INVESTIGATING SUMMER AND AUTUMN ENDOPARASITISM IN FARMED RED DEER, EFFECTS OF WEANING DATE, ANTHELMINTIC TREATMENT AND FORAGE SPECIES

A thesis in partial fulfilment of the requirements for the degree of Masters of Science in Animal Science at Massey University, Palmerston North. New Zealand

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DECLARATION

The studies presented in this thesis were completed by the author while a post-graduate student in the Institute of Veterinary, Animal and Biomedical Sciences, College of Sciences, Massey University, Palmerston North, New Zealand. This is all my own work and the views presented are mine alone. Any assistance received is acknowledged in the thesis.

I officially state that the contents of the thesis have not been submitted for any other degree and are not currently being submitted for any other degree. I certify that to the best of my knowledge, any help received in preparing this thesis, and all sources used, have been acknowledged in the thesis.

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ABSTRACT

Previous research has shown that even small numbers of lung and gastro-intestinal (GI) nematodes cause sub-clinical infections during autumn and can reduce voluntary feed intake (VFI) and liveweight gain (LWG) post-weaning in farmed deer. However, little is known about the effect of parasitism on growth of young farmed deer prior to weaning (summer and early autumn). At present, the control of deer parasites is largely by anthelmintic treatment. Alternatively, chicory sown as a pure sward has been shown to reduce parasitism and increase post-weaning growth of deer compared with perennial ryegrass-based pasture, although, neither forage plantain nor the inclusion of chicory in a pasture mix have yet been evaluated in this context. In addition, it has been suggested that to achieve a high pregnancy rate early in the mating season, deer calves should be weaned prior to mating to optimise nutrition and body condition of the hinds. At present there is no adequate evidence in the published literature to justify this.

Two experiments were conducted in 2005 and 2006 respectively. The first experiment investigated the impact of early or late pre-rut weaning, with and without anthelmintic treatment, on parasitism and growth (LWG g/day) of deer calves during summer and early autumn. Weaning date effects on hind reproductive parameters were also investigated. The second experiment was a preliminary investigation to compare the effect of grazing permanent perennial ryegrass pasture (*Lolium perenne*) with chicory (*Cichorium intybus*) narrow-leaved plantain (*Plantago lanceolata*) and pasture mixes based on short-rotation tetraploid ryegrass (TSR-mix; nil endophyte) or long-rotation tetraploid ryegrass (TLR-mix; low endophyte), with both mixes sown with the same clover (white and red) and chicory, on post-weaning growth and endoparasitism of weaned farmed red deer calves in autumn.

In 2005, seventy-six deer calves were randomly allocated in a 2x2 factorial design, involving sex, genotype, weaning date (February 17 or March 17), treatment with either topical moxidectin (0.5mg/kg) on January 14 and February 25 or no anthelmintic treatment. Liveweight gain, faecal gastrointestinal egg counts (FEC) and lungworm larval counts (FLC), haematological parameters and serum proteins concentrations (i.e., total protein, albumin and globulin) of calves were measured. Mixed-age adult hinds (64) were used to investigate the effect of weaning date on internal parasitism, conception date and pregnancy rate determined by ultrasound scanning. These hinds were not given anthelmintic treatment, but FLC and FEC were determined on

January 12, February 17, March 17, March 31 and May 4. All deer rotationally grazed permanent perennial ryegrass-based pasture (*Lolium perenne*) together until weaning at which point calves were removed to separate but similar pasture.

Calves weaned in March had a higher LWG to March 31 than those weaned in February ($P<0.0001$). Faecal larval count in treated calves was zero, but FEC remained similar to the untreated control calves, regardless of when they had been treated (average 136 epg, range 0-600 epg in mid February and average 92, range 0-350 epg at the end of March). Treated calves had higher serum albumin, and lower serum globulin concentrations than the untreated control group (albumin, 36.2 ± 0.3 vs 35.2 ± 0.3g/L; $P<0.001$; globulin, 23.9 ± 0.4 vs 25.5 ± 0.4g/L; $P<0.005$). In hinds, FLC averaged 5 lpg (range 0 – 122) and FEC averaged 26 (range 0- 200) with no significant relationship between weaning date and either FLC or FEC. No effect of weaning date on conception rate or date was observed.

This study showed that pre-rut weaning date, (although confounded by weaning process management) and sub-clinical parasitism during summer and early autumn may influence LWG in young farmed deer. The failure of moxidectin to reduce FEC to zero raises the question of the efficacy of this macrocyclic lactone anthelmintic against GI nematodes in farmed deer and/or emergence of farmed deer GI nematode resistance. Potential diagnostic parameters such as serum albumin concentration, which was reduced in untreated control deer, warrant further investigation for clinical diagnostic use in farmed deer. The study also highlighted the need for further research to demonstrate the advantages or disadvantages of pre-rut weaning on growth of deer calves and hind reproduction.

The 2006 grazing experiment investigated the effect of pasture species grazed on post-weaning growth and endoparasitism of farmed red deer from 3- 6 months of age. Ninety-five red deer calves were randomly allocated to five groups based on sex, LW, FEC and FLC. These calves rotationally grazed either a permanent pasture based on perennial ryegrass, chicory, narrow-leaved plantain, or one of two pasture mixes based on either a short-rotation tetraploid ryegrass (TSR-mix) or long-rotation tetraploid ryegrass (TLR-mix). Both mixes included the same white clover, red clover and chicory. All deer were initially treated with an anthelmintic (albendazole), with subsequent trigger treatment withheld until weight loss or clinical parasitism was observed. Anthelmintic trigger treatment (albendazole) was given on an individual animal basis.

The anthelmintic trigger treatment and LWG data in this study suggest that plantain, TLR- and TSR-mixes and chicory may all have a role in aiding control of endoparasitism in young growing deer in autumn in deer production systems based on permanent perennial ryegrass-based pasture with low anthelmintic input. However, any potential effects of forage feeding value and anti-parasitic plant compounds of chicory, plantain and pasture-forage mixes on parasitism and growth could not be separated in this study. It is therefore acknowledged that these observations are preliminary and based on a design intended only to establish whether further replicated studies are warranted, particularly with plantain and pasture mixes. However, the study has shown that pasture species, either sown as a pure crop or in a pasture mix can influence LWG, resilience to internal parasitism and requirement for anthelmintic use in young farmed deer.

Data from the first experiment (2005) has shown that pre-rut weaning date and sub-clinical parasitism during summer and early autumn can influence LWG in young farmed deer while the trigger treatment and LWG data from the second experiment (2006), suggest that plantain, TLR- and TSR-mixes and chicory may have a role in aiding control of internal parasitism in young deer in autumn. The outcome of the second experiment has application to deer production systems with low anthelmintic input. Serum protein and haematological parameters investigated in both studies demonstrated the need for further research to establish diagnostic markers for both sub-clinical and clinical internal parasitism in farmed young deer.

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LIST OF ABBREVIATIONS AND CODES

$/kg	dollar per kilogram
%	percentage
<	greater than
>	less than
≤	less than or than
≥	greater than or equal to
µg	microgram
^{0}C	degrees Celsius
AGR	albumin to globulin ratio
AA	amino acids
ATP	adenosine tri- phosphate
BCS	body condition score
BZ	benzimidazoles
cm	centimetre
CO2	carbon dioxide
CP	crude protein
Cr2O3	chromium sesquioxide
CT	condensed tannins
cv.	cultivar
D	digestibility
d	day
DM	dry matter
DMI	dry matter intake
DOMD	digestible organic matter as a % of the dry matter
EAA	essential amino acids
EDTA	ethylenediamine tetraacetic acid (chelating agent)
epg	eggs per gram
et al.	and others
FEC	faecal egg count per gram of fresh faeces
FECDM	faecal egg count per gram of dry matter
Fig.	figure
FLC	faecal larval counts per gram of fresh faeces

FLCDM	faecal larval counts per gram of dry matter
FO	faecal output
FOR	fractional outflow rate
FV	feeding value
g	gram
g/d	grams/day
g/l	gram/litre
GE	gross energy
GENMOD	general mode
GI	gastro-intestinal
GLM	general linear mode
ha	hectare
HB	haemoglobin
HCT	haematocrit
hd	head (animal)
HGB	haemoglobin count
hr (s)	hour (s)
HT	hydrolysable tannins
HWSC	hot water-soluble carbohydrates
IGF-1	insulin-like growth factor-1
IVM	injectable ivermectin
Kg	kilogram
kg0.75	metabolic weight
L	litre
L1	first stage larvae
L2	second stage larvae
L3	third stage larvae (infective stage)
LOG	logarithm
lpg	larvae per gram
Ltd	limited
LWG	liveweight gain
LW	liveweight
MCHC	mean corpuscular haemoglobin count

MCV	mean corpuscular volume
ME	metabolisable energy
MEg	metabolisable energy for growth
MEI	metabolisable energy intake
MEm	metabolisable energy for maintenance
mg	milligram
Mg	milligram
Min	minute
MJ	mega joule
ML	macrocyclic lactones
MP	metabolisable protein
MPV	mean platelet volume
MRT	mean rumen retention time
n	numbers
N	nitrogen
N %	percentage of neutrophils
NAN	non-ammonia nitrogen
NDF	neutral detergent fibre
NH3	ammonia
NS	not statistically significant at $P<0.05$
NSC	non-structural carbohydrates
NUM	numbers
NV	nutritive value
NZ	New Zealand
NZ$	New Zealand dollar
OM	organic matter
OMD	organic matter digestibility
P	probability statistic
PCV	packed cell volume
Pers.comm.	personal communication
pH	measure of the acidity or alkalinity of a solution
PLT	platelet cell count
PRG	perennial ryegrass

RBC	red blood cell count
RFC:SC	ratio of readily fermentable to structural carbohydrates
SAS	statistical analysis system
SD	standard deviation
SEM	standard error mean
SL	sesquiterpene lactones
spp.	species
Sq metre	square metre
TLR	long-rotation tetraploid ryegrass
TSR	short-rotation tetraploid ryegrass
VFA	volatile fatty acids
VFI	voluntary feed intake
WBC	white blood cell count
WC	white clover
WM	wet matter

CHAPTER 1:

LITERATURE REVIEW

1.1. Introduction

The objective of this review of literature is to briefly outline the history and current status of deer farming in New Zealand (NZ) with an emphasis on venison production. The factors affecting production such as seasonality of temperate deer species, feed requirements, pasture production and supply and internal parasitism will also be reviewed. Finally, conclusions and recommendations for further research on the impact of internal parasitism, specialised forages and improved pastures on venison production in NZ will be made.

1.2. The history of the New Zealand farmed deer industry

1.2.1. Introduction of deer to New Zealand

Eight species of deer were introduced to NZ between 1861 and 1910 and successfully established in the wild (Challies, 1985). Predominant were red deer (*Cervus elaphus scoticus*), then fallow deer (*dama dama*), wapiti (or elk; *Cervus elaphus canadensis*), moose (*Alces alces andersoni*), sambar (*Cervus u. unicolor*), rusa (*Cervus timorensi russa*), sika (*Cervus nippon*) and white-tailed deer (*Odocoileus virginianus borealis*). New Zealand had a feral venison export trade during the 1960s and early ‘70s. Feral venison export peaked in 1972 at 4,400 tonnes (Spiers, 1987). The farming of deer behind fences became legal in NZ in 1969 and the first commercial deer farm was set up in 1970 (Yerex, 1982).

Deer farming in NZ has developed as a market-led industry (Yerex, 1982). The current population of deer farmed in NZ is approximately 1.6 million, which is about half of the worlds farmed deer population on an estimated 3,762 farms (Anon., 2007). These farms range in size from smaller lifestyle properties to extensive stations. Generally, deer are farmed as part of a diversified livestock portfolio with other species including sheep and cattle. New Zealand remains the world's largest and most advanced deer farming industry (Anon., 2007).

There are three basic types of operation in deer farming which include breeding, venison finishing and velvet production. Deer breeding involves breeding and selling either as stock animals soon after weaning or taken through and finished for venison. Rising one year hinds not selected as replacement breeding stock are finished for venison or sold live. Breeders can focus on breeding for venison or velvet production. Venison finishing involves purchasing all stock as weaners in March or May and selling as finished stock to a minimum carcass weight of 55-65kg specifically for the chilled venison trade which peaks from October through to January.

Table 1.1: Volume of the deer industry exports for the last five years (Anon., 2006).

July Years	**2002**	**2003**	**2004**	**2005**	**2006** *Provisional*
Venison (Kg)	16,158,092	16,668,589	22,045,882	26,214,424	26,509,313
Velvet (Kg)*	203,224	165,665	211,630	240,549	234,550
Hides (Num)	334,246	310,586	460,427	386,666	473,894
Co-Products (Kg)**	798,033	468,607	989,536	2,301,857	2,350,766
Leather (Sq Metre)	48,796	90,881	102,406	215,137	216,788
Live Exports (Num)	42	256	22	28	1,506

** Dried equipment* *** Tails, pizzles, sinews, others*

Tables 1.1 and 1.2 show the volume and value of deer industry exports for the last five years respectively. The development and marketing of NZ deer products is the mandate of Deer Industry NZ (DINZ), a statutory producer board that currently has five representatives from the Deer Farmers Association, four from the Deer Industry Association, and one appointed by the Minister of Agriculture to represent consumers. It does not trade in deer products.

Table 1.2: Value (NZ$) of the deer industry exports for the last five years (Anon., 2006).

July Years	**2002**	**2003**	**2004**	**2005**	**2006** *Provisional*
Venison	212,779,111	156,628,218	178,190,719	206,273,543	225,881,769
Velvet	36,299,348	28,886,902	27,411,739	24,082,378	20,419,356
Hides	8,806,311	7,764,587	10,929,922	8,950,282	10,303,899
Co-Products	8,373,296	9,533,922	8,751,412	7,121,227	9,496,004
Leather	2,510,344	5,650,690	5,462,321	11,516,088	10,492,110
Live Exports	15,900	315,949	9,600	87,154	1,362,564
TOTAL	268,784,310	208,780,268	230,755,713	258,030,672	277,955,702

1.2.2. Venison production

Worldwide, NZ is the primary producer of farm-raised venison. The major market for NZ venison is Europe and Scandinavia, accounting for approximately 84% of total venison exports in 2006 season (Fig. 1.1). Since 2002 the proportion of NZ venison exports going to Germany has remained stable at slightly over 40%. Today Germany alone imports some 11,000 tonnes of venison. Ten years ago, total exports to all markets were 11,500 tonnes (O'Connor, 2006). However, the volume of exports has increased greatly (Tables 1.1 and 1.2). Belgium and the Netherlands remain important entry points for large volumes of NZ venison into the European Union, while alternative markets in Scandinavia and North America have taken increased volumes of venison. However, new outlets are being developed (including the local market) to increase demand ahead of NZ's ability to supply.

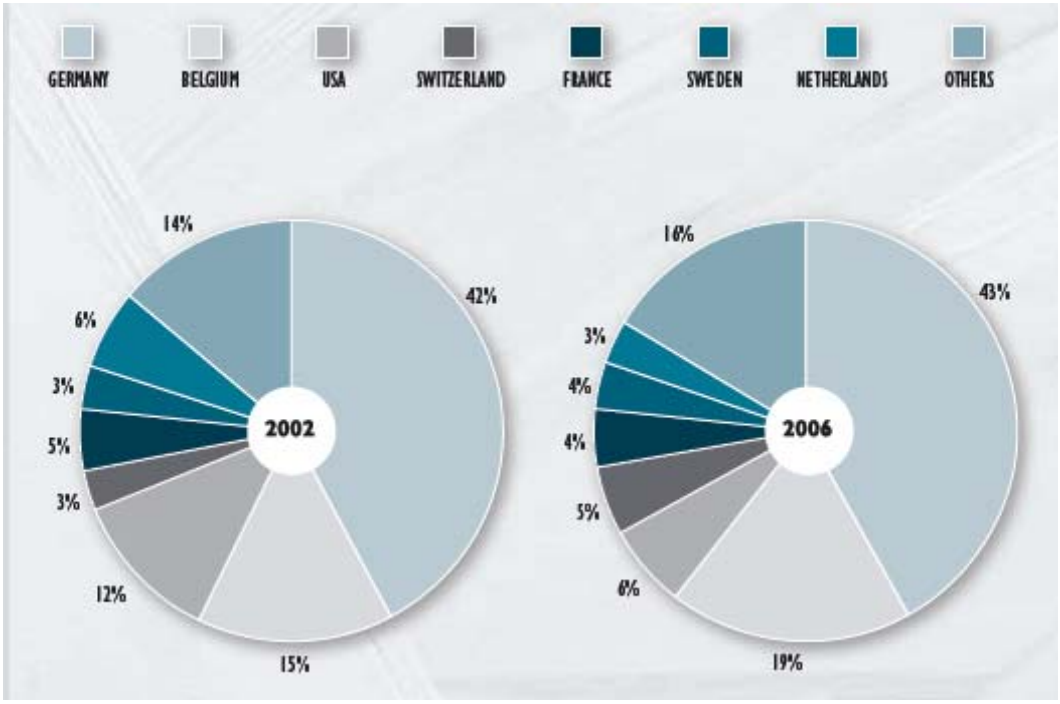

Figure 1.1: New Zealand venison export by market (Anon., 2007)

Venison price schedules in the major markets are seasonal (Fig. 1.2). The objective of venison production systems is to produce the desired carcass weight in the shortest possible period of time (Barry & Wilson, 1994). Most NZ farmers specialising in venison production produce stags for slaughter at an age of 12-24 months (Barry & Wilson, 1994) or even longer (15-27 months; Drew, 1985). However, it is economically preferable to produce carcass weights of 50-60kg at one year of age or less (during August-November), to coincide with peak market demands.

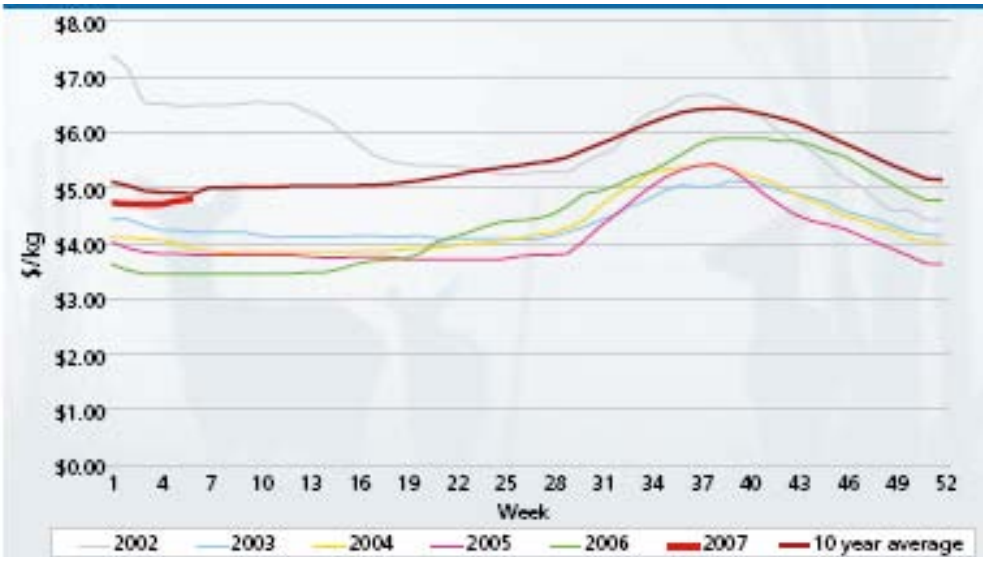

Figure 1.2: Seasonal fluctuations in venison weekly average schedule prices ($/kg carcass) for the last five years (Anon., 2007)

Due to the strong seasonality of voluntary feed intake (VFI) and liveweight gain (LWG; section 1.3), optimum carcass weights (standard 50kg carcass or 92kg Liveweight; LW) are not easily achievable in red deer before 12 months of age. Thus to maintain and improve production performance in deer, gains must be made through improved genetics, nutrition and health management in a sustainable manner without reliance on feed additives and drugs (Parker & Loza, 2002). Red hinds hybridise with animals from other sub-species (Archer, 2003). Where wapiti are used as terminal sires, the superior growth rates of hybrid progeny from red hinds and the ability to reach target weights earlier results in efficient systems, both biologically (Fennessy & Thompson, 1988) and economically.

A national performance database and genetic evaluation for deer has recently been developed to advance productivity by providing objective scientific tools to assist selection decisions and to quantify genetic performance in terms which can be related to commercial deer production (Archer, 2005). Another option is growing pasture species that produce high dry matter (DM) and have a high feeding value (FV; Section 1.4.5) during summer-autumn or winter, when perennial ryegrass/white clover (PRG/WC) pastures are unable to provide the quantity and quality of feed required for good deer growth rates (Section 1.4.3)

1.2.3. Annual farming calendar

Figure 1.3 is a generalised schematic representation of the annual major activities on a deer farm in NZ. However, it is essential that an individualised management plan is drawn for a given property because the circumstances and risks vary greatly between farms. Of particular importance is the timing of calving, mating and weaning in comparison to NZ seasonal pasture production (Section 1.4.3) and venison production and demand (Section 1.2.2).

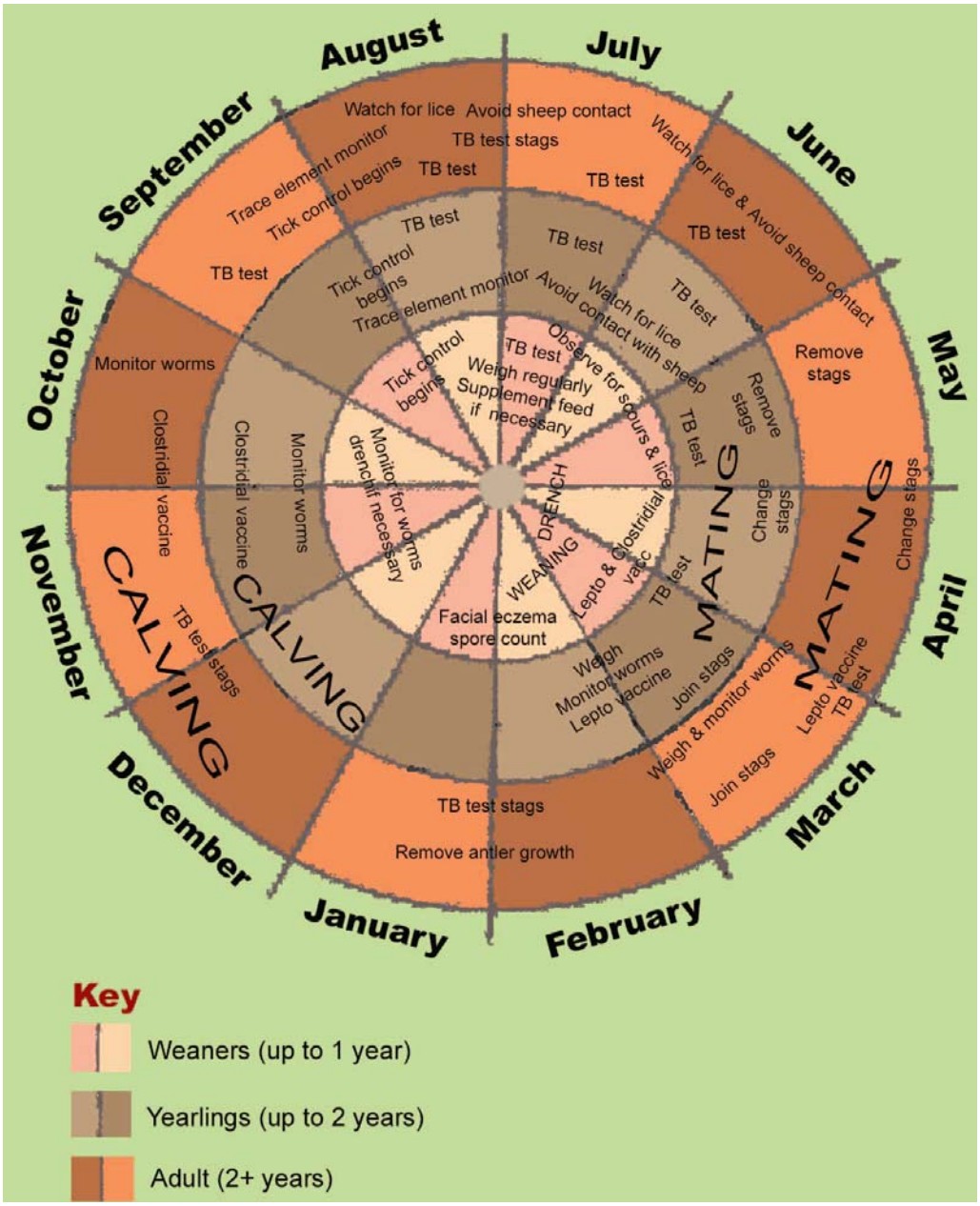

Figure 1.3: A generalised annual disease prevention, monitoring and management plan for deer farms in NZ categorised into weaner (from calving up to 1 year), yearling (up to 2 years) and adult (2+ years) deer by age.

1.3. Seasonality of temperate deer species

Temperate deer species (i.e. red, fallow deer and wapiti) have well developed strong seasonal patterns of VFI, body growth rates, metabolic rate and reproductive activity with high values in spring and summer and low values in autumn (reproductive activity is high in autumn) and winter (Suttie *et al.,* 1983; Semiadi *et al.,* 1993; Barry *et al.,* 1991). This is an adaptation to forage availability with flush of new plant growth in spring and forage abundance in summer providing high quality herbage, but with the onset of winter, plant growth ceases (Barry *et al.,* 1991; Section 1.4.3).

Endogenous mechanisms are cued or timed to photoperiod by the hormone melatonin (Barry & Wilson, 1994; Semiadi *et al.,* 1994, Loudon, 1994) and therefore related to changes in daylight-length (Kay, 1979). The timing of these cycles can be altered by changing photoperiod (Suttie *et al.,* 1992) and by administration of melatonin (Asher *et al.,* 1988). In addition, melatonin treatment advances several aspects of seasonal physiology in deer such as seasonal pattern of LWG and onset of the breeding season (Heydon *et al.,* 1993; Fisher *et al.,* 1988).

1.3.1. Seasonality in reproduction and breeding

In stags and hinds, the pattern of reproductive activity is directly related to photoperiod which governs the secretion of melatonin that is released during the periods of dark (Fisher & Fennessy, 1985; Lincoln, 1985). The physiological steps are tied to gestation length which is about 233 days in red deer (Guinness & Lincoln, 1971; Lincoln & Guinness, 1973; Fisher & Fennessy, 1985).

The seasonal reproductive cycle in temperate deer is affected by endogenous recognition of phototrophic changes (Fisher *et al.,* 1986). Mating activity starts with the decreasing daylight-length of late summer and autumn (Lincoln & Short, 1980), with conception occurring in autumn and parturition in summer (Chapman & Chapman, 1975). Providing pubertal hinds reach a suitable bodyweight (Kelly & Moore, 1977; Fisher & Fennessy, 1985), these photoperiodic changes during autumn induce the onset of the breeding season (Fisher *et al.,* 1986).

