally or were induced, the predominant species were *O. ostertagi* and *C. oncophora.* In most of the natural and several of the induced infections, animals were infected with both species. As might be expected from its pathogenicity, infections with *O. ostertagi* alone can cause marked anorexia (Fox et al., 1989a; Xiao and Gibbs, 1992; Fox et al., 2002), but *C. oncophora* alone has been reported to cause inappetence (Armour et al., 1987) and there is evidence from several studies that in mixed infections with *O. ostertagi*, *C. oncophora* can also contribute to the observed reduction in feed intake (van Adrichem and Shaw, 1977b, a; Randall and Gibbs, 1981; Parkins et al., 1990). Nevertheless, there were also some studies in which no significant effect of infection on feed intake were observed (Parkins et al., 1990; Taylor et al., 1989; Xiao and Gibbs, 1992).

Ostertagiosis

One of the most detailed studies on the effects of *O. ostertagi* on appetite in housed calves is described in the seminal paper by Fox *et. al* (Fox et al., 1989a). Three groups, each comprising four 3-month-old calves, were used in a study that lasted for 10 weeks. Group 1 calves were fed *ad lib* and infected with the equivalent of 10,000

_____

O. ostertagi L3/day given 3 times per week from days 0-45; Group 2 calves were pair fed according to the intake of the corresponding infected replicate calf from Group 1; Group 3 was not infected and fed *ad lib*. All groups were treated with fenbendazole on Day 46.

Group 1 calves had soft faeces periodically during the patent phase of the infection and one calf had transient diarrhoea in the 6th week of the study. The mean feed intakes of Groups 1 and 3 diverged from around 28 days onwards and were significantly (P<0.05) reduced in Group 1 from Day 37. The maximum depression in appetite was recorded on Day 44 when intake in Group 1 was 23.2% of that in Group 3. Though the appetite in Group 1 increased from Day 46 following anthelmintic treatment, it remained significantly (P<0.01) lower than Group 3 at the end of the study (Day 66) when the calves' intake was 71.5% that of the uninfected controls.

The growth rate of Group 3 calves increased steadily throughout the study and was significantly (P<0.01) greater than that of Groups 1 and 2 from day 46 onwards. Through comparison with the pair-fed control group (2), it was calculated that 72% of the growth depression in Group 1 up to day 46 was accounted for by its lower feed intake.

Cooperiosis

A study with a mono-infection with *C. oncophora*, given at 10,000 larvae per day for 6 weeks, clearly showed significant effects on histopathology, pathophysiology and live weight gain in the infected, untreated calves (Armour et al., 1987). Although inappetence was reported by the authors, its magnitude could not be determined from the data provided in the paper, but as can be seen in the next section, *C. oncophora* can contribute to the reduction in intake seen in mixed infections.

Mixed infections of *O. ostertagi* + *C. oncophora*

In a study carried out by researchers at the University of Maine, pathophysiological aspects of both clinical and sub-clinical infections with *O. ostertagi* and *C. oncophora* in calves were examined (Randall and Gibbs, 1981). Three groups of four 5- to 6-month-old calves were used in a study that lasted for 5 weeks after the initial infection. Group 1 calves (clinical) were infected once with 600,000 mixed *O. ostertagi* and *C. oncophora* L_3; Group 2 calves (sub-clinical) were infected with 60,000 *O. ostertagi* and *C. oncophora* L_3 and Group 3 calves (controls) were not infected. Feed intake was measured pre-infection and during weeks 3 and 5 post-infection.

_____

Calves in groups 2 and 3 remained clinically normal throughout the study. Calves in Group 1 first exhibited clinical signs of ostertagiosis, manifest primarily as diarrhoea, 3 weeks after the initial infection and the signs persisted intermittently throughout the experiment. Mean abomasal worm counts at the end of week 5 were 56,005 (89% *C. oncophora*) in group 1; 6,265 (89% *C. oncophora*) in Group 2 and 0 in Group 3. Mean faecal egg counts in the 3rd week after infection, when feed intake differed were 3656, 285 and 0 epg in Groups 1, 2 & 3 respectively.

In the third week of infection, the Gross Energy intake of Group 1 calves was significantly less (-57.9%) than Group 3, as was that of Group 2 (-23.6%) and they were significantly different from each other. By week 5, there were no significant differences in intake, though that in Group 1 was lower than the other Groups. Liveweight gains over the 5 week study were 10.7[a], 34.7[b] and 41.4[b] kg for Groups 1, 2 & 3 respectively, with differences significant at $P<0.05$.

Other nematode species

The only other common nematode species, for which some data are available on its effects on appetite, is *Trichostrongylus axei* (Ross et al., 1970). This was a very small study with only 3 infected and one control calf, but the calf infected once with 500,000 *T. axei* larvae, which proved to be a lethal dose, experienced a dramatic anorexia within 3 weeks when its feed intake had dropped by 79%.

Quantitative aspects

The data presented in Table 1.7 indicate that anorexia appears to be a common, but not universal, feature of mild/clinical parasitism (scores 2 & 3) in infections with *O. ostertagi* and/or *C. oncophora*: the magnitude of the reduction in feed intake ranges from -4 to -77%. With infections defined as sub-clinical, there is a less consistent response with several studies showing no significant effect and others showing reductions of between -24 and -27% (Ames et al., 1969; Randall and Gibbs, 1981).

An important observation is that there does not appear to be a strong quantitative association between worm burdens at necropsy and the magnitude of the reduction in feed intake (Table 1.8). Relatively small burdens – 710 *O. ostertagi* and 5555 *C. oncophora* – were associated with a 24% reduction in intake in one study (Randall and Gibbs, 1981) whilst in the remainder, worm burdens associated with inappetence were generally of the order of 10^4 and in some cases 10^5. This suggests that factors other than the intensity of parasitism can modify the expression of appetite in infected animals. For example, the immune response has previously been shown to be a key

_____

element as appetite depression was effectively abolished in heavily parasitized lambs treated with immunosuppressants, but inappetence was present in untreated lambs with relatively small worm burdens (Greer et al., 2005b).

_____

Table 1.8. Summary of Effects on Feed Intake in Cattle relative to Worm Burdens.

Infection			Necropsy counts		Parasitism	Effect on Intake	Reference
Natural	**Treatment**	**Induced**	*O. ostertagi*	*C. oncophora*			
		1x 60,000 L$_3$ *O. ostertagi* & *C. oncophora*	710	5555	1	-24%	(Randall and Gibbs, 1981)
		1x 600,000 L$_3$ *O. ostertagi* & *C. oncophora*	5960	50,045	3	-58%	
Ostertagia Cooperia	Untreated (1x salvage)		247,100*	-	3		(Entrocasso et al., 1986)
Ostertagia Cooperia	Fenbendazole every 2 weeks		0*	-	1	NS	
Ostertagia Cooperia	Morantel bolus at turnout		3200*	-	1	NS	
Ostertagia Cooperia	Untreated		218,530	15,750	3	-18%	(Bell et al., 1988)
Ostertagia Cooperia	Morantel bolus at turnout		27,979	1875	1		
		42x 2000 L$_3$ *O. ostertagi*/day	33,750*(d42) 8117 (d84)		2/3	NS	(Taylor et al., 1989)
	Morantel bolus at start	42x 2000 L$_3$ *O. ostertagi*/day	16,000*(d42) 243 (d84)		2	NS	
Ostertagia Cooperia	Untreated		9889	-	1	NS	(Bell et al., 1990)
Ostertagia Cooperia	Morantel bolus at turnout		4915	-	1	NS	

	42x 2,000 L$_3$ *O. ostertagi*/day 42x 10,000 L$_3$ *C. oncophora*/day	19,983**	16,017**	3	-4%	(Parkins et al., 1990)
Morantel bolus at start	42x 2,000 L$_3$ *O. ostertagi*/day 42x 10,000 L$_3$ *C. oncophora*/day	1457**	386**	2	NS	
	Uninfected control	0	-	0		(Xiao and Gibbs, 1992)
	98x 1,428 L$_3$ *O. ostertagi*/day	21,144	-	1	NS	
	98x 14,285 L$_3$ *O. ostertagi*/day	74,010	-	3	-50%	
	Uninfected control	0	-	0		(Fox et al., 2002)
	1x 200,000 L$_3$ *O .ostertagi*	14,516		2	-38%	

*   One animal per group only.

**   Counts on Day 84; higher counts 54,200-71,800 *O. ostertagi* and 6,800-128,200 *C. oncophora* on Days 21 & 42.

.

Conclusions

Overall, it can be concluded that under a variety of experimental conditions, clinical, and in some circumstances, sub-clinical PGE associated with *O. ostertagi* +/- *C. oncophora* in calves <1 year of age can result in significant inappetence with affected animals eating between 23-96% of the intake of their respective controls.

Only one study was conducted with adult (dairy) cows and no significant effect on feed intake was observed (Fox et al., 1985), although there was evidence that, despite anthelmintic treatment, the treated group still had patent infections, which could have reduced the potential differences in the responses between the two groups of cows.

In the only study in which herbage intake was estimated in cattle grazing pastures (Bell et al., 1988), there were no significant effects of subclinical PGE on feed intake from June to August, (nor any effects on liveweight), although the control group had significantly elevated plasma pepsinogen values over this period. However, an 18% reduction in intake was reported in the control calves when they had clinical PGE in September. The morantel-treated animals showed no clinical signs of PGE, yet worm counts at necropsy indicated that they had burdens of >25,000 *O. ostertagi*, so it is possible that their appetite was also impaired and this could have lessened any differences between the two groups. Other considerations that limit the value of this study are that the two groups of cattle grazed two separate pastures throughout the grazing season, therefore there could have been cumulative confounding effects on the swards in each paddock, and furthermore there was no replication of the paddocks. In addition, nutritional interactions were present insofar as there was a shortage of grass in September, which led to a severe loss in liveweight in both groups and this was shortly followed by the outbreak of clinical PGE in the control group.

1.8. Grazing behaviour in ruminants parasitized with gastrointestinal nematodes

The role played by parasites in modifying host behaviour has been documented extensively, particularly in the literature on ecology and evolutionary biology (Moore, 2002), but in this literature the importance of host inappetence as a mediator of some of the consequences of parasitism appears often to have been overlooked. Equally in the veterinary and agricultural literature, there are surprisingly few references to the interaction between PGE in ruminants and reduced feed intake under pastoral conditions.

_____

In one study on the parasitological significance of grazing behaviour in cattle the author concluded that selective grazing mitigates the ingestion of lungworm (*Dictyocaulus viviparus*) larvae from pasture: no details were provided on quantitative aspects of herbage intake nor on the parasitological status of the animals (Michel, 1955).

There have been a number of studies in sheep in which several aspects of grazing behaviour and parasitism have been examined, variables have included: parasite status (infected, immune and unparasitized); feed motivation (*ad lib* or 60% of requirements prior to behavioural measurements); different levels and patterns of faecal contamination of pasture and various components of the sward such as height and composition. The main objectives of these experiments were to study aspects of optimal foraging, trade-offs between nutrient intake and faecal avoidance and the influence of parasite status in sheep in grazing behaviour. Most of these studies were relatively short term and some were conducted inside using artificial 'sward boards'; generally inappetence *per se* did not appear to be a strong focus, though it was frequently mentioned in the discussions (Hutchings et al., 1998; Hutchings et al., 1999; Cooper et al., 2000; Hutchings et al., 2000a; Hutchings et al., 2000b; Hutchings et al., 2001a; Hutchings et al., 2001b; Hutchings et al., 2002; Hutchings et al., 2003; Hutchings et al., 2007).

In two studies in which data were collected in free-ranging sheep, grazing time was measured. In one no significant differences were observed between immune, parasitized and non-parasitized sheep (Hutchings et al., 2001a); in the other study, the parasitized sheep grazed for significantly less time than the uninfected controls (590 vs. 629 minutes/day $P<0.05$) and herbage intake was correspondingly reduced (1.17 vs. 1.68 kg DM/day $P<0.01$) (Hutchings et al., 2000a).

1.9. Overall Conclusion

It is clear from the previous sections of this literature review that, whilst the importance of reduced feed intake in bovine gastrointestinal parasitism is well recognised, very little information is available on the manifestation of inappetence under field conditions, particularly in terms of grazing behaviour, nor is the neuroendocrine basis for the observed decrease in appetite precisely known. A more complete understanding of these subjects will not only allow veterinarians, parasitologists and farmers to understand the importance and mechanism for poor

_____

animal performance in naturally parasitized animals and the rationale for its prevention, but it may also lead to novel methods for mitigation of such effects.

_____

1.10. References

Ames, E.R., Rubin, R., Matsushima, J.K., 1969, Effects of gastrointestinal nematode parasites on performance in feed lot cattle. J Anim Sci 28, 698-704.

Angus, K.W., Coop, R.L., Sykes, A.R., 1979, the rate of recovery of intestinal morphology following anthelmintic treatment of parasitised sheep. Res Vet Sci 26, 120-122.

Armour, J., Bairden, K., Holmes, P.H., Parkins, J.J., Ploeger, H., Salman, S.K., McWilliam, P.N., 1987, Pathophysiological and parasitological studies on *Cooperia oncophora* infections in calves. Res Vet Sci 42, 373-381.

Armour, J., Jennings, F.W., Murray, M., Selman, I., 1973. Bovine Ostertagiasis. In: Helminth Diseases of Cattle, Sheep and Horses in Europe, University of Glasgow, pp. 11-22.

Baker, R.D., 2004, Estimating herbage intake from animal performance, In: P.D.Penning (Ed.) Herbage Intake Handbook. British Grassland Society.

Banks, W., Tschop, M., Robinson, S., Heiman, M., 2002, Extent and Direction of Ghrelin Transport Across the Blood Brain Barrier is Determined by its unique Primary Structure. J Pharmacol Exp Ther 302, 822-827.

Bargo, F., Muller, L.D., Delahoy, J.E., Cassidy, T.W., 2002a, Milk Response to Concentrate Supplementation of High Producing Dairy Cows Grazing at Two Pasture Allowances. J Dairy Sci 85, 1777-1792.

Bargo, F., Muller, L.D., Delahoy, J.E., Cassidy, T.W., 2002b, Performance of High Producing Dairy Cows with Three Different Feeding Systems Combining Pasture and Total Mixed Rations. J Dairy Sci 85, 2948-2963.

Bargo, F., Muller, L.D., Kolver, E.S., Delahoy, J.E., 2003, Invited Review: Production and Digestion of Supplemented Dairy Cows on Pasture. J Dairy Sci 86, 1-42.

Bartha, T., Sayed-Ahmed, A., Rudas, P., 2005, Expression of leptin and its receptors in various tissues of ruminants. Domest Anim Endocrinol 29, 193-202.

Bell, S.L., Thomas, R.J., Ferber, M.T., 1988, Feed intake of grazing calves exposed to trichostrongyle infection and treated with the morantel sustained release bolus. Vet Parasitol 28, 125-135.

Bell, S.L., Thomas, R.J., Ferber, M.T., 1990, Appetite, digestive efficiency, feed utilization and carcass evaluation of housed calves naturally infected with gastrointestinal nematodes. Vet Parasitol 34, 323-333.

Bradford, B.J., Allen, M.S., 2008, Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows. Domest Anim Endocrinol 34, 196-203.

Cawdery, M.J., Conway, A., 1971, Production effects of the liver fluke, *Fasciola hepatica*, on beef cattle. Vet Rec 89, 641-643.

Chaudhri, O., Small, C., Bloom, S., 2006, Gastrointestinal hormones regulating appetite. Philos Trans R Soc Lond B Biol Sci 361, 1187-1209.

_____

Chilliard, Y., Delavaud, C., Bonnet, M., 2005, Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Domest Anim Endocrinol 29, 3-22.

Church, D.C. 1988. The Ruminant Animal, Digestive Physiology and Nutrition (Prentice Hall), p. 564.

Colditz, I.G., 2002, Effects of the immune system on metabolism: implications for production and disease resistance in livestock. Livest Prod Sci 75, 257-268.

Coop, R.L., Field, A.C., 1983, Effect of phosphorus intake on growth rate, food intake and quality of the skeleton of growing lambs infected with the intestinal nematode *Trichostrongylus vitrinus*. Res Vet Sci 35, 175-181.

Coop, R.L., Sykes, A.R., Angus, K.W., 1977, The effect of a daily intake of *Ostertagia circumcincta* larvae on body weight, food intake and concentration of serum constituents in sheep. Res Vet Sci 23, 76-83.

Cooper, J., Gordon, I.J., Pike, A.W., 2000, Strategies for the avoidance of faeces by grazing sheep. Appl Anim Behav Sci 69, 15-33.

Dargie, J.D., Berry, C.I., Parkins, J.J., 1979, The pathophysiology of ovine fascioliasis: studies on the feed intake and digestibility, body weight and nitrogen balance of sheep given rations of hay or hay plus a pelleted supplement. Res Vet Sci 26, 289-295.

Delagarde, R., Peyraud, J.L., Delaby, L., Faverdin, P., 2000, Vertical distribution of biomass, chemical composition and pepsin--cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Anim Feed Sci Technol 84, 49-68.

Delavaud, C., Ferlay, A., Faulconnier, Y., Bocquier, F., Kann, G., Chilliard, Y., 2002, Plasma leptin concentration in adult cattle: effects of breed, adiposity, feeding level, and meal intake. J Anim Sci 80, 1317-1328.

Dove, H., Mayes, R.W., 1991, The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. Aust.J.Agric.Res 42, 913-952.

Dynes, R.A., Ankersmit, A.E.L., Poppi, D.P., Barrell, G.K., Sykes, A.R., 1990, Studies on the physiological basis of appetite depression in nematode infection in sheep. Proc NZ Soc Anim Prod 50, 249-253.

Entrocasso, C.M., Parkins, J.J., Armour, J., Bairden, K., McWilliam, P.N., 1986, Metabolism and growth in housed calves given a morantel sustained release bolus and exposed to natural trichostrongyle infection. Res Vet Sci 40, 65-75.

Exton, M.S., 1997, Infection-Induced Anorexia: Active Host Defence Strategy. Appetite 29, 369-383.

Fantuzzi, G., Faggioni, R., 2000, Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol 68, 437-446.

Forbes, J.M., 1995, Voluntary Food Intake and Diet Selection in Farm Animals. CAB International, Wallingford, 532 p.

_____

Forbes, J.M., 1999, Minimal total discomfort as a concept for the control of food intake and selection. Appetite 33, 371.

Forbes, J.M., 2001, Consequences of feeding for future feeding. Comp Biochem Physiol A Mol Integr Physiol 128, 463-470.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Gale, D.L., 1989a, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. Res Vet Sci 47, 294-298.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Simmonds, A.D., 1989b, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on the hormonal control of digestive and metabolic function. Res Vet Sci 47, 299-304.

Fox, M.T., Gerrelli, D., Shivalkar, P., Jacobs, D.E., 1989c, Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. Res Vet Sci 46, 280-282.

Fox, M.T., Hutchinson, M., Riddle, A., Forbes, A.B., 2007, Epidemiology of subclinical dairy cow nematode infections on five farms in England in 2002 and a comparison with results from 1978 to 1979. Vet Parasitol 146, 294-301.

Fox, M.T., Jacobs, D.E., 1980, Factors influencing uptake of nematode larvae in adult dairy cattle during the grazing season and sources of pasture contamination. Vet Rec 107, 575-578.

Fox, M.T., Jacobs, D.E., Campling, R.C., Pocknee, B.R., Clampitt, R., Hart, I.C., 1985, Effect of thiabendazole treatment on feed intake, digestibility and selected blood values in lactating dairy cows. Vet Rec 116, 257-260.

Fox, M.T., Reynolds, G.W., Scott, I., Simcock, D.C., Simpson, H.V., 2006, Vagal and splanchnic afferent nerves are not essential for anorexia associated with abomasal parasitism in sheep. Vet Parasitol 135, 287-295.

Fox, M.T., Uche, U.E., Vaillant, C., Ganabadi, S., Calam, J., 2002, Effects of *Ostertagia ostertagi* and omeprazole treatment on feed intake and gastrin-related responses in the calf. Vet Parasitol 105, 285-301.

Freer, M., Dove, H. 2002. Sheep Nutrition (CSIRO/CABI), p. 385.

Friedman, J.M., Halaas, J.L., 1998, Leptin and the regulation of body weight in animals. Nature 395, 763-770.

Fukumoto, K., Nakahara, K., Katayama, T., Miyazatao, M., Kangawa, K., Murakami, N., 2008, Synergistic action of gastrin and ghrelin on gastric acid secretion in rats. Biochem Biophysical Res Commun 374, 60-63.

Garcia, M.R., Amstalden, M., Williams, S.W., Stanko, R.L., Morrison, C.D., Keisler, D.H., Nizielski, S.E., Williams, G.L., 2002, Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J Anim Sci 80, 2158-2167.

Gentry, P., Willey, J., Collier, R., 2003, Ghrelin, a growth hormone secretagogue, is expressed by bovine rumen J Anim Sci 81, Suppl 1, 123.

_____

Gibb, M., Huckle, C., Nuthall, R., 2002a, Effects of level of concentrate supplementation on grazing behaviour and performance by lactating dairy cows grazing continuously stocked grass swards. Anim Sci 74, 319-335.

Gibb, M.J., 1996. Technology for animal grazing/intake studies. In: Occasional Publication No.3, Proceedings of a Workshop for Concerted Action, Dublin.

Gibb, M.J., 1998. Animal Grazing/Intake Terminology and Definitions. In: Pasture Ecology and Animal Intake, Dublin, pp. 21-35.

Gibb, M.J., Huckle, C.A., Nuthall, R., 1998, Effect of time of day on grazing behaviour and intake rate by lactating dairy cows. Grass Forage Sci 53, 41-46.

Gibb, M.J., Huckle, C.A., Nuthall, R., 2002b, Effect of type of supplement offered out of parlour on grazing behaviour and performance by lactating dairy cows grazing continuously stocked grass swards. Anim Sci 75, 153-167.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1997, Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass Forage Sci 52, 309-321.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1999, The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl Anim Behav Sci 63, 269-287.

Gordon, I.J., Illius, A.W., 1988, Incisor arcade structure and diet selection in ruminants. Funct Ecol 2, 15-22.

Greenwood, G.B., Demment, M.W., 1988, The effect of fasting on short-term cattle grazing behaviour. Grass Forage Sci 43, 377-386.

Greer, A.W., McAnulty, R.W., Stankiewicz, M., Sykes, A.R., 2005a, Corticosteroid treatment prevents the reduction in food intake and growth in lambs infected with the abomasal parasite *Teladorsagia circumcincta*. Proc NZ Soc Anim Prod 65, 9-13.

Greer, A.W., Stankiewicz, M., Jay, N.P., McAnulty, R.W., Sykes, A.R., 2005b, The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunologically naive and competent sheep. Anim Sci 80, 89-99.

Grouselle, D., Chaillou, E., Caraty, A., Bluet-Pajot, M.-T., Zizzari, P., Tillet, Y., Epelbaum, J., 2008, Pulsatile Cerebrospinal Fluid and Plasma Ghrelin in Relation to Growth Hormone Secretion and Food Intake in the Sheep. J Neuroendocrinol 20, 1-9.

Hafez, E.S.E., Schein, M.W., Ewbank, R., 1969, The Behaviour of Cattle, In: Hafez, E.S.E. (Ed.) The Behaviour of Domestic Animals. Baillière, Tindall & Cassell, London.

Hancock, J., 1953, Grazing behaviour of cattle. Animal Breeding Abstracts 21, 1-13.

Hart, B.L., 1990, Behavioral adaptations to pathogens and parasites: five strategies. Neurosci Biobehav Rev 14, 273-294.

_____

Hayashida, T., Murakami, K., Mogi, K., Nishihara, M., Nakazato, M., Mondal, M.S., Horii, Y., Kojima, M., Kangawa, K., Murakami, N., 2001, Ghrelin in domestic animals: distribution in stomach and its possible role. Domest Anim Endocrinol 21, 17-24.

Hodgson, J., 1990, Grazing Management. Longman Scientific & Technical, 203 p.

Hossner, K., 1998, Cellular, molecular and physiological aspects of leptin: Potential application in animal production. Can J Anim Sci 78, 463-472.

Houdijk, J.G., Jessop, N.S., Kyriazakis, I., 2001, Nutrient partitioning between reproductive and immune functions in animals. Proc Nutr Soc 60, 515-525.

Hutchings, M., Gordon, I., Kyriazakis, I., Robertson, E., Jackson, F., 2002, Grazing in heterogeneous environments: infra- and supra-parasite distributions determine herbivore grazing decisions. Oecologia 132, 453-460.

Hutchings, M.R., Athanasiadou, S., Kyriazakis, I., Gordon, I.J., 2003, Can animals use foraging behaviour to combat parasites? Proc Nutr Soc 62, 361-370.

Hutchings, M.R., Gordon, I.J., Kyriazakis, I., Jackson, F., 2001a, Sheep avoidance of faeces-contaminated patches leads to a trade-off between intake rate of forage and parasitism in subsequent foraging decisions. Anim Behav 62, 955-964.

Hutchings, M.R., Gordon, I.J., Robertson, E., Kyriazakis, I., Jackson, F., 2000a, Effects of parasitic status and level of feeding motivation on the diet selected by sheep grazing grass/clover swards. J Agric Sci 135, 65-75.

Hutchings, M.R., Knowler, K.J., McAnulty, R., McEwan, J.C., 2007, Genetically resistant sheep avoid parasites to a greater extent than do susceptible sheep. Proc Biol Sci 274, 1839-1844.

Hutchings, M.R., Kyriazakis, I., Anderson, D.H., Gordon, I.J., Coop, R.L., 1998, Behavioural strategies used by parasitised and non-parasitised sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. Anim Sci 67, 97-106.

Hutchings, M.R., Kyriazakis, I., Gordon, I.J., 2001b, Herbivore physiological state affects foraging trade-off decisions between nutrient intake and parasite avoidance. Ecology 82, 1138-1150.

Hutchings, M.R., Kyriazakis, I., Gordon, I.J., Jackson, F., 1999, Trade-offs between nutrient intake and faecal avoidance in herbivore foraging decisions: the effect of animal parasitic status, level of feeding motivation and sward nitrogen content. J Anim Ecol 68, 310-323.

Hutchings, M.R., Kyriazakis, I., Papachristou, T.G., Gordon, I.J., Jackson, F., 2000b, The herbivores' dilemma: trade-offs between nutrition and parasitism in foraging decisions. Oecologia 124, 242-251.

Ingvartsen, K.L., Boisclair, Y.R., 2001, Leptin and the regulation of feed intake, energy homeostasis and immunity with special focus on periparturient ruminants. Domest Anim Endocrinol 21, 215-250.

_____

Jennings, F.W., Armour, J., Lawson, D.D., R., R., 1966, Experimental *Ostertagia ostertagi* in calves: Studies with abomasal cannulas. Am J Vet Res 27, 1249-1257.

Johnson, R.W., 1998, Immune and endocrine regulation of food intake in sick animals. Domest Anim Endocrinol 15, 309-319.

Johnson, R.W., 2002, The concept of sickness behavior: a brief chronological account of four key discoveries. Vet Immunol and Immunopathol 87, 443-450.

Kalra, S., Dube, M., Pu, S., Xu, B., Horvath, T., Kalra, P., 1999, Interacting Appetite-Regulating Pathways in the Hyopthalamic Regulation of Body Weight. Endocrine Rev 20, 68-100.

Kimambo, A.E., MacRae, J.C., Dewey, P.J., 1988, The effect of daily challenge with *Trichostrongylus colubriformis* larvae on the nutrition and performance of immunologically-resistant sheep. Vet Parasitol 28, 205-212.

Kolver, E.S., Muller, L.D., 1998, Performance and nutrient intake of high producing Holstein cows consuming pasture or a total mixed ration. J Dairy Sci 81, 1403-1411.

Konsman, J.P., Parnet, P., Dantzer, R., 2002, Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci 25, 154-159.

Kopin, A., S., Lee, Y., M., McBride, E.W., Miller, L.J., Lu, M., Lin, H.Y., Kolakowski, L.F., Jr., Beinborn, M., 1992, Expression cloning and characterization of the canine parietal cell gastrin receptor. Proc Natl Acad Sci USA 89, 3605-3609

Krysl, L.J., Hess, B.W., 1993, Influence of Supplementation on Behaviour of Grazing Cattle. J Anim Sci 71, 2546-2555.

Kulcsár, M., Jánosi, S., Lehtolainen, T., Kátai, L., Delavaud, C., Balogh, O., Chilliard, Y., Pyörälä, S., Rudas, P., Huszenicza, G., 2005, Feeding-unrelated factors influencing the plasma leptin level in ruminants. Domest Anim Endocrinol 29, 214-226.

Kyriazakis, I., Anderson, D.H., Oldham, J.D., Coop, R.L., Jackson, F., 1996, Long-term subclinical infection with *Trichostrongylus colubriformis:* effects on food intake, diet selection and performance of growing lambs. Vet Parasitol 61, 297-313.

Kyriazakis, I., Oldham, J.D., Coop, R.L., Jackson, F., 1994, The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. Br J Nutr 72, 665-677.

Kyriazakis, I., Tolkamp, B.J., Hutchings, M.R., 1998, Towards a functional explanation for the occurrence of anorexia during parasitic infections. Anim Behav 56, 265-274.

Laca, WallisDeVries, 2000, Acoustic measurement of intake and grazing behaviour of cattle. Grass Forage Sci 55, 97-104.

_____

Lantinga, E.A., Neuteboom, J.H., Meijs, J.A.C., 2004, Sward Methods, In: P.D.Penning (Ed.) Herbage Intake Handbook. British Grassland Society, pp. 23-52.

Lewis, R.M., Magadlela, A.M., Jessop, N.S., Emmans, G.C., 2003, The ability of the n-alkane technique to estimate intake and diet choice of sheep. Anim Sci 77, 319-327.

Leyva, V., Henderson, A., Sykes, A.R., 1982, Effect of daily infection with *Ostertagia circumcincta* larvae on food intake, milk production and wool growth in sheep. J Agric Sci 99, 249-259.

Liefers, S.C., Veerkamp, R.F., te Pas, M.F.W., Delavaud, C., Chilliard, Y., van der Lende, T., 2003, Leptin Concentrations in Relation to Energy Balance, Milk Yield, Intake, Live Weight, and Estrus in Dairy Cows. J Dairy Sci 86, 799-807.

Lochmiller, R.L., Deerenberg, C., 2000, Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88, 87-98.

Marsh, R., Campling, R.C., 1970, Fouling of pastures by dung. Herbage Abstracts 40, 123-130.

McDonald, P., Edwards, R.A., Greenhalgh, J.F.D., Morgan, C.A., 2002, Animal Nutrition. Prentice Hall, 693 p.

McKellar, Q., Duncan, J.L., Armour, J., Lindsay, F.E., McWilliam, P., 1987, Further studies on the response to transplanted adult *Ostertagia ostertagi* in calves. Res Vet Sci 42, 29-34.

McKellar, Q., Duncan, J.L., Armour, J., McWilliam, P., 1986, Response to transplanted adult *Ostertagia ostertagi* in calves. Res Vet Sci 40, 367-371.

Meeusen, E.N.T., 1999, Immunology of helminth infections, with special reference to immunopathology. Vet Parasitol 84, 259-273.

Meister, B., 2007, Neurotransmitters in key neurons of the hypothalamus that regulate feeding behavior and body weight. Physiol Behav 92, 263-271.

Mendieta-Zerón, H., López, M., Diéguez, C., 2008, Gastrointestinal peptides controlling body weight homeostasis. Gen Comp Endocrinol 155, 481-495.

Michel, J.F., 1955, Parasitological significance of bovine grazing behaviour. Nature 175, 1088-1089.

Miura, H., Tsuchiya, N., Sasaki, I., Kikuchi, M., Kojima, M., Kangawa, K., Hasegawa, Y., Ohnami, Y., 2004, Changes in plasma ghrelin and growth hormone concentrations in mature Holstein cows and three-month-old calves. J Anim Sci 82, 1329-1333.

Moore, J., 2002, Parasites and the Behavior of Animals, 1st Edition. Oxford University Press, 315 p.

Moran, T.H., 2004, Gut peptides in the control of food intake: 30 years of ideas. Physiol Behav 82, 175-180.

_____

Murphy, K., Bloom, S., 2006, Gut hormones and the regulation of energy homeostasis. Nature 444, 854-859.

Mutch, D.M., Clément, K., 2006, Unravelling the Genetics of Human Obesity. PLoS Genetics 2, 1956-1963.

Näslund, E., Hellström, P.M., 2007, Appetite signaling: From gut peptides and enteric nerves to brain. Physiol Behav 92, 256-262.

Olszewski, P.K., Cedernaes, J., Olsson, F., Levine, A.S., Schiöth, H.B., 2008, Analysis of the network of feeding neuroregulators using the Allen Brain Atlas. Neurosci Biobehav Rev 32, 945-956.

Orr, R.J., Rutter, S.M., Penning, P.D., Rook, A.J., 2001, Matching grass supply to grazing patterns for dairy cows. Grass Forage Sci 56, 352-361.

Orr, R.J., Rutter, S.M., Yarrow, N.H., Champion, R.A., Rook, A.J., 2004, Changes in ingestive behaviour of yearling dairy heifers due to changes in sward state during grazing down of rotationally stocked ryegrass or white clover pastures. Appl Anim Behav Sci 87, 205-222.

Parkins, J.J., Taylor, L.M., Holmes, P.H., Bairden, K., Salman, S.K., Armour, J., 1990, Pathophysiological and parasitological studies on a concurrent infection of *Ostertagia ostertagi* and *Cooperia oncophora* in calves. Res Vet Sci 48, 201-208.

Penning, P.D., 1983, A technique to record automatically some aspects of the grazing and ruminating behaviour in sheep. Grass Forage Sci 38, 89-96.

Penning, P.D., 2004a, Animal-based techniques for estimating herbage intake In: P.D.Penning (Ed.) Herbage Intake Handbook. British Grassland Society, p. 191.

Penning, P.D. 2004b. Herbage Intake Handbook (British Grassland Society), p. 191.

Penning, P.D., Rutter, S.M., 2004, Ingestive Behaviour, In: P.D.Penning (Ed.) Herbage Intake Handbook. British Grassland Society, p. 191.

Penning, P.D., Steel, G.L., Johnson, R.H., 1984, Further development and use of an automatic recording system in sheep grazing studies. Grass Forage Sci 39, 345-351.

Phillips, C.J.C., 1993, Cattle Behaviour, 1st Edition. Farming Press.

Poulin, R., 1995, "Adaptive" changes in the behaviour of parasitized animals: A critical review. Int J Parasitol 25, 1371-1383.

Pulido, R.G., Leaver, J.D., 2003, Continuous and rotational grazing of dairy cows - the interactions of grazing system with level of milk yield, sward height and concentrate level. Grass Forage Sci 58, 265-275.

Purewal, A., Fox, M.T., Shivalkar, P., Carroll, A.P., Uche, U.E., Vaillant, C., Watkinson, A., 1997, Effects of *Ostertagia ostertagi* on gastrin gene expression and gastrin-related responses in the calf. J Physiol 498, 809-816.

_____

Randall, R.W., Gibbs, H.C., 1981, Effects of clinical and subclinical gastrointestinal helminthiasis on digestion and energy metabolism in calves. Am J Vet Res 42, 1730-1734.

Roberts, H.C., Hardie, L.J., Chappell, L.H., Mercer, J.G., 2000, Parasite-induced anorexia: leptin, insulin and corticosterone responses to infection with the nematode, *Nippostrongylus brasiliensis*. Parasitology 118, 117-123.

Roche, J.R., Sheahan, A.J., Chagas, L.M., Berry, D.P., 2006, Short Communication: Genetic Selection for Milk Production Increases Plasma Ghrelin in Dairy Cows. J Dairy Sci 89, 3471-3475.

Roche, J.R., Sheahan, A.J., Chagas, L.M., Berry, D.P., 2007, Concentrate Supplementation Reduces Postprandial Plasma Ghrelin in Grazing Dairy Cows: A Possible Neuroendocrine Basis for Reduced Pasture Intake in Supplemented Cows. J Dairy Sci 90, 1354-1363.

Rook, A.J., Huckle, C.A., 1997, Activity bout criteria for grazing dairy cows. Appl Anim Behav Sci 54, 89-96.

Rook, A.J., Huckle, C.A., Penning, P.D., 1994, Effects of sward height and concentrate supplementation on the ingestive behaviour of spring-calving dairy cows grazing grass-clover swards. Appl Anim Behav Sci 40, 101-112.

Ross, J.G., Todd, J.R., Purcell, D.A., 1970, Nutritional balance trials with calves experimentally infected with *Trichostrongylus axei*. Br Vet J 126, 393-400.

Rutter, S.M., 2000, Graze: a program to analyze recordings of the jaw movements of ruminants. Behav Res Methods Instrum Comput 32, 86-92.

Rutter, S.M., Champion, R.A., Penning, P.D., 1997, An automatic system to record foraging behaviour in free-ranging ruminants. Appl Anim Behav Sci 54, 185-195.

Rutter, S.M., Orr, R.J., Penning, P.D., Yarrow, N.H., Champion, R.A., 2002, Ingestive behaviour of heifers grazing monocultures of ryegrass or white clover. Appl Anim Behav Sci 76, 1-9.

Sandberg, F.B., Emmans, G.C., Kyriazakis, I., 2006, A model for predicting feed intake of growing animals during exposure to pathogens. J Anim Sci 84, 1552-1566.