In NZ, the onset of first oestrus marks the beginning of the rut (period when deer breed), which can occur in late February, March or early in April. Duration of the oestrus cycles varies from 18

to 21 days in red deer (Adam *et al.,* 1985). During the breeding period oestrus cycles are uniform in length (18 days) and are repeated regularly. Some animals cease to cycle earlier than others, but all activity appears to cease by the spring equinox or a little before (Lincoln & Guinness, 1973; Fisher & Fennessy, 1985). Farmed red deer in NZ normally calve early in summer (Fisher *et al.*, 1986), normally November-December.

1.3.2. Seasonality in voluntary feed intake

Figure 1.4 shows the seasonality of dry matter intake (DMI) of indoor red deer stags and hinds. Voluntary feed intake may be limited by rumen capacity and digestibility of the feed on offer (Forbes, 1995; Haddad & Grant, 2000). However, red deer can compensate for a diet that is low in energy by increasing intake if available (Webster *et al.,* 2000).

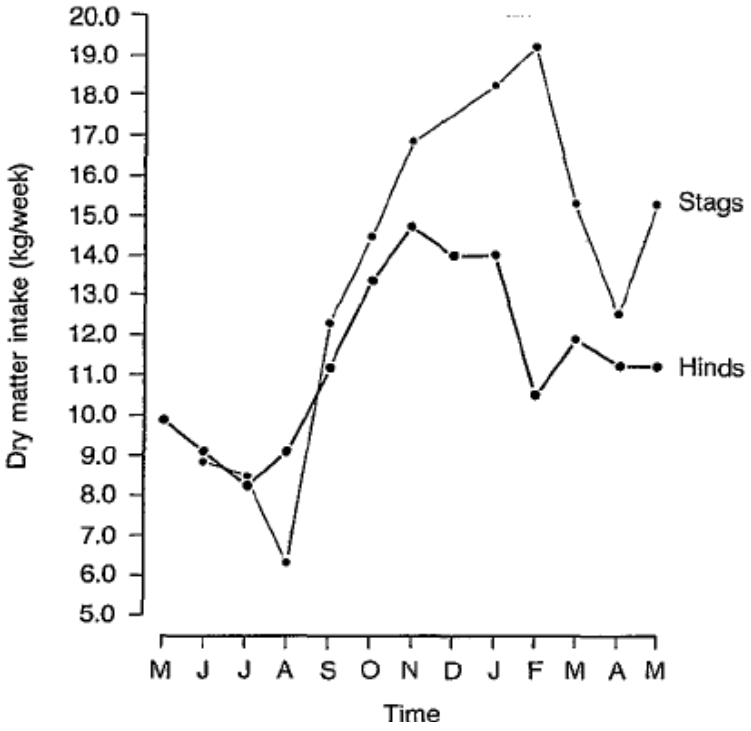

Figure 1.4: Mean monthly dry matter intake (DMI) of stags and hinds fed to appetite on a pelleted diet indoors for one year**.** The ration supplied 11 MJ ME/kg DM and 26g N/kg DM (Suttie *et al.,* 1987).

Feed intake is governed by both appetite and feed availability (Heydon *et al.,* 1993). Temperate species young deer show seasonality in VFI with high values in spring and summer, and low values in winter (Fennessy *et al.,* 1981; Suttie *et al.,* 1987; Barry *et al.,* 1991) resulting in

corresponding fluctuations in body weight and LWG (Adam, 1991). This is economically undesirable in farmed deer. The seasonal pattern of VFI is more evident in stags from two years old and older and less expressed by young stags (Fennessy & Milligan, 1987), castrated stags and hinds (Suttie & Simpson, 1985; Suttie *et al.*, 1987).

There is a distinct voluntary appetite cycle in temperate deer species (Adam, 1988) which tends to influence the magnitude of the VFI cycle. The appetite cycle may be regarded as a response to the sum of the nutrient demands arising from the other seasonal cycles (Barry *et al.*, 1991); a consequence of seasonal changes in metabolic rate (Simpson *et al.*, 1978; Blaxter & Boyne, 1982). These nutrient demands match the VFI cycle such that energy requirements for peak lactation in females and deposition of fat reserves before the rut in stags coincide with peak VFI (Loudon, 1994). However, it is the seasonal changes in daylight-length, acting through melatonin that determine changes in the appetite drive (Heydon *et al.*, 1993) and influence feeding behaviour through direct stimulus of daylight (Sibbald, 1994). Therefore, although VFI has a pattern similar to growth (Suttie *et al*., 1983), it is considered to be a consequence of the seasonal growth cycle rather than the cause (Kay, 1988).

The phasing of the seasonal VFI cycle of deer is independent of seasonal changes in gonadal steroid secretion associated with re-activation of the reproductive axis, since, castrated stags also exhibit seasonal VFI cycles (Domingue *et al.*, 1992; Loudon, 1994). However, entire red deer stags have larger amplitude to their VFI cycle than do castrates of similar age especially during the breeding season in autumn (Milne *et al.*, 1978; Kay, 1985) even when high quality feed is provided *ad libitum* (Fennessy & Milligan, 1987). Semiadi *et al.* (1995) reported that red stags expressed a sharp decline in their VFI of up to 57% during the breeding season compared with up to 32% in hinds. Thus the VFI cycle may be regarded as a combination of two cycles; one directly mediated by photoperiod and the other by sex hormones especially testosterone (Suttie *et al.*, 1984; Barry *et al.*, 1991).

1.3.3. Seasonality of digestion

The digestive efficiency of red deer does not change between winter and summer even though there is a marked increase in VFI from winter to summer (Milne *et al.*, 1978; Barry *et al.*, 1991; Sibbald & Milne, 1993; Freudenberger *et al.*, 1994). This increase in VFI without depressing

apparent digestibility, is because of a large increase in rumen pool size (51%) probably caused by hypertrophy of the rumen (Milne *et al.,* 1978, 1980), coupled with a reduction in rumen outflow rate in summer (Barry *et al.,* 1991; Domingue *et al.,* 1991, 1992); resulting in an increase both in the DM content of the digesta and rumen fill (Sibbald & Milne, 1993).

Hypertrophy of the digestive tract could arise from endocrine changes associated with changes in daylight-length (Milne *et al.,* 1978), which is independent of the seasonal changes in VFI (Sibbald & Milne, 1993). This may explain why seasonal changes in digestive function have been found which are not influenced by the seasonal changes in VFI (Freudenberger *et al.,* 1994), indicating a direct seasonal change in digestive physiology (Webster *et al.,* 2000) of temperate deer. It has been suggested that the effect of the increased levels of prolactin with the increasing daylight-length may directly or indirectly alter the response of stretch receptors in the rumen wall, allowing greater distension of the rumen and increased rumen capacity (Milne, 1980).

There is also an increase in volatile fatty acid (VFA) and rumen ammonia (NH_3) production in summer compared with winter, that are independent of changes of VFI (Milne *et al.,* 1978, 1980; Freudenberger *et al.,* 1994). These indicate increase in mean rumen retention time (MRT) in summer due to a slowing down of fractional outflow rate (FOR) and may function to maintain rumen fibre digestion rates when VFI normally increases during summer (Domingue *et al.,* 1991, 1992; Freudenberger *et al.,* 1994). The ability to decrease the rumen fractional outflow rate of liquid and particulate matter (increased rumen MRT) and increased rumen capacity and pool size in summer enables deer to maintain apparent digestibility in summer when VFI rapidly increases (Milne, 1980; Barry *et al.,* 1991; Domingue *et al.,* 1991, 1992; Freudenberger *et al.,* 1994).

1.3.4. Seasonality of growth

Figure 1.5 shows mean seasonal growth rates of young red deer from birth to slaughter. Temperate deer have seasonal pattern of growth (Fennessy *et al.,* 1981; Moore *et al.,* 1988), with maximum growth occurring in summer and minimum growth occurring in winter (Fig. 3.2; Barry *et al.,* 1991). The growth pattern also varies with feed availability, the age and the sex of the animal (Fennessy et al., 1981; Moore *et al.,* 1988). The potential for red deer calves to grow

is highest during lactation (Stevens & Corson, 2003). The depression in VFI in older stags during the rut in autumn is reflected in considerable body weight losses (Adam, 1988).

Figure 1.6 shows mean monthly LW of hinds and stags fed indoors for one year. The growth of young red deer slows during their first winter even when adequate feed is provided and accelerates in spring (Fennessy *et al.*, 1981; Suttie *et al.*, 1983). Studies conducted by Moore et al (1988) found young males gained weight at a greater rate than females. However, much like the patterns in VFI, seasonal growth differences are less pronounced in age-matched hinds when offered identical diets (Fig. 1.6; Suttie *et al.*, 1987). The seasonal growth pattern of young red deer is related to photoperiodic timing of growth via insulin-like growth factor-1 (IGF-1; Suttie & Webster, 1995) and melatonin levels (Barry *et al.*, 1994). Thus, treatment of red deer with melatonin alters the annual pattern of growth (Loudon, 1994) especially during the first two years of growth (Duckworth & Barell, 1989).

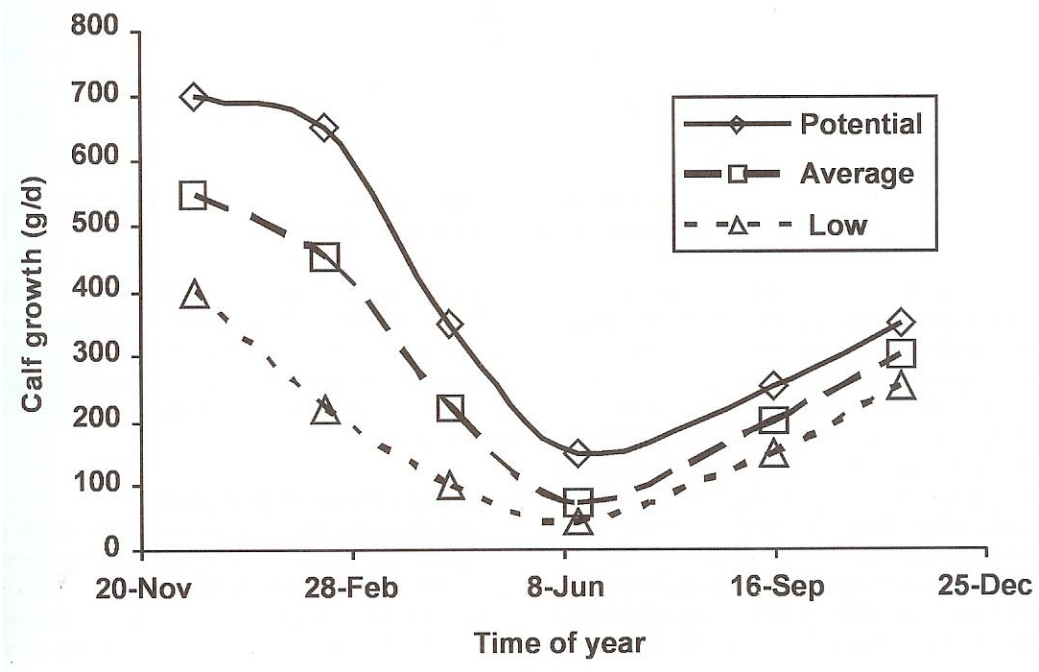

Figure 1.5: Mean seasonal growth rates of young red deer from birth to slaughter (Stevens *et al.*, 2002)

Seasonal cycles of LWG are not simply a direct consequence of changing VFI. In red deer, when the spring rise in VFI was largely suppressed by treatment with the dopamine agonist bromocriptine (i.e. which reduces plasma prolactin concentrations), the treated animals still showed a seasonal rise in body weight (Curlewis *et al.*, 1988). Studies conducted by Suttie *et al.*

(1983) with deer fed to appetite throughout the year, show that the cycle of LW persists and that deer voluntarily reduce their appetite during winter. In addition, skeletal growth continued during the first year of life irrespective of photoperiod or plane of nutrition (Simpson *et al.*, 1984). Thus the growth seasonality in deer is explained by two cycles; growth potential and VFI cycles (Suttie & Corson, 1991).

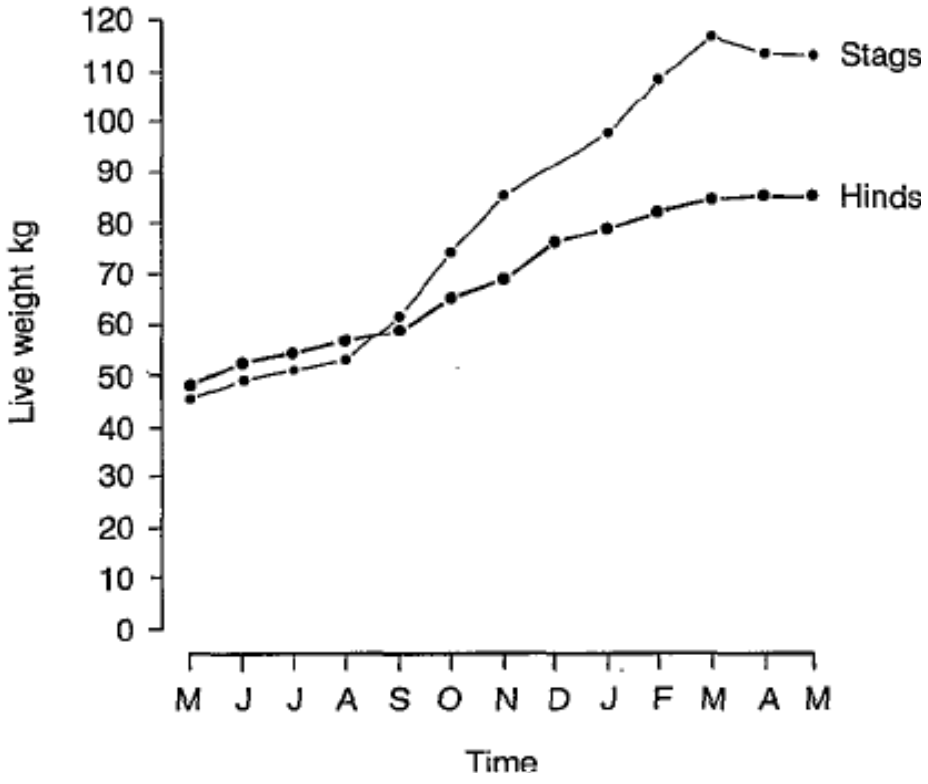

Figure 1.6: Mean monthly live weight (kg) of hinds and stags fed indoors for one year (Suttie *et al.*, 1987)

1.4. Feed requirements of temperate deer species and seasonal feed supply

1.4.1 Nutrition of temperate deer species

Nutritional requirements of temperate deer species vary according to reproductive status and season of the year. Although, deer can eat and digest any of the range of feedstuffs used for domestic ruminants (Shin *et al*., 2000) the type, the quality and quantity of the diet has a significant effect on their performance. Nutritional requirement changes that occur with growth, gestation, lactation, breeding and antler growth should be coordinated with seasonal changes in nutrient availability from forage plants (Shin *et al.,* 2000) and supplement availability such as silage and hay.

1.4.2 Feed requirements of the temperate deer

The peak feed requirements for temperate deer occur during spring and summer, coinciding with high VFI and calving. While this has ensured survival of red deer and wapiti at far north latitudes, there is a mismatch in NZ's warmer environment, where forage production commences in early spring instead of early summer (Barry & Wilson, 1994) with calving in red deer occurring in late spring and early summer (November/December.), after the spring peak in pasture production (October.; Adu *et al.,* 1998). Peak lactation thus occurs during summer (January/February), by which time PRG/WC herbage is maturing and is of reduced DM yield and digestibility due to moisture stress (Adam, 1988) and potential accumulation of seed heads and dead material (Nicol & Barry 2002). Therefore, more careful management of the forage species for farmed deer in NZ can increase production efficiency (Barry *et al.,* 1998, 1999).

1.4.2.1 Metabolisable energy requirements for growth and maintenance

The metabolisable energy (ME) requirement of grazing young red deer stags is estimated at 16, 20.9, 27 and 26.5 MJ ME/hd/day for the periods of autumn, winter, spring and summer, respectively (Fennessy *et al.,* 1981). And the estimated energy requirements for maintenance (ME_m) of red deer based on a relationship between LWG and metabolisable energy intake (MEI) of stags fed indoors ME_m = 0.57MJ ME/$kg^{0.75}$/day and for stags fed outdoors in winter ME_m = 0.85MJ ME/$kg^{0.75}$/day (Fennessy *et al.,* 1981). The estimated ME_m for stags grazing outdoors is 30, 50, 20 and 10% above that of stags indoors during autumn, winter, spring and summer respectively (i.e. 0.74, 0.85, 0.68 and 0.63MJ ME/$kg^{0.75}$/day) while the ME requirements for growth is ME_g = 37MJ ME/kg LWG and for suckling calves is 53MJ ME/kg LWG (Fennessy *et*

al., 1981). Suttie *et al.* (1987) found similar, requirements for hinds fed indoors; ME_m = 0.52MJ ME/$kg^{0.75}$/day and production = 55MJ ME/kg LWG.

1.4.2.2 Metabolisable energy requirements for pregnancy and lactation

Requirement for ME above maintenance for pregnancy in red deer increases from 1.7 to 5.0 MJ in the last three months of gestation (Adam, 1988). Red deer hinds require approximately 47.5MJ ME per day for maintenance and lactation (Fennessy & Milligan, 1987) in summer, more than twice their feed requirement in any other season (22.5-24.0 MJ ME/hd/day). However, exact data on feed requirement for lactation has not been collected for red deer hinds (Mulley, 2002). Peak daily lactation yield seen at about 40 days in well nourished hinds is about 2 kg containing about 10.5 MJ of energy (Arman *et al.,* 1974). This requires an increase above maintenance of 17.2 MJ ME in the diet if no weight loss is incurred (Shin *et al*., 2000). To achieve this level of intake, forages available must be palatable, of sufficient bulk on offer, and of high energy content (Wilson *et al.*, 1991) otherwise bulk becomes a limiting factor.

1.4.2.3 Metabolisable protein requirements

No studies have been done of the metabolisable protein (MP) requirements of farmed red deer (Adam, 1991). It is possible that, as with early-weaned lamb (Barry, 1981; Fraser *et al.*, 1990), the MP supply from PRG/WC pasture may be insufficient to meet the MP requirements of young deer below perhaps 40kg (Nicol *et al.*, 2003). However, protein requirements for adult red deer can range from 12- 16% DM assuming amino acid supply is adequate and depending on the stage of production cycle, with higher requirements in spring and summer for both stags and hinds (Mulley, 2002). The objective of protein supplementation is to precisely meet the animal's needs for amino acids since above this level, feed protein (generally, an expensive feed ingredient) is used as an energy source.

1.4.3 The seasonality of pasture production in New Zealand

The seasonality of pasture growth in NZ varies according to geographic region and season. The major limitations are temperature and moisture, and the interaction of both determines the annual pasture production and seasonal distribution of pasture yield (Scott *et al.*, 1985). Subsequently, the quantity and quality of pastures available to grazing deer varies markedly as a result of these factors and influence feed planning decisions by deer farmers.

1.4.3.1. Ryegrasses

The ryegrasses used in NZ are perennial ryegrass (*Lolium perenne*), Italian ryegrass (*Lolium multiflorum*) and their hybrids (*Lolium hybridum*). Perennial ryegrass is persistent, commonly lasting 5–20 years in mixed swards. It is easily established; is best suited to moist, medium high soil fertility environments; is tolerant of treading damage and hard grazing; and is compatible with white clover (*Trifolium ripens*; Lambert *et al.,* 2004). Thus perennial ryegrass/white cover (PRG/WC) pasture is common. Ryegrasses are natural diploids but tetraploids have been created, and these tend to be of higher quality and more palatable than diploids of equivalent parentage (Easton *et al.,* 2002). Tetraploids are best suited to higher fertility, summer moist or irrigated environments and lax or rotational grazing (5-7cm post-grazing) while diploids are best suited to lower fertility sites, drier environments and low (2-3cm) post grazing masses (Lambert *et al.,* 2004).

1.4.3.2. Alternative forages

A number of special purpose forages are grown in NZ to align deer feed requirements with forage DM production. Such forages are of high feeding value and highly preferred by grazing deer (Nicol & Barry, 2002). These forages include legumes such as red clover (*Trifolium pratense*), white clover, sulla (*Hedysarum coronarium*), lucerne (*Medicago sativa*) and herbs such as chicory (*Chicorium intybus*) and narrow-leaf plantain (*Plantago lanceolata*). Summer brassica crops, particularly leafy turnips (intraspecific hybrid of *Brassica campestris x B. napus* origin) such as the cv. Pasja and Hunter (Charleton & Stewart, 2000) are also used.

Red clover and chicory are both tap-rooted plants that grow from a crown. They can be sown as pure swards or a pasture mix and rotationally grazed for periods of approximately one week at 3-5 week intervals depending on the time of the year. Both are dormant during winter and grazing at this period may damage plant crowns (Nicol & Barry, 2002). Red deer calves, reared on hinds grazing pure red clover swards during lactation, had significantly higher growth rates and weaning weights compared with control animals, grazing conventional PRG/WC pasture (Niezen *et al.,* 1991).

The chicory has been found to have a higher feeding value for deer than PRG/WC pasture (Kusmartono *et al.,* 1996). Chicory produces large quantities of DM during spring, summer and autumn which coincide well with feed requirements and the peak in the seasonal VFI cycle of

temperate deer species (Milne *et al.,* 1978; Domingue *et al.,* 1991, 1992). Therefore, chicory has been recommended to improve summer and autumn energy levels and to increase the growth potential of red deer calves (Stevens & Corson, 2003).

Narrow-leaved plantain is a forage herb suitable for deer production due to a seasonal growth pattern, like chicory, aligned with deer feed requirements (Stewart, 1996; Barry, 1998). Feeding value of plantain compared with PRG/WC pasture has been reported to be higher for lambs in summer (Moorhead *et al.,* 2002), and higher for weaner deer in autumn, but similar in spring (Hoskin *et al.*, 2005a).

These specialised forages often provide a higher proportion of green leaf (Barry *et al.*, 1998), greater digestibility (Niezen *et al.*, 1993) and lower rumen retention time (Freudenberger *et al.*, 1994; Kusmartono *et al.,* 1997), all contributing to a potentially higher feed intake and LWG (Kusmartono *et al.*, 1996; Barry *et al.,* 2000) and therefore provide the opportunity to improve calf growth in summer and autumn (Nicol & Barry, 2002). However, no research has been done to investigate animal production when these specialized forages are established as pasture-forage mixes.

1.4.3.3. Sward height and herbage allowance

Grazing behaviour of deer responds to changes in pasture height, mass and the effect of pasture variables (Nicol & Barry, 2002). Consequently, changes in pasture allowance affect the growth responses of weaner red deer (Fig 1.7). Judson and Nicol (1997) demonstrated a greater LWG response to increasing pasture allowance for wapiti-hybrid compared to red deer during spring, but differences during winter and summer were small. Hybrid deer showed a greater seasonal change in LWG at all pasture allowances compared with red deer. Therefore, it is important to increase pasture allowance in early spring and to offer hybrids greater pasture allowances than red deer for them to reach their potential. A study by Adam (1988) showed rising 6-month-old red stags on 7-day allocations required a pasture allowance equivalent to 5kg DM/day to achieve daily gains of 200g in autumn after weaning.

Grazing systems based on PRG/WC and annual ryegrass/white clover pastures (Ataja *et al.,* 1992) showed lower growth rates in set stocked young red deer grazed at 5cm than 10cm surface heights. However, even when grazing 10cm grass-based pastures during winter and spring, only

42-50% of young stags attained the desired slaughter criteria (Barry *et al.,* 1999). Therefore, there is a need to feed alternative forages to attain target weights in deer production.

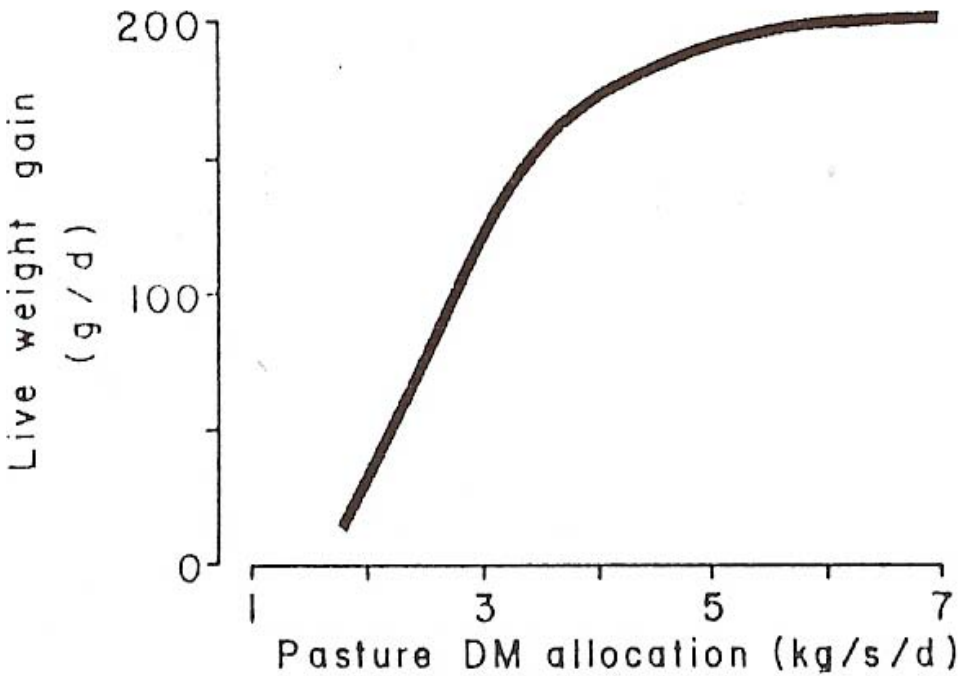

Figure 1.7: Growth responses of weaner red deer stags to changes in pasture allowance over autumn (Adam, 1988).

1.4.4 Matching grazing deer feed requirements with feed supply in New Zealand

Pasture production in NZ often does not coincide with feed energy requirements of breeding hinds and calves for a good supply of high quality feed (Fig 1.8; Nicol & Barry, 2002). For better performance year-round in deer production, feeds with an optimal combination of ME, protein and trace elements content must be provided and matched with stage of production and time of the year requirements (Mulley, 2002). The use of strategic supplementation or alternative forages/specialist pastures (Section 1.4.3.2) and crops may help ensure that calf LWG is maximised when pasture quality is low (Barry & Wilson 1994; Stevens *et al.*, 2002). Due to seasonal differences in LWG, it is essential to provide high pasture allowances in spring (Nicol & Barry 2002) to facilitate LWG of weaners.