Sansinanea, A.S., Cerone, S.I., Zonco, I., García, C., Auza, N., 2001, Serum leptin levels in cattle with different nutritional conditions. Nutr Res 21, 1045-1052.

Sheldon, B.C., Verhultst, S., 1996, Ecological immunology: costly parasite defences and trade-offs in evolutionary ecology. Trends Ecol Evol 11, 317-321.

Simcock, D.C., Joblin, K.N., Scott, I., Burgess, D.M., Rogers, C.W., Pomroy, W.E., Simpson, H.V., 1999, Hypergastrinaemia, abomasal bacterial population densities and pH in sheep infected with *Ostertagia circumcincta*. Int J Parasitol 29, 1053-1063.

Simpson, H.V., 2000, Pathophysiology of abomasal parasitism: is the host or parasite responsible? Vet J 160, 177-191.

_____

Small, C.J., Bloom, S.R., 2004, Gut hormones and the control of appetite. Trends Endocrinol Metabol 15, 259-263.

Smith, G., 2000, The Controls of Eating: A Shift from Nutritional Homeostasis to Behavioral Neuroscience. Nutrition 16, 814-820.

Smith, M.S., Grove, K.L., 2002, Integration of the regulation of reproductive function and energy balance: lactation as a model. Front Neuroendocrinol 23, 225-256.

Stephens, D., Brown, J., Ydenberg, R. 2007. Neuroendocrine Mechanisms of Energy Regulation in Mammals. In Foraging Behavior & Ecology, Woods, S., Castonguay, T., eds. (The University of Chicago Press), pp. 223-230.

Stricklin, W.R., Wilson, L.L., Graves, H.B., 1976, Feeding behavior of Angus and Charolais-Angus Cows During Summer and Winter. J Anim Sci 43, 721-732.

Sugino, T., Hasegawa, Y., Kurose, Y., Kojima, M., Kangawa, K., Terashima, Y., 2004, Effects of ghrelin on food intake and neuroendocrine function in sheep. Anim Reprod Sci 82-83, 183-194.

Sykes, A.R., Coop, R.L., 1977, Intake and utilisation of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. J Agric Sci. 88, 671-677.

Symons, L.E., 1985, Anorexia: occurrence, pathophysiology, and possible causes in parasitic infections. Adv Parasitol 24, 103-133.

Taylor, L.M., Parkins, J.J., Armour, J., Holmes, P.H., Bairden, K., Ibarra-Silva, A.M., Salman, S.K., McWilliam, P.N., 1989, Pathophysiological and parasitological studies on *Ostertagia ostertagi* infections in calves. Res Vet Sci 46, 218-225.

Ungar, E.D., Rutter, S.M., 2006, Classifying cattle jaw movements: Comparing IGER Behaviour Recorder and acoustic techniques. Appl Anim Behav Sci 98, 11-27.

Vadiveloo, J., Holmes, W., 1979, The prediction of the voluntary feed intake of dairy cows. J Agric Sci 93, 553-562.

Vagenas, D., Bishop, S.C., Kyriazakis, I., 2007a, A model to account for the consequences of host nutrition on the outcome of gastrointestinal parasitism in sheep: logic and concepts. Parasitology 134, 1263-1277.

Vagenas, D., Bishop, S.C., Kyriazakis, I., 2007b, A model to account for the consequences of host nutrition on the outcome of gastrointestinal parasitism in sheep: model evaluation. Parasitology 134, 1279-1289.

van Adrichem, P.W., Shaw, J.C., 1977a, Effects of gastrointestinal nematodiasis on the productivity of monozygous twin cattle. I. Growth performance. J Anim Sci 45, 417-422.

van Adrichem, P.W., Shaw, J.C., 1977b, Effects of gastrointestinal nematodiasis on the productivity of monozygous twin cattle. II. Growth performance and milk production. J Anim Sci 45, 423-429.

van Soest, P.J., 1994, Nutritional Ecology of the Ruminant. Cornell University, 476 p.

Viney, M.E., Riley, E.M., Buchanan, K.L., 2005, Optimal immune responses: immunocompetence revisited. Trends Ecol Evol 20, 665-669.

_____

WallisDeVries, M.F., Laca, E.A., Demment, M.W., 1998, From feeding station to patch: scaling up food intake measurements in grazing cattle. Appl Anim Behav Sci 60, 301-315.

Wertz-Lutz, A.E., Daniel, J.A., Clapper, J.A., Trenkle, A., Beitz, D.C., 2008, Prolonged, moderate nutrient restriction in beef cattle results in persistently elevated circulating ghrelin concentrations. J Anim Sci 86, 564-575.

Wertz-Lutz, A.E., Knight, T.J., Pritchard, R.H., Daniel, J.A., Clapper, J.A., Smart, A.J., Trenkle, A., Beitz, D.C., 2006, Circulating ghrelin concentrations fluctuate relative to nutritional status and influence feeding behavior in cattle. J Anim Sci 84, 3285-3300.

Woodward, S.J.R., 1998, Bite mechanics of cattle and sheep grazing grass-dominant swards. Appl Anim Behav Sci 56, 203-222.

Xiao, L., Gibbs, H.C., 1992, Nutritional and pathophysiologic effects of clinically apparent and subclinical infections of *Ostertagia ostertagi* in calves. Am J Vet Res 53, 2013-2018.

Yang, C., Gibbs, H.C., Xiao, L., Wallace, C.R., 1993, Prevention of pathophysiologic and immunomodulatory effects of gastrointestinal nematodiasis in calves by use of strategic anthelmintic treatments. Am J Vet Res 54, 2048-2055.

Yonekura, S., Kitade, K., Furukawa, G., Takahashi, K., Katsumata, N., Katoh, K., Obara, Y., 2002, Effects of aging and weaning on mRNA expression of leptin and CCK receptors in the calf rumen and abomasum. Domest Anim Endocrinol 22, 25-35.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I., 2008a, Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. J Anim Sci 86, 1891-1903.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I., 2008b, Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitized ewes of two breeds. Br J Nutr (accepted).

Zieba, D.A., Amstalden, M., Williams, G.L., 2005, Regulatory roles of leptin in reproduction and metabolism: A comparative review. Domest Anim Endocrinol 29, 166-185.

Objectives

_____

The preceding literature review has shown that grazing behaviour in cattle is an important determinant of feed intake and performance, which is therefore of economic, as well as scientific, interest. Modern technology has allowed grazing behaviour to be recorded and dissected in sophisticated ways, so that it is possible to study some of the intricate behaviour patterns and relationships that exist in the grazing animal.

Parasitic gastroenteritis is ubiquitous in grazing ruminants and is commonly present as a sub-clinical infection, but it is known, mainly from studies in housed animals, that PGE can cause inappetence, a reduction in feed intake and inferior performance. There is nevertheless a paucity of information and literature on the effects of PGE on grazing behaviour under natural conditions, as it relates to appetite and feed intake, in ruminants in general and cattle in particular. In addition, the relationship between the pathophysiology of PGE and the manifestation of inappetence is not fully understood and further studies to illuminate this relationship will allow for a greater understanding of the phenomenon.

The overall objective of this thesis is to determine if modern technologies for the study of grazing animal behaviour could be applied in cattle to determine the manifestations of inappetence in parasitized animals at pasture.

The specific objectives are:

- To determine if changes in grazing behaviour can account for productivity losses in young cattle, naturally infected with parasitic nematodes, but showing no overt clinical signs of parasitism (Chapter 2).

- To determine if changes in grazing behaviour can account for productivity losses in adult, lactating dairy cattle with naturally acquired subclinical parasitism and to compare the responses of lactating heifers and cows (Chapter 3).

- To evaluate the effect of different grazing practices on behavioural responses in adult dairy cows in the presence of subclinical parasitism (Chapter 4).

- To determine if subclinical parasitism in young cattle affects their dietary preference when grazing (Chapter 5).

- To explore various endocrine interactions in experimentally parasitized calves in order to help understand the cascade of events that could result in inappetence (Chapter 6).

Chapter 2: Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake, pasture characteristics and growth in young grazing cattle

_____

2.1. Introduction

Studies on the reduction in voluntary food intake in parasitized ruminants have been largely conducted in housed animals infected artificially with mono-specific infections of various nematodes (Sykes and Coop, 1976; Coop et al., 1979; Fox et al., 1989). Results from these and other workers lend support to the hypothesis that inappetence is one of the main factors which leads to reduced performance in ruminants with parasitic gastroenteritis, even when sub-clinically infected (Coop and Holmes, 1996). Quantitative trials on appetite in grazing ruminants are less easy to conduct, mainly because of the difficulties in measuring food intake in free-ranging animals. There have been some studies on behavioural responses in grazing, parasitized sheep, but these have not focussed on intake appetite *per se* (Hutchings et al., 1998) and there appear to be no papers on the quantitative effects of nematode parasitism on grazing behaviour or herbage intake in cattle. The development and refinement of an automatic system to record foraging behaviour in free-ranging ruminants (Penning, 1983; Penning et al., 1984; Rutter et al., 1997) has provided a valuable tool for field studies. This technology was used in association with faecal markers (Dove and Mayes, 1991) to monitor feeding behaviour and herbage intake in dairy heifers at pasture, naturally infected with parasitic nematodes.

2.2. Materials and Methods

Animals

Twenty autumn-born Holstein-Friesian female calves, with no previous experience of grazing and weighing approximately 200 kg at the start of the trial were used. All animals were trained to use the recording equipment prior to the first measurement period.

Allocation

The calves were ranked in order of live weight and blocked in pairs. Each animal within a pair was randomly assigned to one of two treatment groups. Within each experimental group, calves were ranked in order of descending live weight and paired to provide five pairs in each treatment group for assignment to the replicated measurement paddocks. Calves were paired because previous studies at the Institute of Grassland and Environmental Research (IGER) had shown that herd animals pastured individually do not express normal grazing behaviours because of interactions with other animals within the vicinity.

Treatment

One sustained-release bolus (IVOMEC® SR Bolus, Merial Animal Health) was administered to each heifer in the treated group on 21st April, (10 days before turnout). This bolus delivers 40 µg ivermectin per day over a period of 135 days. No anthelmintic treatments were given to the heifers in the untreated control group.

Pasture management

The animals were turned out onto pasture on 1st May and the study ended on 21st July. The field in which the study was conducted had been grazed by cattle from July to October of the previous year. To augment the population of nematodes on pasture, four Charolais cross heifers, each infected with 10,000 third stage larvae (L$_3$) of *Cooperia oncophora* and 10,000 L$_3$ of *Ostertagia ostertagi* were used to seed the pasture. They grazed the principal pastures from 24th March until 2nd June.

Untreated and treated groups were set stocked at an initial rate of 9.4 animals/ha on two adjacent, predominantly perennial ryegrass paddocks. The two groups grazed separate paddocks in order to minimise social facilitation of grazing behaviour and so that any differences in pasture quality could be evaluated. During intake and behaviour measurement periods, the calves were moved to a separate area divided into ten paddocks (one pair from its respective treatment group on each paddock). These small paddocks were managed by cutting to ensure similar sward height and structure at the start of the two measurement periods in May (11th-22nd) and July (6th-17th). Use of these paddocks provided replication and allowed the direct effect of parasitism on intake and behaviour to be separated from indirect cumulative effects resulting from differences in sward conditions in the principal paddocks.

Sampling and Measurements

Animals were weighed at the start and end of the trial and at 28-day intervals during the study. Faecal samples were collected from each animal *per rectum* shortly before turnout and thereafter at each weighing (every 28 days) and at the end of the trial in July. Faecal egg counts (fec) were carried out on each sample using the Improved, Modified McMaster technique, with a sensitivity of 50 eggs per gram (epg) (MAFF, 1986). The presence of lungworm larvae in faeces was demonstrated using the Baermann apparatus; quantification was not undertaken (MAFF, 1986). Larval culture was conducted on faeces bulked from samples from the July collection from the untreated heifers to identify the nematode species present.

_____

On 26th June, herbage samples were cut to ground level within five 15 cm x 15 cm quadrats located randomly on each of the two main paddocks, for assessment of the botanical composition of the sward. On three occasions during each of the two intake measurement periods, three quadrat samples were cut to ground level in each of the replicated paddocks for measurement of herbage Organic Matter (OM) mass.

Sward surface height (SSH) was measured in the replicated paddocks during each measurement period using a sward stick with a 1 cm × 2 cm 'window' (HFRO, 1986). Herbage intake was measured using n-alkane markers (Dove and Mayes, 1991) for two 5-day periods beginning 11th May and 6th July. Each animal was dosed with a controlled release n-alkane capsule 7 days before intake measurements commenced. Faecal samples were collected for n-alkane analysis by disturbing the animals and sampling fresh dung pats. Herbage samples representative of the material grazed by the animals were collected concurrently for n-alkane analysis.

Grazing behaviours were recorded over a 24-hour period using IGER jaw-movement recorders (Rutter et al., 1997) in the measurement paddocks during the week preceding the intake measurements. The recordings were analysed using the software 'Graze' (Rutter, 2000). Use of these recorders and associated software allowed for quantification and differentiation of total grazing time (TGT), eating time (TET) and ruminating time (TRT) and also parameters such as total grazing jaw movements (TGJM), bites, meals, feeding/ruminating bouts and the rates at which some of these activities took place (see Table 2.1). TET is TGT minus intra-meal intervals of <5 minutes, which comprise searching behaviour or moving from one patch of sward to another (Gibb, 1996).

Statistical methods

Data were analysed, using Genstat 4.1 for Windows, through analysis of variance using a randomised block design, using liveweight at the start of the trial as a covariant for animal measurements. The pair of animals on each measurement paddock formed the unit of replication for intake and grazing time. One animal from the treated group suffered a jaw abscess during the trial and was excluded from all analyses.

2.3. Results

Parasitology

There were no clinical signs of parasitic gastroenteritis in any of the animals over the duration of the trial. The level of infection with gastrointestinal nematodes in the untreated group (as judged by fec) was relatively low, with a mean faecal egg count of 120 epg. in July and a maximum individual count of 450 epg; at this time 5/10 animals had measurable worm egg counts. Counts in the treated group were all <50 epg. Culture of faeces samples in July revealed a species composition comprising 91% *C. oncophora* and 9% *O. ostertagi*. In June and July, 8/10 and 9/10 faecal samples respectively from the untreated heifers were positive for lungworm (*Dictyocaulus viviparus*) larvae. Two of the untreated animals coughed intermittently, in July, presumably because of parasitic bronchitis, but otherwise no clinical signs were observed.

Grazing behaviour

Details of the grazing behaviour parameters in May and July are shown in Table 2.1.

> **May**

No differences were anticipated in May as the animals had only been grazing the principal paddocks for 10 days and the controls would not have had time to acquire patent infections with *C. oncophora* or *O. ostertagi*, though some larvae could have been developing within the alimentary tract during this time. In fact, two of the parameters were significantly ($P \leq 0.05$) different at the May recording, bites per grazing jaw movement (GJM) and the number of ruminating bouts/day. Whether these were chance effects or the result of recently acquired larval infections in the control calves cannot be determined, but the direction of both these effects was similar in the July recording and the latter remained significant ($P=0.018$).

> **July**

In July, the total time spent grazing each day (TGT) by the control animals was 442.6 minutes per day, significantly ($P=0.002$) shorter than the treated heifers, which grazed for 540.2 minutes/day, a difference of 97.6 minutes. Correspondingly, total eating time (TET) was significantly shorter in the controls: 391 minutes compared to 462 minutes ($P=0.025$) in the treated group. There were no differences between the groups in the total time spent ruminating (TRT), but the 'idling' time (24 hours minus total time spent grazing and ruminating) was increased ($P=0.036$) to 564 minutes/day in the controls compared to 483 minutes per day in the treated animals. Other observations

_____

of note were relative decreases in the controls in the total number of grazing jaw movements (P=0.052), total number of bites (P=0.062), the ruminative mastication rate (P=0.058) and the number of ruminative bouts (P=0.018).

Table 2.1. Grazing and ruminating behaviour measurements in May and July
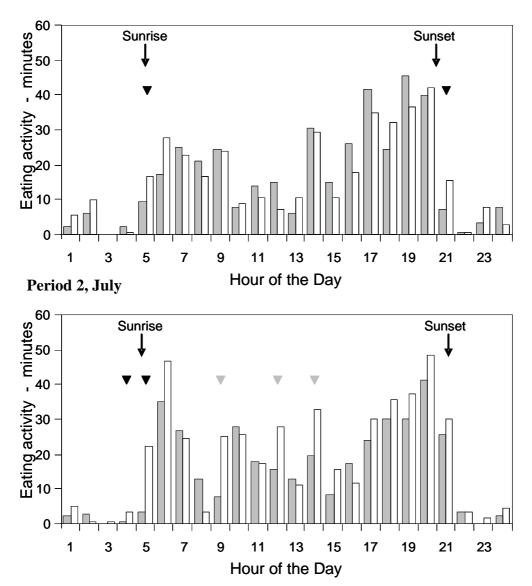
Re-analysis of 24-h grazing data (2007)

Parameter	Treatment		s.e.d.	Signif (*P=*)
	Control	Treated		
Period 1 (May)				
TGT (min)	475	467	24.6	0.775
TET (min)	395	399	24.3	0.899
TGJM	31162	32068	2561.4	0.741
Bites	19238	17956	1868.1	0.530
Bites/GJM	0.614	0.558	0.0194	0.045
TGJM rate /min	78.6	80.5	2.90	0.539
Bite rate /min	48.3	44.9	2.95	0.315
Meals/24h	11.90	13.60	1.454	0.307
Meal duration (min)	33.5	30.4	4.92	0.568
TRT (min)	387	416	33.7	0.443
Ruminating bouts	13.3	16.9	1.298	0.050
Ruminating bout duration (min)	29.1	25.6	2.61	0.252
Boli	530	566	56.0	0.558
Ruminative mastications	28533	32224	3098.9	0.299
Mastication rate /min	73.0	77.3	2.36	0.143
Mastications/bolus	53.5	58.8	4.43	0.300
TIT	657	625	33.1	0.389
Period 2 (July)				
TGT (min)	443	540	14.2	0.002
TET (min)	391	462	20.5	0.025
TGJM	30568	37037	2361.3	0.052
Bites	18286	21503	1249.7	0.062
Bites/GJM	0.598	0.581	0.0093	0.147
TGJM rate /min	78.2	80.1	2.15	0.432
Bite rate /min	46.75	46.45	1.022	0.784
Meals/24h	10.60	12.40	1.546	0.309
Meal duration (min)	36.8	38.6	3.58	0.640
TRT (min)	485	494	26.6	0.750
Ruminating bouts	14.10	17.20	0.797	0.018
Ruminating bout duration (min)	34.8	29.4	0.274	0.120
Boli	535	613	43.1	0.144
Ruminative mastications	36225	39633	2314.2	0.215
Mastication rate /min	74.4	80.2	2.18	0.058
Mastications/bolus	68.5	64.9	7.95	0.676
TIT	564	483	25.9	0.036

_____
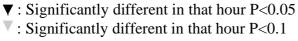
Circadian pattern

The 24-hour eating patterns are shown in Figure 2.1. As can be seen, grazing and eating were largely diurnal activities that occurred between sunrise and sunset, with more grazing in the afternoon than the morning. In July there were significant differences between the treated and control heifers in the time spent eating during some morning and afternoon hours, which contributed towards the significant difference in the total eating time between the two treatments in July.

Figure 2.1. Time spent eating during each hour (GMT) of the day by heifers receiving either no anthelmintic (grey fill) or ivermectin (no fill)

Period 1, May

Period 2, July

▼ : Significantly different in that hour $P<0.05$
▽ : Significantly different in that hour $P<0.1$

Herbage intake and Sward measurements

       Herbage intake

There were no significant differences in herbage intake during the first recording period in May when cattle in the treated and untreated groups consumed 3.53 kg and 3.43 kg Dry Matter (DM)/day, respectively (Table 2.2). In July, the herbage intake of the treated animals was 4.66 kg DM/day, whilst that of the untreated animals was 3.88 kg DM (P=0.166).

Table 2.2. Herbage Intake in May and July

	Treated	*Untreated*	*s.e.d*	*Signif (P=)*
Period 1 (May)				
Herbage Intake (kg DM/day)	3.53	3.43	0.433	0.835
Period 2 (July)				
Herbage Intake (kg DM/day)	4.66	3.88	0.428	0.166

s.e.d = standard error of the difference based on individual animals in measurement paddocks

       Principal paddocks

The pastures on which each group of heifers grazed could be readily distinguished visually (Figure 2.2). That grazed by the treated cattle had a short, dense green sward, while that grazed by the untreated animals had a longer sward, which included a lot of brown stem material. These observations were corroborated by the botanical composition measurements (Table 2.3) which showed that there were differences in sward quality between the paddock grazed by untreated cattle, in which there was more dead material and more flowering stem and pseudostem material than in the paddock grazed by treated animals.

_____

Table 2.3: Botanical Composition Main Paddocks in July

	Treated	*Untreated*	*s.e.d.*	*Signif (P=)*
Live Leaf (kg/ha)	1746	1929	509.0	0.725
Flowering Stem (kg/ha)	169	492	231.1	0.183
Pseudostem (kg/ha)	1703	2195	434.2	0.276
Dead (kg/ha)	1211	1947	243.7	0.009

Figure 2.2. Visual comparison of the herbage height and composition in the two principle paddocks in July. The paddock grazed by treated heifers is on the left and the control heifers grazed the one on the right

Replicated measurement paddocks

During the measurement period in May, there was no significant difference between the mean organic matter mass measured on the paddocks grazed by the control and treated heifers, either on Day 1 or Day 11 (Table 2.4). Similarly, there was no significant difference between the treatments in the mean sward surface heights measured on Days 1 and 12 (Table 2.5).

Table 2.4. Organic Matter Mass (kg/ha)

	Treated	*Untreated*	*s.e.d*	*Signif (P=)*
Period 1 (May)				
Start of Intake Period (Day 1)	6966	7589	782.6	0.448
Day 11	7011	7742	907.8	0.444
Period 2 (July)				
Start of Intake Period (Day 1)	7946	7055	877.2	0.339
Day 11	5814	6089	837.1	0.751

During the measurement period in July, organic matter mass declined by 2132 and 966 kg OM/ha between days 1 and 11 in paddocks grazed by treated and untreated animals, respectively. Swards showed a significantly (P=0.026) different decrease in height of 11.5 and 8.2 cm between days 1 and 12 in paddocks grazed by treated and untreated heifers, respectively (Figure 2.3).

Table 2.5. Sward Surface Heights (cm) on measurement paddocks in May and July

	Treated	*Untreated*	*s.e.d*	*Signif (P=)*
Period 1 (May)				
Start of Intake Period (Day 1)	24.07	24.16	0.590	0.885
Day 12	17.37	17.34	0.580	0.957
Difference Day 1 – Day 12	6.70	6.82	1.025	0.910
Period 2 (July)				
Start of Intake Period (Day 1)	22.96	21.68	1.241	0.333
Day 12	11.43	13.46	1.020	0.081
Difference Day 1 – Day 12	11.53	8.22	1.218	0.026
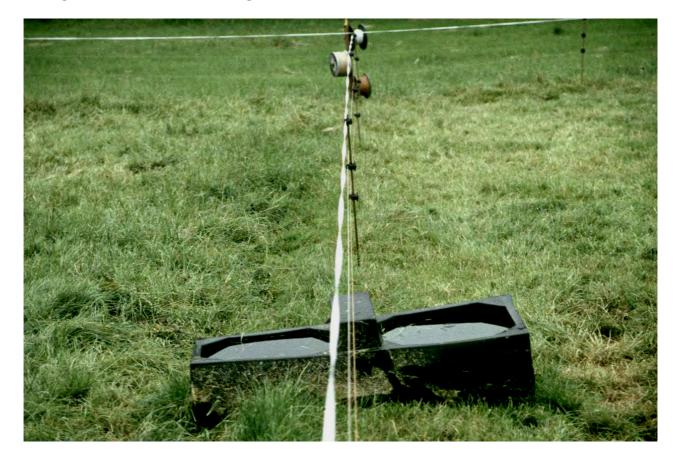
Liveweight Gain

The mean growth rate of the treated group from turnout to mid July was 0.78 kg LW/day compared with 0.68 kg LW/day for the untreated group (s.e.d. 0.042).

2.4. Discussion

This study demonstrated that after ~2½ months of grazing from May to July, calves in the group treated with ivermectin, in a sustained-release bolus formulation administered prior to turnout, which showed no evidence of infection with parasitic nematodes, grazed significantly longer (97.6 minutes/day) than animals in the parasitized, untreated group. Consistent with this, the treated cattle ate for 71 minutes more per day, had more total grazing jaw movements, more bites and idled for 81 minutes less per day. Most of the time spent grazing and eating took place during

_____

daylight hours, with peak levels of activity typically occurring in the few hours before
sunset. Hourly comparisons of the time spent eating by the treated and control animals
showed that the overall behavioural patterns were similar and consistent with previous
observations (Hodgson, 1990). Significant differences between the groups (treated
heifers grazed longer) were confined to periods in the morning and early afternoon,
but, though evident during peak times, were not significantly different then.

**Figure 2.3. Visual comparison of the herbage height and composition in two of
the measurement paddocks in July. The paddock grazed by treated heifers is on
the right and the control heifers grazed the one on the left.**

There appeared to be several consequences of the differences in grazing behaviour
between treated and control groups, manifest in both the paddocks and the animals. At
the end of the measurement period in July, the replicated paddocks, which had
uniform swards when the animals started grazing, showed observable differences in
sward surface height and mass. The swards that had been grazed by the untreated,
parasitized heifers was 3.3 cm taller and contained 1166 kg more organic matter (OM)
per hectare (ha) compared to the paddocks grazed by treated animals.   These

_____

differences were consistent with the observations on herbage intake at this time, when the untreated animals consumed 0.78 kg less DM/day (P=0.166).

In the principal paddocks it was evident from visual observation in July that the reduced grazing time on the paddock containing the untreated heifers had resulted in a taller sward which contained more flowering stems and dead material, compared to the other paddock and this was confirmed in the analysis of herbage composition. The sward grazed by the control animals is typical of one that has been relatively under-grazed and which has deteriorated in quality due to insufficient grazing and the accumulation of senescent herbage during periods of rapid grass growth (Hodgson, 1990). It is also possible that the infected control heifers exhibited more avoidance behaviours of the rank grass associated with faecal deposits, as has been demonstrated in sheep (Cooper et al., 2000).

The rate of daily liveweight gain in the treated heifers from turnout in May until mid-July was 0.78 kg/day, a growth rate compatible with recommendations for dairy replacement heifers calving at 2 years of age (Van Amburgh et al., 1998). The untreated heifers grew at 0.68 kg/day over the same period, a difference of 0.1 kg/day, and they had fallen behind such target rates of gain.

Ivermectin is not known to have any intrinsic effects on appetite at recommended doses and studies in parasite-free animals have not revealed any effects on feed intake. In this study, the differences between the two groups appeared to have arisen as a consequence of low-grade infections with *C. oncophora, O. ostertagi* and *D. viviparus* that were present in heifers in the untreated group, but not detected in the treated animals. The relative importance of each species in determining this outcome cannot be determined from this trial. It is known that *Cooperia* spp. are responsible for the majority of faecal egg output in young cattle during the first months after turnout (Kloosterman, 1971). *C. oncophora* is considered to be of only moderate pathogenicity (Coop et al., 1979), but mono-specific infections can cause clinical and pathological changes in calves (Armour et al., 1987). These workers reported inappetence in calves infected with 10,000 larvae daily for six weeks, but no values for feed intake were provided; the calves also displayed some clinical signs, manifest as softening of the faeces. Based on egg recovery and culture, *O. ostertagi* appeared to be a minor component of the nematode fauna in the heifers in the current study. The pathological effects and impact on food intake of *O. ostertagi* have been well documented (Fox et al., 1989).

_____

The evidence that subclinical lungworm infections could result in reductions in feed intake is limited and equivocal (Boon and Verstegen, 1987). In two similar experiments with artificial infections comprising 640 *D. viviparus* larvae administered 2x per week for 8 weeks, there was a 4% reduction in voluntary feed intake in infected animals compared to uninfected controls in one study and a 5% increase in the other. An effect on liveweight gain was observed in a field trial in which one group of cattle showed evidence of patent lungworm infection, with mild clinical signs and low faecal egg counts, compared to a group with minimal infections (Taylor, 1987). By the end of the 150-day grazing season, the group infected with *D. viviparus* was on average 20kg lighter than the uninfected group.

The mechanism for inappetence in parasitized hosts has not been fully elucidated and will doubtless vary according to a number of factors, such as the predilection site and the severity and type of pathological changes that are induced. Amongst gastrointestinal nematodes, the reduced feed intake associated with *O. ostertagi* has been shown to be correlated with hypergastrinaemia that results from the pathophysiological changes in the abomasum of infected animals (Fox, 1997). A corresponding mediator role for cholecystokinin in parasite-induced inappetence has been suggested for intestinal species of nematode (Symons and Hennessy, 1981).

The reason why parasitized animals eat less has been the subject of much speculation. It is generally assumed that reduced intake may confer a functional advantage to either the parasite, the host or possibly even both, but it is also possible that it is of no specific benefit to either party and is merely a consequence of the infection (Minchella, 1985). In a recent analysis of this phenomenon five potential functional benefits from parasite-induced anorexia were proposed (Kyriazakis et al., 1998). The most supported and plausible of these, particularly for infections acquired by the oral route, is that the reduction in intake limits the acquisition of further infections and it may also allow the host to be more selective in its diet. This is partially supported by recent work in sheep, in which parasitised animals showed enhanced avoidance behaviours to faeces compared to uninfected controls (Cooper, 1996; Hutchings et al., 1998). It has also been shown that nutritional factors, particularly protein, can mitigate against some of the effects of parasitism (Coop and Holmes, 1996; Coop and Kyriazakis, 1999) and more selective feeding behaviours to increase protein intake could enhance host resilience.

2.5. Conclusion

This study has provided evidence that reductions in grazing and eating times are important determinants of reduced feed intake in pastured cattle infected sub-clinically with parasitic nematodes. This change in behaviour produced effects in both the grazing animals and the sward. In the animals a reduction in liveweight gain was observed and this could result in subsequent sub-standard performance. At set stocking rates, swards respond to reduced grazing pressure during the spring and early summer by progressing from the vegetative state, where tillering and leaf growth predominate, to the reproductive state, characterised by the appearance of stems, florets and early senescence. These changes in sward characteristics, which result in reduced nutritional value, could further compromise animal performance.

_____

2.6. References

Armour, J., Bairden, K., Holmes, P.H., Parkins, J.J., Ploeger, H., Salman, S.K., McWilliam, P.N., 1987, Pathophysiological and parasitological studies on *Cooperia oncophora* infections in calves. Res Vet Sci 42, 373-381.

Boon, J.H., Verstegen, M.W.A., 1987, Energy and nitrogen metabolism of growing calves continuously infected with *Dictyocaulus viviparus*, In: Verstegen, M.W.A., Henken, A.M. (Eds.) Energy metabolism in farm animals. Martinus Nijhoff, Dordrecht, Netherlands, pp. 372-387.

Coop, R.L., Holmes, P.H., 1996, Nutrition and parasite interaction. Int J Parasitol 26, 951-962.

Coop, R.L., Kyriazakis, I., 1999, Nutrition-parasite interaction. Vet Parasitol 84, 187-204.

Coop, R.L., Sykes, A.R., Angus, K.W., 1979, The pathogenicity of daily intakes of *Cooperia oncophora* larvae in growing calves. Vet Parasitol 5, 261-269.

Cooper, J., 1996. The behavioural control of helminth infection by sheep. PhD. Aberdeen Scotland,

Cooper, J., Gordon, I.J., Pike, A.W., 2000, Strategies for the avoidance of faeces by grazing sheep. Appl Anim Behav Sci 69, 15-33.

Dove, H., Mayes, R.W., 1991, The use of plant wax alkanes as marker substances in studies of the nutrition of herbivores: a review. Aust.J.Agric.Res 42, 913-952.

Fox, M.T., 1997, Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. Vet Parasitol 72, 285-297; discussion 297-308.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Gale, D.L., 1989, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. Res Vet Sci 47, 294-298.

Gibb, M.J., 1996. Technology for animal grazing/intake studies. In: Occasional Publication No.3, Proceedings of a Workshop for Concerted Action, Dublin.

HFRO 1986. Biennial Report 1984-1985 (Hill Farm Research Organisation), pp. 29-30.

Hodgson, J., 1990, Grazing Management. Longman Scientific & Technical, 203 p.

Hutchings, M.R., Kyriazakis, I., Anderson, D.H., Gordon, I.J., Coop, R.L., 1998, Behavioural strategies used by parasitised and non-parasitised sheep to avoid ingestion of gastro-intestinal nematodes associated with faeces. Anim Sci 67, 97-106.

Kloosterman, A., 1971. Observations on the epidemiology of trichostrongylosis of calves. PhD. Wageningen, Netherlands,

Kyriazakis, I., Tolkamp, B.J., Hutchings, M.R., 1998, Towards a functional explanation for the occurrence of anorexia during parasitic infections. Anim Behav 56, 265-274.

MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationary Office, London, 160 p.

Minchella, D.J., 1985, Host life-history variation in response to parasitism. Parasitology 90, 205-216.

Penning, P.D., 1983, A technique to record automatically some aspects of the grazing and ruminating behaviour in sheep. Grass Forage Sci 38, 89-96.

Penning, P.D., Steel, G.L., Johnson, R.H., 1984, Further development and use of an automatic recording system in sheep grazing studies. Grass Forage Sci 39, 345-351.

Rutter, S.M., 2000, Graze: a program to analyze recordings of the jaw movements of ruminants. Behav Res Methods Instrum Comput 32, 86-92.

Rutter, S.M., Champion, R.A., Penning, P.D., 1997, An automatic system to record foraging behaviour in free-ranging ruminants. Appl Anim Behav Sci 54, 185-195.

Sykes, A.R., Coop, R.L., 1976, Food intake and utilization by growing lambs with parasitic damage to the abomasum or small intestine. Proc Nutr Soc 35, 13A-14A.

Symons, L.E., Hennessy, D.R., 1981, Cholecystokinin and anorexia in sheep infected by the intestinal nematode *Trichostrongylus colubriformis*. Int J Parasitol 11, 55-58.

Taylor, S.M., 1987. The production impact of *Dictyocaulus* in cattle. In: MSD AGVET Symposium, XXIII World Veterinary Congress, Montreal, pp. 81-84.

Van Amburgh, M.E., Galton, D.M., Bauman, D.E., Everett, R.W., Fox, D.G., Chase, L.E., Erb, H.N., 1998, Effects of Three Prepubertal Body Growth Rates on Performance of Holstein Heifers During First Lactation. J Dairy Sci 81, 527-538.

Chapter 3: Impact of eprinomectin on grazing behaviour and performance of dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management

_____

3.1. Introduction

Reduction in appetite and feed intake are important factors that contribute to reduced performance in ruminants sub-clinically infected with gastrointestinal nematodes (Coop and Holmes, 1996; Fox, 1997) and has been demonstrated in housed animals infected artificially with mono-specific cultures of various nematodes (Sykes and Coop, 1976; Coop et al., 1977; Fox et al., 1989; Coop and Holmes, 1996). More recently, research in young grazing cattle has shown that a marked reduction in daily grazing time in parasitized animals is associated with a reduction in herbage intake and consequent production losses (chapter 2). Dairy cows are known to be infected with, generally, low numbers of gastrointestinal nematodes (Agneessens et al., 2000; Borgsteede et al., 2000), but have nevertheless frequently shown production responses to anthelmintic treatment (Gross et al., 1999). Studies with eprinomectin have shown milk yield responses following treatment at various stages of lactation (McPherson et al., 2001; Reist et al., 2002; Sanchez et al., 2002) and, additionally, improved fertility has been demonstrated in studies when treatment was given at calving. The precise mechanism underlying milk yield responses to anthelmintic treatment has not been elucidated, but the previous observations in young, non-lactating dairy heifers (Forbes et al., 2000) provide a possible mechanism, hence a study of the grazing behaviour of lactating dairy cows and heifers following treatment with eprinomectin was initiated.

3.2. Materials and Methods

Animals

Forty spring-calving, Holstein-Friesian, dairy cows and heifers (20 of each type), which had calved between 24 Feb and 14 May 2002, were used in the study, which commenced in June 2002, when the cattle were between 6 and 17 weeks into lactation. They had received no anthelmintic treatment the previous grazing season, but the cows had been treated with nitroxynil for liver fluke control at the end of the previous lactation.

Allocation

The animals were blocked according to parity (primiparous v multiparous) and milk yield two weeks prior to allocation to treatment. Within parity, animals were ranked by milk yield, and paired sequentially: within the replicate pairs so formed individuals were randomly allocated to a different treatment group (treated or control). The

_____

groups were checked to ensure no bias in terms of calving date, milk yield or live weight.

Treatment

Ten replicate pairs, each of 2 cows or 2 heifers, were randomly allocated to receive either EPRINEX®[1](eprinomectin) pour-on, administered along the mid line of the back at the rate of 1 ml/10 kg live weight (500 mcg eprinomectin/kg) on 26th June (treatment group), or no treatment for nematode parasites (control group).

Pasture management

Nematode burdens in the study cattle were derived from infections acquired naturally in previous grazing seasons and from nematode larvae ingested on the designated pasture. Grazing of the pasture was limited during the previous year because of restrictions imposed by Foot and Mouth Disease biosecurity precautions and the pastures were grazed by dairy cows only from August to late October. During previous grazing seasons, the pasture had been continually stocked with dairy cattle during the summer and by sheep during the winter. The swards had been established for at least 12 years.

The animals were turned out to pasture, which comprised mainly perennial ryegrass (*Lolium perenne* L.), on 10 May. From turnout to mid-June the animals grazed under a continuous variable stocking system with additional cows being used as necessary to maintain an overall mean sward surface height (SSH) of 7 to 8 cm across the experimental area. On 26th June, the grazing area was sub-divided into 20 replicate paddocks of equivalent size (0.55 ha) and topography. Grazing pairs of either control or treated animals were randomly assigned to graze each paddock over the duration of the study (four weeks).

Pastures were fertilised with nitrogen, equivalent to 240 kg N/ha per annum, in equal applications at intervals of approximately 4 weeks. All animals were maintained on pasture throughout the experiment. All animals received 4 kg/head/day in two equal feeds at milking of a 14% crude protein dairy concentrate, with a calculated metabolisable energy concentration of 12.8 MJ kg/DM. Water was available at all times. Animals within each treatment were grouped together for droving at milking times, and divided into their appropriate grazing pairs for return to their paddocks.

_____

[1] Eprinex® is a registered trademark of Merial Ltd.

_____

The control animals were always handled first to avoid any possible transfer of eprinomectin from the treated animals to the controls (Bousquet-Melou et al., 2004). Following establishment of the SSH between 7 and 8 cm and treatment with eprinomectin, the area of each individual paddock remained unaltered, at 0.55 ha, with only the allocated grazing pairs of animals being present in each paddock until the end of July when the study ended.

Sampling and Measurements

Pastures

At weekly intervals from turnout to the day of treatment, between 600 and 650 SSH measurements were taken at random sites across the entire experimental area. On the day following treatment (27 June) and on 2, 4 and 11 July, SSH was measured at more than 100 sites within each paddock. Plucked samples, representative of the herbage eaten by the animals, were collected from each paddock on 7th July and bulked before freeze drying and subsequent analysis of organic matter (OM) content and OM digestibility (Iowerth et al., 1975). Sub-samples of the 14-day, post-treatment faecal samples were also analysed for residual digestibility.

Parasitology

Samples were taken from freshly deposited faecal pats from each animal on four occasions in May, June and July, prior to allocation and after imposition of treatments. Faecal sub-samples, each of 4.5 g were analysed for counts of nematode eggs at a sensitivity of 50 eggs per gram (epg) using the special modification of the McMaster method (MAFF, 1986). Subsequently, faecal samples with counts of less than 50 epg were further analysed using a technique with a sensitivity of 1 epg, based on the sensitive centrifugal flotation technique, using saturated salt solution as a flotation medium (MAFF, 1986). Sub-samples of the faeces collected 12 days after treatment were bulked by treatment group (treated or control) for culture and nematode identification. Pasture samples for direct larval counts were collected from each paddock at the same time as faecal sampling. The techniques for sampling, larval recovery and larval identification were those described in MAFF Reference Book 418, pages 31-32 (MAFF, 1986). Pasture larval counts were expressed as the number of infective larvae per kilogram of herbage Dry Matter (L_3 kg/DM).

Grazing behaviour

On three occasions, between four and fourteen days after treatment, solid-state behaviour recorders (Rutter et al., 1997) were fitted to each animal to measure grazing

and ruminating behaviour over 24 hours, commencing at 15.00h. Recordings were analysed using the software 'GRAZE' (Rutter, 2000). Total eating time during grazing was calculated as the sum of the periods of grazing jaw movements (GJM), excluding intervals of jaw inactivity >3 seconds. Total grazing time (TGT) was the sum of the periods of GJM activity, including any periods of jaw inactivity <5 minutes. Periods of jaw inactivity greater than 5 min were interpreted as being inter-meal intervals (Rook and Huckle, 1997). The number of meals was calculated as the number of periods of grazing activity separated by intervals of >5 min. Time spent eating the supplement ration was also easily identified from these recordings by the characteristic wave pattern (Gibb et al., 2002) and marked as such. Total idling time was calculated as the time within each 24 hours, when cows were not grazing or ruminating, and included time spent drinking and in social interaction.

The number of bites and non-biting GJM, the number of ruminative mastications and the total number of jaw movements during consumption of the supplements were counted automatically. The term 'non-biting grazing jaw movement' refers to those jaw movements not identified as bites and therefore includes jaw movements which may have a masticative or manipulative function.

Cow performance

Milk yield was recorded daily at each milking. Milk quality was recorded at four consecutive milkings each week starting with the Monday p.m. milking. Solids-corrected milk (SCM) yields were calculated using the equation of Tyrrell & Reid (Tyrrell and Reid, 1965). Animals were weighed and their body condition score (BCS) was assessed, using a 5-point scale (Edmonson et al., 1989), 24 hours before and on the day of allocation, on the day of treatment and thereafter at weekly intervals until the end of the trial, when live weight was recorded on two consecutive days.

Statistical methods

Mean values were calculated for each grazing pair of animals, which was the experimental unit, over the three measurement days for all variates before statistical analysis, because of the lack of independence between animals within each pair (Mead and Curnow, 1983). All data were analysed by two-way analysis of variance. The mean daily SCM yield during the week before the animals were allocated to grazing pairs was used as a covariate in the analysis of SCM yields as there was a significant

_____

(P<0.05) pre-treatment difference in yields from the cows and heifers. Analyses were carried out using GENSTAT 4.1 for Windows.

3.3. Results

Parasitology

All animals in the study remained in good health throughout the trial and there were no clinical signs of parasitic gastroenteritis in any of the animals. Of the 160 samples taken throughout the study for faecal egg counts, 29 were positive at a sensitivity of 50 epg; 8 samples from the cows and 21 from the heifers. Of the remaining 131 samples (< 50 epg), 60 had zero values on re-analysis at a sensitivity of 1 epg; 43 samples from cows and 17 from heifers. Culture of the bulked faecal sample collected from the control group in July revealed the presence of *Ostertagia, Trichostrongylus* and *Cooperia* spp, but very few larvae were recovered. No larvae were cultured from the faecal sample collected from the group treated with eprinomectin. Pasture larval counts were very low in all samples, with a range between 0 and 68 larvae/kg DM: *Ostertagia* and *Cooperia* species were identified.

Sward measurements

The overall mean SSH measured across the experimental area on 17 June was 8.19 ±0.105 cm. Analysis of variance of the mean SSH on the individual paddocks on 27 June, showed no effects of treatment or parity (mean 7.59 ±0.072 cm). Analysis of variance of the mean SSH of the individual paddocks on 2, 4 and 11 July, showed differences between the control- and treated-animal paddocks; 7.69 v 7.39 ±0.081 (*P*=0.020), 7.65 v 7.43 ±0.087 (*P*=0.087) and 7.66 v 7.34 ±0.090 (*P*=0.022) cm, respectively. There were no significant differences between mean SSH of the pastures due to parity (primiparous versus multiparous). The OM content of the bulked herbage sample collected on 7 July was 902 g/kg dry matter, with an OM digestibility of 811 g/kg.

Grazing behaviour

Treatment effects of grazing behaviour are shown in Table 3.1. There were significant (P<0.05) effects of treatment on daily grazing time, eating time, total grazing jaw movements (TGJM), number of bites, idling time and mean meal duration. Treated cows grazed for 47 minutes longer per day than controls, whilst in heifers the comparable figure was 50 minutes (P=0.016). Mean meal duration was extended as a result of anthelmintic treatment by 11 and 38 minutes, in cows and heifers, respectively,

_____

(P=0.012). There were no significant treatment effects on ruminating time, but treated cows and heifers spent significantly less time idling each day: 50 minutes in cows and 110 minutes in heifers (P=0.010).

Cows spent significantly longer grazing and eating and performed more bites per day compared with heifers (P<0.05). The temporal pattern of grazing meals showed no significant difference resulting from treatment or parity, with an overall mean of 6.6 meals/day and 317 ± 4.9 min of grazing activity occurring between afternoon milking and midnight. Compared with the cows, heifers demonstrated higher ruminative mastication rates and a greater number of ruminative mastications per bolus (P<0.01).

_____

Table 3.1. Effect of treatment with eprinomectin and parity on grazing and ruminating behaviour measured over 24 hours.
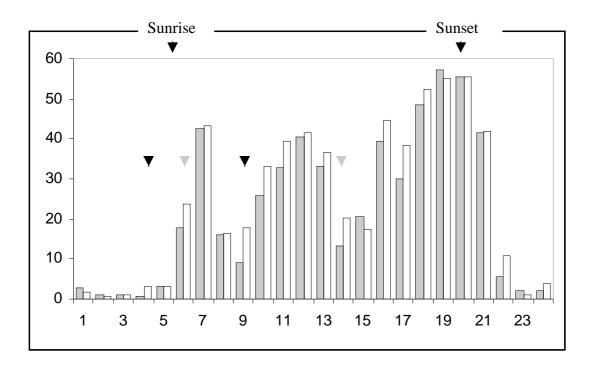
	Control		Eprinomectin		S.E. of T x Parity means	Significance of effect (P=)		
	Heifers	Cows	Heifers	Cows		T	Parity	T x Parity
Ingestive behaviour								
Total Grazing Time (mins)	542	601	592	648	12.9	0.016	0.006	0.920
Total Eating Time (mins)	511	579	573	626	12.3	0.006	0.003	0.684
Evening Grazing [1] (mins)	308	310	305	345	9.8	0.115	0.050	0.065
Total GJM [2] (000)'s	38.77	41.65	45.11	47.24	1.042	<0.001	0.109	0.803
Bites (000)'s	29.19	32.47	34.07	36.65	0.859	0.002	0.028	0.775
Non-biting GJM (000)'s	9.59	9.48	11.04	10.59	0.721	0.178	0.681	0.984
GJM Rate (/min)	75.9	72.2	78.6	75.6	1.49	0.161	0.123	0.867
Bite Rate (bites/min)	57.2	56.3	59.2	58.5	1.59	0.356	0.729	0.968
Non-biting GJM Rate(/min)	18.7	15.8	19.4	17.1	1.06	0.533	0.098	0.662
Bites/GJM	0.753	0.778	0.753	0.772	0.0128	0.888	0.241	0.867
Meals	7.9	6.1	6.1	6.3	0.44	0.219	0.219	0.118
Meal duration (min)	72.1	105.4	110.5	116.5	6.14	0.012	0.038	0.136
Ruminative behaviour								
Ruminating time (mins)	400	430	448	431	12.4	0.170	0.710	0.204
Mastications (000)'s	25.27	25.88	30.00	25.52	1.019	0.149	0.096	0.199
Number of boluses	463	545	524	489	17.1	0.924	0.348	0.029
Mastication rate (/min)	64.8	58.9	66.5	59.7	1.21	0.493	0.002	0.786
Mastications per bolus	56.9	47.2	58.7	52.5	1.95	0.215	0.010	0.535
Ruminating bouts	11.0	11.3	11.2	11.3	0.47	0.900	0.774	0.850
Idling behaviour								
Idling Time[3] (mins)	529	432	419	382	19.3	0.010	0.025	0.282

[1] Time spent eating between afternoon milking and midnight
[2] Total Grazing Jaw Movements
[3] 24 hours minus grazing time minus ruminating time

The circadian eating patterns are shown in Figure 3.1. Consistent with other reports (Gibb et al., 1998; Gibb et al., 1999; Gibb et al., 2002), grazing and eating can be seen as being largely diurnal activities in cattle that occur between sunrise and sunset, with more grazing in the afternoon than the morning. The anthropogenic imposition of milking does not seem to alter the patterns seen in dairy cows compared to young stock. There are significant differences between treated and control animals during some of the hourly recordings and these help explain some of the overall differences eating times in the 24-hour recordings, when it appears that the differences occurred mainly during grazing in the morning and early afternoon, similar to previous observations in young, non-lactating cattle (Hodgson, 1990).

Figure 3.1. Time spent eating during each hour (GMT) of the day by dairy cows and heifers receiving either no anthelmintic (grey fill) or eprinomectin (no fill)

▼ : Significantly different in that hour P<0.05
▽ : Significantly different in that hour P<0.1

There was no significant effect of treatment or parity on the residual digestibility of the OM in the faeces; mean 252 ± 6.64 g/kg OM.

_____

Cow performance

      Milk yield

Treatment had no significant effect on milk quality. Analysis of the SCM yields, adjusted by covariance for the yield before allocation, showed a significant (P<0.05) response to eprinomectin treatment in weeks 2 and 3 after administration (Table 3.2). In week 4, the significance of the treatment response was P=0.071. SCM yields were significantly (P<0.001) higher for cows compared with heifers in all weeks.

      Live weight and body condition score

There was a significant effect of treatment on live weight change over the 28 days following treatment (Table 3.2). The differences in total and daily live weight gain over the 28-day measurement period between groups were significant (P<0.037). However, there was an interaction (P=0.087) between treatment and parity, with the heifers gaining more weight as a result of treatment with eprinomectin compared with the cows; equivalent to 0.55 v 0.06 ± 0.134 kg/day. Analysis of variance of the changes in BCS over 28 days showed a significant (P<0.05) treatment effect, with heifers demonstrating a more marked effect than cows.

3.4. Discussion

Parasitology

The faecal egg counts from heifers and cows were very low, consistent with the literature (Fox and Jacobs, 1980; Agneessens et al., 2000; Borgsteede et al., 2000; Eysker et al., 2002). These results confirm the relative insensitivity of the McMaster technique, at the standard sensitivity of 50 epg, in detecting low-level nematode infections in adult dairy cattle. In addition, these data suggest that dairy heifers' faeces have a higher concentration of nematode eggs than multiparous dairy cows, presumably reflecting differences in their levels of acquired immunity to nematode parasites. The very low recoveries of pasture larvae probably resulted from the limited grazing period during the previous year.

Table 3.2. Effect of parity and treatment with eprinomectin on mean daily solids-corrected milk yield, live weight and body condition score (BCS) change.

	Control		Eprinomectin		S.E. of means	Significance of effect (*P*=)		
	Heifers	Cows	Heifers	Cows		T	Parity	T x Parity
Week Beginning								
1-July	17.89	22.14	18.81	21.26	0.663	0.957	<.001	0.199
8-July	17.01	21.67	19.36	21.93	0.571	0.038	<.001	0.091
15-July	15.54	19.97	17.60	21.50	0.797	0.042	<.001	0.748
22-July	16.65	20.61	18.38	21.05	0.556	0.071	<.001	0.268
Change between 26 June and 24 July								
Live weight gain (kg)	0.0	13.7	15.5	15.4	3.76	0.037	0.089	0.087
BCS change	-0.113	-0.025	0.212	-0.025	0.0766	0.050	0.343	0.050

_____

Grazing and ruminating behaviour

Heifers grazed for less time and performed fewer bites, compared with the cows, as might be expected in relation to live weight, and thus maintenance requirements, and their slightly lower SCM yields. This was a result of shorter meal duration rather than any reduction in the number of grazing meals. However, they exhibited higher ruminative mastication rates and more mastications per bolus than the cows, possibly reflecting a lower efficiency of particle size reduction per mastication by the younger animals. No means of direct measurement of digestive efficiency were available during the experiment, however, laboratory determination of the residual digestibility was used to examine whether treatment or parity may have affected digestive efficiency, based on the assumption that there were no significant differences in the digestibility of the herbage selected. The measurements of residual faecal digestibility obtained, showed no significant differences in digestive efficiency because of treatment or parity.

Treatment with eprinomectin significantly affected grazing behaviour, manifested by an overall increase of about 1 h in grazing and eating times, and a commensurate increase in the number of bites. This was achieved by an increase in meal duration rather than an effect on the number of grazing meals. Although treatment with eprinomectin had no affect on ruminating time, the number of ruminative mastications or number of boluses, it produced an overall reduction in idling time of 86 min/day ($P<0.001$). The lack of any significant differences in residual digestibility indicates that, in this study, neither treatment with eprinomectin nor the maturity of the animals significantly affected digestive efficiency.

Despite the treatment and parity effects on grazing time and mean meal duration, there were no significant effects on the number of grazing meals, the temporal patterns of meals or the total duration of intra-meal intervals, with animals having an average of 6.6 meals each day and conducting just over half of their grazing activity between afternoon milking and midnight. The temporal pattern of grazing, combined from the three recordings, is shown in Figure 3.1, which demonstrates the overall similarity in patterns between treated and control animals, although significant differences in the length of grazing were apparent in some hours, particularly in the early morning and early afternoon. The increase in total grazing time in the cows treated with eprinomectin was accompanied by a concurrent significant ($P=0.022$) reduction of mean SSH in the paddocks occupied by the treated pairs of animals, compared with the control animals.

This is indicative of a greater herbage intake in treated cattle over the measurement period.

Under continuous variable stocking management, bite mass and, as a consequence, short-term intake rate (IR) are constrained by sward structure, primarily height. On short swards, as maintained in this experiment, grazing dairy cattle attempt to overcome such constraints on short-term IR by increasing the time they spend grazing (Gibb et al., 1997, 1999). The grazing and eating times recorded in this experiment in the control animals were similar to those previously recorded with dairy cows grazing swards of 7 to 8 cm SSH at this time of the year (Gibb et al., 1999). With these constraints imposed on short-term IR by sward height, any behavioural response by the animals to increase intake as a consequence of reducing their parasitic burden, would be expected to be manifested in the duration of their grazing activity.

Cow performance in relation to feed intake

As a result of the increases in grazing activity following treatment, there were significant effects on SCM yield, live weight gain and BCS. These production responses were more marked in the treated heifers than in the cows, with an SCM yield response up to +2.35 kg/day (over the second and third weeks after treatment) and an improved mean daily liveweight change of +0.55 kg/day over the 28-day observation period. Although the changes in liveweight and BCS appear slightly contradictory, in that liveweight changes were generally positive whilst BCS changes were negative (except for the treated heifers) the responses to treatment with eprinomectin are generally in agreement, i.e. an increase in liveweight and BCS of heifers and a minor effect in cows. In this study the control heifers failed to gain any weight over the 28-day measurement period and this might be considered unusual, but no explanation for this could be found in the data. It can only be assumed that the nutrient intake over this period was insufficient to support significant growth after the requirements for maintenance and lactation had been met.

Previous studies (Gibb et al., 1997) during July have shown that on swards maintained at an overall mean SSH in the range 7 to 8 cm by continuous variable stocking management, cows are able to achieve mean, short-term OM intake rates of approximately 23 g/min. If such rates were achieved in the present experiment, control cows would have achieved a daily intake of 13.32 kg OM. Assuming a metabolisable energy (ME) concentration of approximately 12.0 MJ/kg herbage DM (AFRC, 1993), and a daily intake of 44.4 MJ from the concentrate ration, the total daily intake would have been approximately 221 MJ of ME. According to Woods et

_____

al. (Woods et al., 2003), with an intake of 210 MJ of ME, cows yielding 20 kg milk per day could be expected to produce 0.06 kg milk per MJ increase in ME intake. The observed increase of 47 min in the time spent eating by the cows, would have resulted in an additional intake of 1.1 kg OM, equivalent to 14.4 MJ ME. Using the model of Woods et al. (2003), the predicted increase in daily SCM production would be about 1.0 kg, somewhat greater than the mean increase of 0.34 kg achieved over the 4 weeks following treatment with eprinomectin. Although the control and treated cows appeared to gain weight whilst losing BCS, the very small changes in BCS are difficult to interpret over a period of only 28 days.

Whilst the intake per bite by the heifers would be expected to be smaller than in the cows, some compensation may have been affected by the slightly higher bite rate. Nevertheless, the slightly greater increase in eating time by the heifers, as a result of treatment with eprinomectin, may have allowed them to achieve a similar level of increase in daily intake as the treated cows. Using the same predictive model of Woods et al. (2003), the milk yield response would be expected to be in the order of 1 kg/day. However, this value is lower than the mean increase of 1.77 kg recorded in the heifers over the 4 weeks following treatment. Nevertheless, the overall average increase in milk yield in the treated cattle of ~1.0 kg/day is remarkably close to the theoretical calculation. Taking into account the significant positive responses in live-weight and BCS change over the 4 weeks following treatment, it appears that, particularly in the heifers, the responses in grazing behaviour can only partially account for the increase in milk production and growth rate. A possible explanation of these discrepancies, which requires further investigation, is the possible consequence to the animal, in terms of body energy balance, immune responses and alimentary tissue turnover as a result of gastrointestinal parasitism (Lochmiller and Deerenberg, 2000).

Parasitism and inappetence

The precise mechanism for the increased appetite in naturally infected cattle following eprinomectin treatment is not known. There are no indications of any direct pharmacological effect on feed intake from studies in parasite-free animals treated with eprinomectin or the related compound, ivermectin, (Merial, unpublished data). Additionally, an increase in appetite and feed intake in cattle infected with *O. ostertagi* has been observed following treatment with fenbendazole, an anthelmintic from the

benzimidazole group, differing in mode of action and pharmacokinetics from the avermectins (Fox et al., 1989).

In a previous study (Forbes et al., 2000), in which both existing and new infections with parasitic nematodes were controlled to a high degree over the whole 2-3 month experimental period through the use of an ivermectin sustained release bolus, it could not be determined if the observed increase in appetite was a consequence of short or long-term parasite control. In the current study, the behavioural measurements were made within two weeks of eprinomectin treatment, at which time it can be assumed that the resident populations of gastrointestinal nematodes had been removed, but re-infection would not have taken place due to the persistent activity of the product (Alvinerie et al., 1999; Cramer et al., 2000). Thus, it would appear that it is the presence of adult and immature parasites within the host that exerts an inhibitory effect on appetite. The rapid reversal of this effect following treatment suggests that there may be parasite or host-derived neurochemical mediators that feed back peripherally or centrally to effect the observed changes. Fox et al., 1989b have previously demonstrated such a mediator role for gastrin in the expression of effects on appetite in ostertagiosis in cattle. The speed of response indicates that resolution of macro- or microscopic parasite-induced gut pathology is a less likely explanation for the increase in appetite following anthelmintic treatment.

Regardless of the mechanisms, this study has shown that adult dairy cattle, which typically have high levels of immunity to gastrointestinal nematodes and low parasite burdens, are still subject to nematode-induced inhibitory effects on appetite. Alleviation of these burdens, through eprinomectin treatment, resulted in increased appetite, manifested as increased grazing time, eating time and number of bites.

Thus, a behavioural mechanism has been demonstrated which explains, in part, the previously reported productivity responses to eprinomectin treatment in lactating dairy cows.

3.5. Conclusion

This study demonstrated that when adult dairy cattle grazed in a system that allowed unrestricted access to herbage, albeit in swards shorter than would be expected under rotational grazing management, they expressed a behavioural response to removal of nematode parasites by eprinomectin treatment. This was manifest as an increase in grazing time and a decline in idling time, similar to that seen previously in young, non-

_____

lactating cattle.   These responses resulted in a significant solids-corrected milk yield response in all animals.   In this study, the heifers showed a particularly marked yield response, which possibly reflects their relative immaturity and greater susceptibility to gastrointestinal nematodes.

The observed effects of treatment on live weight and conditions score may also be of importance, as the positive responses to treatment would provide a rational explanation for the improved fertility, which has been reported in some studies with eprinomectin when administered to cows and heifers at calving.

3.6. References

AFRC, 1993, Energy and protein requirements for ruminants. CAB International, Wallingford, UK, 192 p.

Agneessens, J., Claerebout, E., Dorny, P., Borgsteede, F.H., Vercruysse, J., 2000, Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 90, 83-92.

Alvinerie, M., Sutra, J.F., Galtier, P., Mage, C., 1999, Pharmacokinetics of eprinomectin in plasma and milk following topical administration to lactating dairy cattle. Res Vet Sci 67, 229-232.

Borgsteede, F.H., Tibben, J., Cornelissen, J.B., Agneessens, J., Gaasenbeek, C.P., 2000, Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 89, 287-296.

Bousquet-Melou, A., Mercadier, S., Alvinerie, M., Toutain, P.L., 2004, Endectocide exchanges between grazing cattle after pour-on administration of doramectin, ivermectin and moxidectin. Int J Parasitol 34, 1299-1307.

Coop, R.L., Holmes, P.H., 1996, Nutrition and parasite interaction. Int J Parasitol 26, 951-962.

Coop, R.L., Sykes, A.R., Angus, K.W., 1977, The effect of a daily intake of *Ostertagia circumcincta* larvae on body weight, food intake and concentration of serum constituents in sheep. Res Vet Sci 23, 76-83.

Cramer, L.G., Pitt, S.R., Rehbein, S., Gogolewski, R.P., Kunkle, B.N., Langhoff, W.K., Bond, K.G., Maciel, A.E., 2000, Persistent efficacy of topical eprinomectin against nematode parasites in cattle. Parasitol Res 86, 944-946.

Edmonson, A.J., Lean, I.J., Weaver, L.D., Farver, T., Webster, G., 1989, A Body Condition Scoring Chart for Holstein Dairy Cows. J Dairy Sci 72, 68-78.

Eysker, M., van Aarle, D., Kooyman, F.N., Nijzink, A.M., Orsel, K., Ploeger, H.W., 2002, Exposure of dairy cows to nematode infections at the end of the grazing season in The Netherlands. Vet Parasitol 110, 93-100.

Forbes, A.B., Huckle, C.A., Gibb, M.J., Rook, A.J., Nuthall, R., 2000, Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. Vet Parasitol 90, 111-118.

Fox, M.T., 1997, Pathophysiology of infection with gastrointestinal nematodes in domestic ruminants: recent developments. Vet Parasitol 72, 285-297; discussion 297-308.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Gale, D.L., 1989, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. Res Vet Sci 47, 294-298.

Fox, M.T., Jacobs, D.E., 1980, Factors influencing uptake of nematode larvae in adult dairy cattle during the grazing season and sources of pasture contamination. Vet Rec 107, 575-578.

_____

Gibb, M.J., Huckle, C.A., Nuthall, R., 1998, Effect of time of day on grazing behaviour and intake rate by lactating dairy cows. Grass Forage Sci 53, 41-46.

Gibb, M.J., Huckle, C.A., Nuthall, R., 2002, Effect of type of supplement offered out of parlour on grazing behaviour and performance by lactating dairy cows grazing continuously stocked grass swards. Anim Sci 75, 153-167.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1997, Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass and Forage Sci 52, 309-321.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1999, The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl Anim Behav Sci 63, 269-287.

Gross, S.J., Ryan, W.G., Ploeger, H.W., 1999, Anthelmintic treatment of dairy cows and its effect on milk production. Vet Rec 144, 581-587.

Hodgson, J., 1990, Grazing Management. Longman Scientific & Technical, 203 p.

Iowerth, D., Jones, H., Hayward, M.V., 1975, The effect of pepsin pretreatment of herbage on the prediction of dry matter digestibility from solubility in fungal cellulase solutions. J Sci Food Agric 26, 711-718.

Lochmiller, R.L., Deerenberg, C., 2000, Trade-offs in evolutionary immunology: just what is the cost of immunity? Oikos 88, 87-98.

MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationary Office, London, 160 p.

McPherson, W.B., Gogolewski, R.P., Slacek, B., Familton, A.S., Gross, S.J., Maciel, A.E., Ryan, W.G., 2001, Effect of a peri-parturient eprinomectin treatment of dairy cows on milk production. N Z Vet J 49, 106-110.

Mead, R., Curnow, R.N., 1983, Statistical methods in agriculture and experimental biology. Chapman Hall, New York, 400 p.

Reist, M., Medjitna, T.D., Braun, U., Pfister, K., 2002, Effect of a treatment with eprinomectin or trichlorfon on the yield and quality of milk produced by multiparous dairy cows. Vet Rec 151, 377-380.

Rook, A.J., Huckle, C.A., 1997, Activity bout criteria for grazing dairy cows. Appl Anim Behav Sci 54, 89-96.

Rutter, S.M., 2000, Graze: a program to analyze recordings of the jaw movements of ruminants. Behav Res Methods Instrum Comput 32, 86-92.

Rutter, S.M., Champion, R.A., Penning, P.D., 1997, An automatic system to record foraging behaviour in free-ranging ruminants. Appl Anim Behav Sci 54, 185-195.

Sanchez, J., Dohoo, I., Nodtvedt, A., Keefe, G., Markham, F., Leslie, K., DesCoteaux, L., Campbell, J., 2002, A longitudinal study of gastrointestinal parasites in Canadian dairy farms. The value of an indirect *Ostertagia ostertagi* ELISA as a monitoring tool. Vet Parasitol 107, 209-226.

Sykes, A.R., Coop, R.L., 1976, Food intake and utilization by growing lambs with parasitic damage to the abomasum or small intestine. Proc Nutr Soc 35, 13A-14A.

Tyrrell, H.F., Reid, J.T., 1965, Prediction of the energy value of cow's milk. J Dairy Sci 48, 1215-1223.

Woods, V.B., Kilpatrick, D.J., Gordon, F.J., 2003, Development of empirical models to describe the response in lactating dairy cattle to changes in nutrient intake as defined in terms of metabolisable energy intake. Livest Prod Sci 80, 229-239.

Chapter 4: Effects of sequential treatments with eprinomectin on performance and grazing behaviour in dairy cattle under daily-paddock stocking management

_____

4.1. Introduction

Previous work has shown that the use of anthelmintics to control gastrointestinal parasites in dairy cows may lead to an increase in milk yield (Gross et al., 1999). Until the introduction of eprinomectin, it had not been possible to treat lactating dairy cows with broad spectrum parasiticides without having to discard the milk for a pre-determined period. The availability of eprinomectin made it possible to study the mechanisms by which anthelmintic treatment of lactating dairy cows at pasture have lead to increased milk production.

The objective of this experiment was to evaluate the effect of naturally occurring infections of gastrointestinal parasites in grazing dairy cows on milk production and herbage intake and to explore whether a behavioural mechanism might explain the response to anthelmintic treatment in cows grazing ryegrass swards under daily stocking management.

4.2. Material and Methods

Animals

Twenty-four, March-calving, multiparous dairy cows of similar genotype, which had received no anthelmintic treatment the previous grazing season, other than treatment with nitroxynil for liver fluke at drying off, were used. The cows were selected from a group of thirty-six cows according to milk yield during week 2 after turnout, omitting those at the upper and lower extremes of the normal distribution.

Allocation

Cows were ranked according to milk yield, blocked in pairs, and allocated randomly to either of the two treatments. Within treatments, cows were allocated to pairs and balanced as far possible for milk yield, calving date, parity and current live weight. Cows within each treatment were grouped together for droving at milking times and, on return to their paddocks, were separated into their pairs at the paddock entrances. Cows were observed for oestrus during the experiment and all animals seen showing signs of oestrus within the specified service dates were served immediately after the following milking and returned to their paddock with their treatment group.

Treatment

The effect of treatment with an anthelmintic on the grazing and ruminating behaviour by cows managed under a daily paddock stocking regime was examined over an 18-week period. The treated cows received eprinomectin administered topically along the mid-

line of the back, at the rate of 1ml Eprinex$^{®2}$/10 kg live weight (500 mcg eprinomectin/kg) at approximately 7-week intervals on 1 June, 19 July and 7 September. Cows in the control group were given no anthelmintic. Infection with gastrointestinal nematode parasites was acquired naturally from pasture.

Pasture management

The grass swards had been established for at least 12 years and were predominantly of perennial ryegrass (*Lolium perenne*. L.) and had been continuously grazed by dairy cattle during previous grazing seasons and by sheep during the winters. The swards received nitrogen fertiliser in equal applications at approximately monthly intervals and within one week following grazing, equivalent to 240 kg N/ha per annum. All cows were kept at pasture throughout the experiment and received 4 kg/head/day of 18% Crude Protein dairy concentrate in two equal feeds at milking. Water was available at all times except during measurement of intake rates.

The cows were turned out to pasture in late April when ground conditions permitted. From turnout to mid May the cows were grazed as one group across the entire experimental area to ensure equivalent grazing and initial seeding with nematode eggs. Subsequently, the grazing area was sub-divided into two sets of 12 replicate plots of equivalent size and topography (East and South).

At the start of the experiment in mid May, each pair of control or treated cows was randomly assigned to one plot in each set, for the duration of the study. Within each plot, the pair of cows grazed a series of 1-day paddocks of areas calculated to provide 72 kg of herbage dry matter measured to ground level. Each pair of animals was confined between electrified temporary fences on their daily paddocks. The forward wire was moved each day during afternoon milking to the position required to provide the cows with their calculated daily allocation of herbage. Following the initial stocking of these paddocks, subsequent grazing was determined according to herbage growth, with cows returning to paddocks after re-growth periods of 3 to 5 weeks.

Sampling and Measurements

    Pastures

Daily paddock areas were calculated weekly, based on locally derived regressions relating herbage mass (kg DM/ha) measured to ground level and herbage height. Each week, sward plate height was measured using a rising plate meter at a total of two

---

² Eprinex$^{®}$ is a registered trademark of Merial Ltd.

_____

hundred and forty random sites across the twelve plots (20 per paddock), within the areas to be stocked during the subsequent week. Herbage was then cut to ground level within the area encompassed by the plate meter at six of these sites, covering a range of sward heights. The paddock areas for each pair of cows was then calculated from the mean of the twenty height measurements within that plot and the common locally-derived regression relating herbage mass and sward height. Cows were given access to their new daily paddock following afternoon milking (Orr et al., 2001) and were allowed the previous day's paddock as a loafing area, with a back fence to prevent any grazing of herbage re-growth on earlier paddocks.

Measurements of sward surface height (SSH), at the time of 24-hour grazing behaviour measurements, were made at 50 locations on each paddock using a sward stick with a 1 cm x 2 cm window (HFRO, 1986).

Parasitology

Faecal samples were collected from each cow in April, prior to allocation, and every 28 days thereafter. Whenever possible samples were collected from naturally voided faeces, otherwise they were collected per rectum. Samples were analysed for counts of nematode eggs using a technique based on the modified McMaster method (Improved) (MAFF, 1986), with a sensitivity of 1 egg per gram (epg), in which 3 g faeces were added to 42 ml of a saturated solution of sodium chloride (specific gravity 1.204) as a flotation medium. Additional faecal samples were taken on each occasion from all animals and bulked within treatment groups for culture and nematode identification. Pasture samples for direct larval counts were collected on the same days as the faecal samples, as described in MAFF (1986). Before the start of daily-paddock grazing, samples were collected across the whole pasture, and thereafter from each paddock before the animals were introduced.

Grazing behaviour

Measurements of grazing behaviour and short-term intake rate (IR) in grams of herbage organic matter (OM)/min were made between 17 and 30 May, 25 and 31 July and 14 and 26 September (Periods 1, 2 and 3, respectively).

24-h recording of grazing, ruminating and idling behaviour

In each behaviour measurement period, solid-state behaviour recorders (Rutter et al., 1997) were fitted to every cow on two occasions to record their temporal patterns of grazing, ruminating and idling behaviour over 24 h, commencing at 14:30 h. The

recordings were analysed using the software 'Graze' (Rutter, 2000). Total eating time (TET) during grazing was calculated as the sum of the periods of grazing jaw movements (GJM), excluding intervals of jaw inactivity > 3 seconds (Gibb, 1998). Total grazing time (TGT) was calculated as the sum of the periods of GJM activity, including any periods of jaw inactivity <5 min. Periods of jaw inactivity greater than 5 min were interpreted as being inter-meal intervals (Rook and Huckle, 1997). The number of meals was calculated as the number of periods of grazing activity separated by intervals of >5 min. Time spent eating the supplement ration was easily distinguished from grazing and ruminating activity during the recordings by the characteristic wave pattern (Gibb et al., 2002). Total idling time was calculated as the time within each 24 h period, when cows were not eating grass or ruminating, and included time spent drinking, in the parlour and in social interaction.

The number of bites and non-biting GJM, the number of ruminative mastications and the total number of jaw movements during consumption of the supplements were counted automatically. The term 'non-biting grazing jaw movement' refers to those jaw movements not identified as bites and therefore includes jaw movements which may have a masticative or manipulative function.

Short-term intake rate, bite rate and bite mass

In each period, IR by each cow was measured during a morning and afternoon grazing meal using the differential weighing method, corrected for rate of insensible weight loss, as described by (Gibb et al., 1998). Three pairs of cows from each treatment were measured on each occasion. Measurements of intake rate during the afternoon were made immediately after the animals had been turned onto their fresh allocation of pasture. Measurements of intake rate during the morning commenced according to the temporal pattern of meals, determined from the 24-hour behaviour recordings. During measurement of IR, cows were fitted with behaviour recorders to record the time spent eating and the number of grazing jaw movements. IR, bite rate (BR) and GJM rate were calculated per minute of eating time, rather than grazing time, since grazing time would have included intra-meal intervals (Gibb et al., 1997).

During measurement of IR, snip samples of herbage were taken representative of that consumed by the animals, for analysis for dry matter and organic matter content.

Cow performance

Milk yield was recorded daily at each milking. Milk quality was recorded at four consecutive milkings per week starting on the Monday p.m. milking. Solids-corrected

_____

milk (SCM) yields were calculated using the equation of Tyrrell & Reid (Tyrrell and Reid, 1965). Live weight was recorded 24 hours before and on the day of allocation, on the day of initial treatment, and thereafter at weekly intervals until the end of the experiment when live weight was recorded on two consecutive days.