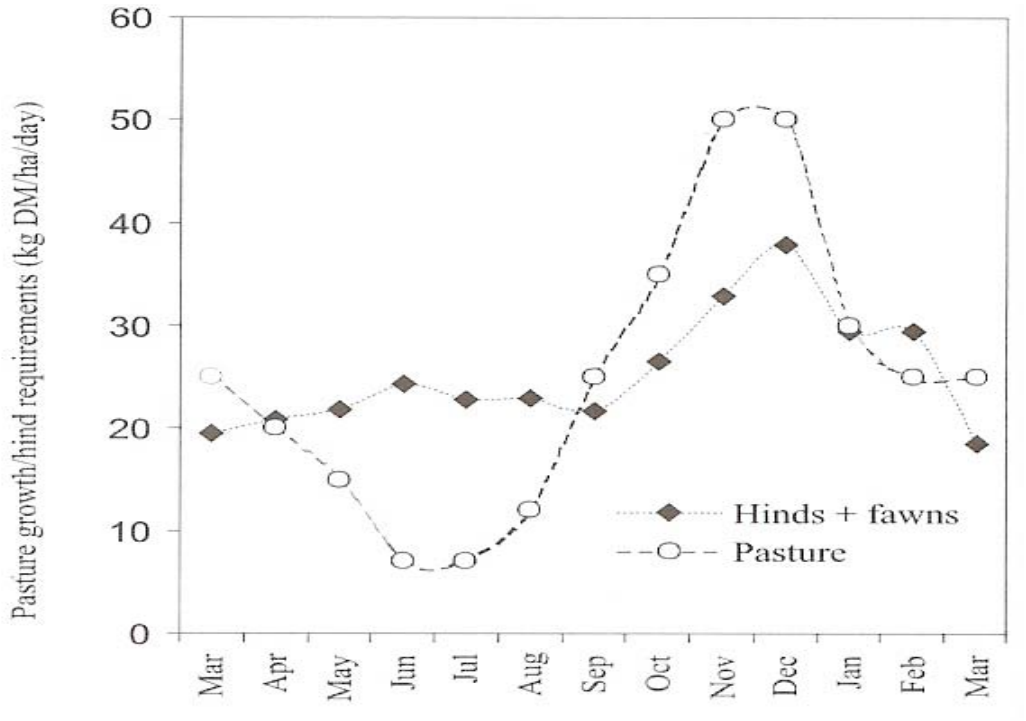

Figure 1.8: The feed demand (kg DM/day) of breeding hinds (5.5/ha) and their offspring (85% weaning) through to slaughter in Oct/Nov in relation to a typical seasonal pattern of pasture growth (Nicol & Barry, 2002).

1.4.5. Principles of forage feeding value

1.4.5.1. The concept of feeding value and nutritive value

Feeding value (FV) has been defined as the animal production obtained from grazing a forage under unrestricted conditions (Ulyatt, 1973), and its components are VFI, the digestive process including percentage of digestion and retention time in each section of the digestive system, and the efficiency of utilisation of digested nutrients (Barry *et al.,* 2002). It includes LWG in growing animals and milk production in lactating animals. For temperate deer species, the growth performance achieved will be a function of the stage of the animal's seasonal cycle as well as the diet (Barry & Wilson, 1994) available.

Nutritive value is a function of the physical and chemical composition of forages, DM digestibility, rate and site of digestion and the efficiency utilization of absorbed nutrients (Ulyatt *et al.*, 1978; Ulyatt, 1981). The NV of forages is often indicated by the ME content, and animal production is influenced by the efficiency with which ME is used (Lambert *et al.*, 2004; Woodfield & Easton, 2004). Therefore, NV is a measure of animal's production response per unit

of DM consumed while the FV of an animal's diet depends on both the rate of VFI and the NV of the feed.

1.4.5.2. Forage physical and chemical composition

Fresh forages typically contain 12-30% DM, but concentrations of crude protein (CP), fibre and non-structural carbohydrate (NSC) vary widely. Forage CP concentrations must exceed 10% of DM for livestock maintenance and about 19% of DM for high-producing animals or young growing stock (Waghorn & Clark, 2004). In young vegetative material the cell wall contents may constitute as much as 70% of the DM (Beever, 1993). Plant fibre or NDF comprises similar proportions of cellulose and hemicellulose, and a lesser amount of lignin (Waghorn & Clark, 2004). The NSC, comprising soluble sugars, fructans, organic acids or starch (in grains), can account for 30% of forage DM though values usually 10-20% of DM (Chaves *et al.,* 2002; Holmes *et al.,* 2002). The concentration of NSC in forages is variable (Waghorn & Clark, 2004).

The leaf component of grasses and legumes is eaten in greater quantities and sustain a higher rate of animal performance than the stem fraction (Minson, 1981). Highly digestible pasture contains a large proportion of green leaf and small proportion of stem and dead material (Holmes *et al.,* 2002). The water content of a forage has a major effect on animal preference and DM intake (DMI). High forage moisture content is associated with lower DMI (John & Ulyatt, 1987), especially when DM content is below 16%.

Pasture management plays a critical role in maintaining pasture quality (Lambert *et al.,* 2004), but genetic improvement has also been important in providing forages with better NV (Woodfield & Easton, 2004). The pasture plants chemical composition and NV can also be improved by fertilizer application to the soil (Ozanne *et al.,* 1976; Rees and Minson, 1978). Available soil N is the primary nutrient deficiency limiting production in NZ's characteristically grass-dominant pastures. N-fertiliser increases CP concentration in the herbage and improves pasture quality through increasing the rate of pasture growth, hence reducing the time between grazing and so reducing average leaf age (Lambert *et al.,* 2004).

1.4.5.3. Digestibility of the ingested feed

Rate of digestion refers to the proportion of feed digested per unit time and is affected by the physical and chemical composition of the forage (Smith *et al.,* 1972), microbial mass, rumen pH, nutrients limiting rumen microbial population growth, the outflow rate of digesta and microbes

from the rumen (Beever *et al.*, 1981). Rumen pH less than 6 generally results in decreases in the rate of fibre digestion, usually occurring in diets with higher starch and readily fermentable substrate content, such as grain or concentrate feeds due to a rapid release of volatile fatty acids (VFA) and inhibition of cellulolytic bacteria (Owens & Goetsh, 1986). Rapid VFA production can also occur when spring pasture has high concentrations of protein and soluble carbohydrates (Waghorn & Clark, 2004).

Rate of passage is a measure of how long digesta is retained in the gut and is dependent on the process of comminution, microbial fermentation, digestion and absorption (Mertens & Ely, 1983). The outflow rate of solutes from the rumen is much faster for deer than for other farmed ruminants, and in deer is affected greatly by season (Domingue *et al.*, 1991, 1992; Section 1.3.3).

1.4.5.4. Site of digestion

Digestion in ruminants is generally centred on the reticulo-rumen, which accounts for 55-65% of the total OM digestion. The small intestine accounts for 25-30% of the total OM digestion with large intestine accounting for 5-15% (Waghorn & Barry, 1987). Digestion in the rumen and caecum is by microbial fermentation and while this process is beneficial, in that it enables ruminants to digest structural carbohydrates, it occurs with a loss of approximately 25% of digested energy as methane and heat. In addition, fermentation of protein in the rumen or caecum causes absorption and loss of N as ammonia (Moore & Hartfield, 1994).

Grasses and legumes are digested initially by microbial degradation in the rumen, after which undigested material together with the microbes are further digested by enzymatic hydrolysis in the intestine (Waghorn *et al.*, 1990). About 90% of the digestible carbohydrate is digested in the rumen (Ulyatt & MacRae, 1974). However, most of the starch escaping rumen fermentation is digested within the small intestine through the activities of pancreatic amylase and intestinal maltase and isomaltase (Nocek & Tamminga, 1991). Undigested starch reaching the large intestine is readily fermented with degradation in the range of 70 to 100% (Hoover, 1986). As the undegradable dietary component is quantitatively small, nutrient supply to the small intestines is greatly dependent on rumen digestion and the efficient capture of degraded N by microbial cell synthesis (Storm & Orskov, 1984).

1.4.5.5. Efficiency of utilisation of digested nutrients

The efficiency of ME utilisation is affected by the type of nutrients absorbed and partitioned between maintenance and production requirements such as lactation and growth (Coleman & Henry, 2002). Nutrients are metabolised during absorption (Lindsay, 1993) and the efficiency of energy capture as high-energy phosphate bonds in adenosine tri-phosphate (ATP) ranges from about 41% from glucose to 29% from amino acids (AA) as the absorption into the blood is considerably less than is apparently absorbed from the gut due to high energy and protein requirements of the gut itself (Waghorn & Barry, 1987).

Thus the efficiency with which absorbed nutrients are utilised is affected by the type of nutrient absorbed and the physiological requirements of the animal (Waghorn & Clark, 2004), as well as the site of nutrient digestion (Ulyatt, 1981; Waghorn & Barry, 1987). A deficiency in protein supply is most likely to occur in ruminants grazing grass based pastures when growth rates are high and when body protein loss is enhanced during severe infestation with internal parasites (Black, 1990). However, energy expenditure is also affected by internal parasites (MacRae *et al.*, 1982).

1.4.5.6. Differences between forages in feeding value

Species differences in forage quality are related to differences in chemical composition usually due to increases in the negative effect of poorly digestible material on intake (Minson, 1981, Ulyatt, 1981). An important distinction between grasses and legumes is that maturation has relatively minor effects on the composition and NV of legumes, whereas changes due to maturation of grasses are profound and detrimental for animal performance (Waghorn & Clark, 2004).

Temperate legumes have a higher FV than grasses because they contain lower concentrations of fibre and higher concentrations of readily fermentable components (sugars, organic acids) and protein (Waghorn & Clark, 2004) and support higher levels of animal production (Ulyatt, 1973; Ulyatt, 1981). Legumes are eaten in greater quantities than grasses of similar energy digestibility (Minson, 1982, Rattray & Clark, 1984). Relative to perennial ryegrass, red clover had higher efficiency of feed utilisation and superior growth rates in red deer during summer and autumn (Niezen *et al.*, 1993; Semiadi *et al.*, 1993).

As the plant matures, the chemical composition of the plant changes with an increase in the proportion of plant stem relative to leaf, and decreases in protein content while structural carbohydrate and lignin contents increase. The stems increase in height and consequently the stem fraction makes up a greater portion of plant DM (Chaves *et al.,* 2001). Due to maturation, the rate of digestion and clearance of residual forage fibre from the rumen is reduced, because mature forages are slower to digest and require more chewing to reduce the particle size of the plant fragment to the critical size (Ulyatt *et al.,* 1986). A slower rate of passage from the rumen reduces feed intake (Chaves *et al.,* 2001).

1.4.5.7. Secondary compounds

The secondary compounds that occur in a range of grazed temperate grasses, legumes and herbs are summarised in Table 1.3. Of these, the condensed tannins (CT) and their effects upon ruminant nutrition have been the most extensively studied in the recent years (Ramìrez-Restrepo & Barry, 2005; Hoste *et al.,* 2006). Tannins are naturally occurring phenolic secondary compounds found in plant vacuoles (Reid *et al.*, 1974) in the leaves, bark and reproductive organs of trees, legumes and a few browse forages (Barry, 1989; Min *et al.,* 2003). Tannins exist primarily in CT and hydrolysable forms (HT; Haslam, 1989) and their concentration in the plant can be influenced by environmental factors (Reid *et al.,* 1974). However, HT are rare in temperate region forages (Min *et al.,* 2003).

Condensed tannins form pH-reversible bonds with forage proteins, which reduce degradation of protein to ammonia by rumen microorganisms, yet release protein at the low pH of the abomasum (2.5–3.5; Jones and Mangan, 1977). This releases amino acids for subsequent digestion and absorption in the small intestines (Waghorn *et al.,* 1990). Reactivity of CT differs between species of plant that contain these compounds, and hence CT from some plants increase the net absorption of essential amino acids (EAA) more than others (Min *et al.,* 2003). The limitation to widespread use of forages containing CT is their relatively poor competitive ability in high-fertility soils and a lower net DM yield than grass-dominant pastures (Waghorn *et al.,* 1998).

Table 1.3: Concentration of secondary compounds in temperate forage species with pastoral value for New Zealand farming systems (Ramirez-Restrepo & Barry, 2005)

Forage	Total condensed tannin content (g/kg DM)	Other known plant secondary compounds	Morphology under grazing
Grasses			
Lolium perenne (perennial ryegrass)	1.8	Endophyte alkaloids 12–30 mg/kg DM	Short
Legumes			
Lotus corniculatus (birdsfoot trefoil)	47	0	Medium
Lotus pedunculatus (big trefoil)	77	0	Medium
Hedysarum coronarium (sulla)			
Spring	84	0	Tall
Autumn	51	0	Tall
Trifolium repens (white clover)[a]			
Normal	3.1	Cyanogenic glycosides	Short
High CT selection	6.7		
Trifolium pratense (red clover)	1.7	Iso-flavones 7–14 g/kg DM	Tall
Medicago sativa (lucerne)	0.5	Coumestrol 0–100 mg/kg DM	Tall
Herbs			
Chicorium intybus (chicory)	4.2	Sesquiterpene lactones 3.6 g/kg DM	Tall
Sanguisorba minor (sheeps burnet)	3.4	0	
Plantago lanceolata (plantain)	14	Iridoid glycosides Catalpol 8 g/kg DM Acubin 22 g/kg DM	Medium

1.4.6. Forage intake by grazing animals

Nutrient intake by grazing animals is restricted by three factors; herbage intake, diet digestibility and metabolisability (Hodgson, 1977). There is a positive linear relationship between digestibility of herbage DM consumed and level of VFI of ruminants (Fig 1.9) up to the point where intake is limited by the energy requirements of the animal and its ability to metabolise absorbed nutrients, rather than by physical factors (Holmes *et al.*, 2002). Note that the slope of the line varies in different studies, presumably because of differences between animals and feeds used in different experiments. The compositional and structural changes in pasture associated with changes in digestibility may explain why this is the case (Holmes *et al.*, 2002).

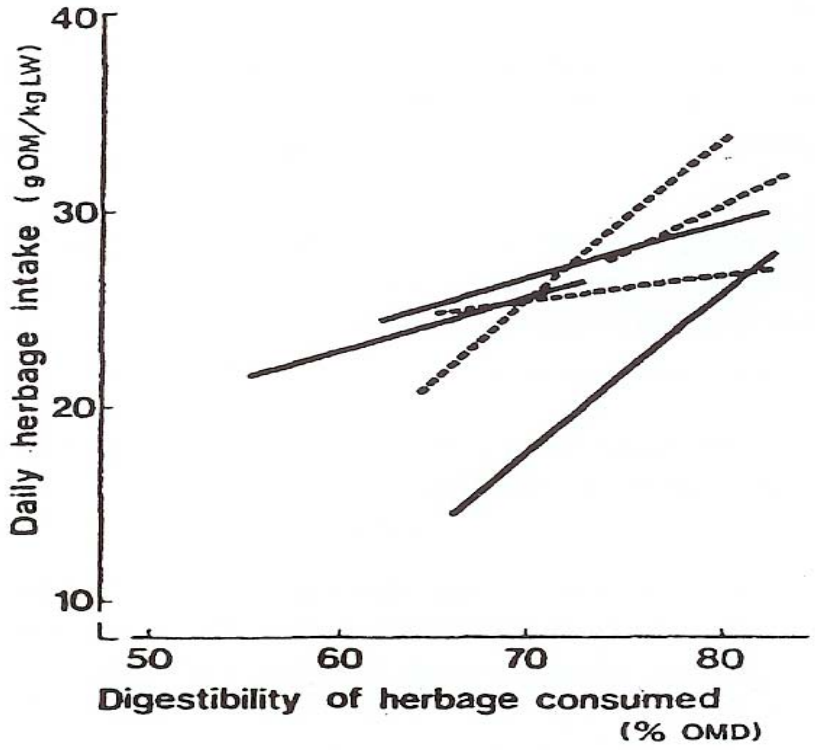

Figure 1.9: Digestibility and intake of herbage organic matter, adapted from Holmes *et al. (*2002)

The quantity of DM eaten per day by a grazing animal is determined by the number of bites per minute of grazing (i.e., bite rates), total grazing time per day and quantity of herbage consumed per bite (Holmes *et al.*, 2002). The DMI is strongly influenced by the height or mass of the pasture on which the animals are grazing (Holmes, 1987). As the herbage becomes defoliated there is reduction in grazing time, number of bites and bite mass (size) due to low leaf density (Forbes, 1995).

Under grazing conditions, between 50-70% of differences in FV can be attributed to VFI (Ulyatt, 1981). Poppi *et al.* (1987) noted that intake by grazing animals is related to pasture allowance by a curvilinear relationship, which is a function of nutritional and non-nutritional factors influencing intake. Non-nutritional factors operate under low herbage allowance and nutritional factors operate under generous herbage allowance. It has been shown that the growth of young deer is relative to level of feed intake (Hamilton & Blaxter, 1981).

Nutritional factors include palatability, digestibility, CP content, rumen mean retention time (MRT) concentrations of blood metabolites and the efficiency of utilisation of digested nutrients (Poppi *et al.*, 1987; Sections 1.4.5.3 and 1.4.5.5). The ability of a grazing animal to harvest

pasture is affected by non-nutritional factors such as environmental conditions (i.e., temperature and daylight-length), grazing behaviour of the animal and the physical structure of the sward canopy (Poppi *et al.*, 1987). Animal genotype, maturity and physiological state will affect animals demand for nutrients, digestive capacity and eating capability, all of which influence intake (Hodgson, 1990). Young and fast-growing animals consume more herbage per unit of LW than mature animals. Consequently, animal production is likely to be more sensitive to variations in sward conditions in high-performance than in low-performance animals (Hodgson, 1990).

Grazing management has an effect on pasture intake through its effects on sward characteristics. As the paddock is grazed down, the sward surface height, the green leaf content and the amount and quality of herbage declines (McGilloway *et al*., 1999). Heydon *et al.* (1993) observed higher intakes by red deer hinds on a 5cm than a 3cm pasture sward and Hamilton *et al*. (1995) found LWG and final LW of yearling red stags to be significantly lower on a 4cm sward than on a 6, 8 or 10cm sward (Section 1.4.3.3).

1.4.7. Methods of measuring feed intake on grazing animals

The actual intake of the grazing animals can be measured indirectly, either by using (a) harness and bag to estimate faecal output (FO) or (b) marker technique in the individual animal, or (c) by using pasture sampling in groups of animals.

1.4.7.1. Indirect animal-based techniques for estimating voluntary feed intake

A common disadvantage of direct animal-based techniques for estimating herbage intake is that the animals grazing behaviour is modified. Disturbance of grazing can often be reduced if herbage intake is estimated indirectly from the animal. Indirect animal-based measurements of herbage intake are based on the expression:

Intake = faecal output/ (1-digestibility)

(Equation 1.1)

Where digestibility = (feed intake – faecal output)/feed intake; Separate estimates of FO and digestibility of herbage consumed are therefore required

1.4.7.1.1. Estimating voluntary feed intake using faecal output

Faecal output can be measured directly by fitting animals with harnesses and collection bags. Meijs (1981) listed the disadvantages of this technique as being: reduced animal performance and modification of grazing behaviour; incomplete collection of faeces through incorrect fitting of bags or damage to bags; difficulties of collecting faeces free of urine from female animals; and high labour requirements. Nevertheless the technique remains the standard method of validating the accuracy of indirect measurement of FO in grazing animals. Results are obtained quickly and only simple low-cost laboratory analyses are required to determine DM and ash content.

1.4.7.1.2. Estimating voluntary feed intake using indigestible markers

Indirect measurements of VFI using an indigestible marker such as chromium sesquioxide (Cr_2O_3) are the most common methods used. As a slow release external marker, intraruminal slow release chromium capsules are used to estimate FO kg OM/day following the equation described by Parker *et al*. (1989). However, markers are often not ideal and faecal recoveries are variable (Dove & Mayes, 1996). A further difficulty is that an *in vivo* or *in vitro* estimate of digestibility must be applied to the forage consumed and may not take into account individual animal variability in digestibility, diet selection and changing forage chemical composition.

$$FO = X/Y$$

(Equation 1.2)

Where, X = Cr_2O_3 release rate from the capsule (mg/day) and Y = Cr_2O_3 concentration in faeces (mg/g OM). Voluntary feed intake (kg OM/day) is then estimated involving *in vitro* OM digestibility (D) thus

$$VFI = FO/ (1-D)$$

(Equation 1.3)

1.4.7.1.3. Estimating voluntary feed intake using the double η-alkane technique

The double η-alkane technique, using saturated long-chain hydrocarbons of plant cuticular wax (alkane) has been proposed as a reliable alternative to estimate DMI (Dove & Mayes, 1996). This technique is often used in conjunction with rumen controlled release capsules to administer the even-chain synthetic alkanes (Ulyatt *et al.*, 2002). Alkanes are not completely recoverable in faeces, but this does not matter if the estimate is based on a pair of alkanes (one natural, one dosed) adjacent in chain length, since their recoveries should be similar (Ulyatt *et al.*, 2002).

Advantages of the double η-alkane technique are firstly that alkane faecal recoveries need not be complete, provided samples accurately represent faecal alkane content, and secondly, this method allows for individual variation between animals as it does not require a common digestibility value to be applied to all animals (Dove & Maye, 1996). However, Swainson *et al*. (2005) reported that double η-alkane technique might not be the ideal technique for estimating DMI of deer grazing different fresh pastures or forages, especially when comparisons between forage species are required. The predicted DMI of deer using the η-alkane technique was underestimated up to 27.6% for deer fed ryegrass-based pasture and overestimated up to 17.2% for deer fed plantain when compared with actual DMI (Swainson *et al*., 2005).

The technique and equations as described by Swainson *et al.* (2005) to validate the double η-alkane technique to estimate the DMI of red deer fed fresh ryegrass-based pasture or plantain are as follows;

$$Intake = \frac{F_i}{F_j} D_j \Big/ \left(H_i - \frac{F_i}{F_j} H_j \right)$$

(Equation 1.4)

Where:
Fi = faecal concentration of the odd-chain plant alkane (mg/kgDM)
Fj = faecal concentration of the even-chain dosed alkane (mg/kgDM)
Dj = dose rate of the synthetic, even-chain alkane (mg/day)
Hi = herbage concentration of the odd-chain plant alkanes (mg/kgDM)
Hj = herbage concentration of the even-chain plant alkanes (mg/kgDM)

Dry matter intake is estimated, using Equation 1.4, from the daily dose rate (*Dj*) and the herbage and faecal concentrations (*Hj* and *Fj*, respectively) of the dosed even-chain alkane (C32), and the adjacent natural odd-chain alkane (C31 and C33) (*Hi* and *Fi*, respectively; Swainson *et al.*, 2005). From the equation 1.4 it can be seen that when using a pair of alkanes to estimate intake, only the faecal ratio of the natural to dosed alkanes is needed.

1.4.7.2 Sward technique

This measurement of VFI is based on the difference in the herbage mass (kg DM/ha) between pre- and post-grazing as described by Kusmartono *et al.* (1996) where estimates of DMI are based on:

$$\text{DMI (kg /head/day)} = \frac{\text{pre-grazing DM (kg)} - \text{post-grazing DM (kg)}}{\text{Number of animals grazing days}}$$

(Equation 1.5)

Limitations to the sward technique are that herbage may accumulate or decompose between the cuts for pre- and post grazing and the fact that values for individual animals cannot be calculated. A correction factor needs to be applied to account for this herbage accumulation if grazing periods are longer than three to four days. Lower values (30 to 40%) for VFI estimated using the sward technique compared to that estimated using animal methods have been reported (Ulyatt *et al.*, 1974).

1.5. Internal parasitism of temperate deer species

There is little information on the epidemiology of internal parasites in deer and no data on the relative importance of lungworm *vs* gastrointestinal parasitism (Castillo-Alcala *et al.,* 2007). The relative pathogenicity of parasite infestations in different organs of the body by different parasite species has not been investigated in deer (Hoskin et al., 2007). The deer most susceptible to internal parasites are calves during the first autumn and early winter when both lungworm and GI parasite larval availability is maximised on pasture due to favourable conditions (Mason, 1994; Charleston, 2001). By one year of age, young deer appear to have acquired some level of resistance to all internal parasites to which they have been exposed (Corrigall *et al.*, 1980; Mason, 1981) and this resistance persists in healthy, unstressed animals.

1.5.1. Lungworms (*Dictyocaulus spp*.)

Lungworm (*Dictyocaulus* spp.) is the parasite of most concern in the NZ deer industry (Mason, 1985; Johnson *et al.,* 2003) and is capable of causing high mortality rates in young deer if not controlled (Mason & Gladden, 1983; Mackintosh *et al.,* 1984; Orr, 1991). Lungworm infection is caused by the deer adapted *Dictyocaulus eckerti* (Johnson *et al*., 2001). Cattle lungworm (*Dictyocaulus viviparus)*, has also been shown to affect deer through cross transmission (Corrigal *et al.,* 1988; Mason, 1994; Johnson *et al.*, 2003) causing some sub clinical effects (Johnson *et al.,* 2003).

1.5.1.1. Epidemiology of lungworm infections

In NZ, most severe outbreaks of lungworm infections occur under intensive farming conditions with high stock densities (Charleston, 1980). *Dictyocaulus* spp is most important in warm moist climates where microclimatic conditions on the pasture are suitable for development. Adult lungworms live in the air passages in the lungs and commence laying eggs around 20 days after infective third stage larvae (L3) are ingested. Females lay eggs for about 30 days; the eggs hatch in the lungs shortly after being laid and L1 larvae are expelled from the lungs and pass down the GI tract of the host and are shed in the faeces. These larvae develop on pasture (i.e.,without feeding) to infective L3 larvae in 5-7 days under optimum conditions (i.e., 18-21 ^{0}C and moist). Cold weather arrests their development, but they can overwinter in cold climates, and can withstand a temperature of 4-5 ^{0}C for a year. Deer become infected when they ingest L3. These penetrate the intestinal wall, and migrate to the mesenteric lymph nodes where they undergo a

moult to L4. They then travel via the lymphatic drainage to the venous system, then to the heart and pulmonary artery to the lungs where they migrate from the capillaries into the alveoli and thence to the bronchi to complete the life cycle (Mason, 1980, 1985, 1994; Mackintosh *et al.*, 1984; Haigh *et al.*, 2002; Mackintosh & Wilson, 2002).

Trial studies indicate that the minimum prepatent period (i.e.,time from ingestion of infective L3 to maturation of adults to egg laying stage) for *D. viviparus* in red deer is around 20 days (Corrigall *et al.*, 1980; Mason, 1985; Mason, 1994). The only recorded duration of patency in deer with shedding of larvae is at least 30 days to 10 months (Haigh & Hudson, 1993) and therefore more research is required in this area.

Young deer of 3 to 5 months of age are the most susceptible to infection. Adult deer appear largely resistant, and have lower faecal larval count (FLC) than calves (Mason, 1994; Audige *et al.*, 1998). This immunity is probably related to age, exposure to infective larvae and nutritional status of the animal (Corrigall *et al.*, 1980; Mason, 1981). Low-grade infections in overwintered animals have the potential to disseminate the parasite to susceptible calves in the following summer (Haigh *et al.*, 2002). Calves over two months shed more larvae than their dams and thus become the major source of infection (Mason, 1985; Audige *et al.*, 1998).

1.5.1.2. Clinical signs and pathogenicity of lungworm infections

Early signs of lungworm infection in red deer are vague, including loss of condition, retarded growth and roughened coat (Mason, 1994). Coughing is not a common sign, but a soft bronchial cough may be heard, especially after exercise (Mackintosh & Wilson 2002). Severely affected animals will die (Charleston, 1980; Orr, 1994) due to asphyxiation caused by physical blockage of the air passages by worms which can pack the larynx, trachea and lower bronchial tree in a frothy exudate (Corrigall *et al.*, 1980). Damage to the lungs caused by *Dictyocaulus* spp increases the susceptibility to secondary bacterial infection which may result in pneumonia (van Reenen, 1982).

1.5.1.3 Diagnosis of lungworm infections

It is often difficult to confirm lungworm infection in red deer by clinical examination (Section 1.5.1.2). On post-mortem examination the trachea and bronchi are usually packed with worms (Charleston, 1980). Confirmation of diagnosis depends upon examination of faecal samples by

the Baermann technique (Hendriksen, 1965) for the characteristic first stage larvae, (Corrigall, 1985; Haigh *et al.,* 2002). However, where susceptible animals have been recently challenged by massive numbers of infective larvae, there may be large numbers of immature lungworm present, which do not lay eggs, thus FLC may be low or absent (Mackintosh & Wilson, 2002).

Necropsy of infected deer can show bronchitis, eosinophilic infiltration of the lung tissue and patchy, red consolidation of parts of the cardiac and apical lobes (Mason, 1981). The degree of risk depends on a number of factors including climate (temperature and moisture), stocking density, weaning practices, pasture type and length, grazing frequency, stress, and genotype. Lungworms are easily controlled by treatment with an appropriate anthelmintic at appropriate intervals to reduce the risk to the animal and/or to break the life cycle (Charleston, 2001; Mackintosh & Wilson, 2002).

1.5.2. Gastrointestinal parasite infections

A variety of GI nematodes have been recorded from deer (Mason, 1994, 1997; McKenna, 1997; Hoskin *et al.,* 2000) but only some are common, mainly the deer-specific abomasal species. The important genera of parasite affecting deer are *Ostertagia*—type and Trichostrongylidae including *axei, colubriformis* and *vitrinus*. There are a number of species of GI worms of sheep and cattle that infect deer (Mackintosh & Wilson, 2002). At least 15 species of GI nematode parasites of sheep or cattle have been recorded in deer in NZ or overseas. These include nematodes of major economic importance in sheep and cattle such as *Haemonchus contortus, Ostertagia* spp. *Nematodirus* spp. and *Trichostrongylus* spp. (Johnston *et al*., 1984; Hoskin, 1998; Hoskin *et al*.; 2000). It appears that wapiti and wapiti-hybrids may be more susceptible than red deer to nematode infections (Parsons *et al.,* 1994)

1.5.2.1. Epidemiology of gastrointestinal nematode infections

Most GI nematodes have similar life cycles with two distinct phases; the free-living phase and the animal or parasitic phase (Johnston, 1982). The mature mated female nematode deposits large quantities of eggs into the GI tract of its host. These eggs pass out of the body in the faeces. The L1 larval stage develops inside the egg under favourable moisture and temperature. The L1 larvae hatches and develops further to the L2 larval stage. Both the L1 and L2 stage larvae feed on bacteria and other micro-organisms found within the faeces. The L3 (infective stage) develops 5-7 days after eggs are deposited on the pasture depending on the prevailing conditions (Johnston,

1982). The larvae may have a period of arrested development associated with winter, or if ingested, may continue to develop in the gut of the host. The spring egg rise known in other domestic stock, associated with this winter inhibition phase, has been observed in deer (Haigh *et al.,* 2002).

The larval stages of these parasites require high humidity and warmth in the microclimates in forage to complete their development in optimum time (Haigh *et al.,* 2002). Migration of the infective L3 to a position where ingestion by the potential host is possible relies on water films and adequate temperature. After ingestion, L3 enter the mucosa of the section of the gut normally inhabited by that species and continue to develop through L4 stage larvae to become adults, which normally takes 7-10 days (Johnston, 1982). Once within the digestive tract the L3 larvae exsheath. The stimulus to exsheath occurs in the section of the digestive tract anterior to the site of infection. Abomasal parasites normally exsheath in the rumen whereas small intestine parasites exsheath in the abomasum. This process is responsive to local changes in carbon dioxide (CO_2) level, temperature and pH (Familton & McAnulty, 1997).

1.5.2.2. Clinical signs and pathogenicity of gastrointestinal nematode infections

Acute heavy burdens manifest clinical signs such as weight loss, or failure to thrive, staring coat, soft faeces or diarrhoea, and soiled tail and perineum. The syndrome akin to type II Ostertagiasis, where immature larvae affect the abomasums, is characterised by anorexia, weight loss, weakness, dull hair coat, diarrhoea and low blood protein (hypoproteinaemia), sometimes resulting in oedema under the jaw, referred to as bottle jaw (Mason, 1997; Wagner & Mackintosh 1993). Cross transmission of the sheep parasite *O. circumcincta*, *H. contorus* and cattle parasite *O. ostertagi* to deer is possible (Johnston *et al.,* 1984).

Pathology is similar in deer to that in other ruminants. Generalised signs of debilitation or condition loss may be evident and the characteristic ‘Morocco leather’ appearance of the mucosa has been seen in Ostertagia infections (Dunn, 1988; Mason 1994). Worm counts are usually modest, but figures as high as 90 000 parasites have been reported in individual animals, with L4 larvae being approximately 80% of the total (Munro, 1994).

1.5.2.3. Diagnosis of gastrointestinal nematode infections

Diagnosis of GI parasites in FEC from the live animal is difficult because the correlation between FEC and total worm number is not well researched or understood; the interpretation of FECs and their relevance as indicators of potential disease have not been adequately evaluated in deer species (Watson & Charleston, 1985; Mason, 1994). In addition, the pathogenic stage tends to be the immature stage in the abomasal lining before they produce eggs (Haigh *et al.,* 2002; Mackintosh & Wilson, 2002).

However, clinical signs, combined with positive FEC, may be taken as positive indicators of the presence of GI nematode clinical parasitism in the absence of evidence of other specific conditions (Haigh *et al.*, 2002), like response to treatment. Although, the heavy winter coat of red deer can readily mask the fact that they are in poor body condition, and suffering severe weight loss (Haigh *et al.,* 2002). In addition, plasma pepsinogen measurement has been used to confirm abomasal Ostertagiasis in cattle but may not be reliable to detect abomasal parasitism in red deer (Johnson *et al.,* 1984; Mason 1994; Hoskin *et al.*, 2000).

At post mortem, abomasal damage includes varying degrees of hyperaemia, oedema, pitting and thickening of the abomasal mucosa or ‘morocco leather appearance’ (Waldrup & Mackintosh, 1993); ulceration and fluid in the abdominal cavity (Prestwood & Kellogg, 1971); and haemorrhages (Conti & Howerth, 1987) and elevated abomasal pH (Connan, 1991; Wagner & Mackintosh, 1993). Hypobiotic larvae of cervid *Ostertagia*-type nematodes have been found in the abomasal walls of red deer (Connan, 1991; 1997) as occurs in pre-type II Ostertagiasis in cattle.

Post-mortem worm counts are the only means of determining the numbers and identities of GI worm burdens in grazing animals (McKenna, 1997; Hoskin *et al.,* 2000). However, because the level and composition of infection may vary considerably between individuals, worm counts need to be performed on several animals in order to obtain meaningful information on the parasites status of the herd as a whole (Brunsdon, 1970).

1.5.3. Impact of internal parasites on farmed deer

Adoption of preventive drenching, as more effective anthelmintics became available, has markedly reduced the clinical significance of lungworm infections (Mason 1997; Charleston, 2001) and doubtless the sub clinical significance of nematode parasitism in general (Charleston & McKenna, 2002). However, the impact of clinical and sub clinical internal parasitism upon deer productivity is not well understood, but may be substantial (Mackintosh & Wilson, 2003; Castillo-Alcala *et al.,* 2007). Lungworm infections limit productivity of red deer which is associated with lowered VFI (Corrigall *et al.,* 1982) resulting in reduced LWG including retarded growth and loss of condition (Mason, 1994). Damage to the lungs caused by *Dictyocaulus* spp. increases the susceptibility to secondary bacterial infection which may result in pneumonia (van Reenen, 1982). In severe cases sudden death is possible. Concurrent infections of lungworm and GI nematodes may be more pathogenic than single-species infections (Hoskin *et al.,* 2007)

In ruminants, clinical infections of GI nematodes are an obvious source of economic loss, while sub-clinical infection results in poor animal production, poor breeding performance and premature culling (Parkins & Holmes, 1989). There is increased loss of N from the body of the host animal either as endogenous proteins (e.g. mucus, plasma leakage or sloughed cells) or excretion as N in urine and faeces (Sykes & Coop, 1976; MacRae, 1993), although this has not been investigated in deer. The hosts response to the infection includes altered metabolism of protein, carbohydrates and minerals (Nielsen, 1982) resulting in changes in LW and body composition (Parkins & Holmes, 1989). Loss of weight has been demonstrated in deer with clinical parasitism (Hoskin *et al.,* 1999, 2000; 2003b), resulting in reduced ability of young deer to reach slaughter weight by one year of age (Hoskin *et al.,* 1999). This is potentially of considerable economic significance to deer farmers.

1.5.4. Control of internal parasites in farmed deer

The main aim is to break the life cycle by minimising both pasture contamination with nematode larvae and contact between susceptible stock and pasture contaminated with infective L3 (Mason, 1980). In NZ, control of deer parasites is by anthelmintic treatment (Waldrup *et al.,* 1998; Charleston, 2001; Castillo-Alcaca *et al.,* 2005; Castillo-Alcaca *et al.,* 2007) together with grazing management (Wilson, 1984a; Haigh *et al.,* 2002) to increase production. Strategic rotation of animals on pasture will also reduce the availability of the infective larvae (Dunn, 1988). In addition, routine monitoring of faecal samples should be carried out in early, middle and late

summer especially where the climate is moist (Haigh *et al.,* 2002) to provide information on FEC and FLC.

There is a risk that development of resistance to anthelmintics, especially avermectins and milbemycins will cause major problems for farmed deer as it has for other ruminants (Mackintosh, 2001). Recent research indicates poor efficacy of new-generation macrocyclic lactone anthelmintics in farmed deer (Hoskin *et al.,* 2005b) suggestive of anthelmintic resistance. Thus, alternative control measures are required that are more sustainable (Mackintosh & Wilson, 2003), including speciality forage crops containing CT and other plant secondary metabolites in the farming system (Niezen *et al.,* 1996, 1998; Barry *et al.,* 2002; Min *et al.,* 2003; Min & Hart, 2003; Hoste *et al.,* 2006). They have been suggested to provide an alternative control of parasitic infections.

Beneficial effects on host physiology and performance under parasitic challenge have been found with the consumption of these bioactive plants when compared to control herbage such as ryegrass, white clover or lucerne in sheep, goats and deer (Niezen *et al.,* 1998, 2002; Hoskin *et al.,* 1999, 2000, 2003b; Paolini *et al.,* 2003; Ramìrez-Restrepo *et al.,* 2004, 2005a). Although, the use of such forages has been shown to be effective experimentally, further research is required.

Other management options may also assist with parasite control. These include alternatives such as cross-grazing with cattle or sheep, grazing and nutritional management, lowering stocking rates, selective breeding for more resistant animals and the use of “natural” means of parasite control (Niezen *et al.,* 1996; Mackay, 2001) such as biological control and nutritional supplementation (Stear *et al.,* 2007). Such practices have been adopted in organic farming systems for other ruminants with a degree of success (Stear *et al.,* 2007). However, little has been published on the practicalities and economics of organic livestock production, especially for deer farming. Vaccination against lungworm could be possible to control nematode infections in deer. Although, trials of “Bovilis Huskvac” vaccine, in parasite naïve red deer, provided a degree of protection, it was not effective enough to recommend its use (Johnson *et al.,* 2003).

1.5.4.1. Use of anthelmintics

Regular treatments at intervals short enough to control pasture contamination over the late summer/autumn period when larval populations would be expected to peak and when obtaining good growth rates of deer calves are of critical importance (Charleston, 2001). The anthelmintics currently on the market with a label claim for use in deer in NZ are listed in Table 1.4 and fall into two categories, benzimidazoles (BZ's) and macrocyclic lactones (ML's). However, a recent survey (Castillo-Alcala *et al.,* 2007) reported considerable variation in most aspects of parasite control between farms, including variation related to the age and sex of deer, and variation in treatment programmes, choice of anthelmintic, decision-making strategies, and knowledge and beliefs about the value of control practices.

Table 1.4: Anthelmintic formulations with a label claim for use against nematode parasites of deer (Charleston *et al.,* 2001).

Drug	Product Name ®	Company	Dose Rate
Albendazole	Albendazole C	Ancare	10 mg/kg
	Valbazen	Schering-Plough	10 mg/kg
Fenbendazole	Axilur	Intervet	7.5 mg/kg
	Panacur [including mineralised forms	Intervet	7.5 mg/kg
Oxfendazole	Bomatak-C [incl. Mineralised forms]	Bomac	4.5 mg/kg
	Oxfen [incl. mineralised forms]	Ancare	4.5 mg/kg
	Spectre [± Se]	Schering-Plough	4.5 mg/kg
	Systamex Low Dose [± Se, minerals]	Schering-Plough	4.5 mg/kg
Ivermectin	Ivomec Pour-on for cattle and deer	Merial	0.5 mg/kg
Eprinomectin	Ivomec Eprinex Pour-on for cattle and deer	Merial	0.5 mg/kg
Moxidectin	Cydectin Pour-on	Fort Dodge	0.5 mg/kg
	Vetdectin Pour-on	Fort Dodge	0.5 mg/kg
Abamectin	Genesis Pour-on for cattle and deer	Ancare	0.5 mg/kg

The BZ's; albendazole, fenbendazole and oxfendazole have a broad spectrum of activity against nematodes in ruminants. All are formulated as oral drenches (Charleston, 2001). Albendazole at the recommended dose-rate (10 mg/kg) will substantially reduce the shedding of lungworm larvae in red deer to very low levels (Anderson & Wilson, 1984; Mackintosh *et al.,* 1984; Mason & Beatson, 1985), but in wapiti the efficacy against both adult and larval lungworm was zero (Waldrup *et al.* 1997). Thus albendazole is generally more effective against GI nematodes

(Anderson & Wilson, 1984) but only moderately effective against lungworm in red deer (Charleston, 2001). It is notable that this is the same dose rate recommended for cattle (10mg/kg) but higher than for sheep (4.75mg/kg).

Fenbendazole has been widely used on deer farms (Mason & Gladden 1983). The recommended dose rate is 7.5 mg/kg compared to 5mg/kg in sheep and cattle. Treated animals did not shed larvae for up to 21 days after treatment suggesting that the drug removed both adult and larval lungworm (Mackintosh *et al.,* 1984; Mason & Beatson, 1985). However, there is no published data on the efficacy of fenbendazole on GI nematodes in red deer in NZ (Charleston, 2001).

Oxfendazole at the recommended dose rate of 4.5 mg/kg effectively reduced the shedding of lungworm larvae to very low levels with most animals not shedding larvae for about three weeks after dosing, suggesting successful removal of adult and larval lungworm (Mackintosh *et al.,* 1984; Mason & Beatson, 1985; Bowie *et al.,* 1987; Mackintosh *et al.,* 1990; Parsons *et al.,* 1994). However, in most trials, larval excretion was not totally eliminated in all animals even, in some cases, with double or repeat dosing (Mason & Beatson, 1985; Bowie *et al.,* 1987).

Subcutaneous injection of ivermectin (IVM) at the recommended dose of 200µg/kg was effective in eliminating shedding of lungworm larvae for at least three weeks after treatment in deer (Mackintosh *et al.,* 1984; Mackintosh & Mason, 1985; Mackintosh *et al.,* 1993). The same dose rate given orally was also effective in reducing FLC (Mackintosh *et al.,* 1984). Although low level larval excretion continued in a few treated animals; the IVM treatments were effective against lungworm (Bowie *et al.,* 1987). Slaughter trials have shown total removal of both immature and mature lungworm at 200µg/kg by injection (Mackintosh *et al.,* 1985, 1993). However, other reports indicated poor efficacy based on continued shedding of GI nematode eggs in faeces (Andrews & Lancaster, 1988; Andrews *et al,* 1993; Hoskin *et al.,* 2005b).

Injectable IVM at 400µg/kg was effective against adult and developing abomasal nematodes and against inhibited abomasal L4 larvae of red deer, removing virtually 100% of adult and developing abomasal nematodes and >95% of inhibited (hypobiotic) L4's (Connan, 1997). The pour-on formulation of IVM at a dose rate of 0.5mg/kg has been shown to be effective against *Dictyocaulus* (Mackintosh *et al.,* 1990) and has a persistent effect that protects against new infection for at least 28 days (Rehbein & Visser, 1997). However, recently Hoskin *et al.* (2005b)

found sub-optimum efficacy against *Ostertagia*-type nematodes with IVM pour-on (0.5mg/kg) in deer.

Moxidectin applied topically is effective against both lungworm and GI nematodes at the standard dose rate of 0.5mg/kg (Mackintosh *et al.*, 1993; Middleberg, 1994). Comparison of efficacy in red deer with wapiti hybrids indicated similar activity in both (Waldrup *et al.*, 1998). However, recently, the failure of moxidectin to reduce FEC to zero could be interpreted as sub-optimal efficacy and/or anthelmintic resistance (Charleston & McKenna, 2002; Hoskin *et al.*, 2005b). Although, moxidectin has the longest recognised period of protection (Mackintosh *et al.*, 1997; Charleston, 2001), it would also be expected to have the longest half-life meaning that partly resistant parasites could have a considerable period of advantage if drenching intervals are extended to take advantage of this long period of activity (Pomroy, 2006).

Nevertheless, moxidectin was the most commonly used anthelmintic (46%) as identified in the national survey in 2004 (Castillo-Alcala *et al.*, 2007) with other MLs accounting for a further 31%. Presumably most of these treatments were given by topical application and thus expected to have some degree of extended half-life compared to oral formulations (Pomroy, 2006). Although, there have been no reports of anthelmintic resistance in deer nematodes, this may reflect a lack of investigation since FEC reduction tests on a small number of farms have revealed three farms with probable resistance or inefficacy issues (i.e., at the standard dose rate) in one or more class of anthelmintics (Dodunski, 2006). Levamisole is largely ineffective in deer (Charleston, 2001; Dodunski, 2006). Sub-optimal efficacy of IVM and moxidectin against abomasal parasites of deer has been found, particularly for oral IVM (Hoskin *et al.*, 2005b). Therefore, drenches currently being used need further scrutiny in terms of appropriate dose rates, and the opportunity to use combinations to delay resistance (Dodunski, 2006).

1.5.4.2. Specialized forage crops and internal parasites

It is desirable to minimise anthelmintic usage, to lower costs and to reduce the risk of development of anthelmintic resistance and risk of carcass chemical residues (Hoskin *et al.,* 1999). Grazing systems that include legumes and herbs with CT such as sulla, red clover and chicory can be used to substantially increase the growth of weaner deer for venison production whilst maintaining deer health and reducing requirements for chemical inputs (Barry *et al.,* 2002; Section 1.4.5.5).

Condensed tannins have been shown to improve amino acid supply to the small intestine (Bermingham *et al*., 2001; Coop & Kyriazakis, 1999) in parasitized sheep by shifting digestion of plant protein from the rumen to small intestines resulting in increased LWG thus having the potential to enhance the tolerance of ruminants to internal parasitism (Hoskin *et al.,* 2000, 2003b). This could counteract protein losses caused by gut parasitism (Kimambo *et al*., 1988) that lead to reduced N retention (Bown *et al*., 1991) and also better meet the AA demands of the immune system. Increased resilience and immunogenic responses of sheep to internal parasitism whilst fed sulla compared with lucerne has been demonstrated (Niezen *et al*., 1995, 2002). Similar observations have been recorded for young deer fed chicory and sulla (Hoskin *et al.,* 1999, 2000, 2003b).

Hoskin *et al.* (1999) found that untreated weaner deer grazed on chicory did not have impaired growth rates and FEC were lower compared to similar animals grazed on PRG/WC pastures. A similar study found 35% of trigger-treated deer grazing pasture required treatment for clinical parasitism, but no anthelmintic was required for trigger-treated deer grazing chicory (Table 1.5; Hoskin *et al.,* 2003b). Grazing chicory reduced the development of deer lungworm L2 larvae to infective L3 larvae in weaner deer (Schreurs *et al.,* 2002). Chicory has been shown to reduce the number of infective larvae found on the forage, this being attributed to plant morphology and sward structure (Moss & Vlassoff, 1993). These findings suggest that grazing chicory is a potential tool for controlling internal parasites in deer with reduced anthelmintic input.

In-vitro studies have shown both CT and sesquiterpene lactones (SL) from chicory to reduce larval motility and in addition, CT also reduced the development of lungworm larvae to the infective L3 stage (Molan *et al.,* 2003; Schreurs *et al.,* 2002). These findings offer a sustainable means of controlling lungworms. Molan *et al*. (2000a) by *in-vitro* assay showed that CT from

sulla and birdsfoot trefoil have anthelmintic properties against deer GI infective L3 larvae and inactivated deer L1 lungworm larvae. Similar studies by Molan *et al.* (2000b) found that CT from *Lotus pedunculatus, Lotus Corniculatus,* sulla and sainfoin (*Onobrychus viciifolia*) were all able to reduce the viability of deer lungworm and GI nematode larvae.

Table 1.5: Summary data from Massey University trials with weaner deer grazing perennial ryegrass (cv. Nui) based pasture or chicory (cv. Grasslands Puna) and regularly treated with anthelmintic (treated) or anthelmintic was withheld until trigger-treatment criteria were reached (trigger) (Hoskin *et al*., 1999a[1]; 2003a[2]).

	Pasture		Chicory		SE
	Treated	Trigger	Treated	Trigger	
Feed intake (kgOM/d)					
Autumn[1]	1920[a]	835[b]	1015[a]	1150[a]	127.3
Liveweight gain (g/d)					
Autumn[1]	217[a]	125[b]	184[a]	212[a]	8.7
Autumn[2]	134[a]	60[b]	208[c]	175[c]	11.8
Carcass weight (kg)					
12 months of age[1]	58[a]	51[b]	57[a]	57[a]	1.4
6 months of age[2]	31[a]	30[a]	37[b]	37[b]	2.3
Clinical parasitism %					
Autumn[2]	-	35	-	0	-
Internal parasites (No)					
Lungworm (No)[2]	1	643	0	311	115.2
GI nematodes (No)[2]	0	2642	52	2240	373.7

a, b, c: different letters denote significant differences within rows($P < 0.05$)

Relative to young deer fed fresh lucerne that contained 1 g CT/kg DM, feeding fresh sulla containing 35 g CT/kg DM reduced the establishment of abomasal nematodes and tended to reduce faecal excretion of lungworm larvae, following trickle-infection for 6 weeks with a mixture of L3 lungworm and GI nematode larvae of deer origin (Hoskin *et al.,* 2000). In controlled indoor studies, involving trickle infections with mixed L1 lungworm and GI nematode larvae, weaner deer fed legume sulla had fewer parasites established and greater growth than young deer fed lucerne (Hoskin, 1998).

1.6. Conclusion and requirements for further research on internal parasitism and specialist forages

1.6.1. Control of internal parasites in young deer is by preventive drenching. However, recent studies have indicated poor efficacy of common deer anthelmintics which is suggestive of a possible development of resistance. This needs further investigation in farmed deer to identify the resistant parasites and ecological distribution within the deer farming community.

1.6.2. Little is known about the epidemiology, pathology and diagnostic markers of lungworm and GI nematode infections in young deer. Research is required into the clinical and sub-clinical effects of internal parasitism of young deer on production. The interpretation of FEC, FLC and changes in serum proteins and haematology parameters as significant indicators of potential clinical and sub-clinical disease in farmed deer need to be satisfactorily investigated.

1.6.3. Specialist forage crops evaluated for deer include chicory, birdsfoot trefoil, red clover and sulla. However, other specialist forages, especially those containing CT such as plantain and sainfoin are yet to be evaluated. In addition, no research has been done to assess the productivity and effect of these specialized forages when established as pasture-forage mixes with grasses.

1.6.4. Studies are required to assess the impact of internal parasitism on deer grazing on the new tetraploid ryegrass cultivars which have higher FV and low or no endophyte. These tetraploid ryegrasses have also not been investigated against the specialised forages or pasture-forage-mix in terms of productivity and feeding value in parasitized farmed deer

1.6.5. Specialised forages containing CT and SL have been shown to significantly increase the performance of parasitized lambs and deer. However, more research on the effect of CT and other plant secondary metabolites on the internal parasitism of young deer with limited anthelmintic input is required including effects on the parasites, animal production and health, the actual amounts of forage and period required to feed or graze the animals for improved performance.

CHAPTER 2:

IMPACT OF PARASITISM AND WEANING DATE ON GROWTH OF DEER CALVES, AND BODY CONDITION AND REPRODUCTIVE PERFORMANCE OF HINDS

2.1. ABSTRACT

There is little informationn on the epidemiology and pathogenicity of lungworm and GI nematode infections of farmed deer and little is known about the effect of parasitism on growth of young farmed deer in summer and early autumn prior to weaning. Pre-rut weaning has been shown to result in lower calf growth rates during autumn compared with growth rates of calves which remained with their mothers until post-rut; however, there is a lack of information on the effect of pre-rut weaning date on LWG of young farmed deer. Finally to achieve a high pregnancy rate early in the mating season, one industry recommendation has been that farmers should wean calves before the mating season to optimise nutrition and body condition of the hinds but to date, yet recommendation has been unsupported by evidence from the published literature.

This study investigated the impact of weaning date on parasitism and liveweight gain (LWG) of deer calves and hinds during summer and early autumn as well as reproductive performance of hinds. Seventy-six deer calves were randomly allocated in a 2x2 factorial design, involving sex, genotype, weaning date (February 17 or March 17, 2005) and either treatment with topical moxidectin (0.5mg/kg) on January 14 and February 25, or no anthelmintic treatment. Liveweight (LW), faecal gastrointestinal nematode egg counts (FEC) and lungworm larval counts (FLC), blood haematological and biochemical parameters of calves were measured on January 12, February 17, March 17 and 31.

Sixty-four mixed-age adult hinds (dams of the above calves) were used to investigate the effect of weaning date on internal parasitism, conception date and pregnancy rate as determined by ultrasound scanning. These hinds were not given anthelmintic treatment, but FLC and FEC were determined on January 12, February 17, March 17, March 31 and May 4. All deer rotationally

grazed permanent perennial ryegrass-based pasture (*Lolium perenne*) together until weaning when the calves were removed to separate but similar pasture.