Statistical methods

Due to the lack of independence between cows within each pair (Mead and Curnow, 1983), the pair mean values were treated as the unit of replication in the statistical analyses. Within each measurement period, IR data were analysed by two-way analysis of variance to examine the effects of treatment and time of day. All other data were analysed by one way analysis of variance within each period. Analysis of intake and behaviour data used the mean values of the two days' behavioural measurements for each of the pairs of animals allocated to each small paddock as replicates. Milk yield results were expressed as Solids Corrected Milk (SCM) (Tyrrell and Reid, 1965). Mean daily milk yield during the week prior to the imposition of treatments was used as a covariate in subsequent milk yield analyses. Analyses were carried out by ANOVA using the statistical package GenStat 2002.
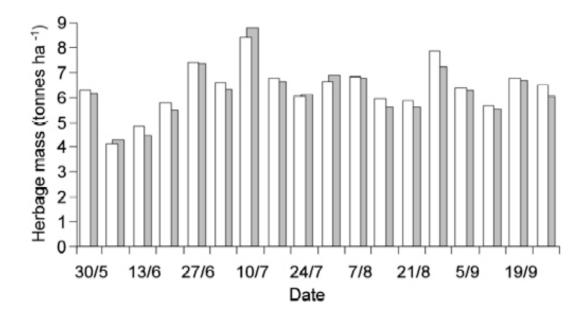
4.3. Results

All cows in the study remained in good health throughout the trial with the exception of animals 1188 (control), which was removed from the trial on 2 August 2000 because of traumatic injury, and 1481 (treated), which was removed from the trial on 15 August 2000 because of suspected pneumonia. Comparable animals from the main dairy herd replaced these cows in order to maintain group size, but were excluded from data analyses.

Sward measurements

There were no significant differences between paddocks grazed by the different treatment groups in herbage mass or sward heights. The overall mean values for herbage mass during the months May to September were 5296 ±118.6, 5449 ±194.8, 6893 ±123.2, 6446 ±127.7, 6210 ±91.7 kg DM/ha, respectively; the weekly pattern is shown in Figure 4.1. The overall mean SSH measurements before and after grazing during behaviour recording periods 1, 2 and 3 were 20.94 ±0.614 v 9.67 ±0.400, 17.20 ±0.218 v 7.77 ±0.121 and 16.29 ±0.442 v 8.23 ±0.268 cm, respectively.

Figure 4.1. Mean estimated herbage mass (DM tonnes/ha) measured to ground level at weekly intervals on paddocks before grazing by control (grey fill) and treated cows (no fill).

Parasitology

No lungworm (*Dictyocaulus viviparus*) larvae were recovered from any of the faecal samples throughout the study. The pre-treatment values for the faecal egg counts were low with less than half the animals having detectable eggs an arithmetic mean of <1 epg in the positive animals. The values remained low over the grazing season and the numbers excreted were similar in both groups. Minor peaks in values were observed in samples taken in July, September and October: peak individual counts were in the range 20-23 epg.

Faecal culture revealed the presence of the following genera: *Cooperia*, *Oesophagostomum*, *Ostertagia* and *Trichostrongylus*. In the pre-treatment and control cultures, a mixed population was present, with *Ostertagia* predominating in all but the final sample in October. In the treated group, only 40% of post-treatment faecal cultures yielded nematode larvae and these were mostly *Ostertagia*.

No nematode larvae were recovered in the pasture samples taken in May. Low numbers of larvae were found in some of the paddocks in June (4/12) and July (2/12). Peak values were observed in August when 5/6 of control paddocks were positive, with a mean of 162 larvae/kg herbage DM in the positive paddocks, and 4/6 of paddocks grazed by treated cows were positive with a comparable mean count of 87 larvae/kg herbage DM. Thereafter the counts in the control paddocks declined, with 3/6 having positive recoveries in September and October with means of 42 and 37 larvae/kg herbage DM respectively.

_____

The decline in pasture infectivity was more marked in the paddocks grazed by treated cows, where only one pasture had a positive count in September (83 larvae/kg herbage DM) and no larvae were recovered from any paddock in October.

Grazing behaviour

      24-h recording of grazing, ruminating and idling behaviour

The results of the analysis of the 24-h behaviour recordings are shown in Tables 4.1 and 4.2. There were no significant differences between the two groups prior to treatment (Period 1), or subsequent to treatment (Periods 2 and 3), in the times spent grazing and eating, the total number of GJM or bites, bite rate, the number of bites per GJM, the number of grazing meals or the mean meal duration. Analysis of variance of the results obtained in period 2 and 3, adjusting the values by covariance analysis for the results obtained in Period 1, similarly, showed no significant treatment effects.

There was no significant effect of treatment on ruminating behaviour (Table 4.2.), although analysis of the results adjusted by covariance analysis using the values obtained in Period 1, showed a slight increase in ruminating time and the number of ruminative mastications by cows receiving the eprinomectin treatment compared with the control treatment during Period 2; 536 v 502 ± 10.6 min (P=0.052) and 33,500 v 30,700 ± 500 mastications (P=0.005), respectively. No significant effects were found in period 3.

Cows in the control group spent more than 30 minutes idling compared with the treated group. However, this pattern of behaviour was apparent during Period 1, before the different treatments were imposed. Adjustment by covariance analysis for the idling times recorded in Period 1 failed to demonstrate any significant difference between the control and treated cows in the time they spent idling.

There was no significant effect of treatment on the number of grazing meals over 24 hours. During Period 1, cows had 10 to 11 grazing meals each day (Table 4.1). However, by Periods 2 and 3 the number had declined, with fewer, but longer meals occurring, particularly between afternoon milking and midnight (4.4 ±0.28, 2.8 ±0.16 and 3 ±0.23 meals during Periods 1, 2 and 3, respectively). The times spent grazing during each hour of the day, during the 24-hour recordings, are shown in Figure 4.2.

Table 4.1. Effect of no treatment (Control) or treatment with eprinomectin on grazing behaviour by Holstein-Friesian dairy cows measured over 24 h.

	Treatment		s.e. of treatment mean	Significance of treatment effect
	Control	Eprinomectin		
Period 1 (May, pre-treatment)				
Grazing time (min)	497	533	18.9	0.201
Eating time (min)	487	498	15.4	0.615
Evening grazing [1]	287	273	6.5	0.147
Total GJM[2] ('000)	35.7	36.2	1.33	0.800
Bites ('000)	24.9	25.0	0.92	0.922
Bite rate (bites/min)	51.2	50.4	0.86	0.506
Bites per GJM	0.70	0.69	0.013	0.729
Grazing meals	11.0	10.5	0.65	0.659
Mean meal duration (min)	49.0	53.6	4.02	0.438
Period 2 (July)				
Grazing time (min)	494	496	14.7	0.946
Eating time (min)	461	470	13.4	0.645
Evening grazing	328	334	7.8	0.562
Total GJM ('000)	34.0	34.7	1.14	0.668
Bites ('000)	23.6	24.1	0.87	0.668
Bite rate (bites/min)	51.1	51.4	0.88	0.840
Bites per GJM	0.69	0.69	0.010	0.939
Grazing meals	7.3	7.7	0.34	0.561
Mean meal duration (min)	70.5	68.6	2.76	0.626
Period 3 (September)				
Grazing time (min)	530	556	15.5	0.269
Eating time (min)	493	523	17.4	0.254
Evening grazing	298	321	8.1	0.081
Total GJM ('000)	36.1	37.2	1.54	0.641
Bites ('000)	24.9	25.9	1.10	0.533
Bite rate (bites/min)	50.4	49.4	0.73	0.362
Bites per GJM	0.69	0.70	0.013	0.740
Grazing meals	8.4	7.6	0.64	0.377
Mean meal duration (min)	68.3	79.1	5.99	0.230

[1] Time spent eating between afternoon milking and midnight
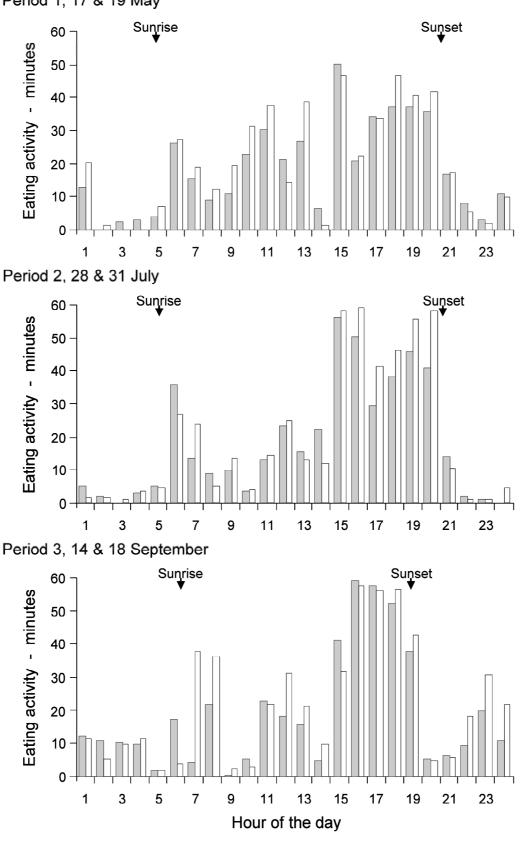[2] Total Grazing Jaw Movements

_____

Figure 4.2 Time spent eating during each hour (GMT) of the day by cows receiving either no anthelmintic (grey fill) or treatment with eprinomectin (no fill).

Period 1, 17 & 19 May

Period 2, 28 & 31 July

Period 3, 14 & 18 September

Table 4.2. Effect of no treatment (Control) or treatment with eprinomectin on ruminating and idling behaviour by Holstein-Friesian dairy cows measured over 24 h.

	Treatment		s.e. of treatment mean	Significance of treatment effect
	Control	Eprinomectin		
Period 1 (May, pre-treatment)				
Total ruminating time (min)	468	470	16.3	0.937
Total boluses	597	625	27.9	0.499
Time spent per bolus (sec)	48.7	45.3	1.76	0.214
Total mastications ('000)	29.5	29.1	1.40	0.819
Mastications per bolus	50.9	46.6	1.97	0.154
[1] Total idling time (min)	475	437	11.4	0.039
Period 2 (July)				
Total ruminating time (min)	502	536	11.6	0.064
Total boluses	619	663	27.9	0.290
Time spent per bolus (sec)	49.8	49.1	1.85	0.782
Total mastications ('000)	30.9	33.4	0.88	0.067
Mastications per bolus	51.0	51.2	2.20	0.966
Total idling time (min)	444	408	13.8	0.099
Period 3 (September)				
Total ruminating time (min)	426	456	13.9	0.157
Total boluses	526	538	20.5	0.704
Time spent per bolus (sec)	49.0	51.0	1.31	0.298
Total mastications ('000)	25.3	26.6	1.01	0.404
Mastications per bolus	48.3	49.9	1.53	0.488
Total idling time (min)	484	428	25.6	0.154

[1] 24 hours minus grazing time and ruminating time

Short-term intake rates

The results of the IR measurements made during the three periods are shown in Table 4.3. There were no significant differences in IR, GJM rate, bite rate, bites per GJM or bite mass between the two groups, either prior to treatment (Period 1) or following treatment (Periods 2 and 3). There was, however, in each period a significant effect of time of day, with significantly greater bite masses and IR being achieved during the afternoon compared with the morning measurement periods. There were no significant interactions between treatment and time of day on the IR measurements.

Table 4.3. **Effect of treatment with eprinomectin and time of day (ToD, a.m./p.m.) on short-term intake rate (IR) of herbage, rate of insensible weight loss (IWL) and grazing behaviour by grazing Holstein-Friesian dairy cows measured over 1 h.**

	Treatment		s.e. of treatment means	Significance of treatment effect	ToD		s.e.of Tod means	Significance of ToD effect
	Control	Eprinex			a.m.	p.m.		
Period 1 (May)								
Rate of IWL (g per min)	15.4	18.9	1.32	0.077	15.3	19.0	1.32	0.066
IR (g OM[1] per min)	38.3	36.9	2.33	0.660	22.1	53.1	2.33	<0.001
Total GJM[2] rate (GJM per min)	76.0	76.7	0.88	0.666	75.6	77.1	0.88	0.233
Bite rate (bites per min)	53.2	55.0	1.35	0.365	52.1	56.1	1.35	0.046
Bites per GJM	0.701	0.716	0.0161	0.528	0.688	0.729	0.0161	0.090
Bite mass (g OM per bite)	0.713	0.664	0.0388	0.385	0.427	0.950	0.0388	<0.001
Period 2 (July)								
Rate of IWL (g per min)	18.8	20.7	2.31	0.560	21.4	18.1	2.31	0.329
IR (g OM per min)	22.5	23.5	2.25	0.748	10.4	35.6	2.25	<0.001
Total GJM rate (GJM per min)	78.4	79.6	1.94	0.688	76.3	81.7	1.94	0.062
Bite rate (bites per min)	58.7	54.5	1.91	0.133	54.8	58.4	1.91	0.198
Bites per GJM	0.746	0.688	0.0198	0.050	0.716	0.718	0.0198	0.948
Bite mass (g OM per bite)	0.390	0.438	0.0516	0.514	0.210	0.617	0.0516	<0.001
Period 3 (September)								
Rate of IWL (g per min)	15.1	14.7	2.04	0.889	14.5	15.3	2.04	0.785
IR (g OM per min)	26.0	25.7	3.04	0.939	12.4	39.4	3.04	<0.001
Total GJM rate (GJM per min)	75.5	75.5	0.95	0.953	73.8	77.2	0.95	0.019
Bite rate (bites per min)	54.7	54.8	1.49	0.984	50.7	58.8	1.49	0.001
Bites per GJM	0.738	0.726	0.0189	0.649	0.704	0.760	0.0189	0.047
Bite mass (g OM per bite)	0.458	0.466	0.0536	0.918	0.247	0.677	0.0536	<0.001

[1] Organic matter
[2] Total Grazing Jaw Movements
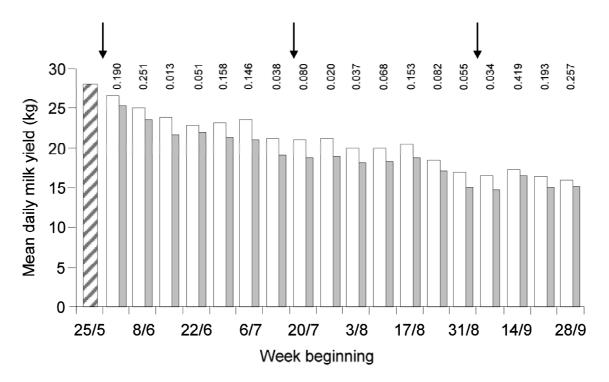
Cow performance

Milk composition and yield

There was no significant effect of treatment on milk fat, protein or lactose content in any week of the experiment, with the overall weekly mean values ranging between 3.97 (wk 3) and 4.96 (wk 20), 2.94 (wk 2) and 3.63 (wk 20), and between 4.21 (wk 17) and 4.59 (wk 1) g/kg milk, respectively.

The weekly mean daily solids-corrected milk (SCM) yields, adjusted by covariance analysis using the mean daily yield during the week prior to the first treatment are shown in Figure 4.3. Although the treated cows consistently yielded more milk than the control cows, statistically significant benefits were generally limited to within the first 4 weeks after treatment. The overall increase in mean daily milk yield over the 18 weeks was 1.68 kg/day; 20.56 v 18.88 ($\pm$ 0.444) kg/day (P=0.026), for the treated and control cows, respectively.

Figure 4.3. Mean daily milk yield (kg) of cows in control (grey fill) and treated (no fill) groups. Numbers above columns indicate significance (P) of treatment effect. Arrows indicate times of treatment.

_____

Live weight

The live weights of the control and treated cows, adjusted by covariance analysis for live weight before the imposition of treatments, showed no significant differences between groups at any time: the growth patterns 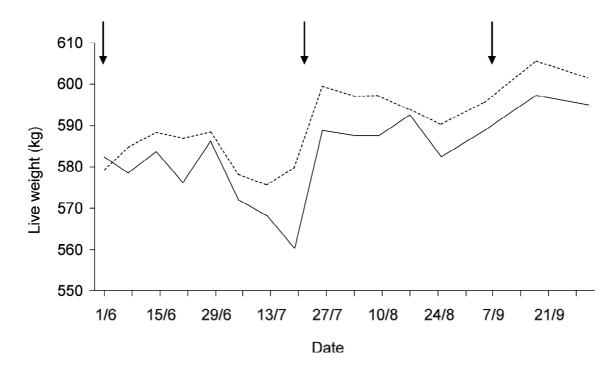are shown in Figure 4.4. The mean daily live-weight gains by the control and treated cows from the start of treatment until the end of the experiment did not differ significantly at 0.10 and 0.18 kg/day, respectively.

Figure 4.4. Live weight (kg) of cows in control (solid line) and treated (dashed line) groups. Arrows indicate time of treatment.

4.4. Discussion

Parasitology

Consistent with the literature, the faecal egg counts in this trial with adult dairy cows were very low. If the usual McMaster technique, with a sensitivity of 50 epg had been employed rather than that in the present study with a sensitivity of 1 epg, then the majority of samples would have been recorded as <50 epg. There are some anomalies between the results of the faecal egg counts and subsequent faecal culture. The faecal egg counts would suggest little difference between the two groups of cattle in terms of nematode egg excretion, but, whereas all the faecal cultures from control cows yielded positive larval recoveries, only two of five post-treatment cultures from the faeces of treated cows produced larvae. *Ostertagia* larvae predominated in all but one of the pre-

treatment and control faecal cultures; *Oesophagostomum* was the next most common genus followed by *Trichostrongylus* and *Cooperia*. These results are consistent with other work in adult dairy cows, which demonstrated the importance of *Ostertagia* as a parasite of adult cattle (Agneessens et al., 2000; Borgsteede et al., 2000).

The faecal cultures may reflect more accurately the potential for pasture contamination with parasitic nematode larvae, because the density of pasture larvae on the control paddocks was consistently higher than that on the paddocks grazed by treated cows from August onwards.

Grazing behaviour and herbage intake

The differences in bite mass, bite rate and IR between the measurements made during the afternoon and morning were mainly in response to the modification of sward structure by the cows during the period that they were present on the paddock. Bite mass, bite rate and IR are constrained by sward height (Gibb et al., 1999) and the effect of the reduction of mean sward height over the period of stocking on daily paddocks in reducing bite mass, bite rate and IR in daily paddocks has been demonstrated (Barrett et al., 2001). However, with the exception of an apparent reduction in the proportion of bites/GJM as a result of treatment with eprinomectin in Period 2, treatment had no effect on any of the parameters derived during measurements of IR.

Similarly, there was no evidence of any treatment effect on the time spent grazing and eating, the number of GJM and bites, or the number of grazing meals. Examination of the temporal patterns of grazing activity (Figure 4.1) showed an approximately 8-hour periodicity, similar to that shown in sheep (Champion et al., 1994) and cattle (Deswysen et al., 1993), with peaks of activity around 08:00 h, 16:00 h and 24:00 h. The reduction in grazing activity around midnight in Period 2, compared with Periods 1 and 3, was probably the result of the 24-h recordings being made during a period of the new moon, whereas Periods 1 and 3 recordings were made close to the full moon phase of the lunar cycle. Such effects of the lunar cycle on the temporal pattern of grazing activity have been reported previously in cattle (Hein, 1935; Vilela et al., 1974), although there is no evidence that such patterns necessarily affect total grazing time over 24 hours. Comparison of the temporal patterns of grazing activity by cows on the two treatments showed a significant effect of treatment in the hours commencing at 14:30 h and 05:30 h within each period. These differences and the occasional treatment effects in subsequent hours are attributable to the control cows always being milked before the treated cows. For example, the enforced reduction in grazing time by the treated cows during the hour following afternoon

_____

milking appears to have been compensated for by subsequent increases in the grazing time during the evening. Likewise, the reduction in grazing time following morning milking appears to have been compensated for by an increase in grazing time during the following two hours. The net result, nevertheless, appears to have been that the total grazing time in 24 hours was not affected by treatment.

Under continuous variable-stocking management, total daily herbage intake may be estimated from measurements of IR and total time spent eating, despite the small changes in IR over the day (Gibb et al., 1998). However, because of the rapid and relatively greater modification of sward structure and consequent effects on IR under daily paddock stocking management, it is not appropriate to attempt to calculate daily herbage intake unless more frequent measurements of IR are made than in the present experiment over the course of the day. What may be concluded, however, is that, because treatment did not significantly affect IR or eating time, it is unlikely that treatment significantly affected daily herbage intake. The similarity in the mean SSH on the paddocks before grazing and after grazing and the proportional reduction in SSH over 24 h grazing also provide evidence of a lack of treatment effect on herbage intake.

This apparent lack of effect on grazing behaviour and herbage intake is in contrast to the results of an experiment in which cows grazed under continuous variable-stocking management (Forbes et al., 2004). Under continuous variable-stocking management, although sward state constrains bite mass and intake rate, cows are able to increase their daily herbage intake by extending their grazing time, consuming herbage from a sward in which the structure is altered little over the course of a day. In the present experiment, where daily grazing paddocks were provided, the restrictive daily herbage allowance will have imposed a limitation on the herbage available.   In these circumstances, if cows attempt to increase their intake of herbage, they are confronted with a rapidly modified and depleted sward, from which they may be reluctant to graze further.

Although the increase in ruminating time by the treated cows compared with the control cows was not statistically significant, the increase of 30 minutes may provide some partial explanation of the origin of the improved milk production and live-weight performance. Shorter ruminating times by the parasitised cows may have resulted in a decreased digestibility of the forage, as has been previously observed in parasitized steers and cows (Fox et al., 1985; Entrocasso et al., 1986).

_____

Sward measurements

The daily herbage allowance provided in the experiment was chosen to achieve a moderately high level of utilization (c. 40%) of the herbage measured to ground level and accepting that daily intake by the cows would be restricted. The imposed daily allowance of 36 kg DM (measured to ground level)/cow approximated to 60 g DM/kg liveweight. The results of Combellas and Hodgson (1979) and Le Du et al. (1979) had demonstrated the intake response to increasing herbage allowance by grazing dairy cows, which suggested that at a daily herbage DM allowance of 60 g/kg liveweight an intake of approximately 25 g/kg liveweight would be expected (Combellas and Hodgson, 1979; Le Du et al., 1979). Such a level of intake would be equivalent to a daily herbage intake of 14.5 kg OM by the cows used in this experiment.

The rising plate meter heights on the swards did not differ between the paddocks occupied by the control and treated cows. Thus, given the limitation that a common, weekly-derived regression was used to estimate herbage mass, no differences existed between the estimated herbage mass present on the control and treated paddocks. Overall mean SSH measured at the time of the behaviour recordings did not differ between paddocks grazed by the control and treated cows, either before the cows entered the paddocks or when they left. In all cases, mean SSH was reduced by between 50 and 55 per cent.

Cow performance

Eprinomectin treatment resulted in a consistent and, during 6 weeks, significant improvement in milk yield, which over the 18 weeks of the experiment represented an overall daily increase of 1.68 kg SCM/day. At the levels of production obtained, the model of Woods et al. (2003) would suggest an increase of about 24 MJ of metabolizable energy being necessary; somewhat in excess of that possibly supplied by increased digestibility as a result of improved dietary comminution (Woods et al., 2003). In addition to possible implications of behaviour on the digestive efficiency of the treated cows, the diminution of the nematode burden should have reduced the turnover of their gastrointestinal tissue (Entrocasso et al., 1986), and possibly the physiological cost of the cow's immune response resulting from infection (Yang et al., 1993).

The non-significant differences in live-weight gain in favour of the treated cows can be considered in the context of the significant differences reported in another study with eprinomectin-treated dairy cows (Forbes et al., 2004) and a study on autumn-born dairy heifers in their first grazing season (Forbes et al., 2000). In both examples it was shown

_____

that subclinical nematode infections were responsible for reductions in weight, when compared with anthelmintic-treated animals. However, in both studies, the improved liveweight performance appeared to have been mediated by increased grazing time and herbage intake.

4.5. Conclusions

The experiment demonstrated that a reduction in subclinical gastrointestinal nematode infections in adult dairy cattle can benefit milk yield, even when cows may be unable to respond by increasing their daily intake of herbage because of restrictive daily herbage allowances. In the absence of evidence for increased intakes, it must be concluded that increased milk production may have resulted from improved digestive efficiency and/or partitioning of nutrients towards production and away from immunological/pathophysiological demands, subsequent to the removal of parasites.

_____

4.6. References

Agneessens, J., Claerebout, E., Dorny, P., Borgsteede, F.H., Vercruysse, J., 2000, Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 90, 83-92.

Barrett, P.D., Laidlaw, A.S., Mayne, C.S., Christie, H., 2001, Pattern of herbage intake rate and bite dimensions of rotationally grazed dairy cows as sward height declines. Grass Forage Sci 56, 362-373.

Borgsteede, F.H., Tibben, J., Cornelissen, J.B., Agneessens, J., Gaasenbeek, C.P., 2000, Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 89, 287-296.

Champion, R.A., Rutter, S.M., Penning, P.D., Rook, A.J., 1994, Temporal variation in grazing behaviour of sheep and the reliability of sampling periods. Appl Anim Behav Sci 42, 99-108.

Combellas, J., Hodgson, J., 1979, Herbage intake and milk production by grazing dairy cows 1. The effects of variation in herbage mass and daily herbage allowance in a short-term trial. Grass Forage Sci 34, 209-214.

Deswysen, A.G., Dutilleul, P., Godfrin, J.P., Ellis, W.C., 1993, Nycterohemeral eating and ruminating patterns in heifers fed grass or corn silage: analysis by finite Fourier transform. J Anim Sci 71, 2739-2747.

Entrocasso, C.M., Parkins, J.J., Armour, J., Bairden, K., McWilliam, P.N., 1986, Metabolism and growth in housed calves given a morantel sustained release bolus and exposed to natural trichostrongyle infection. Res Vet Sci 40, 65-75.

Forbes, A.B., Huckle, C.A., Gibb, M.J., 2004, Impact of eprinomectin on grazing behaviour and performance in dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management. Vet Parasitol 125, 353-364.

Forbes, A.B., Huckle, C.A., Gibb, M.J., Rook, A.J., Nuthall, R., 2000, Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. Vet Parasitol 90, 111-118.

Fox, M.T., Jacobs, D.E., Campling, R.C., Pocknee, B.R., Clampitt, R., Hart, I.C., 1985, Effect of thiabendazole treatment on feed intake, digestibility and selected blood values in lactating dairy cows. Vet Rec 116, 257-260.

Gibb, M.J., 1998. Animal Grazing/Intake Terminology and Definitions. In: Pasture Ecology and Animal Intake, Dublin, pp. 21-35.

Gibb, M.J., Huckle, C.A., Nuthall, R., 1998, Effect of time of day on grazing behaviour and intake rate by lactating dairy cows. Grass Forage Sci 53, 41-46.

Gibb, M.J., Huckle, C.A., Nuthall, R., 2002, Effect of type of supplement offered out of parlour on grazing behaviour and performance by lactating dairy cows grazing continuously stocked grass swards. Anim Sci 75, 153-167.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1997, Effect of sward surface height on intake and grazing behaviour by lactating Holstein Friesian cows. Grass Forage Sci 52, 309-321.

_____

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1999, The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl Anim Behav Sci 63, 269-287.

Gross, S.J., Ryan, W.G., Ploeger, H.W., 1999, Anthelmintic treatment of dairy cows and its effect on milk production. Vet Rec 144, 581-587.

Hein, M.A., 1935, Grazing time of steers on permanent pasture. J.Am.Soc.Agron. 27, 675-679.

HFRO 1986. Biennial Report 1984-1985 (Hill Farm Research Organisation), pp. 29-30.

Le Du, Y.L.P., Combellas, J., Hodgson, J., Baker, R.D., 1979, Herbage intake and milk production by grazing dairy cows 2. The effects of level of winter feeding and daily herbage allowance. Grass Forage Sci 34, 249-260.

MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationary Office, London, 160 p.

Mead, R., Curnow, R.N., 1983, Statistical methods in agriculture and experimental biology. Chapman Hall, New York, 400 p.

Orr, R.J., Rutter, S.M., Penning, P.D., Rook, A.J., 2001, Matching grass supply to grazing patterns for dairy cows. Grass Forage Sci 56, 352-361.

Rook, A.J., Huckle, C.A., 1997, Activity bout criteria for grazing dairy cows. Appl Anim Behav Sci 54, 89-96.

Rutter, S.M., 2000, Graze: a program to analyze recordings of the jaw movements of ruminants. Behav Res Methods Instrum Comput 32, 86-92.

Rutter, S.M., Champion, R.A., Penning, P.D., 1997, An automatic system to record foraging behaviour in free-ranging ruminants. Appl Anim Behav Sci 54, 185-195.

Tyrrell, H.F., Reid, J.T., 1965, Prediction of the energy value of cow's milk. J Dairy Sci 48, 1215-1223.

Vilela, E., de Menezes, J.O.B.X., Schiffler, E.A., Grilo, R.C., 1974, Behaviour of dry cows. Anais da Escola de Agronomia e Veterinaria 4, 67-79.

Woods, V.B., Kilpatrick, D.J., Gordon, F.J., 2003, Development of empirical models to describe the response in lactating dairy cattle to changes in nutrient intake as defined in terms of metabolisable energy intake. Livest Prod Sci 80, 229-239.

Yang, C., Gibbs, H.C., Xiao, L., Wallace, C.R., 1993, Prevention of pathophysiologic and immunomodulatory effects of gastrointestinal nematodiasis in calves by use of strategic anthelmintic treatments. Am J Vet Res 54, 2048-2055.

Chapter 5: Evaluation of the effect of eprinomectin in young dairy heifers sub-clinically infected with gastrointestinal nematodes on grazing behaviour and diet selection

_____

5.1. Introduction

Previous studies in free-ranging young and adult cattle have shown that, when parasitic nematode burdens are controlled through the use of anthelmintics, grazing behaviour is modified (Forbes et al., 2000; Forbes et al., 2004). A significant decrease in the duration of daily grazing time was observed in the untreated control animals and was considered to be an expression of the reduced appetite that is commonly associated with parasitism (Kyriazakis et al., 1998). It has been hypothesised that parasite-induced inappetence is a host adaptation that allows for greater dietary selectivity (Kyriazakis et al., 1998). A 12-week study of housed lambs offered a choice of high or low protein diets and subsequently challenged with 2500 L_3 of *Trichostrongylus colubriformis* daily showed that parasitized animals suffered a reduction in feed intake but consumed a higher proportion of the high protein ration, which enabled them to maintain a level of protein intake similar to that of non-parasitized animals (Kyriazakis et al., 1994). In a second, similar, but more complex study, housed lambs were offered a choice of high or low protein diets and challenged with 2500 L_3 of *Trichostrongylus colubriformis* per day over a 24 week period. In this study there were no significant parasitism x feed interactions, though in most of the comparisons, the parasitized lambs consumed a higher proportion of the high protein diet than the uninfected controls (Kyriazakis et al., 1996).

A study of lambs grazing on grass/clover pastures with naturally acquired infections of gastrointestinal nematodes was undertaken in New Zealand: comparison of effects was through suppressive drenching with ivermectin every two weeks in one group and in the other group 'trigger' drenching when mean faecal egg counts (fec) were >1000 eggs per gram (epg) (Cosgrove and Niezen, 2000). The swards were of either perennial ryegrass (*Lolium perenne*) and white clover (*Trifolium repens*) or browntop (*Agrostis capillaris*) and white clover (*T.repens*). There was some evidence in this experiment that lambs that grazing on mixed swards could modify the composition of their diet as a consequence of gastrointestinal parasitism, but there was no significant depressive effect of parasitism on feed intake and the partial preferences for clover differed according to the species of grass in the sward: 51% of the intake was clover in the ryegrass paddocks compared to 27% in the brown top pastures. In another study in sheep grazing grass/clover swards, parasitized lambs spent significantly less time grazing per day and had a lower daily herbage dry matter (DM) intake compared to control lambs (Hutchings et al., 2000). There were no significant effects of parasitism

_____

on diet selection inasmuch as the parasitized lambs showed no increase in the proportion of clover in their diet compared to controls, however the magnitude of the parasite burden, as reflected by faecal egg counts, was positively correlated with the proportion of clover in the diet.

The results of the two studies in grazing lambs may have been compromised because of the spatial heterogeneity in mixed grass/clover swards and other confounding factors under the experimental conditions. Providing grazing animals with adjacent swards of monocultures of ryegrass and white clover allows for the measurement of dietary preference between grass and clover without constraint (Parsons et al., 1994). The results of such studies in grazing sheep and cattle have recently been reviewed (Rutter, 2006) and in the majority of published reports partial preference for clover was 70±10%. This ratio coincides with the calculated optimum clover content (60-70%) in the diet of housed dairy cows for milk yield, when fed grass and white clover in various proportions (Harris et al., 1998). There is also a consistent diurnal pattern with a marked preference for clover (legume) in the morning and an increased preference for grass in the afternoon. Comparisons between lactating and non-lactating animals show preferences of 75±5 and 65±5% respectively, indicating that preference may be affected by physiological status and nutrient demands.

The similarities in the partial preferences in cattle and sheep for clovers/legumes over grass and also in the observed diurnal patterns in such studies point to the possibility of a common underlying biological basis for such selective feeding behaviour. The theories for such a basis include:

   a)   Maximisation of intake rates

   b)   Sampling to assess relative quality of herbage

   c)   Maintaining rumen function (micro-flora and fauna)

   d)   Balancing Carbon and Nitrogen (energy and protein) intakes

   e)   Conditioned taste aversion

   f)   Anti-predator behaviour

Of these, e) and f) have merit because they offer an explanation of both the observed dietary preferences and the diurnal patterns, however, there is no intuitive reason why several or all of these criteria could not be important components in determining animals' grazing behaviour as they are not mutually exclusive (Rutter, 2006). Notwithstanding evolutionary perspectives, for domestic livestock, balancing energy and protein intakes and maintaining rumen function provide attractive hypotheses.

_____

There is support for d), the principle of self-regulation of protein intake according to requirements in studies in housed animals offered choices of feeds with different crude protein (CP), metabolizable protein (MP) or rumen degradable protein (RDP) levels (Tolkamp et al., 1998a; Tolkamp et al., 1998b; Scott and Provenza, 2000). There is some evidence in support of c) from *in vitro* and *in vivo* studies where increased efficiency in microbial protein synthesis in the rumen and increased flows of microbial nitrogen into the duodenum were observed when the activity of mixtures of grass silage and red clover silage was compared to grass or legume silages provided singly (Merry et al., 2006).

Superimposing differences in the level of parasitism in the grazing animal introduces an additional level of complexity into the grass/legume matrix because the clover content of pasture has been shown to be positively related to the growth rate of lambs grazing mixed grass/clover swards (Niezen et al., 2002). Furthermore, comparisons between monocultures comprising various species of grasses and legumes showed that recovery of infective larvae (*Ostertagia* and *Trichostrongylus* spp.) was generally higher on the grasses than the legumes and a subsequent study showed that recovery of larvae from ryegrass/clover mixes was intermediate between ryegrass and white clover (Niezen et al., 1998). Additionally parasitism can affect foraging behaviour and faecal avoidance in grazing animals (Cooper et al., 2000; Hutchings et al., 2003).

To date, all of the studies that have investigated the impact of gastrointestinal parasitism on dietary preference have been conducted in sheep and none in cattle. The current study was conducted to examine the impact of anthelmintic control of gastrointestinal parasitism on dietary preference in first-grazing season dairy heifers grazing contiguous monocultures of perennial ryegrass and white clover.

5.2. Materials and Methods

Animals

Sixteen autumn-born dairy heifer calves weighing approximately 235 kg, which had not grazed previously, participated in the study. The heifers were acclimatised to grazing adjacent monocultures prior to the behavioural recording in order that novel, exploratory behaviours did not confound any subsequent responses (Rutter, 2006). All animals were trained to wear jaw-movement recorders and faecal collection bags prior to undertaking these measurements.

Allocation

The animals were blocked according to liveweight and assigned at random within blocks to either the control or treated groups. Within each treatment group, calves were ranked in order of descending live weight and paired to provide four pairs in each treatment group for assignment to the replicated measurement paddocks. Calves were paired because previous experience has shown that herd animals pastured individually may not express normal grazing behaviours because of interactions with other animals within the vicinity.

Treatment

Eprinomectin (Eprinex® Pour-on) was administered topically to the animals in the treated group at the recommended dosage of 500 mcg/kg liveweight on Day 1 and again on days 43, 64 and 92 in order to keep nematode worm burdens low. The control group received no anthelmintic.

Parasite infections

To initiate infections with parasitic gastrointestinal nematodes, each heifer was dosed at turnout and again four weeks later with 2000 third stage infective larvae of *Cooperia oncophora* and 2000 *Ostertagia ostertagi*. The larvae were supplied by Merck Research Laboratories and were derived from 1994 isolates from a UK field outbreak of parasitic gastroenteritis (PGE); they had been passaged through calves 5-6 times since collection. Worm burdens were additionally acquired naturally from the pasture during grazing. The whole grazing area had been grazed the previous year by adult dairy cows and was therefore assumed to be lightly contaminated with infective parasitic nematode larvae. Prior to turnout, all the heifers received the recommended two doses of a vaccine comprising irradiated larvae of *Dictyocaulus viviparus* to provide protection against parasitic bronchitis.