Calves weaned in March had a higher LWG to March 31 than those weaned in February ($P<0.0001$). The advantage in LWG of calves weaned in March compared with February could be associated with increased milk intake and/or with age-related stress associated with weaning process management. A significant weaning by anthelmintic treatment interaction was found ($P<0.02$), with LWG higher in treated calves weaned in March ($P<0.02$), but not February ($P>0.1$). Faecal larval counts in treated calves were zero, but FEC remained similar to the untreated control calves regardless of when they had been treated (average 136 epg, range 0-600 epg in mid February and average 92, range 0-350 epg at the end of March).

Weaning date and anthelmintic treatment significantly influenced changes in serum total protein concentrations, but not differential white blood-cell counts. Treated calves had higher serum albumin, and lower serum globulin concentrations than the untreated control group (albumin, 36.2 ± 0.3 vs 35.2 ± 0.3g/L; $P<0.001$; globulin, 23.9 ± 0.4 vs 25.5 ± 0.4g/L; $P<0.005$). Calves weaned in March had higher serum total protein concentrations than those weaned in February (61.3 ± 0.4 vs 59.6 ± 0.4g/L; $P<0.001$). However, there were no significant relationships found between either FLC or FEC and serum albumin, globulin or total protein concentrations.

In hinds, FLC averaged 5 lpg (range 0 – 122) and FEC averaged 26 (range 0- 200) with no significant relationship between weaning date and either FLC or FEC. No effect of weaning date on conception rate or date was shown. This study has shown that weaning date and sub-clinical parasitism during summer and early autumn can influence LWG in young farmed deer. Potential diagnostic parameters such as serum albumin concentration, which was reduced in untreated control deer, warrant further investigation for clinical diagnostic use in farmed deer.

2.2. INTRODUCTION

2.2.1. Parasitism

Lungworm (*Dictyocaulus* spp.) is considered the parasite of most concern in NZ farmed deer (Mason, 1985, Johnson *et al*., 2003). Deer less than 6-months of age in their first autumn are most susceptible to infection (Mason, 1997) because they have not yet developed immunity. In addition, the environment in autumn favours development and survival of endoparasite larvae. Adult farmed deer usually carry low worm numbers, but appear to be largely resistant to heavy infestation under optimum management conditions (Mason, 1985; Audige *et al.,* 1998a; Mackintosh & Wilson, 2002). Nevertheless, calves over two months of age shed more larvae than their dams (Audige *et al.,* 1998a) and are likely to be the major source of re-infection in autumn (Mason, 1985).

Little is known about the epidemiology and pathogenicity of lungworm infections, and even less is known about GI nematode infections of farmed deer. However, investigations have shown that even small numbers of lung and GI nematodes cause sub-clinical infections during autumn and can reduce VFI and LWG post-weaning (Hoskin *et al.,* 2000). Investigations have shown that although GI parasites seldom cause clinical disease or deaths, they can have a significant effect on growth of young deer (Charleston, 2001). Therefore, lungworm and GI nematode parasitism can potentially have a marked effect on the ability of young deer to reach slaughter weight by one year of age (Hoskin *et al.,* 1999). This is because GI nematodes of ruminants in general appear to cause feed intake depression and body protein loss with a consequent reduction in energy utilisation (Poppi *et al.,* 1990). Little is known about the effect of parasitism on growth of young farmed deer in summer and early autumn prior to weaning.

Internal parasites result in clinical and sub-clinical production losses. The costs of control and production losses to the NZ deer industry in 2002 were estimated by Mackintosh & Wilson (2003), to be approximately $12.8 million annually. Lungworm is easily controlled by treatment with an appropriate anthelmintic at appropriate intervals either to reduce the risk to the animal and preferably, to break the life cycle and reduce pasture larval contamination (Charleston, 2001; Mackintosh & Wilson, 2002). However, a research survey has indicated large variation in parasite control programmes on farms concluding that a high proportion failed to achieve optimum success and therefore cost effectiveness (Audige *et al.,* 1998a; Castillo-Alcala *et al.,*

2005; 2007). In addition, recent research indicates possible development of anthelmintic resistance in some deer parasites (Hoskin *et al*., 2003b).

Audige *et al.* (1998a) found a statistically derived positive relationship between date of first anthelmintic treatment and LWG in young deer. In addition, pre-rut weaning resulted in lower calf growth rates during autumn compared with growth rates of calves which remained with their mothers until post-rut (Pollard *et al.,* 2002). This was attributed to mother's milk (Arman, 1974; Loudon *et al.,* 1983) as the major source of nutrition up until 90 days of age. In Iberian red deer, calf growth for the 10-week period after weaning was higher for the calves which had a longer suckling period (i.e., 3 months) compared to the control calves (Gomez *et al.*, 2002). However, there is a lack of information on the effect of pre-rut weaning date on LWG of young farmed deer.

2.2.2. Reproduction and weaning date

Body weight of hinds influences both their ability to conceive and the date of conception (Guiness *et al.,* 1978; Hamilton & Blaxter, 1980; Audige *et al.,* 1998b). Through effects on hind LW, nutrition influences fertility and calving date (Moore *et al.,* 1985). The fertility of primiparous hinds that weighed 61-65kg at mating was 50% and at 70kg fertility was over 90% (Kelly & Moore, 1977; Hamilton & Blaxter, 1980). Therefore, good nutrition prior to mating is vital for high fertility and compact calving.

The ability of a yearling red deer hind to conceive is conditional upon the achievement of a weight threshold or 70% of mature body weight (Kelly & Moore, 1977). However, in adult hinds, body weight and BCS influences the timing of conception as well as conception *per se* (Guiness *et al*. 1978) resulting in optimisation of conception rate for the hinds with body condition score (BCS) set around 2.5 (scoring range 1-5; Audigé *et al.*, 1999). Mixed-age hinds with a low BCS ($\leq$ 2.5) are less likely to conceive than hinds of a higher body condition ($\geq$ 3) while hinds of BCS greater than 3.5 were no more likely to conceive than those at 3-3.5 (Beatson *et al.,* 2000). Therefore a threshold may exist around BCS of 2.5.

Majority of hinds with BCS between 2.5 and 3.0 at mating were identified as rising three year-olds (Beatson *et al.,* 2000). This suggests that BCS at mating may also be related to age. However, age may not have an effect over and above that of LW as suggested by Bray and Kelly

(1979) who found the difference in fertility between 2-year-old and older hinds to be at least partly due to a difference in LW influenced by the level of nutrition. Age of the hind was without effect once the effect of age on body weight was discounted (Blaxter & Hamilton, 1980).

Weaning of calves before mating of hinds has been reported to result in reduced spread of calving without any effect on fertility (Hamilton & Blaxter, 1980). Weaning before mating resulted in a more compact calving because suckling can delay calving through later mating, in addition to any effects of LW alone (Loudon *et al.,* 1983). Pollard *et al* (2002) reported an earlier median conception date in hinds weaned prior to the rut compared with hinds weaned post-rut. Pre-rut weaning in NZ is often practiced due to feed shortage in late summer and early autumn (Nicol & Barry, 2002), otherwise delayed calving dates in the following season (Adam, 1988) may occur due to late conception. Therefore, to achieve a high pregnancy rate early in the mating season, one recommendation has been that farmers should wean calves before the mating season to optimise nutrition and body condition of the hinds.

This study focused on breeding hinds and their offspring prior to and shortly after weaning and examined the impact of anthelmintic treatment and early or late pre-rut weaning on parasitism, growth and reproductive outcomes in a breeding herd during autumn using single-sire mating. The specific study objectives were to examine the impact of early or late pre-rut weaning with and without anthelmintic treatment on growth of calves, and weaning date effects on hind reproductive parameters.

2.3. MATERIALS AND METHODS

2.3.1. Experimental design

The study was undertaken at the Massey University Deer Research Unit in 2005 (Palmerston North, NZ). Observations began January 14 and concluded for weaners on March 31 and for hinds on June 3. On 12 January, 76 deer calves were randomly allocated to a 2x2 factorial design based on sex, genotype and LW. One group was weaned on February 17 and the other on March 17. Moxidectin pour-on (0.5mg/kg, Cydectin®, Fort Dodge Animal Health NZ Ltd) was given to calves on January 14 and February 25, based on the LW of the heaviest calves. Half of the animals within each weaning date group were treated (Treated) and the other half remained as untreated controls (Control).

In addition, 64 red hind dams (mixed-age) weaned either February 17 or March 17 were joined with a single red stag from March 1 to May 4. Measurements investigated included LW, faecal FEC and FLC, blood haematology and serum biochemistry, BCS and conception rate for hinds only.

2.3.2. Animals

Seventy six deer calves born between November 12-December 23 comprising forty seven pure red deer (15 female + 32 male) and twenty nine hybrid ¼ elk and ¾ red deer (17 female + 12 male) were used with a mean LW (± SD) of 32.3 ± 6.13 at 1-2 months of age. Throughout this report the term ‘red calves’ will be used to refer to pure red calves. In addition, a total of 64 mixed-age red hinds with a mean LW (± SD) of 107.9 ±11.03 and a mean age of 7.4 years (range 3 to 15 years) were used to measure effect of weaning date on parasitism and conception date. One red stag was put out with the hinds for single sire mating.

Visual observation and palpation of the udder were used to check that the hinds weaned early had ceased lactating. Conception rate was assessed by a rectal ultrasound (Bingham *et al.,* 1988), performed by a single operator on May 30 and June 3 to assess pregnancy status. Conception date was estimated using foetal age equations of Revol and Wilson, (1990). March 24, when the first conception occurred, is used as the reference (Day 1) for conception dates. LW of the calves and hinds were measured on January 14, February 17, and March 17 and 31, and May 4 for adult hinds only. At the same time, BCS of the hinds was monitored. The BCS system used measures the body condition regardless of body weight by objective appraisal by palpation of the tuber

coxae, sacrum, and rump area of the deer standing straight and quiet, on a score of 1-5, with 5 being the highest score for condition (Audigè *et al.*, 1998b). In this trial BCS estimation was performed by two trained people and the average was used for the purpose of data analysis.

2.3.3. Faecal sampling and Laboratory analysis

Rectal faecal samples of 6-10g were taken from each animal for FLC and FEC on January 14, February 17, March 17 and 31. Hinds were also sampled on May 4. The modified Baermann technique (Hendriksen, 1965) was used to recover lungworm larvae from fresh faeces for FLC. Briefly, Baermann funnels were set up within four hours of faecal collection. These contained 4 grams of fresh faeces suspended in muslin cloth in a 40ml vial of tepid water for 24 hours at room temperature (to allow larvae to migrate to the bottom of the vial). The larvae were counted the following day. Each lungworm larva count represented the number of lungworm per 4 grams of fresh faeces; the count was then divided by 4 to get FLC per gram (lpg). FEC were determined from 2 grams of the refrigerated faeces within 3 days after sampling using a modified McMaster technique (Stafford *et al.*, 1994), where a count of one egg was equivalent to 50 eggs per gram of fresh faeces. Therefore, counts are expressed per gram of fresh faeces.

2.3.4. Blood sampling, serum biochemistry and haematology

Blood samples using personal physical restraint (calves) or a crush device (adult hinds) were taken on January 14, February 17 and March 17 and May 4 (adult hinds only) for serum biochemistry, and haematology analysis. On each sampling occasion 2 x 10ml samples were collected by jugular vene-puncture using a 20 gauge needle and one 10ml plain and one 10ml vacutainer containing 0.5ml of anticoagulant EDTA (Hemograd, Becton-Dickinson, New Jersey, USA).

After centrifugation (Heraeus Multifuge 151s, Roche Diagnostics, GmbH, Germany) serum from the plain blood samples was used to determine total protein, albumin, and globulin concentrations using automated chemistry analyzer (Roche Hitachi 911, Diamond diagnostics, USA). Haematological analysis was done on the EDTA-blood samples within three hours of sampling. White blood-cell counts (WBC), routine differential WBC (neutrophils, eosinophils, basophils, lymphocytes, monocytes), red cell counts (RBC), haematocrit (HCT), corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet cell count (PLT), mean platelet volume (MPV), haemoglobin count (HGB) and packed cell volume (PCV) were

determined using an automatic haematology analyser (Advia ^{TM} 120 Haematology System, Bayer Germany).

2.3.5. Pasture and grazing measurements

Calves and hinds were rotationally grazed on permanent perennial ryegrass-based pasture together until weaning when calves were removed to a separate similar pasture grazed previously on the rotation. Calves weaned in February were grazed together with two 'uncles' (castrated fistulated stags). When the second group of calves was weaned in March, the remaining calves were removed from hinds and put with the group of weaners weaned earlier. Feed allowances (excluding dead matter) were set at 10kg DM/hind/day prior to and after weaning. Feed allowance for calves post weaning was set at 5kg DM/day. The hinds remained together before and during mating. Pre and post grazing pasture masses for hinds were estimated using a rising plate meter during mating (Section 2.3.1) and the pasture residual herbage mass was maintained at 1500kg DM/ha. There were no supplements offered. Occasionally, hinds during mating were used to clean up paddocks grazed by the weaners. The pasture was regularly top dressed with urea 46% N at 20kg N/ha after every grazing.

2.3.5 Statistical Methods

Data analysis was undertaken using SAS (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA). Calf FLC, FEC and LWG were analysed using the MIXED procedure with a linear model that considered the fixed effects of sex, genotype, anthelmintic treatment, weaning date and their interactions and the random effect of animal. Faecal larval count and FEC of hinds were analysed using the GENMOD procedure with a linear model that considered the fixed effects of weaning and sampling dates and their interactions. Faecal larval count and FEC data required log-transformation [LOG_{10} (count + 1)], least squares means are presented as back-transformed means. Accumulated pregnancy curves and BCS of hinds weaned either in February or March were estimated using survival analysis (proc PHREG). Serum proteins and haematology were analysed using MIXED procedure with a linear model that considered the fixed effects of sampling date, weaning date, FLC, FEC (and anthelmintic treatment for calves only) and their interactions and the repeated effect of sampling date. Correlations between traits were obtained using a GLM procedure of multivariate analysis. Significance was declared at $P<0.05$.

2.4. RESULTS

2.4.1. Calf weight

Live weight data are presented in Figure 2.1. Anthelmintic treatment and weaning date had no significant effect on LW *per se*. The interaction between both was significant ($P<0.05$), with calves treated and weaned in March having a significantly higher mean LW (48.3 ± 1.2 kg) on March 31 compared to the calves treated and weaned in February (44.3 ± 1.2 kg). Both sex and genotype had a significant influence on LW ($P<0.0001$ and $P<0.001$ respectively). Male calves averaged 49.8 ± 0.9 kg and females 42.1 ± 1.0 kg, while the hybrid calves averaged 48.1 ± 1.0 kg and red deer calves averaged 43.7 ± 0.8 kg on March 31.

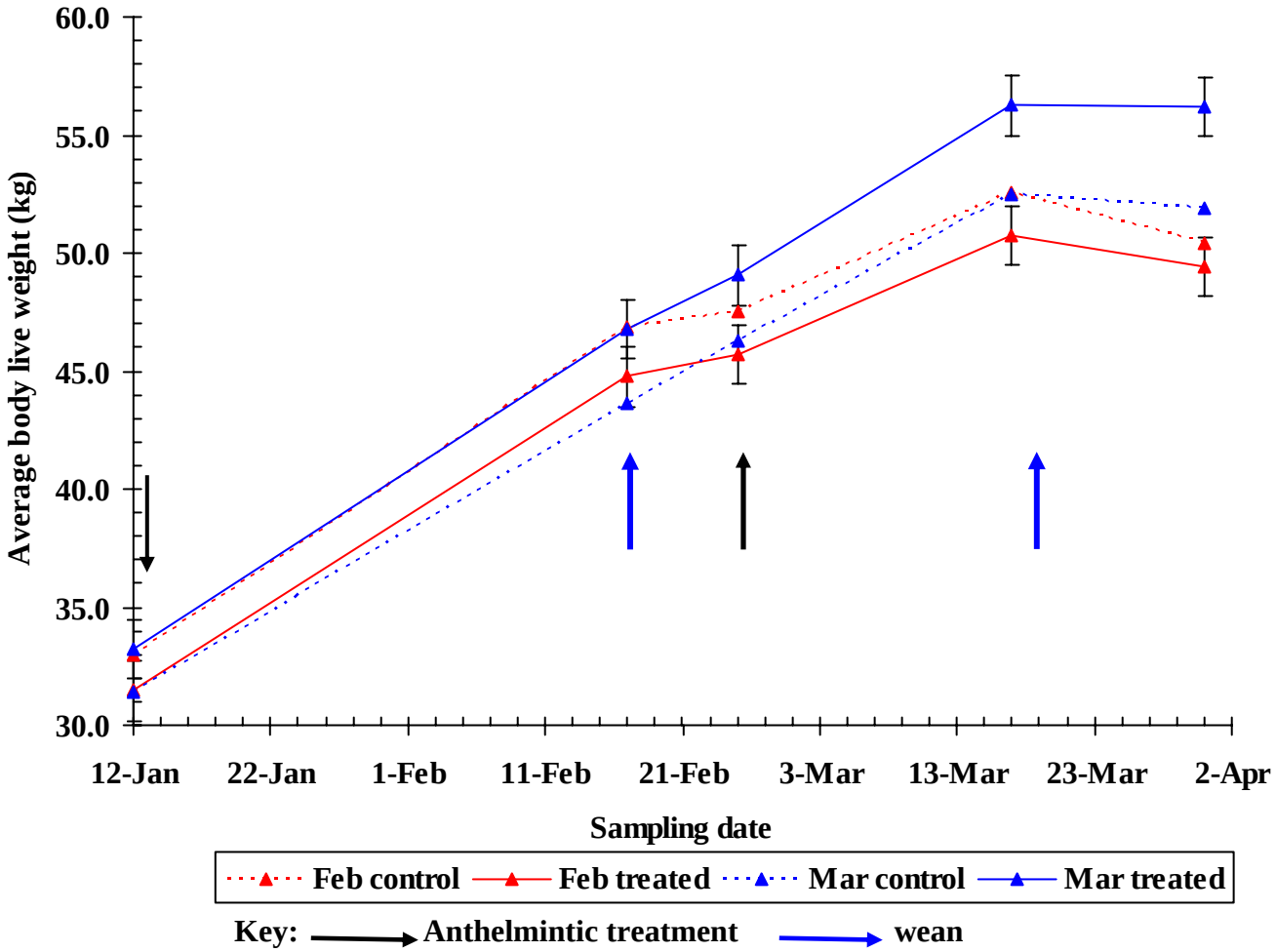

Figure 2.1: Mean (± SEM) body live weight (LW; kg) of calves weaned in mid-February (Feb) or mid-March (Mar) and treated with anthelmintic (treated) on January 14 and February 25 or remaining as untreated controls (control).

Calves weaned in March had a significantly higher mean LWG than those weaned in February (253 ± 17.8 vs.153 ± 17.8g/day, P<0.0001). There was no significant effect of anthelmintic treatment on LWG overall. However, an anthelmintic treatment by weaning date interaction was significant (P<0.05), with higher LWG of anthelmintic treated calves weaned in March (P<0.001) but not in February (P>0.05).

There was no significant effect of sex or genotype on LWG. However, the interaction between sex and weaning date was significant for LWG with the March-weaned male calves having higher LWG than February-weaned male calves (P<0.05; Table 2.1). In contrast, there was no significant difference in LWG between female calves weaned in February and March.

Table 2.1: Mean (±SEM) liveweight gain (LWG; g/day) of male and female calves weaned in February or March and the level of significance due to the interaction between sex and weaning date on LWG**.**

Effect			
Sex	**Weaning date**	**Mean LWG ± SEM**	**Sex*weaning date**
Male	Feb	144 ± 23.4	**
	Mar	259 ± 24.1	
Female	Feb	161 ± 26.7	NS
	Mar	226. ± 26.9	

NS; Not significant, ** P<0.001

Anthelmintic treatment did not have a significant effect on LWG. However, the interaction between anthelmintic treatment and sex was significant (P<0.05) with anthelmintic treated male, but not female, calves having significantly higher LWG compared with the control male calves (Table 2.2).

Table 2.2: Mean (±SEM) liveweight gain (LWG; g/day) of male and female calves either anthelmintic treated (treated) or with treatment withheld (control) and the level of significance due to the interaction between sex and anthelmintic treatment on LWG.

Effect			
Sex	**Treatment**	**Mean LWG± SEM**	**Sex*Treatment**
Female	Control	157 ± 12.4	NS*
	Treated	160 ± 12.4	
Male	Control	148 ± 10.7	*
	Treated	191 ± 10.7	

NS; Not significant, *P<0.05

2.4.2. Calf Parasitology

2.4.2.1 Faecal larval count

The data presented in Table 2.3 shows the effect of genotype, sex, treatment and weaning date on FLC of the calves. The hybrid calves had a higher mean FLC than red deer calves (P<0.0001), but sex had no effect on FLC. Anthelmintic treated calves had lower FLC than the control calves (P<0.001). Moxidectin reduced FLC to zero (P<0.0001). Calves weaned in March had a higher FLC compared with calves weaned in February (P<0.04). In addition, the interaction between anthelmintic treatment and weaning date was significant (P<0.01) with anthelmintic-treated calves weaned either in February or March having significantly lower FLC compared with control calves February- or March-weaned (P<0.0001; Fig. 2.2).

The data presented in Table 2.4 shows the average, range and proportion positive for FLC in anthelmintic treated (treated) or treatment withheld (control) calves by weaning and sampling dates. In mid-March the control group weaned in February had the lowest mean and range for FLC, with 26% positive, whilst in mid-February they had the highest mean and range with 95% positive. March-weaned control calves showed a similar trend, although the proportion positive was higher in mid-February (100 % positive). In both control groups the number of the calves

which had a positive count started increasing towards the end of the trial with March-weaned control calves having the highest number positive (89 vs 32%).

Table 2.3: Mean (±SEM) faecal larval count (FLC; lpg) of deer calves due to effects of genotype, sex, anthelmintic treatment and weaning date by the level of significance of the effects on FLC.

EFFECT	CATEGORY	FLC	SEM	SIGNIFICANCE
Genotype	Red	17		
	Wapiti	24	0.07	**
Sex	Hinds	19		
	Stags	20	0.07	NS
Treatment	Control	29		
	Treated	0	0.07	***
Weaning	February	19		
	March	21	0.04	*

NS; not significant, * P<0.05, ** P<0.001, *** P<0.0001

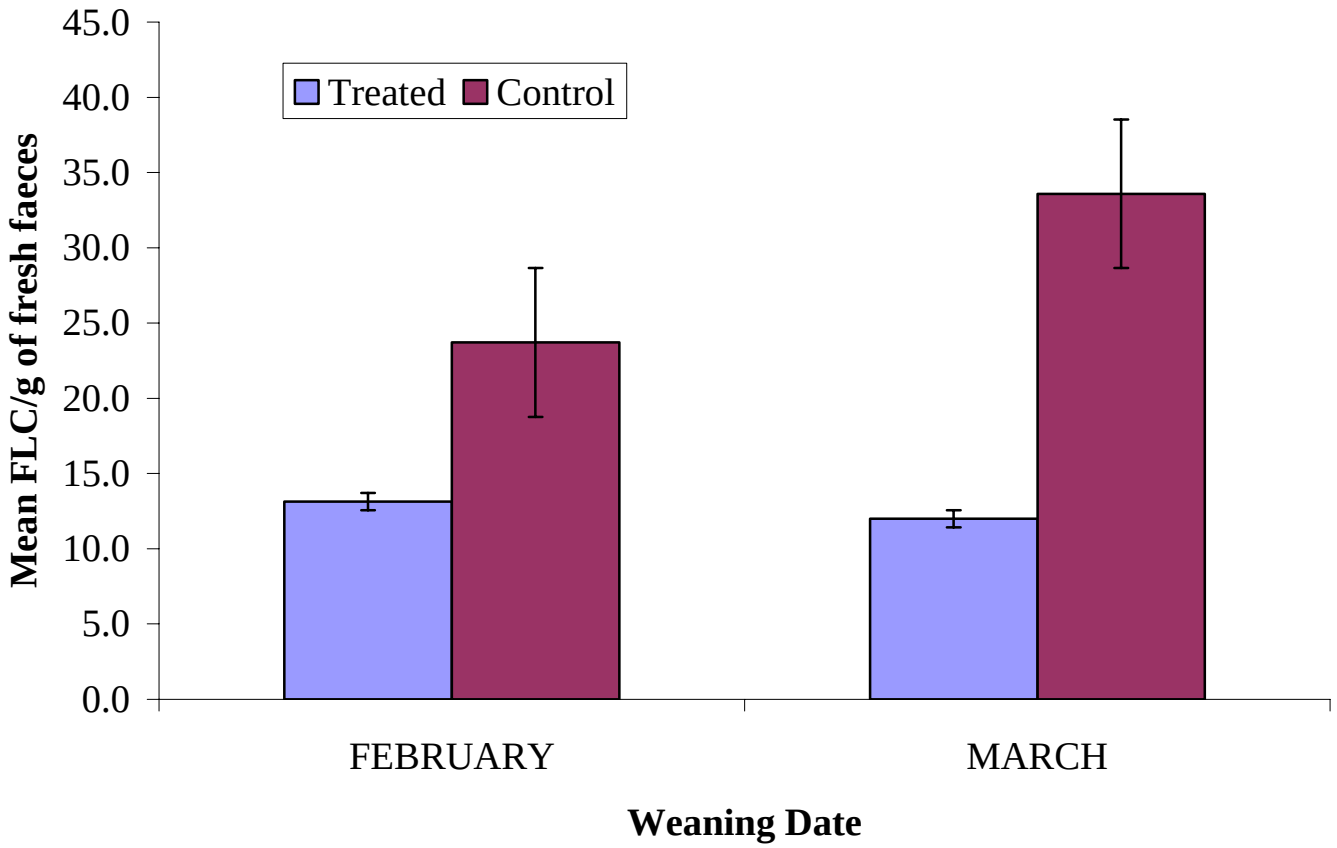

Figure 2.2: Mean (±SEM) faecal larval count (FLC; lpg) of deer calves either given anthelmintic treatment (treated) or with treatment withheld (control). These are shown by weaning date in either February 17 or March 17.

2.4.2.2. Faecal egg count

The data presented in Table 2.5 shows the average, range and proportion positive for FEC in anthelmintic-treated (treated) or treatment-withheld (control) calves by weaning and sampling dates. The FEC in the control calves averaged 108 epg (range 0-850) with the highest counts in February. There was no effect of sex, genotype, anthelmintic treatment or weaning date on FEC. Moxidectin did not reduce FEC to zero as expected, but instead FEC remained similar to the untreated control calves regardless of when they were sampled in relation to treatment. The proportion of positive FECs in the treated calves was high 36 (February 17) and 34 days (March 31) after treatment; average 136, range 0-600 and 92, range 0-350 epg respectively.