Pasture management

The experiment was carried out within a field containing several contiguous monoculture swards of perennial ryegrass (*L.perenne*) and white clover (*T. repens*) established 2 years previously. Four 0.5 ha paddocks, subsequently referred to as the background paddocks, were fenced, each containing equal areas of perennial ryegrass and white clover swards. The perennial ryegrass swards within these paddocks were fertilized to provide 51.75 kg nitrogen (N)/ha in April, June and August.

An additional area, containing adjacent swards of ryegrass and a white clover, each measuring 96 m × 58 m, was also fenced and maintained for subsequent subdivision to

_____

provide eight narrow paddocks, subsequently referred to as measurement paddocks. The ryegrass sward within this area was fertilized to provide 51.75 kg N/ha in April, June and July.   On 6 April, a 1 m wide boundary strip between the grass and clover swards was treated with a solution of sodium chlorate to eradicate all herbage. Both the ryegrass and clover swards within the area were cut on 26 May and 9 July, and the herbage removed. Following the second cut, the area was subdivided and fenced to provide eight paddocks, each containing a 12 m × 58 m area of ryegrass and white clover swards, with a common 1 m × 12 m defoliated boundary between the two swards. Drinking troughs and ball-lick feeders were sited in the boundary areas of each measurement paddock to avoid any biasing effect on herbage selection.   The measurement paddocks were designed to allow the direct effect of parasite burden on grazing behaviour and dietary preference to be studied, without interference due to possible cumulative treatment effects on sward structure in the background paddocks and to provide uniform swards of ryegrass and clover.

The heifers were turned out initially onto a grass sward in early May and were maintained on pasture during the grazing period at a stocking rate of 9.4 animals per hectare. A mixture of molasses and poloxalene (anti-bloat agent) was provided in a ball-lick feeder from the middle of May onwards and at this time an area of white clover sward was incorporated into the paddock area available for grazing. Eprinomectin was administered on 18th May (Day 1) when two pairs from within each experimental group were turned out on onto each of the four background paddocks.

Sampling and Measurements

Pastures

Sward heights were measured weekly at fifty random locations on each of the clover and ryegrass plots in the four background paddocks using a circular rising plate meter graduated at 0.5 cm intervals.   Herbage samples for determination of botanical composition of the ryegrass and clover swards were collected on the background paddocks the day before the heifers were introduced and on three other occasions at approximately monthly intervals. Following measurement of plate height at every tenth location, herbage was cut at ground level for subsequent separation into botanical fractions (*viz.* grass swards – leaf, pseudostem, reproductive stem, dead and weed; clover swards – stolon, petiole, leaf, flower, dead and grass), overnight drying and ashing to determine organic matter (OM) mass (kg/ha).

On the measurement paddocks, rising plate meter heights were measured at forty random sites and two herbage samples were collected on the ryegrass and clover swards in each of the measurement paddocks, on 20 and 27 August, i.e. prior to and following the 24-h behaviour recordings. In addition, forty sward surface height measurements were taken on each sward type within each paddock using a sward stick (HFRO, 1986).

Parasitology

Faecal samples were collected every 28 days from each animal for enumeration of trichostrongyle eggs and lungworm, *Dictyocaulus viviparus,* larvae using standard techniques (MAFF, 1986). Larval cultures were performed on bulk faecal samples collected at the same times to determine nematode species/genus composition (MAFF, 1986). Once a month, in May (prior to turnout), June, July and August, pasture larval counts were conducted on pooled herbage samples taken from the clover and grass monocultures in the four main paddocks using a Standard Operating Procedure, based closely on the MAFF technique (MAFF, 1986).

Grazing behaviour

Times are quoted as British Summer Time (BST) (i.e. Greenwich Mean Time [GMT] + 1h) so the solar zenith is at 13.00 h. Two automatic recordings of behaviour were conducted over 24 hours in late August. On the afternoon of 22 August, all heifers were removed from the background paddocks and fitted with solid-state behaviour recorders (Rutter et al., 1997), before each heifer pair was turned onto a measurement paddock. The recorders were set to record jaw movements between 00.00 and 24.00 on 23 August (observation period 1). The following morning the recorders were removed and the heifers were returned to their background paddocks. The complete procedure was repeated the following afternoon to record jaw movements between 00.00 and 24.00 on 26 August (observation period 2).

The behaviour recordings were analysed using the software 'Graze' (Rutter, 2000). Algorithms within this software enable the operator to distinguish between the jaw-movement patterns associated with grazing and ruminating activities. Total eating time (TET) during grazing activity was calculated as the sum of the periods of grazing jaw movements (GJM), excluding intervals of jaw inactivity >3 s (Gibb et al., 1998). Total grazing time (TGT) was calculated as the sum of the periods of GJM activity, including any periods of jaw inactivity <5 min. Periods of jaw inactivity >5 min were interpreted as being inter-meal intervals (Rook and Huckle, 1997). The number of grazing meals was calculated as the number of periods of grazing activity separated by intervals >5

_____

min. Jaw movements associated with the heifers licking the molasses/poloxalene ball-licks were readily distinguishable by the operator using Graze, because of the absence of the characteristic wave pattern associated with bites and non-biting GJM during grazing. Total idling time was calculated as the time within each 24-h period when heifers were not eating grass or ruminating and included time spent drinking, licking the ball-licks and in social interaction.

The number of bites and non-biting GJM, of ruminative mastications and of boluses regurgitated during rumination were counted automatically. The term 'non-biting GJM' refers to those jaw movements not identified as bites and therefore includes jaw movements which may have had a masticative or manipulative function.

Determination of diet selection

Between 05.30 and 20.30 (i.e. 15 hours per day) during the two 24-h periods of behaviour recording, visual observations were made of the activity (eating, ruminating, idling, social licking, and licking the ball-lick feeders) and whether that activity was conducted on the ryegrass or clover sward or on the sprayed boundary area between the two swards. The observations were carried out by relays of experienced observers located in a tower sited opposite the sprayed boundary between the ryegrass and clover swards. The activity and location of each heifer were recorded throughout the 15-hour observation period at 4-min intervals, with a 15-s interval between each animal in sequence.

Short-term intake rate, bite rate and bite mass

Short-term herbage intake rates (IR, g/min), bite rate (BR, bite/min) and bite mass (BM, g/bite) were measured on the ryegrass and clover swards of the measurement paddocks swards, using a short-term weight change technique (Penning and Hooper, 1985) previously adapted for use with dairy cows (Huckle et al., 1994). Briefly, the technique involves accurate weighing of the animal before and after a period of 1 hour at pasture to measure intake, and applying a correction for the rate of insensible weight loss determined on that animal over the following hour. During each morning and afternoon period, measurements were made on two ryegrass and two clover swards using four randomly selected pairs of heifers, so that over the course of the four days the IR of each pair was measured on both sward types at both times of day. Measurements of IR, BR and BM were conducted over a period of approximately 1 hour, commencing at approximately 10.00 and 15.00 h for the morning and afternoon periods, followed by measurement of rate of insensible weight loss (IWL) over the subsequent hour.

During measurement of Intake Rate, heifers were fitted with behaviour recorders to record the time spent eating and the number of Grazing Jaw Movements. IR, BR and GJM rate were calculated per minute of eating time, rather than grazing time, because the latter would have included intra-meal intervals (Gibb et al., 1999).

During measurement of IR, snip samples representative of the herbage consumed were collected for determination of the dry and organic matter (OM) content of the herbages.

Animal Performance

Live weight was recorded 48 and 24 hours before and on the day of turnout (Day 0) and thereafter at 28 day intervals until the end of the trial when a double weighing was taken.

Statistical methods

Rising plate meter heights and herbage mass of the various botanical fractions on the ryegrass and clover swards were examined by one-way analysis of variance for the effect of treatment at each sampling date. Due to the lack of independence between heifers within each pair, the mean values of measured and derived parameters were treated as the units of replication (Mead and Curnow, 1983). Data derived from the 24-h observation periods were examined by one-way analysis of variance for the effect of treatment with observation day effects removed as block effects. Data derived from the short-term behaviour measurements were examined by three-way analysis of variance (treatment x sward x time of day) with observation day effects removed as block effects.

5.3. Results

Parasitology

All calves in the two treatment groups remained in good health throughout the trial and showed no signs of clinical parasitic gastroenteritis or bronchitis. No lungworm larvae were recovered from any of the faecal samples.

Despite the initial seeding of trial heifers with infective larvae and exposure to previously grazed pasture, the overall level of gastrointestinal parasitism, as reflected in the faecal egg counts, appeared to be very low throughout the study. Less than 50% of the 48 faecal samples from the control animals had values above the detection limit of 50 eggs per gram (epg). Of the 21 samples that were positive, the highest individual value was 300 epg and the highest group mean was during August with an arithmetic mean of 125 epg (range <50 to 300). In the treated group, 8 of 48 faecal samples were positive over the sampling period with an overall arithmetic mean of 20 epg compared with an overall mean of 54 epg

_____

for the untreated group. At the faecal sampling immediately prior to the behaviour recording, 5 of 8 of the control animals had positive (>50) epgs.

Faecal culture showed that *Ostertagia* spp. predominated at all sampling times, representing 69-89% of the larvae identified, with the balance comprising *Cooperia* spp. Consistent with the faecal egg samples, the overall concentration of infective larvae on the background paddocks was low and infective larvae were absent from many herbage samples. Of the 32 pooled samples examined (two from each of the 4 background paddocks per month), 24 (75%) yielded no trichostrongyle larvae at all and the total number of larvae recovered from the other samples was 502. The highest count in the positive samples was 231 trichostrongylid larvae/kg herbage Dry Matter (DM) in June from a clover paddock grazed by untreated heifers and this comprised a significant proportion of the total. Of the 502 trichostrongylid larvae recovered, 466 were from the paddocks grazed by control heifers and 36 from those grazed by treated heifers; additionally 424 larvae were recovered from the clover monocultures compared to 78 from the grass monocultures. Small numbers of *Nematodirus* spp. larvae were recovered sporadically from some paddocks.

Grazing behaviour

Results of the 24-h behaviour recordings are shown in Table 5.1. The heifers treated with eprinomectin ate for 36 minutes longer than the control heifers (P=0.085) and grazed for 56 minutes longer (P=0.054). Consequently, there were significant increases in the total number of grazing jaw movements (GJM) (P=0.015) and the total number of non-grazing GJM (P=0.001) and a significant decrease in the number of bites per GJM (P=0.017) in the treated cattle compared to the controls. The temporal patterns of eating behaviour are illustrated in Figure 5.1 as the time spent eating during each hour of the day. Analysis of variance showed no significant treatment effect during any hour.

Table 5.1. Grazing, ruminating and idling behaviour by untreated (control) and eprinomectin-treated (treated) heifers allowed free access to adjacent monocultures of perennial ryegrass and white clover over 24 hours. Analysis based on pair means as unit of replication.

Treatment	Control		Eprinomectin			Significance of effect of	
					s.e. of	of effect of	
Observation da	1	2	1	2	mean	treatment	day
Eating (min)	390	470	434	498	19.2	0.085	0.003
Grazing (min)	452	506	536	535	26.5	0.054	0.348
Intra-meal intervals (min)	62	36	102	36	14.84	0.192	0.009
Ruminating (min)	455	454	400	451	18.8	0.147	0.207
Idling (min)	594	516	606	491	34.4	0.846	0.016
Total GJM (000)	28.96	35.82	33.34	39.58	1.02	0.015	<0.001
Bites ('000)	18.97	25.53	20.43	25.69	1.146	0.492	<0.001
Non-biting GJM (*)	9.99	10.29	12.91	13.88	0.781	0.001	0.433
Bites/GJM	0.657	0.714	0.607	0.650	0.020	0.017	0.033
Bite rate (per min)	48.9	54.4	46.8	51.6	2.00	0.243	0.025
Total GJM rate (per min)	74.5	76.3	76.9	79.5	1.49	0.083	0.174
Ruminative mastications (*)	31.71	32.48	30.71	33.02	1.289	0.861	0.255
Mastication rate (per min)	69.8	71.6	73.1	73.1	1.36	0.105	0.520
Boli	576	530	608	592	30.8	0.150	0.331
Mastication/bolus	56.1	61.9	53.1	56.8	3.19	0.233	0.161

* = x1000

Analysis of variance of the total time spent eating between 20.30 and 05.30 h (night) showed no effect of treatment or observation day (overall mean 79 ± 4.5 min) and represented proportionately 0.175 of total daily eating activity. Eating activity between 05.30 and 20.30 was longer (P=0.004) during observation day 2 compared with day 1 (398 *v* 341 ± 11.2 min), but was not significantly affected (P=0.062) by treatment (353 *v* 386 ± 11.2 min, control and treated heifers, respectively).
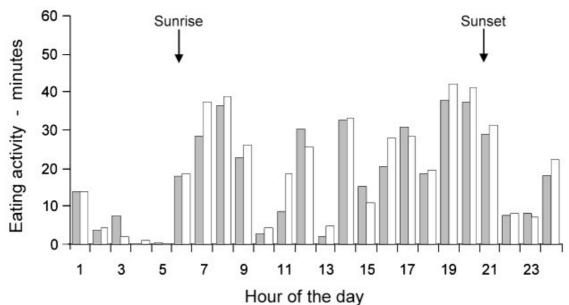
_____

Figure 5.1. Time spent eating during each hour of the day by heifers receiving either no anthelmintic (grey fill) or treatment with eprinomectin (no fill).

Analysis of variance of the results of the visual observations between 05.30 and 20.30 h are presented in Table 5.2. There was a significant (P=0.032) effect of eprinomectin treatment on the number of observations of grazing activity on clover, with the treated animals on average seen grazing on 70.2 occasions and the control heifers on 61.7 occasions between 05.30 and 20.30. The number of observations of eating activity on both the ryegrass and clover swards was significantly greater during the period 13.00 to 20.30 h (afternoon) than the period 05.30 to 13.00 h (morning). The proportion of total grazing activity spent on the clover sward did not differ significantly between treatments (73.0% for the controls, 75.5% for the treated heifers) or the morning and afternoon periods.

Table 5.2. Effect of eprinomectin treatment and time of day (ToD; am 05.30 to 13.00; pm 13.00 to 20.30) on number of observations of grazing activity on grass and clover monocultures during daylight.

	Control		Eprinomectin		s.e. of mean	Significance of effect of	
	am	pm	am	pm		Treatment	ToD
Grass	9.1	14.4	8.1	14.9	1.62	0.878	<0.001
Clover	25.8	35.9	31.6	38.6	1.88	0.032	<0.001
Clover/Total	0.74	0.72	0.79	0.72	0.033	0.407	0.152

Short-term intake rate measurements

The results of the short-term behaviour measurements are presented in Table 5.3. There were significant (P<0.001) reductions in the proportion of bites per grazing jaw movement (as seen in the 24-hour recordings) and in bite rate in the treated animals compared with the controls. There was also a significant treatment × sward interaction on the number of bites per GJM and BR. Intake Rate and Bite Mass of fresh herbage were significantly greater on the clover swards compared with the ryegrass swards, but not when expressed as OM. The proportion of bites per GJM and BR were significantly greater on the clover compared with the ryegrass swards and during the pm compared with the am period.
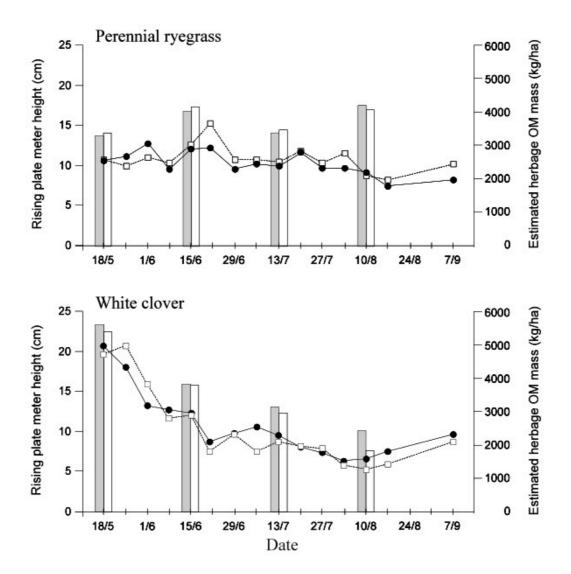
Table 5.3. Effect of sward type (perennial ryegrass *v.* white clover) and time of day (ToD, am *v.* pm) on grazing behaviour and short-term intake rates (IR) by untreated (control) and eprinomectin-treated heifers.

| | Control | | | | Eprinomectin | | | | S.e. of | Significance of effect of | | | | |
| | Grass | | Clover | | Grass | | Clover | | | | | | Trt × | Sward × |
	am	pm	am	pm	am	pm	am	pm	mean	Trt	Sward	ToD	Sward	ToD
FMIR (FM g/min)	141	127	188	170	99	105	192	178	14.9	0.236	<0.001	0.362	0.083	0.577
OMIR OM g/min)	24.0	25.0	24.7	24.5	20.4	20.4	23.9	26.4	2.48	0.319	0.184	0.643	0.199	0.870
Bite mass (FM g/bite)	2.33	2.16	3.04	2.65	1.99	1.96	3.39	2.79	0.251	0.942	<0.001	0.109	0.165	0.281
Bite mass (OM g/bite)	0.399	0.424	0.399	0.381	0.411	0.382	0.422	0.413	0.0441	0.843	0.994	0.812	0.509	0.858
GJM rate (per min)	85.8	83.3	84.7	81.1	81.3	83.2	80.7	84.0	1.52	0.204	0.467	0.844	0.402	0.953
Bite rate (per min)	60.4	59.4	62.0	64.2	50.2	53.9	56.9	64.2	1.55	<0.001	<0.001	0.011	0.025	0.138
Bites/GJM	0.704	0.714	0.735	0.794	0.618	0.648	0.705	0.764	0.0149	<0.001	<0.001	0.001	0.041	0.074
Rate of IWL (g/min)	22.5	24.7	28.1	15.8	20.6	20.0	27.5	22.7	4.53	0.982	0.624	0.238	0.329	0.156

Sward height and composition

Weekly mean rising plate meter heights measured on the clover and grass plots within the background paddocks are shown in Figure 5.2. Analysis of variance of the data on each sampling occasion showed no significant difference between the paddocks stocked by the control and treated heifers.

Figure 5.2. Rising plate meter heights and total herbage OM mass of perennial ryegrass and white clover swards in background paddocks stocked with heifers receiving either no anthelmintic (height ● ; mass (fill) or treated with eprinomectin (height ⬜ ; mass (no fill).
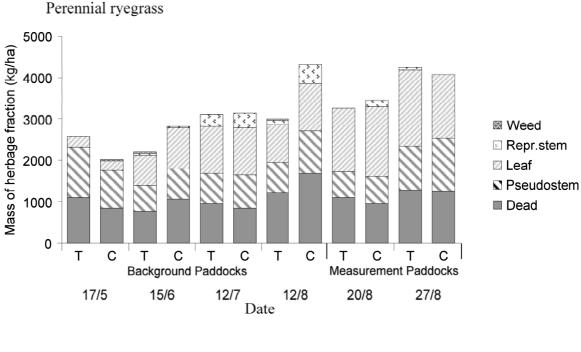
_____

Comparison of local linear regressions of total herbage mass on rising plate height within the background paddocks showed no significant differences due to treatment effects on the grass swards or on the first three sampling occasions on the clover swards. On the fourth sampling occasion, the regression lines on the clover swards on the control and treated paddocks were found to be parallel. Estimates of herbage OM mass from overall mean sward plate height and the local regressions on each sward at each sampling occasion are also presented in Figure 5.3. While grass OM mass generally remained at between 2 and 3 tonne/ha, clover OM mass decreased from about 5 to 2 tonnes/ha during the 12-week period, when the background paddocks were continuously stocked.
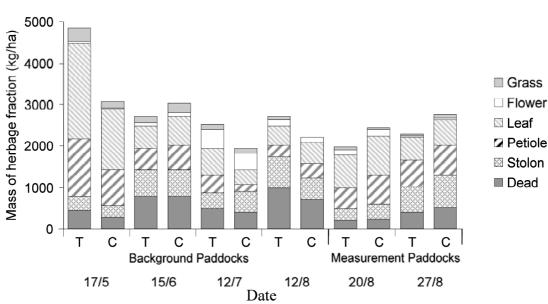
No significant regressions relating total herbage OM mass and rising plate height were established for the samples collected on the grass and clover swards within the measurement paddocks because of the small variation in plate height and OM mass. Sward surface heights on the measurement paddock grass swards decreased from 17.2 to 9.4 cm and on the clover swards from 18.0 to 12.4 cm between 20th and 27th August. The results of the botanical analyses of the ryegrass and clover swards within the background paddocks and the measurement paddocks are represented in Figure 5.3. Analyses of variance of the botanical fractions at each sampling date showed no significant treatment effects on grass or clover fractions with the exception of stolon mass on 12 August and clover flower mass on 27 August.

Animal Performance

The overall mean liveweight of the heifers at the beginning of the experiment was 235.3 ±3.0 kg. At no time during the experiment did the live weights of the control and treated heifers differ significantly, the final weights being 365.6 and 367.7 ±3.2 kg, respectively. Mean daily liveweight gains over the course of the experiment were 1.17 and 1.18 ±0.01 kg, respectively.

Figure 5.3. Organic matter mass (OM g/m2) of botanical fractions on perennial ryegrass and white clover swards stocked with heifers receiving either no anthelmintic (C) or treatment with eprinomectin (T).

_____

5.4. Discussion

Parasitology

The low, inconsistent and sporadic recovery of infective nematode larvae from herbage samples and the concomitant low faecal egg output from the control heifers indicate that the level of gastrointestinal parasitism was low and this is likely to have been an important factor in the failure to discriminate between the growth of the treated and control groups over the first 14 weeks. There are no comparable studies in cattle to determine the importance of the level of parasitism in the expression of dietary preference. In the one study in sheep where partial preference in lambs with parasite-induced inappetence was clearly demonstrated (Kyriazakis et al., 1994), 2500 L_3 *T.colubriformis* were administered daily and this was associated with the erratic passage of soft faeces from the 6th week after the start of infection and worm egg excretion of up to 1400 epg of faeces, hence the infection could be considered borderline clinical. Furthermore, the differences in Crude Protein content of the Low (90 g/kg) and High (214 g/kg) protein rations were very marked and the low-protein feed was considered to be deficient for the lambs used in the study (Cosgrove and Niezen, 2000).

Grazing behaviour

Despite the apparent lack of a treatment effect on liveweight and grazing performance prior to the measurement period, differences in grazing behaviour were observed during the period of intensive recording at the end of August. The increase in daily grazing time in the treated compared to the control heifers just failed to reach significance at the P<0.05 level (P=0.054), but this difference is consistent with the results of two previous experiments at the same location in which young dairy heifers given anthelmintic treatment grazed for 105 minutes longer (P=0.051) than their untreated counterparts (Forbes et al., 2000) and lactating dairy heifers and cows given treated with eprinomectin grazed for 50 and 47 minutes longer (P=0.016) than the controls (Forbes et al., 2004). In the present experiment, the increase in the time spent grazing by the treated heifers allowed them to perform approximately 4,000 additional total Grazing Jaw Movements compared to the controls (P=0.015). Furthermore, analyses of the jaw movements during the 24-h recordings and the measurements of short-term intake rates both demonstrate a significant decrease in the proportion of bites per GJM in the treated heifers compared to the control heifers. Bite rate, non-biting GJM rate and the proportion of bites per GJM have been shown to be influenced by sward surface height (Gibb et al., 1996) and, as shown in the short-term intake rate measurements of the present experiment and previously (Penning et

al., 1995), to differ between ruminants grazing grass and clover swards. The significant effect of time of day on bite rate and bites per GJM recorded during the short-term intake rate measurements are similar to previous reports of subtle changes in the grazing strategy of cattle over the course of a day, despite sward morphology remaining constant, when the proportion of bites per GJM have been shown to increase during late afternoon and evening (Gibb et al., 1998).

The reason why some treatment effects were apparent at this time, despite the fact that the dietary conditions remained essentially the same, may relate to the fact that the faecal egg counts in the control animals showed some signs of increase, in terms of magnitude and morbidity, just prior to the measurement period. Additionally, larval culture revealed a predominance of *O. ostertagi*, which is generally considered to be the most pathogenic of the common, temperate, gastrointestinal nematodes of cattle and which also has a relatively low fecundity and hence produces low epgs. Typically in first grazing season calves, *Cooperia* spp. predominate in larval cultures in the first half of the grazing season (Forbes et al., 2000), but on these pastures, pasture contamination the previous year was from adult dairy cows, in which *O. ostertagi* is the most common gastrointestinal nematode species (Agneessens et al., 2000; Borgsteede et al., 2000).

Whilst the shorter eating time and greater idling time during observation day 1 compared with day 2 may be, in part, due to the heifers' lack of familiarity when first introduced onto these paddocks, the significant differences in these and other grazing parameters during the two 24-h recording periods are commensurate with the reduction in sward surface height on the measurement paddocks as a result of both herbage consumption and trampling of the swards (Gibb et al., 1996). Whilst the intensity of grazing activity, expressed as the number of minutes eating per hour, was never as great as that shown by lactating dairy cows, the overall temporal pattern of grazing was similar to that shown by such cows (Gibb et al., 2005), with the major peak of activity occurring during the late afternoon and evening, a slightly shorter period of peak activity occurring in the first few hours after sunrise and minor peaks around late morning, early afternoon and midnight.

In general, the diurnal patterns of grazing, the partial preference for clover and the differences between observations in the morning and afternoon observed in this study were consistent with the current published literature, which can be summarised as a partial preference for clover of 70±10%, higher grazing activity and intakes in the afternoon compared to the morning and a subtle shift in preference from clover to grass in the afternoon, possibly related to the higher sugar content of grass following photosynthetic

_____

activity earlier in the day (Gibb et al., 1998; Delagarde et al., 2000; Orr et al., 2001; Rutter, 2006).

Diet Selection

There was no significant effect of anthelmintic treatment on the partial preference of clover over grass: in control animals clover comprised 73.0% of the diet, in treated animals 75.5%. Additionally, in the present study treated heifers were observed grazing the clover swards 14.0% (P=0.032) more frequently than the control heifers. This suggests that the parasitized control animals did not express a selective preference for clover, which typically has a higher protein content than ryegrass (Orr et al., 2004), to compensate for their reduced grazing time. This result does not therefore provide support for the original hypothesis (Kyriazakis et al., 1998) that an increased partial preference for protein-rich diets can compensate for parasite-induced inappetence . However, the low level of parasitism in the control cattle and the high growth rates in both groups may have masked possible differences in selective grazing behaviour

Animal Performance

The lack of differentiation in performance between the control and anthelmintic-treated heifers during the ~14 weeks on the background paddocks contrasts with many reports of increased liveweight gain in first season grazing cattle when treated strategically with anthelmintics (Shaw et al., 1998; Epe et al., 1999; Schnieder et al., 1999; Dorny et al., 2000; Forbes et al., 2000; Forbes and Rice, 2000). The absence of any significant differences in herbage height, mass and botanical composition amongst the background paddocks grazed by control and treated animals also differs from previously reported observations in predominantly ryegrass paddocks grazed by untreated and anthelmintic-treated heifers (Forbes et al., 2000).

The reasons for the lack of differentiation cannot be determined retrospectively, but two factors appear to be important:

- the nutritional quality of the swards
- the larval challenge from the pasture and the level of parasitism in the heifers

The relatively high rates of daily liveweight gain in both groups of cattle (>1.1 kg/day) compared to typical gains in this class of cattle on this site of 0.65-0.97 kg/day (Forbes et al., 2000; Rutter et al., 2002; Orr et al., 2004) and under good management conditions on a commercial farm in this region of 0.63 – 0.66 kg/day (Forbes et al., 2000) indicate that nutrition was probably not limiting for growth and that the grass and clover swards provided a highly nutritious diet. It has been demonstrated, mainly in sheep, that

increasing the availability and nutritional quality of a ruminant diet, particularly in terms of its protein content, allows the parasitized host to become more resilient and to compensate for some of the adverse effects of gastrointestinal parasitism (van Houtert and Sykes, 1996; Coop and Kyriazakis, 1999; Houdijk et al., 2005). The liveweight gain of untreated, first-year, grazing heifers, whose diet was supplemented with lucerne (*Medicago sativa*) was comparable to that achieved by heifers treated strategically with anthelmintics (Jorgensen et al., 1992), findings that are consistent with the observations in the current study. Conversely, supplementary feeding failed to mitigate the adverse effects of gastrointestinal parasitism in first-season grazing cattle in a 3-year study in Sweden (Larsson et al., 2006).

5.5. Conclusion

This study provides further evidence in grazing cattle for parasite-induced inappetence, manifest as a reduction in grazing time and in subtle changes in ingestive behaviour. The observed partial preference for clover in both treated and control cattle was not significantly affected by the level of parasitism.

_____

5.6. References

Agneessens, J., Claerebout, E., Dorny, P., Borgsteede, F.H., Vercruysse, J., 2000, Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 90, 83-92.

Borgsteede, F.H., Tibben, J., Cornelissen, J.B., Agneessens, J., Gaasenbeek, C.P., 2000, Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 89, 287-296.

Coop, R.L., Kyriazakis, I., 1999, Nutrition-parasite interaction. Vet Parasitol 84, 187-204.

Cooper, J., Gordon, I.J., Pike, A.W., 2000, Strategies for the avoidance of faeces by grazing sheep. Appl Anim Behav Sci 69, 15-33.

Cosgrove, G.P., Niezen, J.H., 2000, Intake and selection for white clover by grazing lambs in response to gastrointestinal parasitism. Appl Anim Behav Sci 66, 71-85.

Delagarde, R., Peyraud, J.L., Delaby, L., Faverdin, P., 2000, Vertical distribution of biomass, chemical composition and pepsin--cellulase digestibility in a perennial ryegrass sward: interaction with month of year, regrowth age and time of day. Anim Feed Sci Technol 84, 49-68.

Dorny, P., Demeulenaere, D., Smets, K., Vercruysse, J., 2000, Control of gastrointestinal nematodes in first season grazing calves by two strategic treatments with eprinomectin. Vet Parasitol 89, 277-286.

Epe, C., Woidtke, S., Pape, M., Heise, M., Kraemer, F., Kohlmetz, C., Schnieder, T., 1999, Strategic control of gastrointestinal nematode and lungworm infections with eprinomectin at turnout and eight weeks later. Vet Rec 144, 380-382.

Forbes, A.B., Huckle, C.A., Gibb, M.J., 2004, Impact of eprinomectin on grazing behaviour and performance in dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management. Vet Parasitol 125, 353-364.

Forbes, A.B., Huckle, C.A., Gibb, M.J., Rook, A.J., Nuthall, R., 2000, Evaluation of the effects of nematode parasitism on grazing behaviour, herbage intake and growth in young grazing cattle. Vet Parasitol 90, 111-118.

Forbes, A.B., Rice, B.J., 2000, Patterns of parasitic nematode infection and immunity in dairy heifers treated with ivermectin in a sustained-release bolus formulation either at turnout or in the middle of the grazing season. Vet Rec 147, 295-297.

Gibb, M.J., Huckle, C.A., Forbes, A.B., 2005, Effects of sequential treatments with eprinomectin on performance and grazing behaviour in dairy cattle under daily-paddock stocking management. Vet Parasitol 133, 79-90.

Gibb, M.J., Huckle, C.A., Nuthall, R., 1998, Effect of time of day on grazing behaviour and intake rate by lactating dairy cows. Grass Forage Sci 53, 41-46.

Gibb, M.J., Huckle, C.A., Nuthall, R., Penning, P.D., 1996. Can grazed pasture meet the needs of the high genetic merit dairy cow? In: Grass and Forage for Cattle of High Genetic Merit, British Grassland Society, November 1996.

Gibb, M.J., Huckle, C.A., Nuthall, R., Rook, A.J., 1999, The effect of physiological state (lactating or dry) and sward surface height on grazing behaviour and intake by dairy cows. Appl Anim Behav Sci 63, 269-287.

Harris, S.L., Auldist, M.J., Clark, D.A., Jansen, E.B., 1998, Effects of white clover content in the diet on herbage intake, milk production and milk composition of New Zealand dairy cows housed indoors. J Dairy Res 65, 389-400.

HFRO 1986. Biennial Report 1984-1985 (Hill Farm Research Organisation), pp. 29-30.

Houdijk, J.G., Kyriazakis, I., Jackson, F., Huntley, J.F., Coop, R.L., 2005, Effects of protein supply and reproductive status on local and systemic immune responses to *Teladorsagia circumcincta* in sheep. Vet Parasitol 129, 105-117.

Huckle, C.A., Nuthall, R., Gibb, M.J., 1994. The use of short-term weight changes to measure intake rates in grazing cattle. In: Fourth Research Conference, British Grassland Society, Reading, UK, pp. 157-158.

Hutchings, M.R., Athanasiadou, S., Kyriazakis, I., Gordon, I.J., 2003, Can animals use foraging behaviour to combat parasites? Proc Nutr Soc 62, 361-370.

Hutchings, M.R., Gordon, I.J., Robertson, E., Kyriazakis, I., Jackson, F., 2000, Effects of parasitic status and level of feeding motivation on the diet selected by sheep grazing grass/clover swards. J Agric Sci 135, 65-75.

Jorgensen, R.J., Satrija, F., Monrad, J., Nansen, P., 1992, Effect of feeding lucerne pellets on trichostrongyle infection in grazing heifers. Vet Rec 131, 126-127.

Kyriazakis, I., Anderson, D.H., Oldham, J.D., Coop, R.L., Jackson, F., 1996, Long-term subclinical infection with *Trichostrongylus colubriformis:* effects on food intake, diet selection and performance of growing lambs. Vet Parasitol 61, 297-313.

Kyriazakis, I., Oldham, J.D., Coop, R.L., Jackson, F., 1994, The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. Br J Nutr 72, 665-677.

Kyriazakis, I., Tolkamp, B.J., Hutchings, M.R., 1998, Towards a functional explanation for the occurrence of anorexia during parasitic infections. Anim Behav 56, 265-274.

Larsson, A., Dimander, S.O., Rydzik, A., Uggla, A., Waller, P.J., Hoglund, J., 2006, A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle--effects on animal performance. Vet Parasitol 142, 197-206.

MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques. Her Majesty's Stationary Office, London, 160 p.

Mead, R., Curnow, R.N., 1983, Statistical methods in agriculture and experimental biology. Chapman Hall, New York, 400 p.

Merry, R.J., Lee, M.R., Davies, D.R., Dewhurst, R.J., Moorby, J.M., Scollan, N.D., Theodorou, M.K., 2006, Effects of high-sugar ryegrass silage and mixtures with red clover silage on ruminant digestion. 1. In vitro and in vivo studies of nitrogen utilization. J Anim Sci 84, 3049-3060.

_____

Niezen, J.H., Charleston, W.A., Hodgson, J., Miller, C.M., Waghorn, T.S., Robertson, H.A., 1998, Effect of plant species on the larvae of gastrointestinal nematodes which parasitise sheep. Int J Parasitol 28, 791-803.

Niezen, J.H., Robertson, H.A., Sidey, A., Wilson, S.R., 2002, The effect of pasture species on parasitism and performance of lambs grazing one of three grass-white clover pasture swards. Vet Parasitol 105, 303-315.

Orr, R.J., Rutter, S.M., Penning, P.D., Rook, A.J., 2001, Matching grass supply to grazing patterns for dairy cows. Grass Forage Sci. 56, 352-361.

Orr, R.J., Rutter, S.M., Yarrow, N.H., Champion, R.A., Rook, A.J., 2004, Changes in ingestive behaviour of yearling dairy heifers due to changes in sward state during grazing down of rotationally stocked ryegrass or white clover pastures. Appl Anim Behav Sci 87, 205-222.

Parsons, A.J., Newman, J.A., Penning, P.D., Harvey, A., Orr, R.J., 1994, Diet preference of sheep: effects of recent diet, physiological state and species abundance. Journal of Animal Ecology 63, 465-478.

Penning, P.D., Hooper, G.E., 1985, An evaluation of the use of short-term weight changes in grazing sheep for estimating herbage intake. Grass Forage Sci 40, 79-84.

Penning, P.D., Parsons, A.J., Orr, R.J., Harvey, A., Champion, R.A., 1995, Intake and behaviour responses by sheep, in different physiological states, when grazing monocultures of grass or white clover. Appl Anim Behav Sci 45, 63-78.

Rook, A.J., Huckle, C.A., 1997, Activity bout criteria for grazing dairy cows. Appl Anim Behav Sci 54, 89-96.

Rutter, S.M., 2000, Graze: a program to analyze recordings of the jaw movements of ruminants. Behav Res Methods Instrum Comput 32, 86-92.

Rutter, S.M., 2006, Diet preference for grass and legumes in free-ranging domestic sheep and cattle: Current theory and future application. Appl Anim Behav Sci 97, 17-35.

Rutter, S.M., Champion, R.A., Penning, P.D., 1997, An automatic system to record foraging behaviour in free-ranging ruminants. Appl Anim Behav Sci 54, 185-195.

Rutter, S.M., Orr, R.J., Penning, P.D., Yarrow, N.H., Champion, R.A., 2002, Ingestive behaviour of heifers grazing monocultures of ryegrass or white clover. Appl Anim Behav Sci 76, 1-9.

Schnieder, T., Epe, C., Ilchmann, G., 1999, Survey on parasite control in dairy cattle in northern Germany. Vet Rec 145, 704-706.

Scott, L.L., Provenza, F.D., 2000, Lambs fed protein or energy imbalanced diets forage in locations and on foods that rectify imbalances. Appl Anim Behav Sci 68, 293-305.

Shaw, D.J., Vercruysse, J., Claerebout, E., Dorny, P., 1998, Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. Vet Parasitol 75, 115-131.

_____

Tolkamp, B.J., Dewhurst, R.J., Friggens, N.C., Kyriazakis, I., Veerkamp, R.F., Oldham, J.D., 1998a, Diet choice by dairy cows. 1. Selection of feed protein content during the first half of lactation. J Dairy Sci 81, 2657-2669.

Tolkamp, B.J., Kyriazakis, I., Oldham, J.D., Lewis, M., Dewhurst, R.J., Newbold, J.R., 1998b, Diet choice by dairy cows. 2. Selection for metabolizable protein or for ruminally degradable protein? J Dairy Sci 81, 2670-2680.

van Houtert, M.F., Sykes, A.R., 1996, Implications of nutrition for the ability of ruminants to withstand gastrointestinal nematode infections. Int J Parasitol 26, 1151-1167.

Chapter 6: Associations between blood gastrin, ghrelin, leptin, pepsinogen and *Ostertagia ostertagi* antibody concentrations and voluntary feed intake in calves exposed to a trickle infection with *O. ostertagi*

_____

6.1. Introduction.

A reduction in, or loss of, appetite – inappetence or anorexia - is a common characteristic of many diseases, both infectious and non-infectious (Symons, 1985; Hart, 1990; Johnson, 1998, 2002; Sandberg et al., 2006). Inappetence is a common expression of parasite infections in ruminants and has been described consequent to infection with several common helminths, including abomasal nematodes *Ostertagia ostertagi* (Fox et al., 1989a), *Teladorsagia circumcincta* (Coop et al., 1977) and intestinal nematodes *Trichostrongylus colubriformis* (Kyriazakis et al., 1994). The importance of inappetence as a component of production losses resulting from parasitic gastroenteritis (PGE) in young animals has been demonstrated in studies that have shown that between 60-73% of the reduced growth rate in lambs and calves can result from a reduction in feed intake (Sykes and Coop, 1977; Fox et al., 1989a). Despite the importance of a reduction in feed intake and subsequent performance in farm animals, little is known of the precise mechanisms whereby parasitized animals become anorexic. Potential candidates for inducing inappetence in disease include various compounds that are known to regulate appetite in healthy animals, such as insulin, leptin, ghrelin, cholecystokinin (CCK) and somatostatin (Murphy and Bloom, 2006; Stephens et al., 2007). Additionally, the pro-inflammatory cytokines interleukin (IL) 1, IL6 and tumour necrosis factor (TNF)-α (Exton, 1997; Johnson, 1998, 2002; Konsman et al., 2002), which are associated with inflammatory and immune responses, may also be involved with anorexia in helminth infections (Meeusen, 1999; Greer et al., 2005a; Greer et al., 2005b).

Infection with the abomasal nematode, *O. ostertagi*, in the calf results in a loss of acid-producing parietal cells and an increase in gastric pH (Murray et al., 1970), which is accompanied by a marked hypergastrinaemia (Fox et al., 1987). There is a strong association between elevated blood gastrin levels and a reduction in voluntary feed intake in calves exposed to a trickle infection with *O. ostertagi* infective larvae (Fox et al., 1989a; Fox et al., 1989b). This association between gastrin and inappetence was also demonstrated in studies in worm-free animals treated with omeprazole, a proton pump inhibitor that increases gastric pH and hence produces hypergastrinaemia (Fox et al., 1989c; Fox et al., 2002).

Potential neuroendocrine transmitters that could act in concert with gastrin to mediate parasite-associated inappetence include ghrelin and leptin; these are described in more

_____

detail below as, in addition to research in laboratory animals and man, both have also been studied in ruminants.

Ghrelin is a 27/28-amino acid peptide that acts as a Growth Hormone Secretagogue (GHS) and, although it is produced mainly in the oxyntic (parietal) glands of cow and sheep abomasa (Grouselle et al., 2008), it is also found in other organs, including the rumen, intestine, pancreas and immune system (Hayashida et al., 2001; Gentry et al., 2003; Sugino et al., 2004). Ghrelin can cross the blood-brain barrier (Banks et al., 2002) and bind to receptors in the brain; GHS (ghrelin) receptors (GHS-R) are expressed mainly in the arcuate nucleus of the hypothalamus where they are associated locally with neuropeptide-Y (NPY) expression (Olszewski et al., 2008). NPY is a potent stimulator of feed intake that is secreted by neuronal cells in the arcuate nuclei of the hypothalamus (Smith, 2000; Chaudhri et al., 2006; Murphy and Bloom, 2006; Stephens et al., 2007; Olszewski et al., 2008). In general terms, concentrations of ghrelin in the circulation increase prior to scheduled meals and in response to fasting and sub-optimal levels of nutrition, while feeding generally suppresses ghrelin secretion (Wertz-Lutz et al., 2006; Bradford and Allen, 2008; Wertz-Lutz et al., 2008). Work using isolated rat stomachs has shown that ghrelin secretion is inhibited by gastrin (Lippl et al., 2004), which suggests that ghrelin could mediate gastrin-associated inappetence in cattle with abomasal parasites. On the other hand, recent studies have shown that ghrelin receptors in the oxyntic cells are associated with gastrin receptors and there is evidence that gastrin may directly stimulate ghrelin release from the stomach and that both may increase gastric acid synergistically (Fukumoto et al., 2008).

Leptin not only plays a central role in signaling and regulating energy homeostasis (appetite, nutrient partitioning, body composition), but it is also involved in diverse other functions, including reproduction and immunity (Garcia et al., 2002; Smith and Grove, 2002; Liefers et al., 2003; Chilliard et al., 2005; Kulcsár et al., 2005; Zieba et al., 2005). Leptin is a protein that is synthesised primarily in white adipose tissue and adipocytes in other tissues (Bartha et al., 2005), but in ruminants, leptin gene expression has also been demonstrated in other organs, including the rumen, abomasum and duodenum (Yonekura et al., 2002). It is possible that the parasite-induced increase in blood gastrin could up-regulate leptin synthesis by binding with CCK-B/gastrin receptors present on adipocytes (Attoub et al., 1999) and/or gastric epithelial cells. The inceased synthesis would result in an elevation in blood leptin concentrations that could, in turn, down-regulate the synthesis of NPY (Henry et al., 1999).

_____

On a theoretical basis at least, the two peptides, ghrelin and leptin, could operate in concert to mediate the proposed anorexigenic effects of gastrin in ostertagiosis; thus, an increase in leptin and decrease in ghrelin in infected calves would result in a reduction of the release of the orexigenic transmitter, NPY, from the hypothalamus. Preliminary data in support of this hypothesis have been provided by experimental *Ostertagia (Teladorsagia) circumcincta* infection in sheep in which a significant positive correlation was established between blood gastrin and leptin values and a significant negative correlation demonstrated between blood leptin and appetite during the later stages of infection (Fox et al., 2006).

Although feed intake and ingestive behaviour data are generally consolidated into a daily total, in fact cattle, even when offered feed ad lib or with generous pasture allowances, eat in discrete meals during the day. Furthermore, in grouped animals, there is typically a high level of synchronicity in eating activity (Rook and Huckle, 1995), both because of social interactions within the herd and because of management routines, e.g. feeding and milking. The length of time spent eating during a meal is obviously a function of when it starts and when it ends; the start presumably has some neuroendocrine 'trigger' for increased appetite, but there is also a social/synchronicity component, whilst the end of a meal will be effected by satiety signals. In terms of leptin and ghrelin therefore, possible mechanisms for nematode-induced inappetence would be that ghrelin levels might be suppressed such that there would be a lower motivation to start a meal, whilst increased leptin levels might occur prematurely to bring the meal to an end. Data from studies on pastured dairy cows showed a significant effect of parasitism on the length of meal, but it could not readily be determined if the shorter meals in the infected cows resulted from a later start to their meals or an earlier finish or both (Forbes et al., 2004).

The objectives of this study were to measure changes in and define relationships between appetite and blood gastrin, ghrelin, leptin and *O. ostertagi* antibody concentrations in calves experimentally infected with the abomasal nematode, *O. ostertagi*.

6.2. Materials and Methods

Animals

Twenty-five, castrated male Holstein-cross calves, between four and five months of age and weighing 156.5±12.2 kg, were purchased from a commercial dairy farm, after

having been reared under conditions designed to minimise the risk of parasitic infection. The calves were weighed upon arrival at the Royal Veterinary College and treated with fenbendazole at a dosage of 7.5 mg/kg.

Animal Husbandry

The calves were housed in a building with natural light and ventilation, kept in individual pens, in full view of their companions, and bedded on wood shavings. Calves were offered 0.5 kg hay per day per head between 07.30 and 08.30, immediately after the remaining concentrate feed had been removed and weighed. A proprietary 16% protein concentrate feed with a metabolisable energy (ME) content of 11.1 mega joules (MJ)/kg Dry Matter (DM) was provided between 12.00 and 13.00 each day in sufficient quantity to ensure *ad libitum* (ad lib) intake (other than the pair-fed controls). Mains tap water, provided in buckets, was freely available throughout the study.

Allocation

On Study Day 0, the 25 animals were weighed, ranked by bodyweight and randomly allocated to one of the five treatment groups listed below.

Study Design

The study design and treatment groups are shown in Table 1.

Table 6.1. Study design and Treatment Groups

	Group	Description	Challenge	Treatment
T1	ALC	Ad lib fed control	-	No treatment
T2	INF	Ad lib fed infected	+	Eprinomectin Day 56
T3	PFC	Controls, pair-fed with INF	-	No treatment
T4	E-ALC	Ad lib fed, treated controls	-	Eprinomectin Days 0 & 56
T5	E-INF	Ad lib fed, treated, infected	+	Eprinomectin Days 0 & 56

The rationale for the groups was as follows:

ALC: Free expression of appetite without any constraints that might arise through infection or treatment.

INF: Free expression of appetite with the constraint of infection, hence a measure of any appetite depression resulting from infection. Eprinomectin was administered on Day 56 to determine if recovery from any negative effects could be measured.

PFC: Calves fed to the same level as those in the INF group in order to determine what proportion of any changes in performance (growth) or blood parameters could be attributed to the effects of inappetence.

_____

E-ALC: Calves fed ad lib and treated with eprinomectin to determine if there were any direct pharmacological effects on appetite in the absence of infection.

E-INF: Infected calves were treated prophylactically with eprinomectin to determine if any effects observed in the INF group would be mitigated.

The study commenced on Day 0, after an initial acclimatisation period of seven days for the calves, and continued until Day 77.

This study was performed under Home Office Project Licence PPL 70/ 6459 19B14, ensuring that all appropriate animal welfare guidelines and regulations were complied with (in particular the Animal (Scientific Procedures) Act 1986).

Challenge

A non-inhibition-prone strain of *O. ostertagi* infective larvae (L_3) was obtained from a commercial laboratory and stored at 2-8°C, protected from light. The equivalent of 10,000 L_3 daily were administered orally by syringe three times a week (Monday: 20,000 L_3; Wednesday: 20,000 L_3; and Friday: 30,000 L_3) from Day 0 to 55 inclusive to calves in groups INF and E-INF.

Treatment

Each calf in Groups INF, E-ALC and E-INF was treated according to the schedule in 2.4 (shown in Table 6.1) above with a solution containing 0.5% w/v eprinomectin for topical use in cattle (Eprinex® Pour-On, Merial Animal Health). Treatment was administered at a rate of 1ml per 10kg bodyweight to achieve a dose of 500 micrograms eprinomectin/kg. The product was applied topically by pouring along the backline in a narrow strip extending from the withers to the tail head. Treated and untreated animals were handled and penned so as minimise any risk of transfer of eprinomectin between animals through mutual licking or absorption through the skin (Bousquet-Melou et al., 2004).

Observations

      Feed Intake

Ad libitum feed intake was monitored on a daily basis by subtracting the weight of concentrate refusals remaining after 24 hours from the weight of concentrate offered. Animals in group PFC were pair-fed with animals in Group INF and were therefore offered the same weight of concentrate that their infected pair replicate had eaten the previous day.

Body weights

Body weights were measured on arrival and on Study Days 0, 56 and 77.

Blood samples

Pilot study. A pilot study in a sub-set of three calves was conducted prior to the main study in order to determine the magnitude of any diurnal variation in the concentrations of gastrin, ghrelin, leptin and pepsinogen. The ration and feeding regime were the same as that described in the main study. The calves were catheterised to facilitate blood sampling and samples were collected at 09.00, 10.00, 11.00, 12.00, 14.00 and 16.00 over three consecutive days. Samples were subsequently processed according to the protocol described in the main study.

Main study. Jugular blood samples (2 x 10 ml) were collected from each calf between 11.30 and 13.00 three times per week prior to feeding. One 10 ml blood sample was collected in lithium heparin vacutainers containing 200 kallikrein Inactivator Units of proteinase inhibitor per ml of blood for gastrin, ghrelin and leptin assays. The other 10 ml sample was collected in lithium heparin vacutainers without additives for pepsinogen and *O.ostertagi* antibody assays.

Sample tubes were placed on ice immediately after collection and transported to the laboratory for processing. Within an hour and a half of sample collection, two aliquots of plasma were harvested from each blood sample and each aliquot labelled with the study number and sample identity. The two sets of aliquots were stored in separate domestic freezers until transfer to the laboratory for analysis.

Faecal Worm Egg Counts

Faecal samples were collected per rectum on Days 0, 10, 15, 16, 17, 18, 19, 20, 21, 25, 30, 40, 56, 66 and 77. All samples were stored at 2-8°C prior to screening for the presence of nematode eggs using the modified McMaster method with a sensitivity of 50 eggs per gram (epg) (MAFF, 1986).

Laboratory Assays

Blood Pepsinogen

Pepsinogen assays were performed at the Veterinary Laboratory Agency (VLA) Shrewsbury using a modification of the colorimetric method (Mylrea and Hotson, 1969) employing bovine albumin as substrate in a hydrochloric acid buffer. Pepsinogen concentrations were calculated as units of tyrosine (U), where 1 unit=1μM tyrosine released per litre of plasma per minute at 37°C and expressed as U/l.

_____

Blood Gastrin, Ghrelin and Leptin

Blood gastrin, ghrelin and leptin analyses were performed at Hammersmith Hospital using commercially available radioimmunoassay (RIA) and Enzyme-Linked ImmunoSorbent Assay (ELISA) kits previously validated for use in non-human species. Gastrin: RIA from Euro-Diagnostics, Sweden with a sensitivity of 5 pmol/L; ghrelin: RIA from Linco, Missouri USA with a sensitivity of 7.8 pg/ml; leptin: ELISA from DRG Diagnostics Germany with a sensitivity of 1.0 ng/ml Gastrin values are expressed in picomoles (pmol) per litre (L); ghrelin in picograms per millilitre (pg/ml) and leptin in nanograms per millilitre (ng/ml).

O. ostertagi antibodies

The concentration of antibodies in serum was measured using the (Svanovir®), *O. ostertagi* Milk ELISA (Svanova, Sweden). The plasma samples were diluted 1/140 in phosphate-buffered saline-Tween 20. Antibody concentrations were expressed as an optical density ratio (ODR), based on the optical density of the test sample in relation to a positive and negative serum control that were run on each plate.

Statistical Methods

Statistical analysis of data was performed using the SAS for Windows version 9.1 software procedure PROC MIXED. The effects of time (study day) and treatment group were studied by including these as main and first order interaction effects, with gastrin, ghrelin, leptin, pepsinogen, FEC and antibodies as the outcome variable in separate models. To take account of repeated measures, study day was included as a repeated effect and the dependence between observations from the same subject was modelled using either a compound symmetry or first-order autoregressive covariance structure after first using Akaike's Information Criterion (AIC) to assess the different models. Differences between the individual treatment groups and within and between treatment groups on individual study days were established using Bonferroni adjusted post hoc tests. Statistical significance was assessed using a P value of 0.05. Some of the data were also analysed in a similar fashion over three periods within the study: 0-21; 23-56; 58-77 days, corresponding approximately to the prepatent period, patency and post-treatment for groups INF and E-INF.

Since there were no significant differences in feed intake between groups ALC and E-ALC, ghrelin and leptin assays were only conducted on samples from ALC, INF, PFC and E-ALC. *O. ostertagi* antibodies were only measured in the two infected groups, INF

_____

and E-INF and in the uninfected ad lib fed group ALC. Although all groups were included in the statistical analyses of all the other parameters, as there were no significant differences in feed intake between any of the groups, for clarity, the focus of the results and accompanying figures is on groups ALC, INF and E-INF.

6.3. Results

Parasitology

Clinical

There were no clinical signs of parasitic gastroenteritis in any of the (infected) calves throughout the study.

Faecal Egg Counts

Faecal egg counts (FEC) were all negative in the uninfected groups (1, 3 & 4), apart from a single animal in Group ALC which had a count of 100 epg on Day 16, but on no other occasion. No explanation could be found for the anomalous finding in the single animal and it is assumed that it was an artefact, possibly from cross-contamination between samples. The pattern of faecal egg output for groups ALC, INF and E-INF are shown in Figure 6.1. Faecal egg counts changed significantly over time ($P<0.01$) and there was a significant ($P<0.0001$) interaction between treatment group and day.
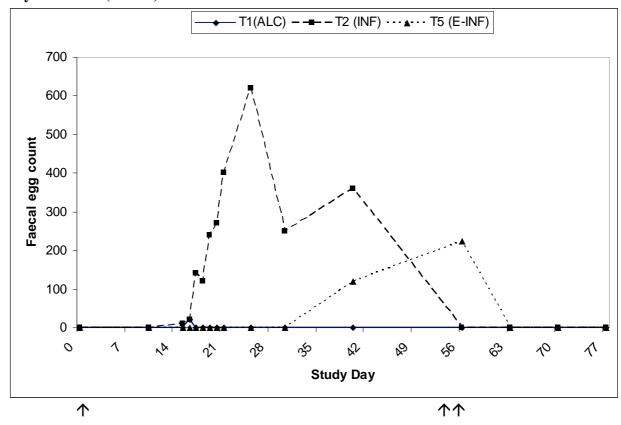
_____

Figure 6.1. Pattern of *Ostertagia ostertagi* faecal egg counts in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin on Days 0 and 56 (E-INF).

↑   Treatment with eprinomectin Day 0 (E-INF)
↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)

In the INF group the first positive sample occurred on Day 15; thereafter the mean epg for the group increased to a maximum of 620 epg on Day 25, after which the counts declined to zero by Day 56. Animals in this group were treated with eprinomectin on Day 56 and all subsequent samples were negative. There was noticeable within-group variability in that three animals developed obvious patent infections from Days 15-17 onwards, whereas one animal had low positive values (50-200 epg) in only four samples between Days 18 and 30. The remaining animal in this group did not develop an identifiable patent infection.

In the E-INF group, the first positive samples were detected on Day 40 (mean 120 epg) after which the counts rose to 225 epg on Day 56, then returned to zero following treatment with eprinomectin on Day 56 and remained at zero until the end of the study on Day 77.

Blood parameters

Pilot study

The results are shown in Table 2. Though there was some variability between individual calves within and over the sampling period, overall the patterns of the various blood parameters were consistent between the hours of 09.00 and 16.00, hence it was determined that blood sampling would be conducted at the same time each day throughout the main study. Thus sampling took place between 11.30 and 13.00 each day, after the calves hade been offered hay and before they were given fresh concentrate feed.

Table 6.2. Diurnal pattern of mean blood concentrations of gastrin, ghrelin, leptin and pepsinogen in three uninfected calves sampled over three consecutive days.

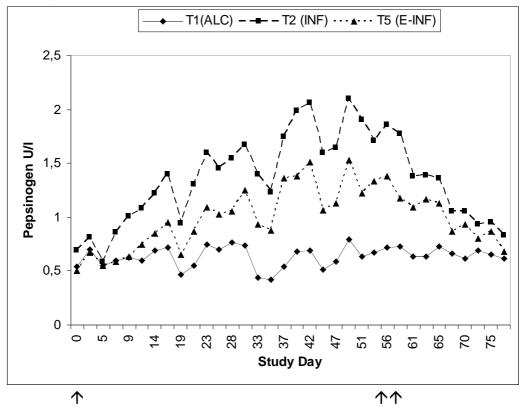|	Time of Day					
Parameter	09.00	10.00	11.00	12.00	14.00	16.00
Gastrin pmol/L	61.49	61.66	66.31	69.39	70.83	78.20
Ghrelin pg/ml	73.33	69.56	71.78	64.89	62.67	68.00
Leptin ng/ml	1.12	1.07	1.09	0.91	1.13	1.07
Pepsinogen U/L	0.55	0.61	0.53	0.51	0.53	0.53

Main study

Plasma Pepsinogen

Pepsinogen changed significantly over time ($P < 0.0001$) and was significantly different between treatment groups ($P < 0.0001$); there was also a significant interaction between day and treatment group ($P < 0.0001$). Overall the ALC group was significantly different from the INF group ($P = 0.0001$) and the E-INF group ($P < 0.05$) while the difference between INF and E-INF was not significant ($P = 0.15$).

In the three uninfected groups (ALC, PFC, E-ALC) the mean pepsinogen levels oscillated around a baseline value of between 0.4 and 0.9 U/l. The mean concentrations in the INF group started to increase from Day 9 (1.0 U/l) and thereafter, with some day-to-day variation, followed a parabolic curve, with peak values of 2.06 & 2.10 U/l occurring on Days 42 and 49 respectively, before declining to 0.83 by Day 77; the start of the decline coincided with eprinomectin treatment on Day 56. There was again some heterogeneity within this group, with the three animals with evident patent infections

_____

having lower pepsinogen concentrations than the two animals with zero or low egg output.

The pattern of pepsinogen values in the E-INF group was intermediate between that of the three uninfected groups and the INF group, though there appeared to be a delay in the increase in plasma concentrations until around Day 23. The peak values of 1.51 and 1.53 U/l in E-INF occurred on Days 42 and 49. Figure 6.2 shows the evolution of the plasma pepsinogen concentrations in groups ALC, INF and E-INF.

Figure 6.2. Pattern of plasma pepsinogen in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin on Days 0 and 56 (E-INF).

↑   Treatment with eprinomectin Day 0 (E-INF)
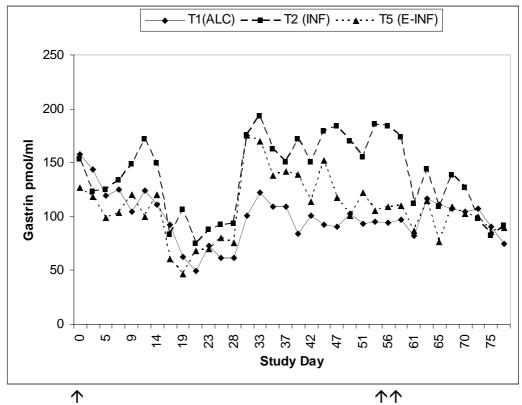↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)

Plasma Gastrin

Over the duration of the study, gastrin changed significantly over time (P < 0.0001) and there was a significant difference between treatment groups (P = 0.045), however there was no significant relationship with feed intake (P = 0.4918). Group ALC plasma gastrin concentrations were significantly different from group INF (P<0.05) values, with

the greatest effect seen between Days 30 and 56 (P=0.015); there were no significant differences between levels in groups ALC and E-INF or between INF and E-INF.

In the second week of the study (Days 7-14), the concentration of plasma gastrin in the INF group increased, while those in the ALC and E-INF groups were relatively stable. Gastrin levels declined in all groups from Days 14 to 28 after which the concentrations of gastrin remained high in the INF calves until the Day 56 treatment with eprinomectin, after which they declined. The calves with low/no patent infections in the INF group had the highest gastrin levels from Day 28 to 56. Gastrin levels in E-INF group declined over the period of 35 to 49 and remained low until the end of the study. The pattern of plasma gastrin for groups ALC, INF and E-INF is shown in Figure 6.3.

Figure 6.3. Pattern of plasma gastrin in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin on Days 0 and 56 (E-INF).
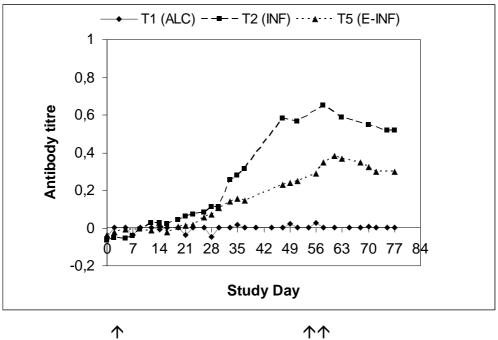

↑   Treatment with eprinomectin Day 0 (E-INF)
↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)

_____

Plasma *O. ostertagi* Antibodies

The evolution of *O. ostertagi* antibodies in the calves in groups ALC, INF and E-INF is shown in Figure 6.4. Antibody levels changed significantly over time ($P < 0.0001$) and were significantly different between treatment groups ($P = 0.0011$); there was also a significant interaction between day and treatment ($P = 0.0049$). The values in the ALC group remained at baseline at all time points. In the INF group, titres started to show a small, steady increase after the first three weeks and then increased from there to peak values of around 0.65 ODR by Day 60 and remained at a high level thereafter. The antibody levels in the E-INF group increased similarly until reaching a lower peak (0.38 ODR) at around Day 60.

Figure 6.4. Evolution of plasma antibodies to *Ostertagia ostertagi* in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin on Days 0 and 56 (E-INF).

↑   Treatment with eprinomectin Day 0 (E-INF)
↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)
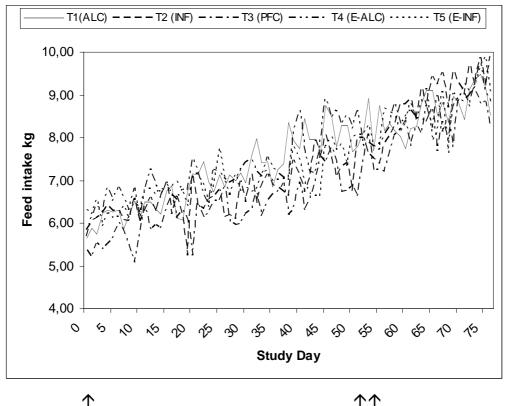
Animal Performance

Live weight Gain

There were no significant differences between any of the groups at any time point in liveweight, total liveweight gain or daily liveweight gain. Mean daily liveweight gain

_____

over the 77 days of the study ranged from 1.21 to 1.35 kg/day amongst the groups, with the gains in the two infected groups being numerically lower.

Feed Intake

The daily feed intake increased from ~6 kg fresh weight per day to ~9 kg/day over the course of the study (Figure 6.5). Feed intake changed significantly over time (P < 0.0001), but there were no significant differences between any of the treatment groups. Equally there were no differences between treatment groups when the data were analysed in the periods from Days 0-21; 23-56 and 58-77, though it was noted that there was a high level of variability in the data. There was however a significant interaction between day and treatment group indicating significant differences within groups and between groups on some days (P = 0.013).

Figure 6.5. Pattern of feed intake in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin on Days 0 and 56 (E-INF).
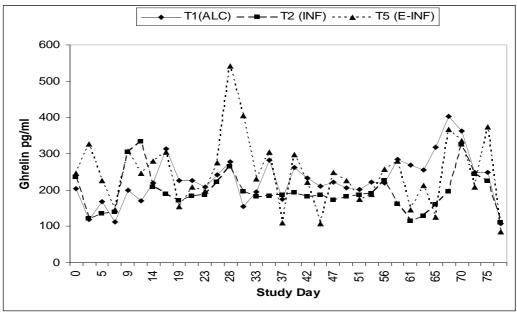

↑   Treatment with eprinomectin Day 0 (E-INF)
↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)

_____

Endocrinology

    Blood ghrelin

Ghrelin changed significantly over time (P < 0.0001) but there were no significant differences between treatment groups or any effect of feed intake (Figure 6.6).

Figure 6.6. Pattern of ghrelin concentration in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin on Days 0 and 56 (E-INF).

↑   Treatment with eprinomectin Day 0 (E-INF)
↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)

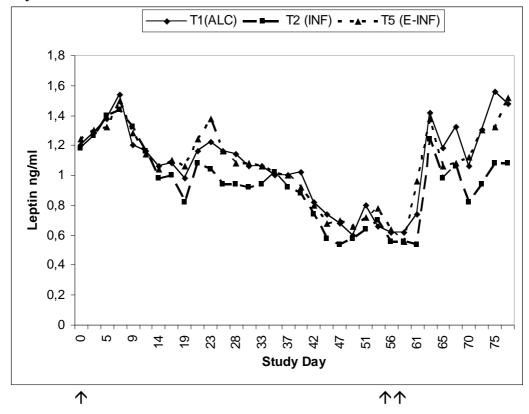   **Blood leptin**

Leptin changed significantly over time (P < 0.0001) and was significantly different between treatment groups (P = 0.013). Leptin concentrations were significantly lower in the INF group than in groups ALC and E-INF, but there were no significant differences between groups ALC and E-INF. The differences were most apparent between Days 19 and 33 and Days 65 and 77 (Figure 6.6).

Figure 6.7. Pattern of plasma leptin concentrations in ad lib fed controls (ALC), infected (INF) calves and those infected and treated with eprinomectin (E-INF) on Days 0 and 56.

↑   Treatment with eprinomectin Day 0 (E-INF)
↑↑   Treatment with eprinomectin Day 56 (INF & E-INF)

6.4. Discussion

This study was designed to be consistent with a previous experiment in which a profound effect on appetite was observed in calves trickle-infected with *O. ostertagi* (Fox et al., 1989a). There were however some differences between these two studies that may have affected the respective outcomes.

Animal movement restrictions delayed the start of the current study somewhat and the calves in the present study were thus slightly older (4-5 vs. 3 months) and heavier (~150 vs. 100 kg) than those used previously. They were still however well within the range of ages/weights of first-season grazing calves, which are known to be susceptible to infections with gastrointestinal nematodes. Because the infective larvae were given as a fixed dose (equivalent to 10,000 per head per day) rather than on a body weight basis, the number of larvae given to the calves in the current study was lower on a liveweight basis (67 vs. 100 L_3/kg), but would still be equivalent to a heavy larval challenge from pasture (>1000 L_3/kg DM of herbage). Calves in the previous experiment showed

_____

mild/intermittent clinical signs of ostertagiosis; those in the current study did not. An important difference appears to be in the ration: in the 1989 experiment, calves were fed hay only with no concentrate resulting in modest feed intakes in the ALC group (1→5 kg/day) and correspondingly low growth rates (0.3 kg/day). In contrast, the calves in the current study were fed a concentrate ration ad lib and the intakes were considerably higher (6→9 kg/day), as were the growth rates (1.21-1.35 kg/day). Differences in the magnitude of gastrin responses in similarly infected calves fed either hay or concentrate-based rations have been reported: peak values in infected calves fed the concentrate diet were 5.2 times higher than those in the pair-fed controls, whereas they were 12.4 times higher in calves fed hay; no data on feed intake were provided (Fox et al., 1988). In another study, calves that were 2½ months old and weighed ~90 kg were on a dietary regime similar to that in the current study and infected with a single dose of 200,000 *O.ostertagi* (Fox et al., 2002). The infected calves showed mild/intermittent clinical signs during the patent phase of the infection and a significant drop in appetite from Day 20 onwards. The lack of any significant differences in feed intake between the INF and ALC groups in the current study may be the result of chance, but it may indicate that this model for inappetence is dependent on the age of the calves, the mode and magnitude of infection and the ration.

Regardless of the reasons, the lack of any consistent pattern or significant differences in feed intake between the groups in the current study limits the extent to which any associations between parasite-induced inappetence and the various biochemical, immunological and neuroendocrine measurements can be interpreted. Nevertheless, the responses to the trickle infection in the INF group, in terms of plasma pepsinogen, gastrin and *O.ostertagi* antibody levels are consistent, at least qualitatively, with the results of several studies in the literature. Hence possible associations between ghrelin and leptin can still be legitimately considered as possible markers for inappetence in ostertagiosis. In addition, the impact of preventative anthelmintic treatment (E-INF) can also be evaluated.

The lack of any significant differences in feed intake and body weight between the groups in general and groups ALC and INF in particular meant that the PFC group provided no useful additional data in this study. The E-ALC group provided strong evidence that there is no direct pharmacological effect of eprinomectin on feed intake in the absence of parasites, providing support for previous argumentation based on indirect

evidence (Forbes et al., 2004). Neither of these groups will be considered further in the discussion.

Preventative treatment with eprinomectin (E-INF) resulted in a delay in the first appearance of positive faecal egg counts to 40 days after the trickle infection commenced, compared to 15 days for the INF group; this is consistent with the persistent activity of this formulation against *O. ostertagi* (Cramer et al., 2000). Values for the other parasitological parameters – gastrin, pepsinogen and *O. ostertagi* antibodies – followed the same patterns as those in the INF group, but with values that were typically intermediate between INF and ALC, though not necessarily statistically distinguishable from either group. Thus it appears that preventative treatment moderates the pathophysiology of infection, the endocrine and the antibody response, consistent with the literature from both artificial and natural infection with gastrointestinal nematode parasites (Claerebout et al., 1998a; Claerebout et al., 1998b).

There was no clear evidence for a relationship between appetite and ghrelin or leptin in either uninfected or infected calves; this may have been influenced by the minor differences in appetite amongst the groups or it may be that these peptides did not mediate changes in appetite under the conditions of this experiment. In addition, in healthy ruminants, plasma ghrelin levels are generally lower and fluctuate much less in animals fed ad lib (Sugino et al., 2002; Wertz-Lutz et al., 2006) and in young calves (compared to adult dairy cows (Miura et al., 2004), thus the conditions of the study may have limited the likelihood of detecting variations and associations within and between the different groups. There are currently no studies in the literature regarding ghrelin in parasitized animals, so more research is required to determine if it plays a role in appetite control under such circumstances.

Several papers have reported on the role of leptin in parasitized animals (cattle, sheep and rat). The study in cattle was carried out in grazing dairy heifers that were naturally parasitized, half of which were treated regularly with ivermectin, and the main objective was to determine the effect of PGE on growth and age at puberty. There was a notable effect of parasite control on the rate of live weight gain (faster) and the age of onset of puberty (younger) and there was a correlation between leptin and liveweight during development, but no association with parasitism per se was reported, nor was there any information regarding feed intake (Diaz-Torga et al., 2001). In a study with sheep infected with a single infection of 100,000 *O. (Teladorsagia) circumcincta* larvae, an increase in leptin was associated with a decline in feed intake, whereas, when appetite

_____

improved, serum leptin levels fell (Fox et al., 2006). In another study using a trickle infection of 7000 *T. circumcincta* larvae/day, the effects of parasitism on feed intake and circulating leptin levels in two different breeds of lambs of differing susceptibility to GI nematodes was examined (Zaralis et al., 2008a). The main findings were that anorexia was only observed in the more susceptible breed and inappetence occurred both during primary infections and during re-exposure. Plasma leptin concentrations were greater in infected than in non-infected lambs at similar levels of feed intake. This apparently contradictory effect was explained by the fact that leptin responses in the infected lambs may have been subject to conflicting influences of reduced appetite (which would typically cause a decrease in leptin) and infection (an increase in leptin), the latter association has been demonstrated in several studies in infectious diseases (Fantuzzi and Faggioni, 2000). In a parallel study by the same authors in periparturient ewes, plasma leptin levels were not associated with the observed feed intake depression in infected ewes, nor with the trickle infection with ~4300 *T. circumcincta* larvae/day, nor by the level of protein supplementation (Zaralis et al., 2008b). Lastly, in rats infected with a single infection with 45 *Nippostrongylus brasiliensis* larvae, leptin concentrations were elevated early in the infection (<2 days) and lower subsequently, compared to uninfected controls (Roberts et al., 2000). In the present study, plasma leptin concentrations were significantly lower in infected calves (INF), noticeably during patency and after anthelmintic treatment. It can be concluded from the evidence to date that the relationship between leptin and parasitism is complex and probably is related to both energy balance and infection, but it seems unlikely that leptin is solely responsible for anorexia, when it occurs.

There remains the possibility that gastrin, which has been previously shown to be strongly correlated with inappetence in calves, with (Fox et al., 1989b) or without ostertagiosis (Fox et al., 1989c), could act independently of other mediators to induce inappetence, as demonstrated in sheep (Grovum and Chapman, 1982). Studies have shown that not only are cholecystokinin (CCK) and gastrin closely related in terms of chemical structure, but also that the CCK-B receptor is highly homologous with, if not identical to, the gastrin receptor (Kopin et al., 1992). Because CCK-B receptors are found not only in parietal cells, but also in the brain, gastrin could perhaps mediate inappetence directly via the central nervous system (Kopin et al., 1992; Olszewski et al., 2008).

_____

The lack of a significant effect of infection on appetite in the infected group (INF) in this study suggests that the high energy and protein content of the concentrate ration, and correspondingly high levels of feed intake in all calves, mitigated the expected effects on appetite, though protein deficit or excess did not affect the expression of inappetence in peri-parturient ewes infected with *T.circumcincta* (Zaralis et al., 2008b). While this was a disappointing result in the context of this study, it is consistent with a body of literature, mainly on sheep, that indicates that some of the detrimental effects of gastrointestinal parasitism in ruminants can be countered by supplementation, particularly with protein rich feeds (Jorgensen et al., 1992; Valderrabano et al., 2002; Houdijk and Athanasiadou, 2003; Sykes and Greer, 2003). It also raises the possibility that nutritional supplementation could be used in worm control, either by itself or as an adjunct to other means of control (anthelmintics, grazing management) under field conditions. Supplementation of grazing cattle over a ~4 week period during the period of peak pasture larval density provided effective control in one study (Jorgensen et al., 1992), but it was ineffective in another (Larsson et al., 2006). Nevertheless, the provision of ad lib, high quality feed should make a positive contribution to the ability of parasitized host to counter the pathogenic effects of infection (Coop and Kyriazakis, 1999).