Table 2.4: Mean, range and proportion positive (%+ve) of faecal lungworm larval counts (FLC; lpg) from calves treated with Moxidectin on January 14 and February 25 (Treated) and untreated controls (Control).

Sampling dates		**Jan-12**			**Feb-17**			**Mar-17**			**Mar-31**	
Weaning date	**Mean**	**Range**	**%+ve**	**Mean**	**Range**	**%+ve**	**Mean**	**Range**	**%+ve**	**Mean**	**Range**	**%+ve**
February-17												
Control	20	0-124	0.63	48	0-300	0.95	<1	0-2	0.26	4	0-19	0.32
Treated	32	0-212	0.58	<1	0-1	0.21	0	0	0.0	0	0	0.0
March-17												
Control	6	0-71	0.44	83	0-320	1.0	3	0-16	0.44	94	0-108	0.89
Treated	12	0-78	0.68	<1	0-1	0.11	0	0	0.0	0	0	0.0

Table 2.5: Mean, range and proportion positive (%+ve) of faecal egg counts (FEC, epg) from calves treated with Moxidectin on January 14 and February 25 (Treated), and untreated controls (Control).

Sampling dates		**Jan-12**			**Feb-17**			**Mar-17**			**Mar-31**	
Weaning date	**Mean**	**Range**	**%+ve**	**Mean**	**Range**	**%+ve**	**Mean**	**Range**	**%+ve**	**Mean**	**Range**	**%+ve**
February-17												
Control	47	0-350	0.26	108	0-850	0.37	32	0-150	0.32	142	0-800	0.68
Treated	58	0-700	0.21	159	0-450	0.58	42	0-200	0.32	74	0-300	0.53
March-17												
Control	31	0-300	0.22	131	0-550	0.50	47	0-200	0.50	114	0-350	0.72
Treated	26	0-150	0.37	136	0-600	0.47	37	0-200	0.37	92	0-350	0.58

2.4.3. Hind parasitology

Mean FEC and FLC from hinds were low with the proportion being positive declining from 62% for FLC and 28% for FEC in January to only 9% for both by the beginning of May (Table 2.6). There were no significant relationships between weaning date of hinds and either FLC or FEC.

Table 2.6: The mean, range and proportion positive for faecal larval count (FLC; lpg) and faecal egg count (FEC; epg) by sampling date from mature mixed-age hinds**.**

	Sampling date	**Jan-12**	**Feb-17**	**Mar-17**	**May-04**
FLC	Average	5	1	<1	<1
	Range	0-122	0-9	0-8	0-12
	% +ve	62	28	6	9
FEC	Average	26	17	16	6
	Range	0-200	0-100	0-150	0-100
	% +ve	28	25	22	9

2.4.4. Hind reproduction

The daily cumulative percentage of hinds conceiving (weaned either February or March) from Day 1 are shown in Figure 2.3. There was no significant difference in overall conception rate (Mean ± SEM = 84.4%) or conception date related to weaning date. However, three hinds in the March weaned group conceived after the last of the February weaned hinds, with the latest being 10 days later.

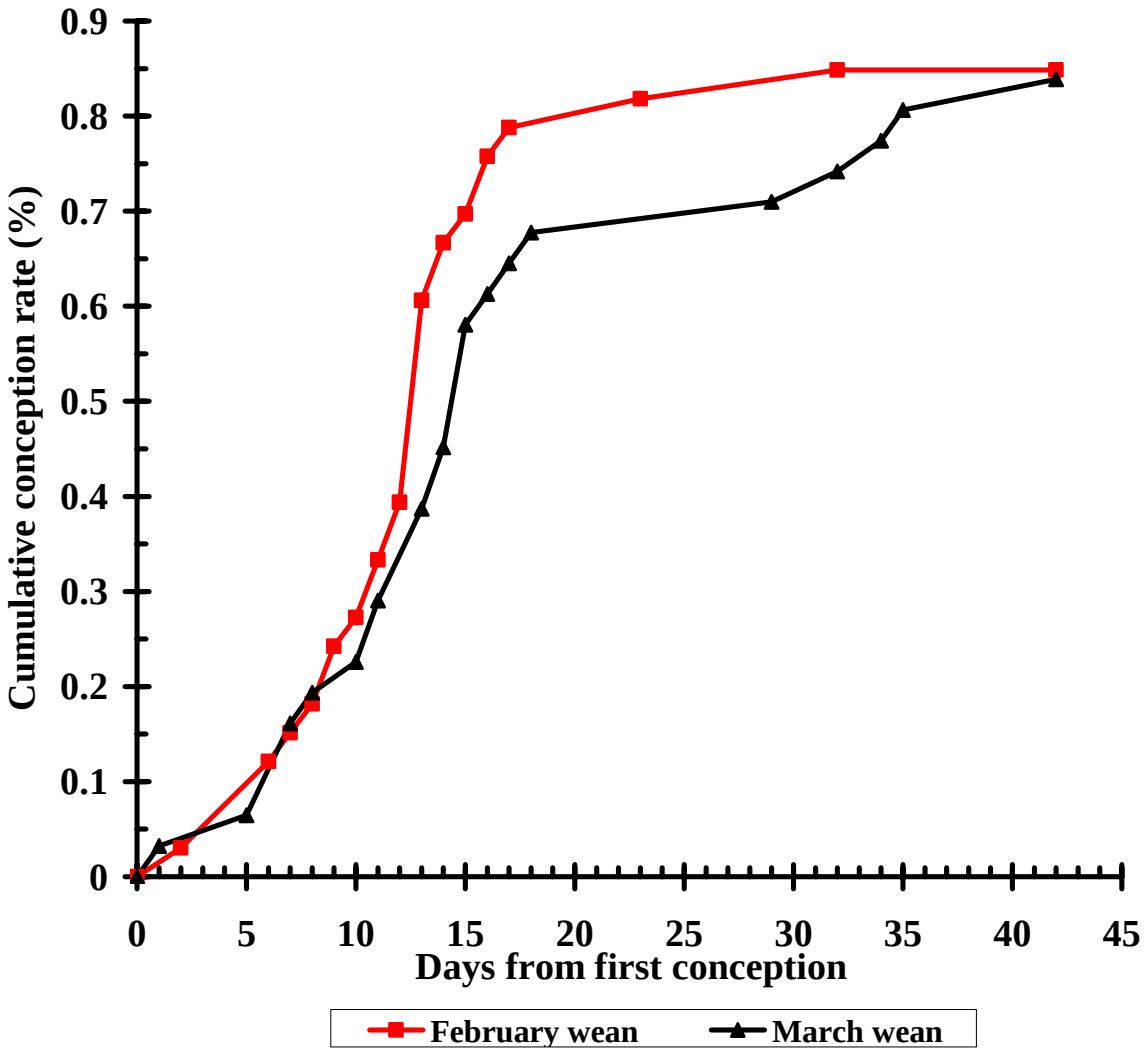

Figure 2.3: The daily cumulative conception rate (%) of hinds weaned in February or March with Day 1 corresponding to March 24**.**

A summary of conception dates (Figure 2.4) shows that the median conception date was day 12 (April 4) and 13 (April 5) for February and March-weaned hinds, respectively. Hinds weaned in February achieved a relatively more compact mating by 10 days compared with March-weaned hinds (Table 2.7). A total of five hinds failed to conceive within the mating period from each group.

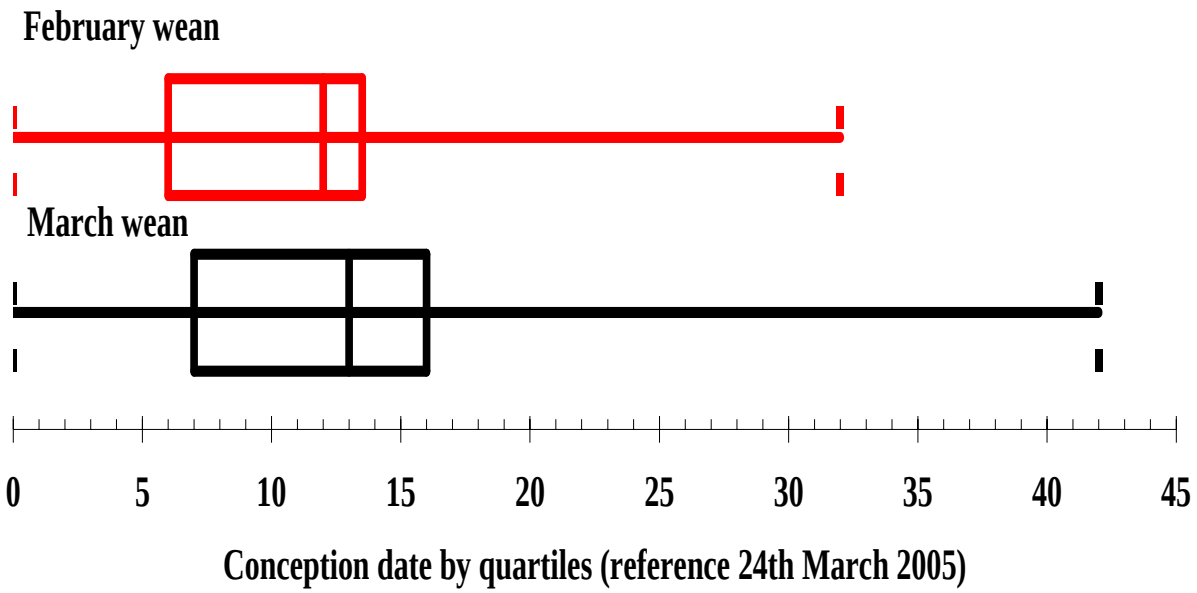

Figure 2.4: Box plot representation of conception date of February and March-weaned hinds (with Day 1 corresponding to March 24th), showing the range of conception dates (line), with the box showing the range over which 75% conceived. The vertical line within each box shows the median conception day**.**

Table 2.7: The five-number summary of conception dates for hinds weaned either in February or March as represented by the box and whisker plot in Figure 2.4 above.

Title	Feb wean	March wean
Minimum	0	0
First Quartile	6	7
Median	12	13
Third Quartile	13.5	16
Maximum	32	42

2.4.5. Body condition score

Weaning date did not have a significant effect on BCS; mean (±SEM) was 2.8 ± 0.07 for each of February- or March-weaned hinds. Table 2.8 presents the grouping of hinds by BCS indicating the number of hinds pregnant and the percentage of hinds which failed to conceive within the mating period. There was no significant effect of body condition score, age or interaction of both on conception rate of the hinds.

Table 2.8: Pregnancy and non-pregnancy rate of mixed-age deer hinds weaned in February and March according to body condition score (BCS) at the start of mating (March 1).

BCS	Total	Pregnant	Not Pregnant	% Not pregnant
2.0	4	2	2	50.0
2.25	4	4	0	0.0
2.5	21	17	4	19.1
2.75	9	8	1	11.1
3.0	17	14	3	17.7
3.25	2	2	0	0.0
3.5	5	5	0	0.0
3.75	2	2	0	0.0
Total/Overall %	**64**	**54**	**10**	**15.6**

2.4.5. Serum biochemistry and haematology

2.4.5.1. Calf serum protein concentrations

Detailed data of calf serum total protein, albumin and globulin concentrations and albumin:globulin ratio (AGR) according to weaning date, anthelmintic treatment, sex and genotype are presented in Tables 2.10 and 2.11 of the appendices. The mean (ranges) serum concentration of the following parameters were: total protein 60.5g/l (49 to 73g/l), albumin 35.6g/l (29 to 44g/l), globulin 24.9g/l (15 to 44g/l) and AGR 1.5 (0.7 to 2.5).

Serum total protein: Mean serum total protein concentrations on each sampling date are shown in Figure 2.5. Total protein significantly increased with time ($P<0.0001$). Overall calves weaned in March had higher total serum protein concentrations compared with those weaned in February (61.3± 0.4 vs 59.6 ± 0.4g/l; $P<0.001$). The interaction between weaning date and time was

significant (P<0.0001) with March weaned calves having higher total serum protein concentrations compared with calves weaned in February (66.7 ± 0.7 vs 62.5 ± 0.7g/l). There was no significant relationship between either FLC or FEC and serum total protein concentrations

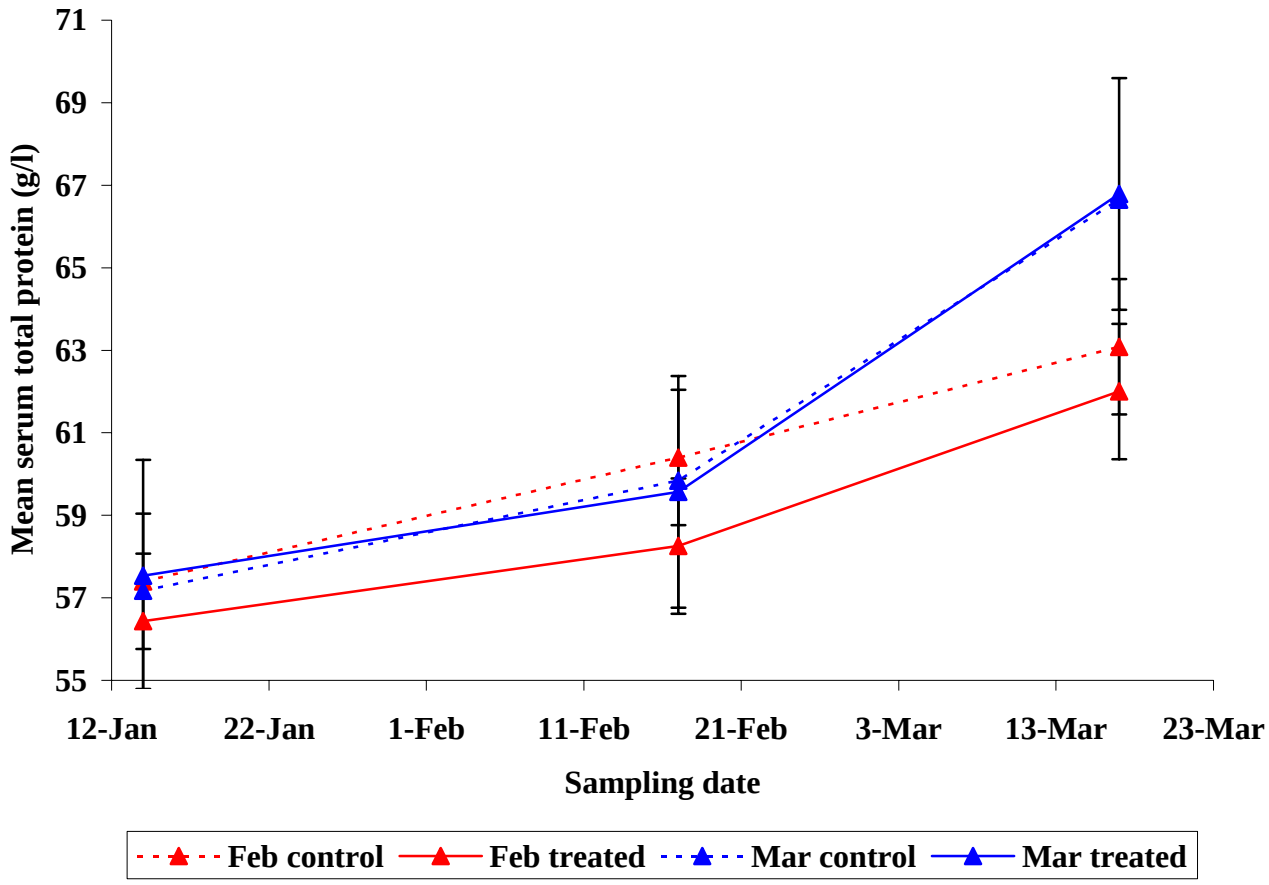

Figure 2.5: Mean (±SEM) serum total protein (g/l) of young deer calves weaned on February 17 or March 17 and treated 6-weekly with moxidectin (treated) or untreated (control)

Serum albumin: Mean serum albumin concentrations (g/l) on each sampling date are shown in Figure 2.6. Serum albumin concentrations significantly increased with time (P<0.0001). Treated calves had higher albumin concentrations compared with control calves (36.2 ± 0.3 vs 35.2 ± 0.3g/l; P<0.001). The male calves had higher serum albumin concentrations compared with that of the female calves (36.1 ± 0.3 vs 35.2 ± 0.3g/l; P<0.005). Hybrid calves had higher serum albumin concentrations compared with the red deer calves (36.5 ± 0.3 vs 34.9 ± 0.3g/l; P<0.001). Weaning date did not have a significant effect on the serum albumin concentrations. There were

no significant interactions found and there were no significant relationships between either FLC or FEC and serum albumin concentrations.

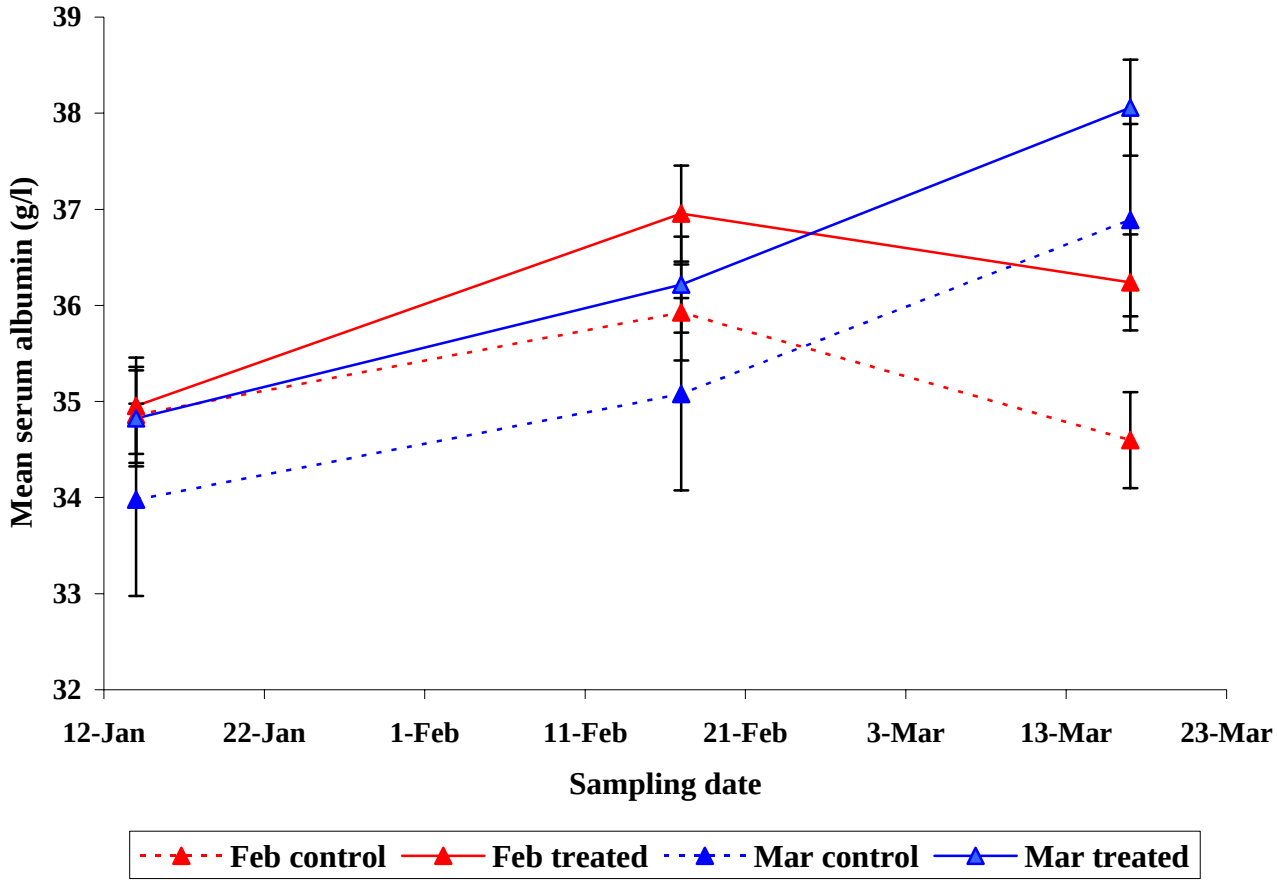

Figure 2.6: Mean (±SEM) serum albumin concentrations (g/l) of young deer calves weaned on February 17 or March 17 and treated 6-weekly with moxidectin (treated) or untreated (control)

***Serum globulin**:* Mean serum globulin concentrations (g/l) on each sampling date are shown in Figure 2.7. Globulin increased over time (P<0.0001). Significant main effects of weaning date, anthelmintic treatment and genotype were found for serum globulin concentrations. Calves weaned in March had higher serum globulin concentrations than calves weaned in February (25.4 ± 0.4 vs 24.0 ± 0.4g/l; P<0.001). Calves treated with moxidectin had lower globulin concentrations compared with control calves (23.9 ± 0.4 vs 25.5 ± 0.4g/l; P<0.001). Red deer calves had higher serum globulin concentrations than hybrid calves (25.8 ± 0.4 vs 23.7 ± 0.4g/l; P<0.0001). There were no significant interactions found and there were no significant relationships between either FLC or FEC and serum globulin concentrations.

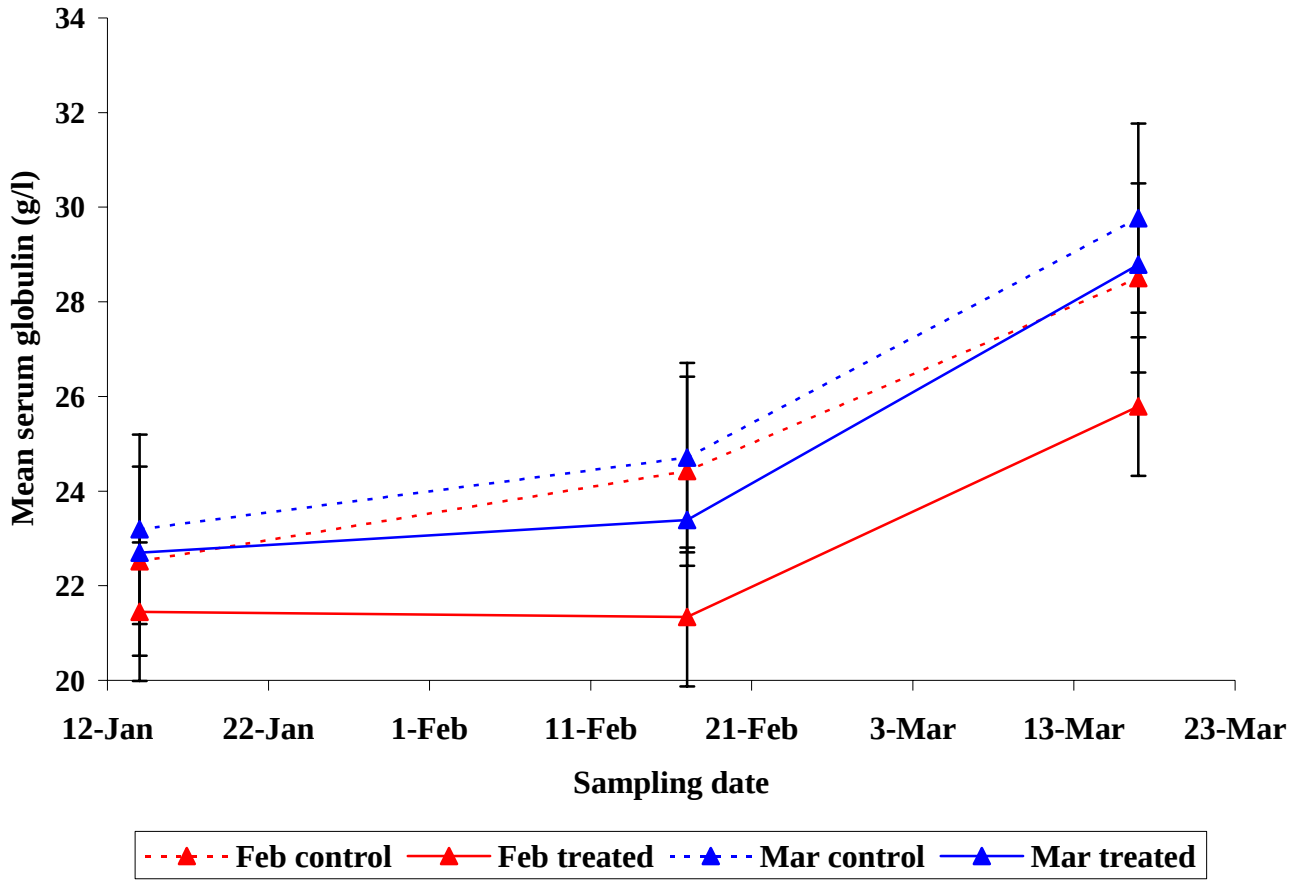

Figure 2.7: Mean (±SEM) serum globulin concentrations (g/l) of young deer calves weaned on February 17 or March 17 and treated 6-weekly with moxidectin (treated) or untreated (control)

Albumin:globulin ratio (AGR); The albumin to globulin ratio increased with time (P<0.0001). Hybrid calves had a higher AGR compared with red deer calves (1.6 ± 0.03 vs 1.4 ± 0.03; P<0.0001). Anthelmintic treated calves had a higher AGR compared with control calves (1.6 ± 0.03 vs 1.4 ± 0.03; P<0.001). There were no significant interactions found and there was no significant relationship between either FLC or FEC and calves serum AGR.

2.4.5.2. Adult hind serum protein concentrations

The mean (±SEM) and range of serum total protein, albumin and globulin concentrations and AGR of the adult mixed-age hinds are presented in Table 2.9. There were no significant effects of weaning date, FLC, FEC or their interactions on any components of serum protein (i.e. total serum protein, albumin and globulin) of the adult hinds. However, there were significant ($P<0.0001$) changes in mean serum total protein, albumin, globulin and the AGR over time.

Total protein concentration decreased ($P<0.0001$) between January 14 and February 17 (78.4 ± 0.7 vs 71.8 ± 0.6g/l) and between March 17 and May 4 (72.5 ± 0.7 vs 71.1 ± 0.7g/l; $P<0.03$) Mean albumin concentrations increased significantly between February 17 and March 17 (34.6 ± 0.5 vs 36.4 ± 0.5; $P<0.0001$) and decreased between March 17 and May 4 (36.4 ± 0.5 vs 35.5 ± 0.5; $P<0.01$) but there were no significant changes in albumin concentrations between January 14 and February 17. Mean globulin concentrations decreased between January 14 and February 17 (44.0 ± 0.6 vs 37.1 ± 0.6g/l; $P<0.0001$) and between March 17 and May 4 (36.7 ± 0.6 vs 35.4 ± 0.6g/l; $P<0.001$). However, the AGR significantly increased overall overtime ($P<0.0001$).

Table 2.9: The mean (±SEM) and range of the serum total protein, albumin, globulin concentrations and the albumin:globulin ratio(AGR) of adult mixed-age hinds by weaning and sampling dates.