6.5. Conclusion

This study was designed to investigate potential biochemical and neuroendocrine mediators for inappetence in calves infected with *O. ostertagi*. The expression of inappetence in the infected calves appeared to be masked through access to a high quality concentrate ration fed ad lib. The lack of significant differences in feed intake between the various treatment groups precluded drawing any conclusions on the relative roles and relevance of the various parameters analysed. Nevertheless, typical profiles of plasma gastrin were generated in the infected calves, which also had reduced plasma leptin concentrations compared to controls, indicative of possible interactions locally and at the level of the central nervous system. Under the conditions of this study, there were no significant relationships between plasma ghrelin concentrations and appetite or parasitism. More research is required to more fully understand the mediation of inappetence in ruminants with parasitic gastroenteritis.

6.6. References

Attoub, S., Levasseur, S., Buyse, M., Goiot, H., Laigneau, J.P., Moizo, L., Hervatin, F., Le Marchand-Brustel, Y., Lewin, J.M., Bado, A., 1999, Physiological role of cholecystokinin B/gastrin receptor in leptin secretion. Endocrinology 140, 4406-4410.

Banks, W., Tschop, M., Robinson, S., Heiman, M., 2002, Extent and Direction of Ghrelin Transport Across the Blood Brain Barrier is Determined by its unique Primary Structure. J Pharmacol Exp Ther 302, 822-827.

Bartha, T., Sayed-Ahmed, A., Rudas, P., 2005, Expression of leptin and its receptors in various tissues of ruminants. Domest Anim Endocrinol 29, 193-202.

Bousquet-Melou, A., Mercadier, S., Alvinerie, M., Toutain, P.L., 2004, Endectocide exchanges between grazing cattle after pour-on administration of doramectin, ivermectin and moxidectin. Int J Parasitol 34, 1299-1307.

Bradford, B.J., Allen, M.S., 2008, Negative energy balance increases periprandial ghrelin and growth hormone concentrations in lactating dairy cows. Domest Anim Endocrinol 34, 196-203.

Chaudhri, O., Small, C., Bloom, S., 2006, Gastrointestinal hormones regulating appetite. Philos Trans R Soc Lond B Biol Sci 361, 1187-1209.

Chilliard, Y., Delavaud, C., Bonnet, M., 2005, Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Domest Anim Endocrinol 29, 3-22.

Claerebout, E., Dorny, P., Vercruysse, J., Agneessens, J., Demeulenaere, D., 1998a, Effects of preventive anthelmintic treatment on acquired resistance to gastrointestinal nematodes in naturally infected cattle. Vet Parasitol 76, 287-303.

Claerebout, E., Vercruysse, J., Dorny, P., Demeulenaere, D., Dereu, A., 1998b, The effect of different infection levels on acquired resistance to gastrointestinal nematodes in artificially infected cattle. Vet Parasitol 75, 153-167.

Coop, R.L., Kyriazakis, I., 1999, Nutrition-parasite interaction. Vet Parasitol 84, 187-204.

Coop, R.L., Sykes, A.R., Angus, K.W., 1977, The effect of a daily intake of *Ostertagia circumcincta* larvae on body weight, food intake and concentration of serum constituents in sheep. Res Vet Sci 23, 76-83.

Cramer, L.G., Pitt, S.R., Rehbein, S., Gogolewski, R.P., Kunkle, B.N., Langhoff, W.K., Bond, K.G., Maciel, A.E., 2000, Persistent efficacy of topical eprinomectin against nematode parasites in cattle. Parasitol Res 86, 944-946.

Diaz-Torga, G.S., Mejia, M.E., Gonzalez-Iglesias, A., Formia, N., Becu-Villalobos, D., Lacau-Mengido, I.M., 2001, Metabolic cues for puberty onset in free grazing Holstein heifers naturally infected with nematodes. Theriogenology 56, 111-122.

Exton, M.S., 1997, Infection-Induced Anorexia: Active Host Defence Strategy. Appetite 29, 369-383.

Fantuzzi, G., Faggioni, R., 2000, Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol 68, 437-446.

Forbes, A.B., Huckle, C.A., Gibb, M.J., 2004, Impact of eprinomectin on grazing behaviour and performance in dairy cattle with sub-clinical gastrointestinal nematode infections under continuous stocking management. Vet Parasitol 125, 353-364.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Gale, D.L., 1989a, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on appetite, digestibility, rate of passage of digesta and liveweight gain. Res Vet Sci 47, 294-298.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Gill, M., Simmonds, A.D., 1989b, *Ostertagia ostertagi* infection in the calf: effects of a trickle challenge on the hormonal control of digestive and metabolic function. Res Vet Sci 47, 299-304.

Fox, M.T., Gerrelli, D., Pitt, S.R., Jacobs, D.E., Hart, I.C., Simmonds, A.D., 1987, Endocrine effects of a single infection with *Ostertagia ostertagi* in the calf. Int J Parasitol 17, 1181-1185.

Fox, M.T., Gerrelli, D., Shivalkar, P., Jacobs, D.E., 1989c, Effect of omeprazole treatment on feed intake and blood gastrin and pepsinogen levels in the calf. Res Vet Sci 46, 280-282.

Fox, M.T., Pitt, S.R., Gerrelli, D., Jacobs, D.E., 1988, Effect of diet on gastrin response to *Ostertagia ostertagi* infection in the calf. Int J Parasitol 18, 547-548.

Fox, M.T., Reynolds, G.W., Scott, I., Simcock, D.C., Simpson, H.V., 2006, Vagal and splanchnic afferent nerves are not essential for anorexia associated with abomasal parasitism in sheep. Vet Parasitol 135, 287-295.

Fox, M.T., Uche, U.E., Vaillant, C., Ganabadi, S., Calam, J., 2002, Effects of *Ostertagia ostertagi* and omeprazole treatment on feed intake and gastrin-related responses in the calf. Vet Parasitol 105, 285-301.

Fukumoto, K., Nakahara, K., Katayama, T., Miyazatao, M., Kangawa, K., Murakami, N., 2008, Synergistic action of gastrin and ghrelin on gastric acid secretion in rats. Biochem Biophysical Res Commun 374, 60-63.

Garcia, M.R., Amstalden, M., Williams, S.W., Stanko, R.L., Morrison, C.D., Keisler, D.H., Nizielski, S.E., Williams, G.L., 2002, Serum leptin and its adipose gene expression during pubertal development, the estrous cycle, and different seasons in cattle. J Anim Sci 80, 2158-2167.

Gentry, P., Willey, J., Collier, R., 2003, Ghrelin, a growth hormone secretagogue, is expressed by bovine rumen J Anim Sci 81, Suppl 1, 123.

Greer, A.W., McAnulty, R.W., Stankiewicz, M., Sykes, A.R., 2005a, Corticosteroid treatment prevents the reduction in food intake and growth in lambs infected with the abomasal parasite *Teladorsagia circumcincta*. Proc NZ Soc Anim Prod 65, 9-13.

_____

Greer, A.W., Stankiewicz, M., Jay, N.P., McAnulty, R.W., Sykes, A.R., 2005b, The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunologically naive and competent sheep. Anim Sci 80, 89-99.

Grouselle, D., Chaillou, E., Caraty, A., Bluet-Pajot, M.-T., Zizzari, P., Tillet, Y., Epelbaum, J., 2008, Pulsatile Cerebrospinal Fluid and Plasma Ghrelin in Relation to Growth Hormone Secretion and Food Intake in the Sheep. J Neuroendocrinol 20, 1-9.

Grovum, W.L., Chapman, H.W., 1982, Pentagastrin in the circulation acts directly on the brain to depress motility of the stomach in sheep. Regul Pept 5, 35-42.

Hart, B.L., 1990, Behavioral adaptations to pathogens and parasites: five strategies. Neurosci Biobehav Rev 14, 273-294.

Hayashida, T., Murakami, K., Mogi, K., Nishihara, M., Nakazato, M., Mondal, M.S., Horii, Y., Kojima, M., Kangawa, K., Murakami, N., 2001, Ghrelin in domestic animals: distribution in stomach and its possible role. Domest Anim Endocrinol 21, 17-24.

Henry, B.A., Goding, J.W., Alexander, W.S., Tilbrook, A.J., Canny, B.J., Dunshea, F., Rao, A., Mansell, A., Clarke, I.J., 1999, Central administration of leptin to ovariectomized ewes inhibits food intake without affecting the secretion of hormones from the pituitary gland: evidence for a dissociation of effects on appetite and neuroendocrine function. Endocrinology 140, 1175-1182.

Houdijk, J.G., Athanasiadou, S., 2003. Direct and indirect effects of host nutrition on ruminant gastrointestinal nematodes. In: Matching herbivore nutrition to ecosystem biodiversity, Yucatan, Mexico, pp. 213-236.

Johnson, R.W., 1998, Immune and endocrine regulation of food intake in sick animals. Domest Anim Endocrinol 15, 309-319.

Johnson, R.W., 2002, The concept of sickness behavior: a brief chronological account of four key discoveries. Vet Immunol and Immunopathol 87, 443-450.

Jorgensen, R.J., Satrija, F., Monrad, J., Nansen, P., 1992, Effect of feeding lucerne pellets on trichostrongyle infection in grazing heifers. Vet Rec 131, 126-127.

Konsman, J.P., Parnet, P., Dantzer, R., 2002, Cytokine-induced sickness behaviour: mechanisms and implications. Trends Neurosci 25, 154-159.

Kopin, A., S., Lee, Y., M., McBride, E.W., Miller, L.J., Lu, M., Lin, H.Y., Kolakowski, L.F., Jr., Beinborn, M., 1992, Expression cloning and characterization of the canine parietal cell gastrin receptor. Proc Natl Acad Sci USA 89, 3605-3609

Kulcsár, M., Jánosi, S., Lehtolainen, T., Kátai, L., Delavaud, C., Balogh, O., Chilliard, Y., Pyörälä, S., Rudas, P., Huszenicza, G., 2005, Feeding-unrelated factors influencing the plasma leptin level in ruminants. Domest Anim Endocrinol 29, 214-226.

Kyriazakis, I., Oldham, J.D., Coop, R.L., Jackson, F., 1994, The effect of subclinical intestinal nematode infection on the diet selection of growing sheep. Br J Nutr 72, 665-677.

Larsson, A., Dimander, S.O., Rydzik, A., Uggla, A., Waller, P.J., Hoglund, J., 2006, A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle--effects on animal performance. Vet Parasitol 142, 197-206.

Liefers, S.C., Veerkamp, R.F., te Pas, M.F.W., Delavaud, C., Chilliard, Y., van der Lende, T., 2003, Leptin Concentrations in Relation to Energy Balance, Milk Yield, Intake, Live Weight, and Estrus in Dairy Cows. J Dairy Sci 86, 799-807.

Lippl, F., Kircher, F., Erdmann, J., Allescher, H.D., Schusdziarra, V., 2004, Effect of GIP, GLP-1, insulin and gastrin on ghrelin release in the isolated rat stomach. Regul Pept 119, 93-98.

MAFF, 1986, Manual of Veterinary Parasitological Laboratory Techniques (Reference Book 418). Her Majesty's Stationery Office, London, 160 p.

Meeusen, E.N.T., 1999, Immunology of helminth infections, with special reference to immunopathology. Vet Parasitol 84, 259-273.

Miura, H., Tsuchiya, N., Sasaki, I., Kikuchi, M., Kojima, M., Kangawa, K., Hasegawa, Y., Ohnami, Y., 2004, Changes in plasma ghrelin and growth hormone concentrations in mature Holstein cows and three-month-old calves. J Anim Sci 82, 1329-1333.

Murphy, K., Bloom, S., 2006, Gut hormones and the regulation of energy homeostasis. Nature 444, 854-859.

Murray, M., Jennings, F.W., Armour, J., 1970, Bovine ostertagiasis: structure, function and mode of differentiation of the bovine gastric mucosa and kinetics of the worm loss. Res Vet Sci 11, 417-427.

Mylrea, P.J., Hotson, I.K., 1969, Serum pepsinogen activity and the diagnosis of bovine ostertagiasis. Br Vet J 125, 379-388.

Olszewski, P.K., Cedernaes, J., Olsson, F., Levine, A.S., Schiöth, H.B., 2008, Analysis of the network of feeding neuroregulators using the Allen Brain Atlas. Neurosci Biobehav Rev 32, 945-956.

Roberts, H.C., Hardie, L.J., Chappell, L.H., Mercer, J.G., 2000, Parasite-induced anorexia: leptin, insulin and corticosterone responses to infection with the nematode, *Nippostrongylus brasiliensis*. Parasitology 118, 117-123.

Rook, A., Huckle, C., 1995, Synchronization of ingestive behaviour by grazing dairy cows. Anim Sci 60, 25-30.

Sandberg, F.B., Emmans, G.C., Kyriazakis, I., 2006, A model for predicting feed intake of growing animals during exposure to pathogens. J Anim Sci 84, 1552-1566.

Smith, G., 2000, The Controls of Eating: A Shift from Nutritional Homeostasis to Behavioral Neuroscience. Nutrition 16, 814-820.

_____

Smith, M.S., Grove, K.L., 2002, Integration of the regulation of reproductive function and energy balance: lactation as a model. Front Neuroendocrinol 23, 225-256.

Stephens, D., Brown, J., Ydenberg, R. 2007. Neuroendocrine Mechanisms of Energy Regulation in Mammals. In Foraging Behavior & Ecology, Woods, S., Castonguay, T., eds. (The University of Chicago Press), pp. 223-230.

Sugino, T., Hasegawa, Y., Kurose, Y., Kojima, M., Kangawa, K., Terashima, Y., 2004, Effects of ghrelin on food intake and neuroendocrine function in sheep. Anim Reprod Sci 82-83, 183-194.

Sugino, T., Yamaura, J., Yamagishi, M., Ogura, A., Hayashi, R., Kurose, Y., Kojima, M., Kangawa, K., Hasegawa, Y., Terashima, Y., 2002, A transient surge of ghrelin secretion before feeding is modified by different feeding regimens in sheep. Biochem Biophysical Res Commun 298, 785-788.

Sykes, A.R., Coop, R.L., 1977, Intake and utilisation of food by growing sheep with abomasal damage caused by daily dosing with *Ostertagia circumcincta* larvae. J Agric Sci. 88, 671-677.

Sykes, A.R., Greer, A.W., 2003, Effects of parasitism on the nutrient economy of sheep: an overview. Aust J Exp Agric 43, 1393-1398.

Symons, L.E., 1985, Anorexia: occurrence, pathophysiology, and possible causes in parasitic infections. Adv Parasitol 24, 103-133.

Valderrabano, J., Delfa, R., Uriarte, J., 2002, Effect of level of feed intake on the development of gastrointestinal parasitism in growing lambs. Vet Parasitol 104, 327-338.

Wertz-Lutz, A.E., Daniel, J.A., Clapper, J.A., Trenkle, A., Beitz, D.C., 2008, Prolonged, moderate nutrient restriction in beef cattle results in persistently elevated circulating ghrelin concentrations. J Anim Sci 86, 564-575.

Wertz-Lutz, A.E., Knight, T.J., Pritchard, R.H., Daniel, J.A., Clapper, J.A., Smart, A.J., Trenkle, A., Beitz, D.C., 2006, Circulating ghrelin concentrations fluctuate relative to nutritional status and influence feeding behavior in cattle. J Anim Sci 84, 3285-3300.

Yonekura, S., Kitade, K., Furukawa, G., Takahashi, K., Katsumata, N., Katoh, K., Obara, Y., 2002, Effects of aging and weaning on mRNA expression of leptin and CCK receptors in the calf rumen and abomasum. Domest Anim Endocrinol 22, 25-35.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I., 2008a, Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. J Anim Sci 86, 1891-1903.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I., 2008b, Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitized ewes of two breeds. Br J Nutr (accepted).

_____

Zieba, D.A., Amstalden, M., Williams, G.L., 2005, Regulatory roles of leptin in reproduction and metabolism: A comparative review. Domest Anim Endocrinol 29, 166-185

Chapter 7: General discussion: the effects of sub-clinical gastrointestinal parasitism on components of grazing behaviour in cattle and their role in inappetence, including observations on possible neuroendocrinological pathways linking abomasal pathophysiology and reduction in feed intake

_____

7.1. Introduction

The main objective of this thesis was to investigate the application of established methodology in the study of grazing behaviour in ruminants to determine the magnitude and expression of inappetence in free-ranging pastured animals naturally infected with parasitic gastrointestinal nematodes and to relate any effects to animal performance. An additional complementary study under controlled experimental conditions using a trickle infection with *Ostertagia ostertagi* was conducted in order to investigate possible neuroendocrine and immunological pathways linking abomasal pathophysiology and inappetence.

The field studies showed that:

1. The IGER behaviour recorders, in association with the software programme 'Graze', provided an effective and efficient means for studying the effects of parasitism on grazing behaviour in cattle.

2. In three of the four field studies, there were statistically significant differences in grazing behaviour between naturally parasitized cattle and anthelmintic-treated animals.

3. Behavioural parameters that were sensitive to gastrointestinal parasitism included total grazing time (TGT), total eating time (TET), total grazing jaw movements (TGJM) and the total number of bites within a 24-hour recording period.

4. In two of the three studies in which significant behavioural effects of parasitism were observed, inappetence appeared to affect animal performance and was associated with a reduction in either milk yield and/or growth rate.

The study with experimental *O. ostertagi* infection indicated that:

1. Despite showing typical profiles of pepsinogen and gastrin, indicative of abomasal pathophysiology, no significant reduction in feed intake was observed in the untreated infected calves.

2. Plasma leptin was significantly lower in the untreated infected calves than in the other groups: the effect was most evident following patency of the infection.

3. There were no differences in the concentrations of plasma ghrelin between the groups.

4. Antibodies to *O. ostertagi* were detected in infected calves from patency onwards and increased linearly thereafter.

_____

5. Preventative treatment of infected calves with eprinomectin moderated the manifestations of parasitism, including faecal egg counts, pepsinogen and gastrin and *O. ostertagi* antibody concentrations.

In this chapter, these results will be discussed in the context of the normal grazing behaviour patterns in cattle; grazing behaviour in parasitized ruminants and the endocrinology of inappetence in parasitic disease. Finally the practical relevance of this thesis will be considered in the context of livestock production, nutrition and veterinary medicine.

7.2. General effects of subclinical PGE on grazing behaviour in cattle.

Table 7.1 comprises a distillation of the principle results of the four studies described in Chapters 2-5 in order that the main effects can be compared. From this compilation it can be deduced that the most consistent effects of subclinical PGE on grazing behaviour in cattle are reductions in total eating time and total grazing time, with correspondingly fewer total grazing jaw movements and bites per day. In only one study were none of these effects statistically significant and in this trial all these parameters were directionally consistent with the other studies. In the two studies in which the parasitized cattle grazed for significantly less time each day, there were also significant increases in 'idling' time (defined as the time remaining within a day when animals are neither eating nor ruminating). The untreated dairy cows grazing rotationally (Chapter 4) showed a trend towards less ruminating and more idling time.

In order to compare and contrast the results of the fours studies in a broader context, they will be considered in three pair-wise comparisons:

1. Calves continuously grazing ryegrass pastures or adjacent monocultures of ryegrass and clover.
2. Adult lactating dairy cattle grazing ryegrass pastures continuously or under daily-paddock stocking management.
3. Calves and adult lactating dairy cattle continuously grazing ryegrass pastures.

_____

Table.7.1. Summary data and statistics from studies described in Chapters 2-5.

	Chapter 2 Calves continuous stocking grass (July)		Chapter 3 Dairy cows continuous stocking grass (June)		Chapter 4 Dairy cows daily paddocks grass (July)		Chapter 5 Calves continuous stocking grass/clover (August)	
	Control	Treated	Control[1]	Treated[1]	Control[2]	Treated[2]	Control[3]	Treated[3]
Behaviour over 24 hours								
Total Grazing Time (TGT) (min)	443	540	571	620	494	496	479	535
Total Eating Time (TET) (min)	391	462	545	599	461	470	430	466
Total Grazing Jaw Movements (TGJM) (000s)	30.6	37.0	40.2	46.1	34.0	34.7	32.4	36.5
Total bites (000s)	18.3	21.5	30.8	35.4	23.6	24.1	22.2	23.1
Bites/Grazing Jaw Movement (B/GJM)	0.598	0.581	0.765	0.762	0.690	0.690	0.685	0.628
Grazing Jaw Movement Rate (GJM/min)	78.2	80.1	74.0	77.1	74.1	74.5	75.4	78.2
Bite Rate (BR) (bites/min)	46.8	46.4	56.7	58.8	51.1	51.4	51.6	49.2
Total Ruminating Time (TRT) (min	485	494	415	439	502	536	454	425
Total Idling Time (TIT) (min) =24hrs - (TET + TRT)	564	483	480	400	477	434	555	548
Performance								
Daily Live Weight Gain (DLWG) (kg) over the duration of the study	0.68	0.78	0.24	0.55	0.10	0.18	1.17	1.18
Daily Solids-corrected Milk (SCM) Yield (kg)	-	-	18.9*	20.0*	18.9*	20.6*	-	-

Red numbers: control vs. treated significantly different at P<0.05
Blue numbers: control vs. treated significantly different at P<0.10
Black numbers: control vs. treated no significant difference P>0.10

[1]Heifers and Cows combined
[2]Results for July recording
[3]Days 1 and 2 combined
*Yield over the duration of the study

It should be remembered however that although the methodology and basic experimental designs were similar for all four studies, they were not contemporary. Each took place in a different grazing season and the pastures used were different, except for the two dairy cows studies, where there was some overlap in the grazing area between each year.

Calves continuously grazing ryegrass pastures or adjacent monocultures of ryegrass and clover.

The results of the two studies in calves differed somewhat in that parasitism had a clear effect on appetite in calves continuously grazing ryegrass pastures and furthermore, the reduced herbage intake appeared to account for the differences in live weight gain between the treated and untreated calves. In calves grazing grass and clover, while some effects on appetite were observed, there were no significant differences in liveweight gain between the two groups of calves. One possible explanation is that free access to abundant highly nutritious food can mitigate some of the negative effects and the expression of parasitism. Other factors that may have limited the differences in performance between the treated and control calves includes the fact that legumes such as clover also contain tannins, which have been shown to have both protein-sparing and anthelmintic properties (Hoste et al., 2006). In addition, the microclimate in legume monocultures has been shown to be generally more hostile to the survival and transmission of infective nematode larvae (Moss and Vlassoff, 1993). The level of anthelmintic control was greater in the calves on grass alone, as they were treated with an ivermectin sustained-release bolus, which provided a very high level of parasite control throughout the study, reflected in the zero faecal egg counts. Eprinomectin administered topically, even at regular intervals, does not control parasites with the same intensity as a continuous-release bolus and therefore some ingested larvae can develop into adult worms between treatments, reflected in the occasional, low faecal egg counts in some animals.
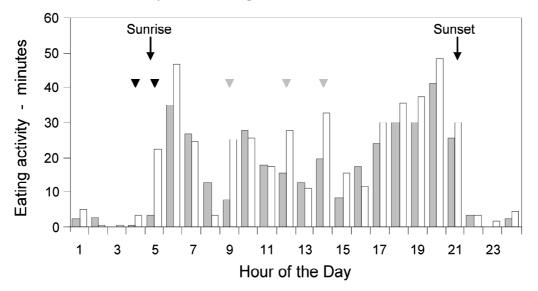
Adult lactating dairy cattle continuously grazing ryegrass pastures or under daily-paddock stocking management.

An important consequence of the differences in grazing management in these two studies is that in the daily-paddock system, the daily herbage allowance was restricted to twice the calculated energy requirements for the cattle, while in the continuous system the herbage is essentially available *ad lib* (generally equivalent to at least four times calculated nutrient requirements). These differences in grazing management and

_____

allowances may account for the observed differences in behavioural responses in the two studies. Nevertheless, in both studies treatment with eprinomectin resulted in significant increases in milk yield, so different mechanisms for this observation must operate. Individual cows responded to each of three successive eprinomectin treatments over the grazing season with a significant yield response under daily-paddock management, yet there were no differences in liveweight gain or condition score in the cows, so the additional milk yield did not apparently result from measurable mobilisation of body tissues. In young parasitized ruminants, nutrients are diverted away from anabolic processes, such as skeletal and muscle growth, into plasma protein synthesis, production of mucus and mucoprotein, repair of gut pathology and maintenance of immune function. It is not unreasonable to suppose that there could be a similar diversion of resources in adult cattle away from lactation despite, or perhaps even because of, their immune status. This would however be inconsistent with the proposed framework, based mainly on studies in sheep, in which it is suggested that in adult reproductive animals, lactation and/or pregnancy would have priority over the expression of immunity in the partitioning of scarce nutrients (Coop and Kyriazakis, 1999).
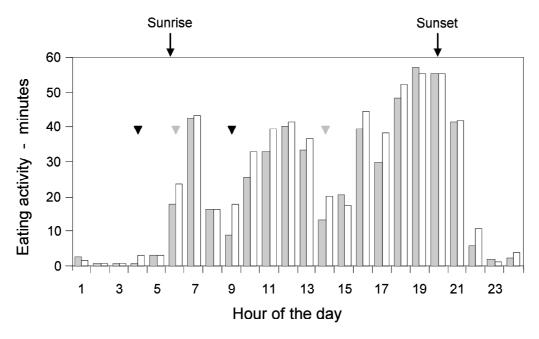
Calves and adult lactating dairy cattle continuously grazing ryegrass pastures.

In both of these studies there was a clear differentiation between sub-clinically infected and treated animals in terms of total daily eating time and there were correspondingly significant effects on performance. Treated young cattle grew some 15% faster than their infected counterparts, while treated dairy cattle experienced a 6% increase in milk yield and a short-term increase in daily growth rate compared to controls. In these studies, the cattle grazed continuously on ryegrass pastures that provided essentially *ad lib* feeding conditions. The additional daily eating times of 71 minutes in the calves and 54 minutes in the cows in the treated animals were of a similar magnitude, which was somewhat surprising, given that the calves were essentially parasite-naïve at the start of the grazing season, whereas adult cattle would be considered to be functionally immune to gastrointestinal nematodes, including *O. ostertagi*. Also it must be remembered that the potential daily grazing time in the cows was less because of the time spent away from the pastures when being milked and that they were fed 4 kg of concentrates while in the milking parlour. These observations suggest that the magnitude of the behavioural responses to parasitism may be independent of the magnitude of the worm burdens.

_____

Figure 7.1 Hourly patterns of eating behaviour over 24-hours in cattle receiving either no anthelmintic (grey fill) or treatment (no fill) with ivermectin (7.1a) or eprinomectin (7.1b).

a) Calves continuously stocked on grass

b) Dairy cows continuously stocked on grass

▼ : Significantly different in that hour P<0.05
▼ : Significantly different in that hour P<0.1

_____

Figure 7.1 shows the eating patterns for cattle in these two studies. Comparisons have been made between the groups in terms of eating time during each hour of the day and where significant differences are present, these have been annotated. It appears that the differences in daily grazing time have resulted from increased eating by the treated animals during the early grazing period from just before sunrise to around 09.00 hours. There is also a suggestion of increased eating in the hours around the middle of the day (12.00-14.00), but no significant differences during the main grazing period from mid-afternoon to sunset. It is tempting to speculate whether this might indicate parasite avoidance behaviour by the parasitized control cattle. There is some evidence for a diurnal pattern of infective nematode larvae on herbage, with the greatest number of larvae on the grass blades in the early morning (Rees, 1950). It is also known that parasitized sheep exhibit more pronounced parasite avoidance behaviours than uninfected animals. However, the visual cues for this appear to be faeces, there being no evidence that sheep could detect infective larvae on the herbage (Cooper et al., 2000).

Although the time spent eating each day is normally expressed as the total number of minutes, in fact feeding takes place in discrete meals interrupted by other behaviours such as ruminating, lying, movement and social interactions. This is an important consideration when considering the control of feed intake and the relative stimuli for appetite that lead to the initiation of a meal and the signals for satiety that cause its end. The treated dairy cows had significantly more meals per day and each was of greater duration, however there was no obvious pattern in terms of start and finish times compared to the infected control cows. The timing of grazing meals in lactating dairy cows is also of course influenced by being removed from pasture twice daily for milking, when additionally they may be fed a concentrate supplement, which could further affect subsequent feed intake (Roche et al., 2007). The number of meals and the meal duration was not significantly different between treated and control calves, though numerically the treated calves ate two more meals per day than the controls.

7.3 Pathophysiology, immunology and neuroendocrinology of ostertagiosis

In the study described in Chapter 6, trickle infection with 10,000 *O. ostertagi* larvae per day elicited the expected increases in plasma and gastrin concentrations and in antibodies to adult *O. ostertagi*, though with noticeable variability between individuals, but the calves experienced no significant decline in feed intake, nor were their growth rates adversely affected. The most likely explanation for the last two observations was

_____

that provision of high energy/high protein ration, fed ad lib, allowed the animals to counter some of the negative expressions of parasitism. The lack of a significant effect on growth rates was also seen in the field study (Chapter 5) where infected and treated calves had access to monocultures of ryegrass and white clover, which offered a high quality diet in abundance and a similar explanation was offered for those results. The concentrations of leptin in this study were significantly lower in infected, untreated calves and associations between parasitism and leptin have been reported previously in studies in rats and sheep (Roberts et al., 2000; Fox et al., 2006; Zaralis et al., 2008a; Zaralis et al., 2008b), but not in cattle (Diaz-Torga et al., 2001). However, the direction of the changes reported to date have not been consistent insofar as sometimes leptin concentrations have increased, in others decreased: in cattle there was an association between leptin and growth rate, which was higher in anthelmintic (ivermectin) treated calves than in controls, but no significant associations between parasitism per se and leptin could be found. This is probably a reflection of the multi-faceted local and central neuroendocrine roles of leptin and in particular the part it plays in the interplay between energy status and immunity. In this study, ghrelin concentrations did not vary at all according to any of the measures of parasitism.

The variation in responses within the untreated infected group appeared to follow a pattern that could help explain some of the differences in the expression of parasitism. Of the five individuals, three developed typical patent infections that led to high faecal egg counts (peak value amongst three calves 1550 epg), while in the remaining two calves, one had only four positive fecs with the highest being 200 epg, while the other never developed a patent infection. There appeared to be an inverse relationship between the magnitude of the faecal egg count and the magnitude of biochemical and immune changes, in that the calves with the higher egg counts had lower levels of pepsinogen, gastrin and *O. ostertagi* antibodies; the converse was true for the calves with low worm egg counts. It would be fascinating to know if these patterns are typical and if they represent trade-offs in biological responses and the extent to which they might affect parasite epidemiology and the expression of parasitism under field conditions. The neuroendocrine control of these responses is likely to be complex and involve mediators of immunity as well as energy and protein balance, but there is evidence that gastrin itself and leptin are strong candidates to play pivotal roles.

_____

7.4. Summary of effects.

Table 7.2 provides summary information on the relationships between parasitism, appetite, feed and animal performance. In the field studies, the effects of parasitism were determined from the differences between treatment with ivermectin (1) or eprinomectin (3) and untreated controls. In the challenge study, the effects are determined from the differences between infected and uninfected control groups and an infected group treated preventatively with eprinomectin. In all the studies, no clinical signs of clinical parasitic gastroenteritis were observed in any animal: all infections were subclinical.

Table 7.2. Summary of the interactions between parasitism, appetite, feed and performance in the five studies.

Study	Cattle	Feed	Availability	Appetite	Performance
Natural infections					
Chapter 2	FGS calves	Ryegrass	Ad lib	Decreased	Decreased
Chapter 3	Lactating cows and heifers	Ryegrass	Ad lib	Decreased	Decreased
Chapter 4	Lactating cows	Ryegrass	Restricted	No effect	Decreased
Chapter 5	FGS calves	Ryegrass + Clover	Ad lib	Decreased	No effect
Induced infection					
Chapter 6	4-5 mo calves	0.5 kg hay/day + concentrate	Ad lib	No effect	No effect

The patterns outlined in Table 7 can be interpreted from a practical perspective as follows:

- Under study conditions that are representative of typical livestock farming (Chapters 2-4), performance in both young FSG calves and lactating heifers and cows was improved by anthelmintic (ivermectin/eprinomectin) treatment; in two of the studies these improvements appeared to largely result from an increase in feed intake relative to the untreated controls.

- No significant improvements in animal performance through anthelmintic treatment were observed in the studies that included feed (concentrate or white clover) of high nutritional quality offered ad lib.

- Appetite was increased by anthelmintic treatment in studies in which herbage was offered ad lib; the response was more evident when the sward comprised ryegrass rather than as monocultures of ryegrass and white clover.

_____

7.5. Relevance of this thesis to parasite control under practical field conditions

The studies that comprise this thesis have provided for the first time evidence that the grazing behaviour of pastured cattle – calves, heifers and cows - can be modified by subclinical gastrointestinal nematode parasitism and that an important manifestation is a reduction in daily grazing time, resulting from a depression in appetite. It has also clearly demonstrated that these effects can be rapidly reversed through effective anthelmintic treatment with consequent improvements in animal performance. The studies have also shown that the relationships between parasitism, grazing management, nutrient quality and animal performance are complex, but nevertheless, certain patterns can be discerned.

The interrelationships between grazing management, food availability, parasitism and animal performance were also investigated in a 2-year study on associations between stocking density and gastrointestinal parasites. At equivalent stocking densities, the herbage height in paddocks grazed by untreated steers was significantly ($P<0.05$) higher (40-88%) than those grazed by (ivermectin) treated cattle (Bransby, 1993). These differences were considered to have resulted from an increase in appetite and forage intake as a result of parasite control in the treated groups of cattle. There were no significant differences in weight gain between treated and control cattle in either year, regardless of stocking rate, and it was deduced from data analysis that reduced forage availability in the paddocks of the treated animals, resulting from their increased intakes, accounted for this. To use the author's words "restricted nutrition (low forage intake) caused by lower forage availability in the treated groups was as limiting to weight gain as were restricted nutrition (parasite-induced anorexia) and presence of parasites in untreated groups." An additional observation of relevance to the current thesis is that worm egg counts were low in all groups (including untreated controls) throughout the grazing seasons and, at necropsy, low worm burdens and limited abomasal pathology were present, leading the author to speculate whether there was a threshold of infection above which inappetence occurred or whether the relationship was quantitative.

The knowledge gleaned from this thesis provides strong evidence that the use of anthelmintics in cattle needs to be reconsidered and extended. Typically and classically anthelmintics are used therapeutically to treat (young) animals affected by clinical PGE, tactically to try and mitigate losses when cattle graze (heavily) infected pasture or

_____

strategically in order to limit faecal egg output and hence diminish the typical increase in pasture infectivity through the grazing season. Examples of the last are fixed-interval early season treatments, or bolus administration at turnout, to FGS (and occasionally SGS cattle) and treatment prior to transfer to a hay or silage aftermath (dose-and-move). With the possible exception of therapeutic treatments, generally all animals in a group receive anthelmintic and indeed this is fundamental and central to classical strategic approaches. By ensuring that parasitism remains at subclinical levels, strategic (and tactical) regimens also ensure that young cattle can perform well and growth rates typically exceed those of untreated and therapeutically treated cattle (Shaw et al., 1998). The field studies in this thesis indicate that most grazing cattle are probably suffering from some level of production loss through the constant intake of infective larvae and the resultant subclinical nematode infections. There is no clear evidence for a threshold effect (Vercruysse and Claerebout, 2001) as it seems that levels of larval challenge and infection that previously might have been considered so low as to be trivial can still result in measurable changes in behaviour, biochemistry and animal performance. This now provides a rational explanation for the production responses to anthelmintics in adult dairy cows, which, whilst being functionally immune, nevertheless harbour small burdens of parasitic nematodes (Agneessens et al., 2000; Borgsteede et al., 2000; Murphy et al., 2006), which are nevertheless sufficient to provoke some of the responses described previously Results from studies in this thesis and related literature suggest that, in addition to local pathophysiological damage, signals from the parasites within the host, probably via biochemical, neuroendocrine or immune mediators invoke responses that alter behaviour. These adaptations may benefit the host, the parasite, both or neither, but from an animal production point of view, any that reduce performance levels in the hosts must be considered as unfavourable.