		February weaned hinds				March weaned hinds			
Sampling date		Jan-14	Feb-17	Mar-17	May-04	Jan-14	Feb-17	Mar-17	May-04
Total protein g/l	Mean	78.0	72.1	74.0	72.0	77.7	71.6	71.8	70.9
	Range	62 - 92	65 - 81	64 - 84	61 -83	60 - 90	62 - 80	47 - 80	50 - 81
	± SEM	0.95	0.89	0.90	0.91	0.99	0.93	0.94	0.94
Albumin g/l	Mean	33.6	34.4	36.2	35.8	34.2	35.1	37.1	35.7
	Range	21 - 40	26 - 43	27 - 44	30 - 43	26 - 48	28 - 44	30 - 46	27 - 43
	± SEM	0.68	0.66	0.66	0.67	0.71	0.68	0.68	0.69
Globulin g/l	Mean	44.4	37.8	37.8	36.0	43.5	36.5	35.8	35.0
	Range	38 - 56	31 - 47	30 - 48	28 - 46	34 - 52	29 - 43	29 - 44	23 - 46
	± SEM	0.80	0.78	0.78	0.79	0.83	0.80	0.81	0.81
AGR	Mean	0.76	0.92	0.97	1.01	0.80	0.98	1.06	1.07
	Range	0.5 - 1.0	0.6 - 1.3	0.7 - 1.4	0.7 - 1.5	0.6 - 1.3	0.7 - 1.4	0.8 - 1.5	0.8 - 1.8
	± SEM	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03

AGR = Albumin:globulin ratio

2.4.6. Haematology

2.4.6.1. Calf haematology

Detailed data of calf haematology; concentrations of the RBC, HGB, HCT, MCV, MCHC, PLT, MPV, WBC and proportions of differential WBC counts including neutrophils, lymphocytes, monocytes, eosinophils and basophils according to weaning date, anthelmintic treatment, sex and genotype are presented in Tables 2.12 and 2.13 of the appendices.

The mean (ranges) concentrations of the following haematology parameters were: RBC 12.0 x 10^{12}cells/l (8.9 to 15.2 x 10^{12}cells/l), HGB 160g/l (114 to 206g/l), HCT 0.4 l/l (0.11 to 0.54 l/l), MCV 35.2fl (28.5 to 42.0fl), MCHC 381g/l (359 to 400g/l), PLT 463 x 10^9 cells/l (98 to 860 x 10^9 cells/l) and MPV 6.2 fl (4.9 to 16.3fl). There were no significant effects of weaning date, anthelmintic treatment, FLC, FEC, sex, genotype and/or their interactions on RBC counts, HCT, MCV, MCHC, PLT or MPV.

The mean (ranges) concentrations of the following haematological parameters were: WBC 5.1 x 10^9cells/litre (2.31 to 9.74 x 10^9cells/litre), percentage neutrophils 33.6% (11.5 to 69.7%), lymphocytes 59.1% (25.5 to 85.6%), eosinophils 2.3% (0 to 13.5%), monocytes 2.9% (0 to 7.8%) and basophils 2.4% (0 to 8.3%). The mean percentage of lymphocytes (L %) was higher than that of neutrophils (N %); 59.1 vs 33.6 %). There was no significant effect of or relationship between FLC or FEC and WBC or differential WBC (neutrophils, lymphocytes, basophils, monocytes and eosinophils).

Mean WBC increased with time ($P<0.01$) and was higher ($P<0.001$) on February 17 than on January 14 and March 17, which were similar. Mean WBC were higher for red deer calves compared with hybrid calves ((5.4 vs 4.6 x 10^9cells/litre; $P<0.001$). Calves weaned in March had higher ($P<0.02$) mean WBC than the calves weaned in February ((5.1 vs 4.8 x 10^9cells/litre). However, there was no significant relationship between anthelmintic treatment, sex or any interactions with WBC.

Mean total neutrophil concentration increased with time ($P<0.001$) being higher on February 17 (1.9 x 10^9cells/l) compared with 1.8 and 1.4 (x 10^9cells/l) for January 14 and March 17, respectively. Red deer calves had higher ($P<0.04$) mean total neutrophil concentrations than

hybrid calves (1.8 vs 1.5 x 10^9cells/l) and March weaned calves tended (P<0.06) to have higher mean total neutrophil concentrations compared with February weaned calves (1.8 vs 1.6 x 10^9cells/l). There were no significant effects of anthelmintic treatment, sex or any interactions on total neutrophil concentrations.

Mean total lymphocyte counts were significantly higher on March 17 (P<0.0001) than on January 14 and February 17. Red deer calves had higher mean total lymphocyte concentrations compared with hybrid calves ((3.2 vs 2.6) x 10^9cells/l; P<0.0001). There were no significant effects of weaning date, anthelmintic treatment, sex or any interactions on total lymphocyte concentrations.

Mean total monocyte and eosinophil concentrations were higher in the hybrid calves than red deer calves (P<0.001) while mean total basophil concentration was significantly higher in red deer calves than in the hybrid calves (P<0.001). There were no significant effects of weaning date, anthelmintic treatment, sex or any interactions on either total monocyte, eosinophil or basophil concentrations.

2.4.6.2. Adult hind haematology

Detailed data of adult mixed-age hind haematology; concentrations of the WBC, RBC, HCT, MCV, MCHC, PLT, MPV and differential WBC counts including neutrophils, lymphocytes, monocytes, eosinophils and basophils according to weaning and sampling dates are presented in Table 2.14 of the appendices.

The mean (ranges) concentrations of the following haematological parameters were: RBC 9.6 x 10^{12}cells/l (3.4 to 12.9 x 10^{12}cells/l), HGB 153g/l (199 to 116g/l), HCT 0.4l/l (0.3 to 0.53l/l), MCV 42.1fl (10.1 to 51.3.0fl), MCHC 379g/l (352 to 409g/l), PLT count 355 x 10^9 cells/l (61 to 618 x 10^9 cells/l) and MPV 7.0fl (4.6 to 15.9fl). The RBC, HGB, HCT, MCV, MCHC, and PLT concentrations significantly changed with time (P<0.0001), but there was no significant effect of weaning date except for RBC and MCHC concentration. RBC were lower (P<0.04) for February-weaned hinds (9.4 x 10^{12}cells/l) compared with March-weaned hinds (9.7 x 10^{12}cells/l). The MCHC concentration was lower (P<0.03) for February-weaned hinds (378g/l) than for March-weaned hinds (380g/l).

The mean (ranges) concentrations of the following haematological parameters were: WBC 4.7 x 10^9cells/litre (1.82 to 8.04 x 10^9cells/litre), percentage neutrophils 42.0% (10.5 to 73.3%), lymphocytes 40.6% (14.4 to 97.3%), eosinophils 10.3% (0 to 32.5%), monocytes 4.2% (0 to 13.1%) and basophils 3.2% (0 to 7.4%). The mean percentage of lymphocytes (L %) was similar to that of the neutrophils (N %); 42.0 vs 40.3 %.

The mean WBC and totals and proportions of differential WBC including neutrophils, lymphocytes, eosinophils, monocytes, and basophils significantly increased with time ($P<0.0001$) but did not vary significantly between treatment groups of adult hinds due to effects of weaning date, internal parasitism or interaction of both. However, there was a significant negative correlation between neutrophils and log-FEC ($y = -2.26x + 42.7$; $r^2 = 0.027$; $P<0.01$) and log-FLC ($y = -1.59x + 42.1$; $r^2 = 0.0026$; $P<0.02$) while lymphocytes had a positive correlation with log-FEC ($y=2.47x + 39.6$; $r^2 = 0.029$; $P<0.02$) and log-FLC ($y = 0.0015x + 0.11$; $r^2 = 0.002$; $P<0.02$).

2.5. DISCUSSION

This is the first investigation of the relationship between pre-rut weaning date, anthelmintic treatment and internal parasitism in young deer. The study has shown that pre-rut weaning and summer anthelmintic treatment influenced growth rate of deer calves. However, the pre-rut weaning date had no effect on reproductive outcomes in hinds. It has previously been shown that calves weaned pre-rut had a lower weight gain from March to June than those weaned after the rut (Pollard, *et al.*, 2002). However, there is limited information on the weight gain of farmed red deer calves weaned prior to 90 days (Bao *et al*., 2004) of age. This is the first research into the impact of pre-rut weaning date on parasitism and LWG of deer calves. The advantage in LWG of calves weaned in March compared with February could be associated with milk intake (Arman, 1974; Loudon *et al.,* 1983), and/or with age-related stress associated with weaning (Pollard *et al.*, 2002; Bao *et al.*, 2004).

Fallow deer calves weaned onto pasture at 12 weeks of age suffered an initial growth check for one or two weeks due to the stress of weaning (Flesch *et al.,* 1999; Bao *et al.,* 2004). The growth check may have been reduced by weaning onto high quality concentrate feed so that calves maintained their weight in the first week after weaning. Dryden (2001) found that feed intake and growth rate were lower in calves weaned at seven weeks than in calves weaned at nine weeks. However, although accustomed to the pasture and the paddock, calves in this study were weaned onto permanent perennial ryegrass-based pasture of low to moderate feeding value under summer dry conditions. This may have reduced VFI, (although not measured in this trial), consequently reducing LWG in the February-weaned calves.

It appears that the experimental design of this study, which did not consider possible effects of animal behaviour and stress associated with weaning management, may have differentially influenced weaner LWG post-weaning between the early and late-weaned groups. Calves weaned in February were removed to a separate paddock, previously grazed with their dams earlier in the rotation, with two 'uncles' (castrated fistulated stags), whereas calves weaned in March were placed with the earlier-weaned calves. This may have possibly decreased the level of stress for the group weaned in March. Differences in LWG here should therefore be interpreted with caution. It is suggested that in further work of this type, groups weaned at different times are weaned into separate paddocks under the same conditions.

Faecal egg and larval counts recorded in this study could be viewed as sub-clinical (parasitic infection which has no recognizable signs or symptoms but may be associated with reduced LWG), since no clinical parasitism (parasitic infection showing signs and symptoms that can be recognized such as coughing, scouring or weight loss) was witnessed among the calves. Faecal larval counts were higher in hybrid than pure red deer indirectly supporting the previous observation of increased susceptibility to lungworm associated with wapiti/elk genes (Mason, 1997). In contrast, Parsons *et al.* (1994) in a study (January 31 to July 4) to determine if breed differences in susceptibility to trichostrongyloid nematodes including lungworms exist, found that, ¾ red ¼ -wapiti hybrids had significantly higher FECs but lower FLCs than pure red deer. Other previous studies have shown that wapiti hybrid weaners are more adversely affected by parasitism than red deer weaners (Waldrup *et al.*, 1994). Therefore the relative susceptibility of red and hybrid deer to internal parasitism warrants further investigation using challenge studies and worm count data rather than FEC and FLC.

A recent survey has shown that moxidectin pour-on was the most commonly used anthelmintic for weaner deer (Castillo-Alcala *et al.*, 2005, 2007). Moxidectin 0.5% pour-on has a claim for minimum persistent activity of 35 to 42 days against re-infection with lungworms (mature and immature) and GI nematodes (Mackintosh *et al.*, 1997; Waldrup *et al.*, 1998), respectively. As the persistent activity of moxidectin against GI nematodes is the same as the treatment intervals, FEC after the first treatment should have remained at zero. The failure of moxidectin to reduce FEC to zero could be interpreted as sub-optimal efficacy and/or parasite anthelmintic resistance (Charleston & McKenna, 2002; Hoskin *et al.*, 2005b). Emergence of deer GI nematode resistance is a concern and may result in major loss of production in young farmed deer (Charleston, 2001), if this issue is not addressed by the deer industry.

A common feature of many parasitic GI infections is an increased loss of endogenous protein into the GI tract, which is partly attributable to increased leakage of plasma protein, increased sloughing of epithelial cells and increased secretion of mucoproteins (MacRae, 1993), resulting in partitioning of nutrients to the repair of damaged tissues and the immune response (Coop & Kyriazakis, 1999) and therefore loss of weight. Haematological and blood biochemical changes which reflect gastric dysfunction are documented in sheep infected with *Ostertagia circumcincta* (Coop *et al.*, 1977; Anderson *et al.*, 1988). In a trickle infection trial, with deer infected with lungworm and GI nematode infective larvae, Hoskin *et al.* (1998) observed elevated serum pepsinogen and gastrin, reduced serum albumin and elevated serum globulin concentrations. Waldrup *et al.* (1994) found decreased serum albumin and increased serum total protein in parasitized red deer, and that serum albumin increased with anthelmintic treatment.

In the current study serum albumin concentrations were significantly higher in the anthelmintic treated calves than in control deer calves. Serum globulin concentrations followed a converse similar pattern as serum albumin in both treatment groups. These results suggest loss of serum albumin in the parasitized control calves due to nematode infection. Anthelmintic treatment decreased serum globulin concentrations. The increase in serum globulin may be explained at least in part by a reduction in immunoglobulins, although these were not directly measured in this study. However, relationships between serum total protein, albumin and globulin and FLC or FEC were not significant in both calves and adult hinds. Further investigations are necessary to

provide more information on these markers of potential benefit for diagnosis of sub-clinical parasitism in deer.

The WBC count and differential WBC counts found in this study are similar to those reported by Wilson & Pauli (1982). The absence of significant differences in haematological parameters between sexes in this study is similar to the reported absence of sex differences for other ruminant and deer species (Pedersen & Pedersen, 1975; Schalm *et al.*, 1975; Wilson & Pauli, 1982). However, WBC and differential WBC counts vary markedly between individuals and between deer species (Chapman, 1977; English & Lepherd, 1981; Wilson & Pauli, 1982). In the current study there was variation between individual deer as indicated by wide range in each of the haematology parameters investigated. There was also variation between deer species. Red deer calves had higher concentrations of WBC counts, neutrophils, lympocytes and basophils compared to hybrid deer calves. But the lack of relationship between WBC counts or WBC differentials and FLC or FEC in this study tend to support the suggestion that deer may have less ability to produce a leucocytosis in response to infection than seen in other ruminant species (Wilson & Pauli, 1982). However, the level of infection in the current study may still have been sufficiently low to trigger an elevation in these haematological parameters. Further investigations on the ability of the deer to produce a leucocytosis in responses to parasitic infection are necessary; the relationship between WBC count and differential WBC counts and lungworm and GI nematode infections in farmed deer.

Low FLC and FEC of mature hinds in the absence of clinical signs indicated low sub-clinical parasitism throughout the trial. These findings support previous research showing that adult deer had lower FEC and FLC than calves (Mason & Gladden, 1983; Parsons *et al*., 1994; Audige *et al.*, 1998a), probably due to acquired immunity. It is possible that climatic and nutritional stress may reduce immunity to internal parasites in adult deer (Audige *et al.*, 1998a). However, there is not available research data to support this.

In this study the hinds weaned in February had a relatively compact mating. Although, the overall difference between the two groups of hinds was not significant, there was about an 80% conception rate in the February-weaned hinds compared with 68% in the March-weaned hinds by day 18 from when the first hind conceived. Previous trials found mean conception dates to be earlier in pre-rut weaned hinds compared with post-rut weaned hinds by 12 and 7 days in 1999

and 2000, respectively (Pollard *et al*., 2002). That study concluded that post-rut weaning was associated with later conception dates and poorer winter hind BCS. Other studies demonstrated that pre-rut weaning brought forward average calving by a week (7 days) in red deer and elk respectively (Hamilton & Blaxter, 1980; Friedel & Hudson 1994).

In contrast, although the design of these previous studies compared pre-and post-rut weaning dates, the current study has shown no difference in reproductive parameters resulting from the two pre-rut weaning dates. However, this study compared the reproduction of two groups of pre-rut weaned hinds and the result seems to suggest a trend that the closer the weaning date is to the rut the more likely fertility of the hinds may be reduced. This supports previous suggestion that the later the hinds are weaned, the less likely they are to have a BCS >2.0 (Hamilton & Blaxter, 1980) and that lactating hinds come into oestrous some days later than non-lactating hinds (Wilson, 1984b), therefore conceiving later. Frequency and intensity of suckling affects onset of oestrus and they are likely to increase in dry years when milk production is low (Loudon *et al.*, 1983) again resulting in late conceptions. Although, this conception rate data had low statistical power (63% at $P<0.05$, $\acute{\alpha}=5\%$) due to small group numbers, it may suggest that pre-rut weaned hinds may conceive earlier and therefore further research is required to validate the advantages of pre-rut weaning on hind reproduction. Power analysis (i.e., the ability of a test to detect an effect, given that the effect actually exists) has indicated that n=48, 51 and 59 will give a power of 78, 80 and 85% respectively $P<0.05$, $\acute{\alpha}=5\%$ (Faul *et al.*, 2007) for conception date.

This study has shown that pre-rut weaning date, (although, possibly confounded by weaning process management) and sub-clinical parasitism during summer and early autumn can influence LWG in young farmed deer. The changes in serum proteins may reflect internal parasitism infection, although the level of infection to cause these changes was not determined. Similarly the level of infection may have been sufficiently low to trigger elevation in the haematology parameters investigated. The failure of moxidectin to reduce FEC to zero raises the question of the efficacy of macrocyclic lactone anthelmintics against GI nematodes in farmed deer and/or emergence of farmed deer GI nematode resistance. This requires urgent investigation as it may result in loss of productivity in young farmed deer. The study has also highlighted the need for further research to demonstrate the possible advantages of pre-rut weaning on hind reproduction and post weaning growth of deer calves.

2.9 APPENDIX

Table 2.10: The mean (range; g/litre) of serum total protein, albumin, globulin, and albumin to globulin ratio (AGR) of February weaned anthelmintic treated (treated) and untreated controls (control) calves by sex and genotype

	Control					**Treated**				
	Sex		**Genotype**			**Sex**		**Genotype**		
	Stags	Hinds	Hybrid	Red	Total control	Stags	Hinds	Hybrid	Red	Total treated
No. of animals	11	8	7	12	19	11	8	7	12	19
Albumin	34.8	35.3	35.9	34.5	35	35.8	35.9	36.5	35.5	35.9
	(29 - 40)	(31 - 39)	(29 - 39)	(30 -40)	(29 - 40)	(32-41)	(32-40)	(33-40)	(32-41)	(32-41)
Total protein	60.9	59.6	61.0	59.9	60.3	59.0	59.2	57.9	59.8	59.1
	(55 - 73)	(52 - 68)	(55 - 73)	(52 - 67)	(52 - 73)	(51-70)	(54-65)	(51-63)	(54-70)	(51-70)
Globulin	26.1	24.3	25.1	25.4	25.3	23.2	23.3	21.4	24.3	23.2
	(20 - 44)	(17 - 30)	(17 - 44)	(20 - 33)	(17 - 44)	(17-32)	(19-30)	(17-29)	(19-32)	(17-32)
AGR	1.4	1.5	1.5	1.4	1.4	1.6	1.6	1.7	1.5	1.6
	(0.7 - 1.8)	(1.2 - 2.2)	(0.7 - 2.2)	(1.0 - 1.8)	(0.7 - 2.24)	(1.2-2.2)	(1.2-2.1)	(1.2-2.2)	(1.2-2.2)	(1.2-2.2)

AGR; Albumin to globulin ratio

Table 2.11: The mean (range) (g/litre) of serum total protein, albumin, globulin, and albumin to globulin ratio (AGR) of March weaned anthelmintic treated (treated) and untreated controls (control) calves by sex and genotype

	Control					**Treated**				
	Sex		**Genotype**			**Sex**		**Genotype**		
	Stags	Hinds	Hybrid	Red	Total control	Stags	Hinds	Hybrid	Red	Total treated
No. of animals	11	7	7	11	18	10	9	8	11	19
Albumin	35.7	34.5	36.0	34.8	35.2	36.7	35.7	37.5	35.3	36.2
	(30-40)	(31-39)	(32 - 40)	(30 - 40)	(30 - 40)	(33-44)	(31-43)	(31-44)	(31-39)	(31-44)
Total protein	61.9	60.3	60.5	61.8	61.3	61.7	61.1	61.0	61.7	61.4
	(53-70)	(49-72)	(49 - 72)	(53 - 71)	(49 - 72)	(54-70)	(52-69)	(52-70)	(54-70)	(52-70)
Globulin	26.2	25.9	24.5	27.0	26.1	25.0	25.4	23.5	26.3	25.2
	(20-32)	(17-35)	(17 - 33)	(20 - 35)	(17 - 35)	(19-35)	(15-35)	(15-30)	(19-35)	(15-35)
AGR	1.4	1.4	1.5	1.3	1.4	1.5	1.5	1.6	1.4	1.5
	(1.1-2.0)	(1.0 - 1.9)	(1.1 - 1.9)	(1.0 - 2.0)	(1.0 - 2.0)	(1.0-2.0)	(1.0-2.5)	(1.1-2.5)	(1.0-2.0)	(1.0-2.5)

AGR; Albumin to globulin ratio

Table 2.12: The mean and range of blood haematology concentrations of the red blood cell (RBC) count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet cell count (PLT), mean platelet volume (MPV) white blood cell (WBC) count and differential WBC counts including neutrophils, lymphocytes, monocytes, eosinophils and basophils of February weaned anthelmintic treated (treated) and untreated controls (control) calves according to weaning date, anthelmintic treatment, sex and genotype

February wean

		Control					Treated				
		Sex		Genotype			Sex		Genotype		
		Stags	Hinds	Hybrid	Red	Total	Stags	Hinds	Hybrid	red	Total
WBC x 10^9 cells/L	Mean	4.5	5.4	4.4	5.3	4.9	5.2	4.6	4.5	5.2	4.9
	Range	2.4 - 7.1	3.4 - 7.2	2.4 - 6.5	3.6 - 7.2	2.4 - 7.2	2.5 - 7.9	2.9 - 6.4	2.9 - 6.3	2.5- 7.9	2.5 - 7.9
RBC x 10^{12} cells/L	Mean	11.7	12.0	11.9	11.9	11.9	12.0	11.6	12.0	11.7	11.8
	Range	10.4 - 15.2	10.1 - 14.4	10.3 - 14.3	10.1-15.2	10.1- 15.2	10.4- 13.9	8.9 - 13.5	9.4- 13.63	8.9-3.9	8.9 - 13.9
Haemoglobin g/L	Mean	162.4	160.9	164.8	159.6	161.5	157.7	159.5	163.0	155.9	158.5
	Range	137 - 193	135 - 181	144 - 183	135 - 193	135 - 193	139 - 182	122 - 187	131 - 187	122-179	122 - 187
Haematocrit L/L	Mean	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	Range	0.4 - 0.5	0.3 - 0.5	0.4 - 0.5	0.3 - 0.5	0.3 - 0.5	0.4 - 0.5	0.3 - 0.5	0.4 - 0.5	0.3- 0.5	0.3 - 0.5
MCV fL	Mean	36.6	35.4	36.7	35.5	35.9	34.5	36.3	35.7	35.0	35.3
	Range	31.9 - 41.9	29.6 – 42.0	30.1 – 42.0	29.6 - 41.9	29.6 – 42.0	28.5 - 40.8	32.1 - 40.1	31.4- 40.8	28.5- 39.2	28.5- 40.8
MCHC g/L	Mean	379.8	382.0	380.4	381.9	381.3	382.8	380.7	380.7	382.6	381.9
	Range	368 - 393	362 - 399	368 - 390	362 - 399	362- 399	363 - 400	363 - 397	367 - 394	363 - 400	363 - 400
PLT x 10^9 cells/L	Mean	483.2	454.0	540.0	423.0	466.1	441.5	494.3	498.1	443.7	463.7
	Range	268 - 706	268 - 780	434 - 706	268 - 780	268 - 780	257 - 710	142 - 760	257 - 718	142 - 760	142 - 760
MPV fL	Mean	6.1	6.0	6.2	5.9	6.0	6.2	6.0	6.3	6.0	6.1
	Range	4.9 - 7.2	5.0 - 7.2	5.2 - 7.2	4.9 - 7.2	4.9 - 7.2	5.0 - 7.3	5.1 - 6.9	5.1 - 7.0	5.0 - 7.3	5.0 - 7.3
Neutrophils %	Mean	32.8	33.3	32.6	33.3	33.1	33.7	30.8	35.7	30.7	32.5
	Range	13.7 – 66.0	13.9 - 57.4	13.7 – 66.0	13.9 - 57.4	13.7 – 66.0	18.0 - 62.2	18.4 - 56.5	18.4 -62.2	18.0 - 58.6	18.4- 62.2
Neutrophils (x 10^9/L)	Mean	1.5	1.8	1.5	1.7	1.6	1.8	1.4	1.7	1.6	1.6
	Range	0.4 - 4.2	0.8 - 3.4	0.5 - 4.2	0.4 - 3.4	0.4 - 4.2	0.5 - 3.6	0.6 - 3.2	0.6- 3.6	0.5- 3.6	0.5 3.6
Lymphocytes %	Mean	60.9	58.9	59.1	60.1	59.7	59.6	60.8	57.0	61.9	60.1
	Range	26.8 - 85.6	38.0 - 76.9	26.8 - 81.9	38.0 - 85.6	26.8 - 85.6	34.4 - 77.7	38.2 - 75.2	34.4- 73.5	35.6- 77.7	34.4 -77.7
Lymphocyte (x 10^9/l)	Mean	2.7	3.1	2.6	3.1	2.9	3.0	2.8	2.6	3.2	3.0
	Range	1.5 - 4.8	2.0 - 5.1	1.5 - 3.7	2.1 - 5.1	1.5 - 5.1	1.8 - 5.5	1.7 - 4.0	1.7- 4.0	1.8- 5.5	1.8- 5.5
Monocytes %	Mean	2.6	2.8	3.2	2.5	2.7	2.4	3.7	3.4	2.7	2.9
	Range	1.0 -5.	1.1 - 6.4	1.0 - 6.4	1.0 - 6.0	1.0 - 6.4	0.6 - 4.6	0.5 - 6.2	0.6 - 5.8	0.5 - 6.2	0.5 - 6.2
Monocytes (x 10^9/L)	Mean	0.1	0.2	0.1	0.1	0.1	0.1	0.2	0.1	0.1	0.1
	Range	0.0 - 0.4	0.1 - 0.4	0.0 - 0.3	0.1 - 0.4	0.0 - 0.4	0.0 - 0.3	0.0 - 0.3	0.0- 0.3	0.0- 0.3	0.0- 0.3
Eosinophils %	Mean	2.1	2.5	3.4	1.8	2.4	1.9	2.6	2.3	2.2	2.2
	Range	0.5 - 5.9	0.2 - 9.3	1.0 - 9.3	0.2 - 5.9	0.2 - 9.3	0.2 - 6.1	0.2 - 13.5	0.2 - 6.1	0.3 - 13.5	0.2 - 13.5
Eosinophils (x 10^9/L)	Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Range	0.0 - 0.3	0.0 - 0.4	0.1 - 0.4	0.0 - 0.3	0.0 - 0.4	0.0 - 0.2	0.0 - 0.7	0.0- 0.3	0.0- 0.7	0.0- 0.7
Basophils %	Mean	2.2	2.3	1.5	2.7	2.3	2.8	1.9	1.5	2.9	2.4
	Range	0.7 - 3.9	0.5 - 4.1	0.5 - 3.5	1.2 - 4.1	0.5 - 4.1	1.0 - 7.7	0.4 - 3.7	0.4 - 3.2	1.2 - 7.7	0.4 - 7.7
Basophils (x 10^9/L)	Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.2	0.1
	Range	0.0 - 0.2	0.0 - 0.3	0.0 - 0.1	0.1 - 0.3	0.0 - 0.3	0.0 - 0.5	0.0 - 0.2	0.0 - 0.2	0.0- 0.5	0.0- 0.5