Thus, providing that clinical PGE remains uncommon in cattle and providing that general control of subclinical infections is adequate, there seems now to be a rationale for an additional category of anthelmintic use and that is solely as a performance booster. Evidence from this thesis shows that young and adult cattle can show beneficial responses to anthelmintic treatment within a week of administration and that the improvements can persist beyond the period of anthelmintic activity, i.e. they are sustainable. These responses may vary according to quality and quantity of feed available, but are not dependent on parasite epidemiology nor do they necessarily have any effect on it. This is because treatments can be given to cohorts or individuals at

_____

times that are driven by performance targets rather than for parasite control in the traditional sense. Naturally, such use of anthelmintics must be justifiable from a cost: benefit perspective and also the farmer needs to consider opportunity costs, that is, are there other ways in which this money could be spent that would lead to better returns? To this end, the mitigating effects of good nutrition, described in Chapters 5 and 6 need to be considered: can additional feed (concentrate, legumes etc) be provided at the same/less cost than anthelmintics and give the same/more return? What are the risks attached to the various options? Modern anthelmintics generally carry a very low risk of adverse effects and are highly effective at controlling parasites, but the spectre of anthelmintic resistance is a potential long-term consideration. In fact because the use of anthelmintics as performance boosters is not based on parasite epidemiology and can be targeted at selected individuals or sub-groups of animals, it is unlikely to increase selection pressure markedly. On the other hand, while the provision of additional feed is unlikely to have unforeseen negative effects in general, the evidence that additional feed alone will adequately control parasites in young grazing cattle and boost performance remains equivocal (Jorgensen et al., 1992; Larsson et al., 2006).

7.6. Conclusions

The information generated by this thesis not only provides a clear demonstration of the mechanisms for production losses in young grazing cattle with subclinical PGE but also helps explain why adult cows can be adversely affected by parasites, despite having a strong functional immunity. It introduces a new concept for the selective use of anthelmintics to achieve production targets, independent of parasite epidemiology. It also challenges the notion that thresholds exist for interventions in PGE in cattle as it has been shown that even adult cows that are exposed to very low levels of pasture larval contamination can respond positively to anthelmintic treatment repeatedly during the same grazing season, resulting in cumulative performance benefits.

_____

7.7 References

Agneessens, J., Claerebout, E., Dorny, P., Borgsteede, F.H., Vercruysse, J., 2000, Nematode parasitism in adult dairy cows in Belgium. Vet Parasitol 90, 83-92.

Borgsteede, F.H., Tibben, J., Cornelissen, J.B., Agneessens, J., Gaasenbeek, C.P., 2000, Nematode parasites of adult dairy cattle in the Netherlands. Vet Parasitol 89, 287-296.

Bransby, D.I., 1993, Effects of grazing management practices on parasite load and weight gain of beef cattle. Vet Parasitol 46, 215-221.

Coop, R.L., Kyriazakis, I., 1999, Nutrition-parasite interaction. Vet Parasitol 84, 187-204.

Cooper, J., Gordon, I.J., Pike, A.W., 2000, Strategies for the avoidance of faeces by grazing sheep. Appl Anim Behav Sci 69, 15-33.

Diaz-Torga, G.S., Mejia, M.E., Gonzalez-Iglesias, A., Formia, N., Becu-Villalobos, D., Lacau-Mengido, I.M., 2001, Metabolic cues for puberty onset in free grazing Holstein heifers naturally infected with nematodes. Theriogenology 56, 111-122.

Fox, M.T., Reynolds, G.W., Scott, I., Simcock, D.C., Simpson, H.V., 2006, Vagal and splanchnic afferent nerves are not essential for anorexia associated with abomasal parasitism in sheep. Vet Parasitol 135, 287-295.

Hoste, H., Jackson, F., Athanasiadou, S., Thamsborg, S.M., Hoskin, S.O., 2006, The effects of tannin-rich plants on parasitic nematodes in ruminants. Trends Parasitol 22, 253-261.

Jorgensen, R.J., Satrija, F., Monrad, J., Nansen, P., 1992, Effect of feeding lucerne pellets on trichostrongyle infection in grazing heifers. Vet Rec 131, 126-127.

Larsson, A., Dimander, S.O., Rydzik, A., Uggla, A., Waller, P.J., Hoglund, J., 2006, A 3-year field evaluation of pasture rotation and supplementary feeding to control parasite infection in first-season grazing cattle--effects on animal performance. Vet Parasitol 142, 197-206.

Moss, R.A., Vlassoff, A., 1993, Effect of herbage species on gastro-intestinal roundworm populations and their distribution. NZ J Agric Res 36, 371-375.

Murphy, T.M., Fahy, K.N., McAuliffe, A., Forbes, A.B., Clegg, T.A., O'Brien, D.J., 2006, A study of helminth parasites in culled cows from Ireland. Prev Vet Med 76, 1-10.

Rees, G., 1950, Observations on the vertical migrations of the third-stage larva of *Haemonchus contortus* (Rud.) on experimental plots of *Lolium perenne* S24, in relation to meteorological and micrometeorological factors. Parasitology 40, 127-143.

Roberts, H.C., Hardie, L.J., Chappell, L.H., Mercer, J.G., 2000, Parasite-induced anorexia: leptin, insulin and corticosterone responses to infection with the nematode, *Nippostrongylus brasiliensis*. Parasitology 118, 117-123.

_____

Roche, J.R., Sheahan, A.J., Chagas, L.M., Berry, D.P., 2007, Concentrate Supplementation Reduces Postprandial Plasma Ghrelin in Grazing Dairy Cows: A Possible Neuroendocrine Basis for Reduced Pasture Intake in Supplemented Cows. J Dairy Sci 90, 1354-1363.

Shaw, D.J., Vercruysse, J., Claerebout, E., Dorny, P., 1998, Gastrointestinal nematode infections of first-grazing season calves in Western Europe: general patterns and the effect of chemoprophylaxis. Vet Parasitol 75, 115-131.

Vercruysse, J., Claerebout, E., 2001, Treatment vs non-treatment of helminth infections in cattle: defining the threshold. Vet Parasitol 98, 195-214.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I., 2008a, Changes in food intake and circulating leptin due to gastrointestinal parasitism in lambs of two breeds. J Anim Sci 86, 1891-1903.

Zaralis, K., Tolkamp, B.J., Houdijk, J.G.M., Wylie, A.R.G., Kyriazakis, I., 2008b, Consequences of protein supplementation for anorexia, expression of immunity and plasma leptin concentrations in parasitized ewes of two breeds. Br J Nutr (accepted).

Summary

_____

Summary

Parasitic gastroenteritis (PGE) is very common in cattle of all ages, under all types of husbandry, except those in which animals never have any access to the outside (fields, exercise paddocks etc) at any time in their lives. The classical clinical disease in first season grazing calves is commonly referred to as ostertagiosis Type I, though this does not take into account the role played by *Cooperia* spp. in some outbreaks of disease. This clinical presentation of bovine PGE has however become much less common over the last 2-3 decades, probably because of a greater understanding of parasite epidemiology, leading to improved control measures, facilitated by the availability of several highly effective anthelmintic products. Subclinical infections have always been regarded as the typical manifestation of PGE in older cattle, but in Western Europe, this is now also the case for many first-grazing season calves. In terms of animal performance and economics, subclinical infections can be important sources of losses in cattle of all ages and one of the most important underlying mechanisms appears to be parasite-associated inappetence. A greater knowledge of this phenomenon in grazing cattle would enable veterinarians and farmers to better understand the complex interactions between the parasites, the pasture and animal performance and thus be able to mitigate such adverse effects.

The literature review that comprises Chapter I is of necessity broad in scope because several apparently disparate topics need to be brought together in order to understand the various elements of behavioural biology, physiology, and pathophysiology, together with some details of the methodology used in other relevant fields of research. Initially the normal grazing behaviour of cattle is described, together with consideration of a number of factors (other than disease) that can affect key parameters, such as daily eating time. The average number of hours spent grazing each day by cattle is around 9 hours but the duration can vary according to several variables, including sward height, supplementation, month, botanical composition of the sward, age and physiological status of the animals and grazing management. Grazing is essentially a diurnal behaviour with prolonged bouts of eating taking place after dawn, in the late morning and from mid-afternoon to dusk. It is practically impossible to measure the feed intake directly of cattle under natural grazing conditions, hence several indirect methods are utilised, frequently more than one is used in individual studies. Estimates of herbage

_____

intake can be based on techniques such as measuring differences in sward height; using inert faecal markers; live weight differences before and after grazing and the use of jaw movement recorders. The last named has the advantage that it also can record grazing and ruminating behaviours. Feed intake in healthy animals is affected by numerous factors, which can be broadly categorised into those pertaining to nutrition, the animal and its environment. An attempt to unify the multitude of influential factors into a discrete model goes under the name of 'Minimal Total Discomfort.' Its underlying principle is that animals will endeavour, through modifications in feeding/grazing behaviour, to ingest an optimal quantity and balance of nutrients, whilst avoiding excesses or deficiencies. Control of appetite ultimately results from an integration and orchestration of the afferent and efferent neuroendocrine signals via the regulating function of the arcuate nucleus in the hypothalamus. In parasitized animals, there are several peptides that are known to change in concentration in the blood subsequent to pathophysiological disturbances in the gut and some of these are candidates for mediating effects on feed intake, but the exact mechanisms have yet to be discovered. Because of the difficulties in measuring intake in the field, inappetence in cattle with PGE has been investigated almost entirely in housed animals and predominantly in calves. Reductions in feed intake associated with parasitism can vary considerably between different experiments over a range of -4 to -77%; the magnitude of the decline in appetite does not appear to be consistently related to the severity of parasitism, whether judged by clinical signs or by worm counts.

In Chapter 2, the effectiveness of jaw movement recorders and agronomic measurements in assessing parasite-associated inappetence in grazing cattle is described for the first time. Twenty-four first-grazing season (FGS) calves were naturally exposed to parasitic nematodes and half were treated with an anthelmintic (ivermectin sustained-release bolus) at the start of the study in May, while the remainder comprised matched, untreated control animals. Each group of calves grazed initially on separate pastures in order to evaluate any effects on growth and herbage characteristics, before transfer in pairs to a suite of identical, replicated paddocks, where the behavioural measurements were made. Over the 2-month course of the trial, the control calves gained weight at the rate of 0.68 kg/day, while the treated cattle grew at 0.78 kg/day, despite their being no evidence of any clinical gastrointestinal parasitism in any animal. Visual assessment of the two main pastures in July showed that in comparison to the relatively short,

_____

uniform, leafy sward in the paddock grazed by treated animals, that grazed by the controls had longer grass, with more dead material and seed heads and a generally uneven sward, suggesting differences in herbage intake and grazing behaviour. The evidence from the behaviour and herbage measurements in the replicated paddocks provided strong support that the underlying functional difference between the two groups of calves was inappetence. Control, parasitized calves grazed for 443 minutes each day, compared to treated calves, which grazed for 540 minutes, a difference of 97 minutes – just over 1½ hours/day. The actual eating (ingestion) time correspondingly differed by 71 minutes per day, in favour of the treated group. The effects on appetite and herbage intake were underlined by significant differences in sward height between the replicated paddocks at the end of the 12-day recording period, when the height was lower in the paddocks grazed by treated animals, indicative of a higher intake.

Having established the value of the methodology for studying the effects of subclinical parasitism on grazing behaviour in young cattle, a similar study in adult, lactating dairy cows and heifers was conducted, and this is described in Chapter 3. Forty spring-calving cows and heifers (20 of each) were allowed to acquire infection with gastrointestinal (GI) nematodes naturally during grazing.   The control group (10 cows and 10 heifers) were compared with 20 similar animals treated once with eprinomectin. The cattle were stocked continuously on a grazing area that was sub-divided into 20 replicated paddocks of equivalent size and topography. Grazing pairs of either control or treated animals were randomly assigned to each paddock over the duration of the study (one pair per paddock). Grazing behaviour was recorded using IGER jaw-movement recorders for both groups over a 10-day period commencing four days after treatment with eprinomectin. Milk yield was recorded daily and milk quality was recorded weekly. Live weight and body condition score were recorded on the day of allocation, the day of treatment and thereafter at weekly intervals. There were no clinical signs of gastrointestinal parasitism throughout the study. Low levels of faecal nematode egg output were present, with the heifers having higher counts than the cows. Pasture larval levels were very low throughout with no value exceeding 68 larvae/kg dry matter (DM) of herbage. There were significant effects of treatment on several behavioural parameters, including grazing time, eating time, idling time and mean meal duration. Treated cows and heifers ate for 54 minutes longer per day respectively, than controls (P=0.006). In the treated cattle, there was an increase in solids-corrected milk yield compared with the

_____

control cattle, which was significant in weeks 2 and 3 after treatment. The response was particularly marked in heifers, where the difference in yield between treated and controls was up to 2.35 kg/day. The differences in live weight gain and condition score over 28 days post-treatment were significant in both cows and heifers, in favour of the treated animals.

Dairy cows are often grazed in a system of daily paddocks, in which cows are given access to fresh grass every day, typically by using an electric fence. The benefits of the system are that utilisation of grassland is optimised and animal performance is high, though there may be disadvantages too, for example high labour requirements. Grazing behaviour in dairy cows has been quite well studied under such systems, but not in relation to parasitism, hence the study described in Chapter 4 was designed to address this topic. Twenty-four spring calving dairy cows were divided into two matched groups; twelve naturally infected control cows were compared with twelve similar animals treated on 3 occasions (in June, July and September) with eprinomectin. The grazing area was sub-divided into two sets of 12 replicated paddocks of equivalent size and topography. Pairs of either control or treated animals were randomly assigned to graze each paddock over the duration of the study. Within each plot, the pair of cows grazed a series of 1-day paddocks, of areas calculated to provide 72 kg of herbage dry matter (twice the daily requirement). Behaviour measurements on all cows were made during three periods, once before the first treatment with eprinomectin and thence after the 2nd and 3rd treatments.   During each behaviour measurement period, grazing and ruminating behaviour were recorded over two 24-h periods and measurements of components of short-term intake rate were made during a morning and a late afternoon grazing meal. Milk yield was recorded daily and milk quality was recorded weekly. Live weight and body condition score were recorded on the day of allocation, the day of initial treatment and thereafter at weekly intervals until the end of the trial. There was no evidence of clinical parasitism in any animal during the study and faecal samples showed low levels of worm egg output throughout the study; pasture larval levels were also low with a peak values of 135 L_3/kg DM herbage. There was no significant effect of treatment on components of grazing or ruminating behaviour recorded over 24 hours or on short-term intake rates. The overall milk yield response to treatment with eprinomectin was +1.68 kg/day solids-corrected milk (SCM) (P=0.026), which included significant increases in mean daily SCM yield following each of the three treatments, indicating a positive

_____

response to repeated treatments at several different stages of lactation. The differences in live weight were not significant, although there was a consistent pattern throughout for the treated cows to be heavier than the controls. The study demonstrated that a reduction in subclinical gastrointestinal nematode infections in adult dairy cattle can lead to an increase milk yield, even when cows may be unable to respond by increasing their daily intake of herbage because of restrictive daily herbage allowances. In the absence of evidence for increased grazing time and herbage intake, it can be surmised that increased milk production in treated cows resulted from differences in digestive efficiency and/or a reduction in the physiological/immunological demands resulting from parasitism.

It has been hypothesised that parasitized animals showing inappetence may feed more selectively in order to proportionally increase the protein content of their diet and thus partially compensate for their reduced feed intake. A study that was designed to investigate this theory is described in Chapter 5. Sixteen autumn-born dairy heifer calves, which had not grazed previously, were matched and assigned to either a control group of 16 or a group of 16 that was treated with eprinomectin. An area that contained adjacent monocultures of grass and clover was used to investigate dietary preference. The area was subdivided into paddocks in which the heifers could become acclimatised to the different types of sward, before being transferred to a suite of replicated paddocks comprising adjacent, equal areas of ryegrass and clover. Grazing behaviour and herbage intake rates in the replicated paddocks were determined through the use of jaw-movement recorders, direct observation and short-term live weight change. Consistent with previous observations and despite evidence that nematode burdens were low in the untreated control heifers, a reduction in daily grazing time of 56 minutes was observed in the control animals. There was however no evidence that the control heifers showed greater preference for clover compared with ryegrass: partial preference for clover was 73.0% in the untreated controls and 75.5% in the treated heifers. Furthermore control heifers were observed grazing the clover swards significantly less frequently than the treated heifers. This study provided additional evidence in grazing cattle for parasite-induced inappetence, manifest as a reduction in grazing time and in subtle changes in ingestive behaviour, however the observed partial preference for clover was not significantly affected by parasitism, hence the hypothesis of increased diet selectivity through parasitized-associated inappetence was not supported.

_____

Chapter 6 differs from the foregoing chapters in that it was conducted inside in calves with artificially induced infections; this was necessary to facilitate frequent sampling in order to monitor various physiological and endocrine changes over the 11 weeks of the study. Twenty five parasite-naive Holstein-cross calves approximately 4 months old and weighing around 150 kg were assigned to five groups of animals. The groups were: *ad libitum* fed control calves (ALC); *ad libitum* fed infected calves (INF); control calves pair-fed with the INF group (PFC); *ad libitum* fed treated control calves (E-ALC) and *ad libitum* fed treated, infected calves (E-INF). The calves in groups INF and E-INF were infected with the equivalent of 10,000 *Ostertagia ostertagi* infective larvae (L_3) per day. The calves were penned individually and feed intake (concentrates) was measured daily; liveweight was measured on Days 0, 56 and 77. Eprinomectin was administered to animals in groups INF on Day 56 and on Days 0 and 56 to groups E-ALC and E-INF. Blood samples were collected from each calf at the same time each day three times a week throughout the study; samples were subsequently analysed for gastrin, pepsinogen, ghrelin, leptin and antibodies to adult *O. ostertagi*. No significant differences in feed intake or liveweight gain were observed between any of the groups, probably as a consequence of the high quality concentrate feed offered. Significant differences between the INF and control groups were however observed in faecal egg counts, plasma pepsinogen, gastrin and *O. ostertagi* antibodies, which were all elevated, and leptin, which was reduced. Values of these parameters for the E-INF group were intermediate between the INF and ALC groups. Plasma ghrelin showed no association with either feed intake or parasitism. Further studies are needed to fully elucidate the roles of various biochemical and neuroendocrine mediators, particularly gastrin and leptin, for inappetence in ruminants with parasitic gastroenteritis.

The results of the aforementioned studies are consolidated in Chapter 7, which also includes additional consideration of their value in understanding parasite-associated inappetence in the context of commercial livestock farming. In three of the four field studies, there were statistically significant differences in grazing behaviour between naturally parasitized cattle and anthelmintic-treated animals, which were consistent with a reduction in appetite and feed intake. Behavioural parameters that were sensitive to parasitism included total grazing time (TGT), total eating time (TET), total grazing jaw movements (TGJM) and the total number of bites within a 24-hour recording period. In

_____

two of the three studies in which significant behavioural effects of parasitism were observed, inappetence appeared to account for reductions in animal performance: milk yield and/or growth rate. Differences in observations and responses amongst the four studies seemed to be related to the quality of herbage available and the grazing management systems used. The study on neuroendocrine control of appetite in artificially infected animals failed to provide clear evidence for the role of the various peptides evaluated.

Subclinical infections with gastrointestinal nematodes are currently considered to be the most common expression of ostertagiosis and cooperiosis in cattle grazing temperate grassland. The fact that such infections are subclinical can mean that their importance as a cause of sub-optimal performance is easy to overlook, particularly in adult animals. The information generated by this thesis not only provides a clear demonstration of the mechanisms for production losses in young grazing cattle with subclinical PGE but also helps explain why adult cows can be adversely affected by parasites, despite having a strong functional immunity. It introduces a new concept for the selective use of anthelmintics to achieve production targets, independent of parasite epidemiology

Samenvatting

_____

Samenvatting

Parasitaire gastro-enteritis (PGE) is een veelvoorkomende aandoening bij runderen van alle leeftijden onder alle types van veehouderij, behalve bij dieren die nooit buitenbeloop hebben gehad. Het klassieke ziektebeeld bij eersteweideseizoenskalveren wordt gewoonlijk ostertagiosis type I genoemd. Nochtans spelen bij sommige ziekteuitbraken *Cooperia* spp. ook een belangrijke rol. Het aantal klinische presentaties van boviene PGE is sterk gedaald in de laatste 20-30 jaar, waarschijnlijk door een betere kennis van de parasitaire epidemiologie en de beschikbaarheid van verschillende hoogeffectieve ontwormingsproducten die geleid hebben tot verbeterde controlemaatregelen. Subklinische infecties werden beschouwd als de typische presentatie van PGE bij oudere runderen, maar in West-Europa zien we deze presentatie nu ook vaak bij eersteweideseizoenskalveren. Deze subklinische infecties kunnen een belangrijke oorzaak zijn van productiviteitsvermindering en één van de waarschijnlijk belangrijkste onderliggende mechanismen is een door de parasitaire infectie geïnduceerde eetlustvermindering. Een verhoogde kennis van dit fenomeen bij grazend rundvee zou dierenartsen en veehouders in staat stellen om de complexe interacties tussen parasiet, weide en dierproductiviteit beter te begrijpen en te modifiëren.

Het literatuuroverzicht in Hoofdstuk I is zeer breed omdat het uiteenlopende aspecten samenbrengt uit de gedragsbiologie, fysiologie en pathofysiologie, samen met enkele methodologische aspecten uit andere relevante onderzoeksgebieden. Eerst wordt het normale graasgedrag van runderen beschreven, samen met enkele niet-ziektegebonden factoren die dit gedrag gaan beïnvloeden. Runderen spenderen dagelijks gemiddeld ongeveer 9 uur door te grazen, maar de graasduur wordt beïnvloed door factoren zoals graslengte, bijvoedering, maand, botanische samenstelling van het gras, leeftijd en fysiologische status van de dieren en weidemanagement. Grazen gebeurt typisch diurnaal met langere graasperioden bij dageraad, in de late voormiddag en tenslotte van halfweg de namiddag tot het schemert. Het is praktisch onmogelijk om de voedselopname tijdens het grazen onder natuurlijke omstandigheden te meten. Daarom worden indirecte methoden gebruikt, die vaak gecombineerd worden binnen eenzelfde studie. Schattingen van grasopname zijn gebaseerd op het meten van het verschil in graslengte, het voederen van inerte stoffen voor concentratiebepaling in de faeces, het meten van gewichtsverschillen voor en na het grazen en het registreren van kaakbewegingen. Deze laatste methode heeft als voordeel dat hiermee ook graas- en herkauwgedrag kan geregistreerd worden. Voedselopname bij gezonde dieren wordt

beïnvloed door tal van factoren die kunnen ingedeeld worden als betrekking hebbende op de voeding, het dier of z'n omgeving. Een poging om deze verschillende factoren in één model te gieten, staat bekend onder de naam 'Minimale Totale Ontbering'. Het onderliggende principe is dat door het veranderen van hun voedings- en graasgedrag, dieren zullen proberen een optimale en evenwichtige hoeveelheid aan voedingsstoffen op te nemen en overmaat of tekorten te vermijden. De regulatie van de eetlust is het resultaat van een integratie en orkestratie van afferente en efferente neuro-endocriene signalen in de nucleus arcuatus hypothalami. Bij geparasiteerde dieren zijn er verschillende peptiden bekend waarvan de bloedconcentratie verandert na het optreden van pathofysiologische verstoringen in de darm. Sommige van deze peptiden beïnvloeden mogelijks de voedselopname, maar de exacte mechanismen werden nog niet ontrafeld. Door de moeilijkheden bij voedselopnamemetingen in natuurlijke omstandigheden werden studies naar eetlustvermindering bij runderen met PGE bijna steeds uitgevoerd bij opgestalde dieren en hoofdzakelijk bij kalveren. Eetlustverminderingen geassocieerd met parasitisme variëren aanzienlijk tussen verschillende experimenten gaande van -4 tot –77%. Bovendien staat de grootte van de eetlustvermindering niet steeds in verband met de ernst van de infectie, gemeten o.b.v. klinische ziektetekenen of wormtellingen.

In Hoofdstuk 2 wordt voor de eerste keer de effectiviteit van kaakbewegingsrecorders en landbouwkundige methoden voor het meten van parasitair geïnduceerde eetlustvermindering bij grazend rundvee beschreven. Eersteweideseizoens(EWS)-kalveren werden op natuurlijke wijze blootgesteld aan parasitaire nematoden en de helft werd behandeld met een anthelminthicum (ivermectine-bolus) bij het begin van de studie in mei, terwijl de andere helft bestond uit gepaarde, onbehandelde controledieren. Beide groepen graasden eerst op aparte weiden om het effect op groei en graskarakteristieken te evalueren. Daarna werden de dieren per 2 uit dezelfde groep (behandeld of controle) verplaatst naar een apart perceel, dat vergelijkbaar was met het perceel van hun gepaarde tegenpolen, waar de gedragsmetingen werden uitgevoerd. Hoewel bij geen enkel dier klinische ziektetekens van gastrointestinaal parasitisme werden waargenomen, hadden de controledieren over de 2 maanden durende studie een gewichtsaanzet van 0,65 kg/dag en de behandelde dieren een gewichtsaanzet van 0,80 kg/dag. Visuele inspectie van de 2 hoofdweiden in juli toonde een relatief kort, uniform en jong grasland in de weide van de behandelde dieren en langer, ongelijk grasland met meer dood materiaal en zaadhoofden in de weide van de controledieren. Dit suggereerde

_____

verschillen in grasopname en graasgedrag. Het bewijs werd vervolgens geleverd door de gedrags- en grasmetingen in de percelen, wat erop wees dat het onderliggende functionele verschil tussen de 2 kalvergroepen een verschil in eetlust was. Controledieren en behandelde dieren graasden gemiddeld respectievelijk 443 minuten en 540 minuten per dag, een verschil van meer dan anderhalf uur per dag. De eigenlijke eetduur (voedselopname) verschilde met 71 minuten, in het voordeel van de behandelde groep. De geobserveerde effecten op eetlust en grasopname werden verder ondersteund door significante verschillen in de graslengte van de gepaarde percelen. Na 12 dagen meten werd vastgesteld dat het gras in de percelen van behandelde dieren korter was, wat wijst op een hogere grasopname.

Nu de waarde van de gebruikte methodologie voor het bestuderen van de effecten van subklinisch parasitisme op graasgedrag bij kalveren was aangetoond, werd beslist om een vergelijkbare studie uit te voeren bij lacterend melkvee (Hoofdstuk 3). Twintig multipare en 20 primipare in de lente gekalfde koeien werden op natuurlijke wijze blootgesteld aan gastrointestinale nematoden tijdens het grazen. De controlegroep (10 multipare en 10 primipare koeien) werd vergeleken met 20 vergelijkbare dieren die één keer behandeld werden met eprinomectine. De dieren verbleven permanent op een weide die onderverdeeld was in 20 percelen, vergelijkbaar qua grootte en topografie. Paren van ofwel controledieren ofwel behandelde dieren werden willekeurig toegewezen aan één perceel gedurende de studieperiode. Het graasgedrag werd geregistreerd met kaakbewegingsrecorders gedurende 10 dagen, te beginnen vanaf de 4e dag na de behandeling met eprinomectine. De melkproductie en melkkwaliteit werden respectievelijk dagelijks en wekelijks gemeten. Het levend gewicht en de lichaamsconditiescore werden gemeten op de dag van allocatie, de dag van eerste behandeling en werd daarna wekelijks herhaald. Er werden geen ziektetekenen van gastrointestinaal parasitisme waargenomen tijdens de studie. Er was een lage fecale uitscheiding van nematodeneieren, met hogere uitscheiding bij de vaarzen dan bij de koeien. De weidebesmetting met parasitaire nematodenlarven was zeer laag gedurende de hele studie en was nooit hoger dan 68 L$_3$ / kg droge stof. Er waren significante effecten van de behandeling op verschillende gedragsparameters zoals graasduur, eetduur, het stilliggen en de gemiddelde duur van een maal. Behandelde koeien aten per dag 54 minuten langer dan controledieren. Bij de behandelde dieren was er een toename in de voor vaste stof gecorrigeerde melkproductie, met significante verschillen in de 2^e en 3^e week na de behandeling. Het verschil was vooral opvallend bij de primipare

_____

koeien waar de melkproductie bij behandelde dieren tot 2,35 kg/dag hoger was dan bij controledieren. Tenslotte waren er ook significante verschillen in het levend gewicht en de conditiescore in het voordeel van de behandelde dieren gedurende 28 dagen na de behandeling.

Een vaak gebruikt weidesysteem bij melkkoeien is het gebruik van met elektrische draad verschuifbare percelen, waar de dieren elke dag toegang krijgen tot vers gras. Het voordeel van dit systeem is dat het grasland optimaal gebruikt wordt, wat samengaat met een hoge dierproductiviteit. Anderzijds zijn er ook nadelen aan verbonden zoals de arbeidsintensiviteit. Er werden reeds veel studies uitgevoerd naar het graasgedrag onder zulke omstandigheden, maar niet in verband met parasitisme, wat het objectief was van Hoofdstuk 4. Vierentwintig in de lente gekalfde melkkoeien werden verdeeld in 2 groepen: 12 op natuurlijke wijze geïnfecteerde dieren (controlegroep) en 12 vergelijkbare dieren die 3 keer behandeld werden met eprinomectine (juni, juli, september). Een weide werd verdeeld in 12 percelen, vergelijkbaar qua grootte en topografie. De dieren werden per paar van ofwel controledieren ofwel behandelde dieren willekeurig toegewezen aan een perceel gedurende de volledige studieperiode. Binnen elk perceel graasden de paren van dieren op een reeks van nog kleinere ééndagspercelen waarvan de grootte berekend was om 72 kg droge stof van gras te voorzien (tweemaal de dagelijkse behoefte). De gedragsmetingen gebeurden op 3 verschillende tijdstippen: één keer voor de eerste behandeling met eprinomectine en verder na de 2e en 3e behandeling. Op elk tijdstip werd het graas- en herkauwgedrag gemeten gedurende 2 perioden van 24 u, terwijl de voedselopnameparameters werden gemeten tijdens een ochtend- en een laat namiddagmaal. De hoeveelheid geproduceerde melk, melkeiwit en melkvet werden respectievelijk dagelijks en wekelijks gemeten. Het levend gewicht en de lichaamsconditiescore werden gemeten op de dag van allocatie, de dag van de eerste behandeling en daarna wekelijks tot het einde van de studie. Er werden geen ziektetekenen van gastrointestinaal parasitisme waargenomen en de fecale uitscheiding van nematodeneieren was laag gedurende de volledige studie. De weidebesmetting met parasitaire nematodenlarven was ook laag met piekwaarden van 135 L_3 / kg droge stof. Er werden geen significante behandelingseffecten waargenomen op de voedselopname noch op het herkauwgedrag. Het gemiddelde behandelingseffect op de voor vaste stof gecorrigeerde melkproductie was 1,68 kg/dag. Bovendien werd er een toename in melkproductie waargenomen na elk van de 3 behandelingen, wat wijst op een positief behandelingseffect in verschillende lactatiestadia. Er was geen verschil

_____

in levend gewicht, hoewel de behandelde koeien consistent een hoger gewicht hadden dan de controlekoeien. Deze studie toonde aan dat het behandelen van subklinische gastrointestinale nematodeninfecties bij melkkoeien kan leiden tot een melkproductieverhoging, zelfs wanneer de dieren niet kunnen reageren door een verhoging van de dagelijks grasopname door de beperking van het aanbod. Er werd geen verhoging van de graasduur en grasopname waargenomen, daarom kunnen we vermoeden dat de melkproductieverhoging van anthelminthische behandeling resulteerde uit verschillen in efficiëntie van verteren en/of een vermindering van de fysiologische en immunologische eisen van het lichaam als reactie op het parasitisme.

Voorheen werd er geopperd dat dieren met eetlustvermindering door een parasitaire infectie hun voedsel selectiever opnemen om het proteïnegehalte van hun voeding te verhogen en dus deels te compenseren voor de verminderde opname. Deze hypothese werd onderzocht in Hoofdstuk 5. Zestien EWS kalveren, in de herfst geboren en zonder voorgaande weidegang, werden gepaard en toegewezen tot ofwel een controlegroep (n= 16) ofwel een groep die behandeld werd met eprinomectine (n= 16). De opnamevoorkeur tussen gras en klaver werd onderzocht a.h.v. aangrenzende monoculturen van deze gewassen. In een eerste weide konden de dieren gewennen aan de 2 gewassen. Daarna werden de kalveren verplaatst naar een reeks van herhaalde percelen, die elk bestonden uit een gelijke oppervlakten van raaigras en klaver. In deze percelen werd het graasgedrag en de grasopname bepaald a.h.v. kaakbewegingsrecorders, directe observatie en kortetermijnsveranderingen in het levend gewicht. Zoals in de vorige studies en ondanks lage parasitaire infectieniveaus bij de controledieren, werd bij deze controledieren een reductie in de dagelijkse graasduur van 56 minuten vastgesteld. Er was echter geen indicatie dat de controlekalveren een grotere voorkeur hadden voor klaver dan de behandelde dieren: de relatieve voorkeur voor klaver was 73,0% bij de controledieren en 75,5% bij de behandelde dieren. Bovendien graasden controlekalveren significant minder frequent op de klaverdelen van de percelen dan behandelde dieren. Deze studie leverde dus bijkomend bewijs van eetlustverminderingen te wijten aan parasitaire infecties bij grazend rundvee door de geobserveerde verschillen in graasduur en subtiele veranderingen in voedselopnamegedrag, maar de relatieve voorkeur voor klaver stond niet in verband met parasitisme. De hypothese van een verhoogde voedselopnameselectiviteit geïnduceerd door een parasitaire infectie werd dus niet ondersteund.

Hoofdstuk 6 verschilt van de voorgaande hoofdstukken omdat het een experiment bij opgestalde kalveren betrof met artificieel geïnduceerde infecties. Deze opzet was nodig om een frequente staalname mogelijk te maken en zo verschillende fysiologische en endocriene veranderingen op te volgen gedurende 11 weken. Vijfentwintig parasiet-naïeve, ingekruiste Holsteinkalveren van ca. 4 maanden oud en met een gewicht rond de 150 kg werden toegewezen tot 5 verschillende groepen van elk 5 dieren. Deze 5 groepen waren: *ad libitum* gevoederde controledieren (ALC); *ad libitum* gevoederde geïnfecteerde kalveren (INF); controlekalveren die hetzelfde rantsoen kregen als de INF-groep (PFC); *ad libitum* gevoederde behandelde controlekalveren (E-ALC) en *ad libitum* gevoederde behandelde geïnfecteerde kalveren (E-INF). De kalveren in de INF- en E-INF-groep werden geïnfecteerd met een dosis van 10 000 infectieve *Ostertagia ostertagi* L_3 per dag vanaf dag 0 tot en met dag 55. De kalveren werden individueel opgehokt en de krachtvoeropname werd dagelijks gemeten. De dieren werden gewogen op dag 0, dag 56 en dag 77. De dieren in groep INF werden behandeld met eprinomectine op dag 56 en in groep E-ALC en E-INF op dag 0 en dag 56. Van elk kalf werden gedurende de volledige studie 3 maal per week en op hetzelfde moment van de dag bloedstalen verzameld voor de bepaling van gastrine, pepsinogeen, ghreline, leptine en *O. ostertagi*-specifieke antistoffen. Er werden geen significante verschillen in voederopname of gewichtstoename vastgesteld tussen de verschillende groepen. Dit kan te wijten zijn aan de hoge kwaliteit van het aangeboden voeder. Er waren nochtans significante stijgingen in de INF-groep voor de fecale eitellingen, gastrine, plasma pepsinogeen en *O. ostertagi*-specifieke antistoffen en een significante daling van leptine. De waarden van deze parameters voor de E-INF-groep waren intermediair ten opzichte van de INF- en ALC-groep. Voor ghreline werd geen significante associatie gevonden met voederopname noch met parasitaire infectie. Er zullen nieuwe studies nodig zijn om de rol van gastrine, leptine en andere biochemische en neuroendocriene mediatoren in de eetlustbeïnvloeding bij PGE bij herkauwers te ontrafelen.

In Hoofdstuk 7 worden de resultaten van de studies in de voorgaande hoofdstukken samengebracht en wordt het belang van parasitair-geassocieerde eetlustverminderingen besproken in het kader van commerciële veehouderij. In 3 van de 4 veldstudies was er een statistisch significante lagere eetlust en voederopname bij op natuurlijke wijze geparasiteerde runderen dan bij runderen behandeld met een anthelminthicum. Gedragsparameters waarop een effect van parasitisme werd vastgesteld omvatten de totale graasduur, de totale eetduur, het totaal aantal kaakbewegingen en het totaal aantal

_____

beten binnen een 24 u–durende meetperiode. In 2 van de 3 studies waar significante gedragsverschillen werden geobserveerd, bleek de eetlustvermindering negatieve gevolgen te hebben voor de dierproductiviteit op het gebied van melkproductie en/of gewichtsaanzet. Verschillen tussen de 4 studies in de geobserveerde effecten van parasitaire infecties op de eetlust leken verband te houden met graskwaliteit of het gebruikte begrazingssysteem. De studie naar de neuroendocriene controlemechanismen van de eetlust bij artificieel geïnfecteerde kalveren, kon geen bewijs leveren van een rol van de geëvalueerde peptiden. Subklinische infecties worden hedendaags gezien als de meest voorkomende vorm van ostertagiose en cooperiose bij grazend rundvee in streken met een gematigd klimaat. Door de subklinische vorm kan het belang van deze infecties als een reden van suboptimale productiviteit gemakkelijk over het hoofd gezien worden. De informatie die gegenereerd werd door deze thesis toont mechanismen aan die aan de basis liggen van een verminderde productiviteit zowel bij grazend jongvee met subklinische PGE als bij volwassen koeien die reeds een functionele immuniteit hebben ontwikkeld. Het introduceert een nieuw concept voor selectief gebruik van anthelminthica om productiedoelen te bereiken, onafhankelijk van de epidemiologie van de parasiet.