Table 2.13: The mean and range of blood haematology concentrations of the red blood cell (RBC) count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet cell count (PLT), mean platelet volume (MPV) white blood cell (WBC) count and differential WBC counts including neutrophils, lymphocytes, monocytes, eosinophils and basophils of March weaned anthelmintic treated (treated) and untreated controls (control) calves according to weaning date, anthelmintic treatment, sex and genotype

		March wean									
		Control					Treated				
		Sex		Genotype			Sex		Genotype		
		Stags	Hinds	Hybrid	Red	Total	Stags	Hinds	Hybrid	red	Total
WBC x 10^9 cells/L	Mean	5.0	5.3	4.6	5.4	5.1	5.5	5.2	4.7	5.8	5.3
	Range	2.6 - 7.1	3.8 - 7.7	2.6 - 5.7	3.2-7.7	2.6 - 7.7	3.3-9.7	2.3 - 9.0	2.3 - 9.0	4.2-9.7	2.3-9.7
RBC x 10^{12} cells/L	Mean	12.2	12.4	12.4	12.0	12.1	12.0	12.1	12.8	11.5	12.1
	Range	10.6-13.9	9.4-14.8	9.4-14.8	10.5-13.2	9.4-14.8	10.1-14.1	9.0-14.7	10.1-14.7	9.0-13.8	9.0-14.7
Haemoglobin g/L	Mean	160.1	162.8	168.9	156.2	161.1	157.1	159.0	161.8	155.7	158.0
	Range	133 - 206	132 - 193	132 - 206	133 - 178	132 - 206	142 - 180	114 - 190	114 - 190	127 - 180	114 - 190
Haematocrit L/L	Mean	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.4	0.4	0.4
	Range	0.3- 0.5	0.4 - 0.5	0.4 - .5	0.3- 0.5	0.3- 0.5	0.4- 0.5	0.1- 0.5	0.1- 0.5	0.3- 0.5	0.1- 0.5
MCV fL	Mean	34.4	35.9	35.9	34.4	35.0	34.5	34.7	33.4	35.5	34.6
	Range	29.5- 40.2	31.4- 41.5	31.4- 41.5	29.5- 38.4	29.5- 41.5	29.8- 39.8	30.3- 40.3	30.3- 37.3	29.8- 40.3	29.8- 40.3
MCHC g/L	Mean	382.3	379.9	379.9	382.3	381.4	381.2	385.3	384.3	382.2	383.1
	Range	368 - 397	359 - 391	359 - 392	371 - 397	359 - 397	363 - 398	363 - 400	363 - 400	363 - 398	363 - 400
PLT x 10^9 cells/L	Mean	469.9	468.1	455.4	478.0	469.2	463.8	443.3	488.3	429.4	454.1
	Range	315 - 810	98 - 783	98 - 783	335 -810	98 - 810	293 - 740	196 - 860	196 - 860	287 - 740	196 - 860
MPV fL	Mean	6.4	6.5	6.5	6.4	6.5	6.1	6.2	6.3	6.0	6.2
	Range	4.9 - 16.3	5.4 - 11.3	5.4 - 11.3	4.9 - 16.3	4.9 - 16.3	5.0 - 9.8	4.9 - 7.2	5.3 - 7.2	4.9 - 9.8	4.9 - 9.8
Neutrophils %	Mean	34.0	37.1	36.4	34.4	35.2	33.2	34.1	30.8	35.7	33.6
	Range	19.9- 60.8	23.5- 60.3	23.5- 60.3	19.9- 16.3	19.9- 60.8	11.5- 64.1	18.4- 69.7	18.1- 50.4	11.5- 69.7	11.5- 69.7
Neutrophils (x 10^9/L)	Mean	1.7	2.0	1.7	1.9	1.8	1.9	1.8	1.4	2.2	1.8
	Range	0.8- 4.3	1.0- 3.9	0.9- 3.4	0.8- 4.3	0.8- 4.3	0.5- 6.2	0.3- 5.8	0.3- 3.5	0.5- 6.2	0.3- 6.2
Lymphocytes %	Mean	57.9	54.5	54.8	57.8	56.6	59.0	60.6	63.2	57.2	59.8
	Range	34.2- 73.4	32.6- 69.2	32.6- 69.2	34.2 -73.4	32.6- 73.4	28.6- 82.8	25.5- 85.2	42.9- 85.2	25.5- 82.8	25.5- 85.2
Lymphocyte (x 10^9/L)	Mean	2.9	2.8	2.5	3.1	2.9	3.2	2.8	2.7	3.2	3.0
	Range	1.4 - 4.6	1.8- 3.9	1.4- 3.3	1.5 - 4.6	1.4 - 4.6	1.8 - 5.1	1.1 - 4.5	1.1 - 3.8	2.0 - 5.1	1.1 - 6.6
Monocytes %	Mean	3.0	3.6	3.8	2.9	3.2	2.8	2.7	2.9	2.6	2.7
	Range	1.2 - 6.2	1.1 - 7.8	2.0 - 6.2	1.1 - 7.8	1.1 - 7.8	1.2 - 5.3	1.2 - 6.6	1.2 - 6.6	1.2 - 5.1	1.2 - 6.6
Monocytes (x 10^9/L)	Mean	0.2	0.2	0.2	0.2	0.2	0.2	0.1	0.1	0.2	0.1
	Range	0.1 -0.3	0.1- 0.7	0.1- 0.3	0.1- 0.7	0.1- 0.7	0.0- 0.3	0.0 - 0.3	0.0 - 0.3	0.1 -0.3	0.0 - 0.3
Eosinophils %	Mean	2.5	1.8	2.9	1.8	2.3	2.6	2.0	2.8	2.0	2.3
	Range	0.3 - 8.4	0.3 - 6.2	0.8 - 8.4	0.3 - 6.3	0.3 - 8.4	0.6 – 10.0	0.3 - 6.7	0.3 - 10.0	0.6 - 4.8	0.3 - 10.0
Eosinophils (x 10^9/lL	Mean	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1
	Range	0.0- 0.3	0.0- 0.3	0.0- 0.3	0.0 -0.3	0.0- 0.3	0.0- 0.5	0.0- 0.3	0.0- 0.5	0.3- 0.3	0.0- 0.5
Basophils %	Mean	2.6	2.5	2.1	2.8	2.6	2.4	2.7	2.6	2.5	2.5
	Range	0.6 - 8.2	0.6 - 6.4	0.6 - 6.4	0.9 - 8.2	0.6 - 8.2	0.7 - 8.3	0.9 - 5.7	0.7 - 5.7	1.0 - 8.3	0.7 - 8.3
Basophils (x 10^9/L)	Mean	0.1	0.1	0.1	0.2	0.1	0.1	0.1	0.1	0.1	0.1
	Range	0.0- 0.4	0.0- 0.3	0.0 – 0.3	0.1- 0.4	0.0- 0.4	0.0 - 0.6	0.0 - 0.3	0.0 - 0.3	0.1 - 0.6	0.0 - 0.6

Table 2.14: The mean and range of blood haematology concentrations of the red blood cell (RBC) count, haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin concentration (MCHC), platelet cell count (PLT), mean platelet volume (MPV) white blood cell (WBC) count and differential WBC counts including neutrophils, lymphocytes, monocytes, eosinophils and basophils of adult mixed-age hinds according to weaning and sampling dates

		February-weaned hinds					**March-weaned hinds**				
		Jan-14	Feb-17	Mar-17	May-04	Entire period	Jan-14	Feb-17	Mar-17	May-04	Entire period
WBC x 10^9 cells/L	Mean	5.5	4.7	4.3	4.8	4.8	5.4	4.5	4.2	4.6	4.7
	Range	3.8- 7.6	2.9 - 6.6	1.8 - 5.6	2.4 - 7.2	1.8 - 7.6	4.0- 7.3	2.4 -6.0	2.0 -5.6	3.1 -8.0	2.0 - 8.0
RBC x 10^{12} cells/L	Mean	9.0	9.0	9.8	10.0	9.4	9.2	9.5	9.9	10.4	9.8
	Range	7.6 -11.1	7.3 - 1.0	7.7 - 11.9	7.8 - 1.6	7.3 - 11.9	3.4 - 12.2	7.4 - 12.3	7.2 -2.4	7.7 -2.9	3.4 - 12.9
Haemoglobin g/L	Mean	147.7	142.9	152.7	164.2	151.9	152.3	149.9	150.8	167.4	155.1
	Range	124 - 172	116 - 171	120 - 179	122 - 185	116 - 185	125 - 179	122 - 170	116 - 171	132 - 199	116 - 199
Haematocrit L/L	Mean	0.4	0.4	0.4	0.5	0.4	0.4	0.4	0.4	0.5	0.4
	Range	0.3- 0.4	0.3- 0.5	0.3 - 0.5	0.3 - 0.5	0.3 - 0.5	0.3 -0.5	0.3 -0.5	0.3 -0.5	0.4 -0.5	0.3 - 0.5
MCV fL	Mean	42.3	42.8	39.1	44.8	42.3	41.5	42.2	40.0	43.8	41.9
	Range	37.2- 48.6	37.1- 49.7	10.1- 46.8	39.4- 51.3	10.1 - 51.3	35.1- 46.0	35.4 -48.9	33.8 -45.0	38.4 -48.9	33.8 - 48.9
MCHC g/L	Mean	389.3	373.9	383.3	366.9	378.4	391.7	375.1	384.1	370.3	380.3
	Range	377 - 409	363 - 384	371 - 404	352 - 379	352 - 409	378 - 402	362 - 392	362 - 408	364 - 379	362 - 408
PLT x 10^9 cells/L	Mean	395.6	378.3	303.4	330.4	351.9	392.8	379.0	331.1	319.3	355.5
	Range	200 - 618	186 - 518	61 - 442	193 - 522	61 - 618	92 - 548	58 - 513	79 - 461	81 - 459	58 - 548
MPV fL	Mean	7.4	6.9	7.4	6.1	6.9	8.0	7.1	7.2	6.2	7.1
	Range	5.3 - 9.0	4.9 - 8.3	6.0 - 15.5	4.6 - 9.6	4.6 - 15.5	6.3 - 15.9	6.1 - 11.1	5.7 – 12.0	4.9 - 12.5	4.9 - 15.9
Neutrophils %	Mean	45.4	43.7	34.9	43.5	41.9	43.8	42.7	38.3	44.1	42.2
	Range	25.9- 67.3	21.5- 59.0	19.1- 50.8	22.0- 57.1	19.0 - 67.3	26.5- 65.6	10.5- 61.8	21.2 -59.4	26.4 - 73.3	10.5 - 73.3
Neutrophils (x 10^9/l)	Mean	2.5	2.1	1.5	2.1	2.0	2.4	2.0	1.6	2.1	2.0
	Range	1.4- 4.9	1.0- 3.4	0.6- 2.3	0.9- 3.8	0.6 - 4.9	1.4 - 4.0	0.7 - 3.1	0.6 - 2.8	0.9 - 5.9	0.6 - 5.9
Lymphocytes %	Mean	39.08	40.33	49.0	36.6	41.3	37.4	41.0	46.5	34.5	39.9
	Range	18.9- 97.3	24.2- 61.9	31.2- 72.7	20.5- 60.3	18.9 - 97.3	22.8- 54.4	26.2- 66.2	29 - 62.8	14.4- 56.3	14.4 - 66.2
Lymphocyte (x10^9/l)	Mean	2.2	1.9	2.1	1.8	2.0	2.0	1.9	1.9	1.6	1.8
	Range	1.0- 5.6	1.0- 3.4	0.8- 3.4	0.6- 3.4	0.6 - 5.6	0.9- 3.0	1.1 - 3.4	1.0 – 3.0	0.9- 2.8	0.9 - 3.4
Monocytes %	Mean	3.2	6.8	3.4	3.6	4.2	3.4	5.3	3.8	4.0	4.1
	Range	0.0 - 7.6	2.1 - 11.5	1.4 - 7.7	1.9 - 9.2	0.0 - 11.5	0.2 - 7.4	0.2 - 13.1	1.4 - 9.0	0.9 - 8.3	0.2 - 13.1
Monocytes (x 10^9/l)	Mean	0.2	0.3	0.1	0.2	0.2	0.2	0.2	0.2	0.2	0.2
	Range	0.1 - 0.4	0.1 - 0.6	0.1- 0.3	0.1- 0.4	0.0 - 0.6	0.0- 0.3	0.0- 0.5	0.1 - 0.3	0.1 - 0.3	0.0 - 0.5
Eosinophils %	Mean	11.2	5.9	9.8	13.4	10.1	12.6	6.7	8.3	14.6	10.6
	Range	0.0 - 24.6	2.0 - 11.3	4.2 - 21.8	5.4 - 26.3	0.0 - 26.3	1.4 - 32.5	2.3 - 13.4	3.7 - 24.7	6.7 - 22.9	1.4 - 32.5
Eosinophils (x 10^9/l)	Mean	0.6	0.3	0.4	0.6	0.5	0.7	0.3	0.3	0.7	0.5
	Range	0.0 - 1.3	0.1 - 0.6	0.2 - 1.1	0.3- 1.2	0.0 - 1.3	0.2- 1.6	0.1 - 0.7	0.1 - 1.0	0.3 - 1.3	0.1 - 1.6
Basophils %	Mean	3.3	3.5	3.3	3.1	3.7	3.1	3.4	2.9	3.2	3.1
	Range	0.5 - 7.4	0.8 - 5.8	0.6 - 6.6	0.7 - 6.8	0.5 - 7.4	0.6 - 5.9	1.9 - 7.0	1.3 - 4.8	0.9 - 6.2	0.6 - 7.0
Basophils (x 10^9/l)	Mean	0.2	0.2	0.1	0.1	0.2	0.2	0.2	0.1	0.1	0.2
	Range	0.0 - 0.4	0.0 - 0.3	0.0- 0.2	0.0- 0.3	0.0 - 0.4	0.0- 0.4	0.1 -0.3	0.0- 0.2	0.0- 0.4	0.0 - 0.4

CHAPTER 3:

PRELIMINARY COMPARATIVE EVALUATION OF FORAGE SPECIES AND COMBINATIONS ON AUTUMN GROWTH AND PARASITISM OF YOUNG FARMED RED DEER

3.1. ABSTRACT

Chicory sown as a pure sward has been shown to increase post-weaning growth and reduce parasitism of deer compared with perennial ryegrass-based pasture. However, neither forage plantain nor the inclusions of chicory in a pasture mix have been evaluated in this context. The aim of this study was to investigate the effect of pasture species, either sown pure or in mixes, upon post-weaning growth and endoparasitism of farmed red deer from 3- 6 months of age.

On 7 March 2006, 95 weaned red deer calves (55 stags and 40 hinds) were randomly allocated to five groups based on sex, live weight (LW), faecal egg count (FEC) and faecal larval count (FLC). These calves were rotationally grazed on either a permanent pasture based on perennial ryegrass (Control; *Lolium perenne*), chicory (Chicory; *Cichorium intybus*), narrow-leaved plantain (Plantain; *Plantago lanceolata*), or pasture mixes based on either a short-rotation tetraploid ryegrass (TSR-mix) or long-rotation tetraploid ryegrass (TLR-mix), with both mixes including the same white clover, red clover and chicory.

Liveweight, FEC, FLC, haematological and serum biochemical parameters were measured fortnightly until May 15. All deer were initially treated with an anthelmintic (albendazole), with subsequent trigger treatment being withheld until weight loss or clinical parasitism (parasitic infection showing signs or symptoms that can be recognized) was observed. Anthelmintic trigger treatment (albendazole) was given on an individual animal basis.

There were significant differences in forage chemical composition. The mean LWG was 130 g/day on TLR mix which was similar to the TSR mix (112 g/d) and plantain (109g/d). However, these were significantly higher than LWG on chicory (69 g/d) and the control (45g/d). Mean FEC

differed between forage types and with time ($P<0.0001$), and the interaction between forage type and time was significant ($P<0.05$). Mean FLC differed between sampling dates ($P<0.0001$) and forage types ($P<0.03$). On May 15, 50% of the plantain-grazed deer and 89% of both the TSR-mix and the TLR-mix grazed deer had been trigger treated compared to one or more treatments for both the control-(142%) and the chicory-(142%) grazed deer. Mean serum albumin concentrations differed between deer grazed on different forage treatments ($P<0.001$) and reduced with time ($P<0.0001$). The deer grazing on control pasture and chicory had low and similar overall serum albumin concentrations compared to deer grazed on plantain, TSR-mix and TLR-mix ($P<0.001$). There were no significant relationships between either FLC or FEC and serum total protein, albumin or globulin concentrations.

White blood cell counts (WBC) of deer grazing different forages did not differ significantly but increased with time ($P<0.0001$). The overall proportion of neutrophils in the blood of deer grazing chicory was higher compared with that of deer grazing plantain ($P<0.001$), TSR-mix ($P<0.01$) or TLR-mix ($P<0.02$) but similar to deer grazing control pasture. The eosinophil count of deer grazing all the pasture types increased with time ($P<0.0001$) and the interaction between time and pasture type grazed was significant ($P<0.001$). However, there were no significant relationships between either FLC or FEC and any haematological parameter investigated.

This study has provided preliminary data suggesting that pasture species, either sown as a pure crop or in a pasture mix can influence LWG, resilience to internal parasitism and requirement for anthelmintic use in young farmed deer. However, any potential effects of forage feeding value and anti-parasitic plant compounds of chicory, plantain and pasture-forage mixes on parasitism and growth could not be separated in this study. It is therefore acknowledged that these observations are preliminary and based on a design intended only to establish whether further replicated studies are warranted, particularly with plantain and pasture mixes.

3.2. INTRODUCTION

The cost to the deer industry of control and production losses associated with deer endoparasitism was estimated in 2002 to be approximately $12.8 million annually (Mackintosh & Wilson, 2003). Young deer are most susceptible during their first autumn, when seasonal conditions appear optimal for these parasites (Mason, 1994; Hoskin *et al.,* 1999) and when weaners have a high growth potential (Stevens & Corson, 2003). Small numbers of lung and gastro-intestinal (GI) nematodes can cause sub-clinical infections and can reduce voluntary feed intake (VFI) and liveweight gain (LWG) post-weaning (Hoskin *et al*., 2000). The objective of parasite control in deer should be to prevent clinical disease and reduce sub-clinical effects (Charleston, 2001).

Control of deer parasites is largely by anthelmintic treatment (Audigé *et al.,* 1998; Castillo-Alcala *et al.,* 2007), sometimes combined with grazing management (Wilson 1984a; Haigh *et al.,* 2002). Recent research indicates poor efficacy or development of resistance to new-generation macrocyclic lactone anthelmintics in farmed deer (Hoskin *et al.,* 2005; Chapter 2). In sheep, GI nematodes have been shown to impair animal productivity through reductions in VFI and/or reductions in the efficiency of food use in the gastrointestinal tract (Coop and Kyriazakis, 2001). This is most pronounced for protein, for which a reduced absorption and / or retention in parasitized animals has been shown (Coop and Kyriazakis, 1999) but also the reduced absorption of minerals, especially phosphorus, is also of high significance (Poppi et al., 1990). Therefore, more sustainable alternative control measures are required, such as forages of high nutritive value with anti-parasitic secondary compounds e.g. chicory (*Cichorium intybus*; Hoste *et al.,* 2006; Hoskin *et al.,* 1999; 2000; 2003).

Beneficial effects on host physiology and performance under parasitic challenge have been found with the consumption of plants such as chicory, sulla and *lotus corniculatus* when compared to control herbage not containing known anti-parasitic secondary compounds such as ryegrass, white clover or lucerne in sheep, goats and deer (Niezen *et al.,* 1998; 2002; Hoskin *et al.,* 1999; 2000; 2003; Paolini *et al.,* 2003; Ramırez-Restrepo *et al.,* 2004). Grazing weaner deer on forage chicory during autumn has negated the requirement for anthelmintic treatment in two previous studies (Hoskin *et al.,* 1999; 2003).

In-vitro research has demonstrated that condensed tannins (CT) extracted from forage legumes and sesquiterpene lactones (SL) from chicory have direct inhibitory activity against deer lung and GI nematode larvae (Molan *et al.*, 2000a, b; 2003). It has been suggested that narrow-leaved plantain (*Plantago lanceolata*) has anthelmintic properties (Knight *et al.*, 1996; Gustine *et al.*, 2001). The major constituents in plantain are mucilage, iridoid glycosides (particularly aucubin and catalpol), and CT and it is higher than ryegrass in concentrations of sodium, cobalt, copper, calcium and zinc (Rumball *et al.*, 1997). However, despite recent widespread use of plantain in commercial pasture mixes (Judson, pers comm.), its potential for parasite control or use in systems with low anthelmintic input has not been investigated. Direct comparisons between grazing of chicory, plantain and other forages on internal parasitism and growth of young deer do not appear in the literature. There is also no published information on the potential of pasture mixes containing these bioactive plants for parasite control especially when they constitute only a small proportion of the sward dry matter (DM).

The aim of this preliminary investigation was to compare the effect of grazing permanent perennial ryegrass-based pasture with chicory, narrow-leaved plantain and pasture mixes based on short-rotation tetraploid ryegrass or long-rotation tetraploid ryegrass, with both mixes sown with the same clover (white and red) and chicory, on post-weaning growth and endoparasitism of weaned farmed red deer calves in autumn. The study was conducted principally to provide data to permit evaluation of whether further study of plantain and/or mixed species swards was warranted, since there has been no investigation of these for deer production with low anthelmintic input.

3.3. MATERIALS AND METHODS

3.3.1. Experimental design and animals

The experiment was conducted at Massey University Deer Research Unit, Palmerston North, New Zealand, from 7 March to 15 May, 2006. On 20 February, the animals were weaned, weighed, ear-tagged and vaccinated against clostridial infections (Clostridial 5 in 1; Ultravac CSL Ltd, NZ) and yersiniosis (Yersiniavax; AgVax, AgResearch, NZ), and treated orally with albendazole at 10mg/kg (LW) (Albendazole C®; Ancare, NZ Ltd) based on the live weight of the heaviest animal. Booster vaccinations were given 30 days later.

On 7 March (Day 1), 95 red deer calves comprising 55 stags and 40 hinds sired by the same stag (mean LW ± SD = 37.1 ± 0.44kg) were randomly allocated to five groups (n = 19) each balanced for sex, LW, faecal egg count (FEC) and faecal larval count (FLC) at weaning. A sub-sample of male calves (n=10) from each treatment group was used for blood sampling. Faecal egg count, FLC, LW of all the calves and blood samples from male calves were monitored fortnightly. Subsequent anthelmintic treatment with albendazole, on an individual animal basis, was withheld until the following trigger treatment criteria were achieved: clinical signs of parasitism (coughing, and/ or laboured breathing at rest) observed during daily monitoring, and/or a weight loss of ≥2kg over a 2-week period), together with either FLC exceeding 500/g, and/or FEC exceeding 1000/g). The number and date of anthelmintic trigger treatments given to individual deer in each treatment group was recorded.

Seven castrated adult stags and one weaner stag, accustomed to intensive handling and wearing harnesses were used to monitor faecal output (FO) using two animals per treatment forage to assess the dilution effect of forages on FLC and FEC. Faecal output was determined by collecting fresh faeces using a harnesses and collection bags on the animals for three consecutive 24-hour periods. The bags were changed twice within each 24-hour period. The FO was measured twice per treatment forage during the entire trial period using the same two animals each time, but FO was not measured on the TLR-mix pasture due to inadequate number of animals. At each collection period the animals were accustomed to carrying the bags for 2 to 3 days before the actual collection. The collection periods were March 31 to April 3 (Session 1) and May 1 to 4 (Session 2).

3.3.2. Forages and grazing management

Chicory (*Cichorium intybus* cv. Grasslands Choice) was sown as a pure crop in spring 2004 (1.32ha; 2 paddocks) and 2005 (0.87ha). Plantain (*Plantago lanceolata* cv. Ceres Tonic) was sown as a pure crop in spring 2002 into 1.85ha comprising three paddocks. Permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) was autumn sown with white clover (*Trifolium repens* cv Huia) in 1992 or before, into 1.69ha comprising three paddocks. The pasture mixes, comprising short-rotation tetraploid ryegrass cv. Delish (TSR-mix; nil endophyte) or long-rotation tetraploid ryegrass cv. Banquet (TLR-mix; low endophyte) were sown in spring 2004. Both mixes included the same white clover (*Trifolium repens* cv. Bounty and Kopu II), red clover (*Trifolium pratense* cv. Grasslands Pawera) and chicory (cv. Grasslands Choice). The TSR-mix was sown into 1.74ha comprising four paddocks and the TLR-mix was sown into 1.89ha comprising four paddocks. All the forages were rotationally grazed by hinds and calves and/or yearling deer for a minimum of five months before the trial in an attempt to establish uniform parasite contamination.

Forages were rotationally grazed according to treatment forage with allowances (excluding dead matter) being set at 5kg DM/deer/day. The time animals grazed each paddock based on the herbage allowance was calculated as follows;

$$\text{Total days} = \frac{\text{Herbage mass (kg DM/ha) x total area of paddock (ha)}}{[(\text{Total animals/group}) \times (\text{Pasture DM allowance/deer/day})]} \quad \text{(Equation 3.1)}$$

Therefore, rotation length was 19-23, 35-41, 20-39, 19-37 and 19-28 days with grazing periods of 18-23, 10-23, 6-22, 6- 16 and 5-15 days/paddock for control, chicory, plantain, TLR- and TSR-mix pastures, respectively. Nitrogen fertilizer 50kgN/ha (Urea; 46%N) was applied to all the forage treatments after the first round of grazing.

3.3.3. Forage sampling and measurements

Pre-grazing herbage mass (kg DM/ha) was measured by taking cuts to soil level from eight quadrats ($0.1m^2$) per paddock for DM determination, enabling the calculation of grazing days (Semiadi *et al.*, 1993) according to the allowance set and taking into consideration the proportion of dead matter obtained from botanical composition determination. Post-grazing herbage mass was recorded similarly to estimate intake (Smit *et al.*, 2005), but pasture growth during grazing

was not measured. Samples of herbage (n=17) on offer were taken from each paddock (by taking cuts to soil level) at the start of grazing, mixed, divided into two 200g portions and stored at -20 °C to be used for determination of nutritive value as described in Section 3.3.6. Similar samples (n=17) for botanical composition were dissected into grasses (ryegrass was not distinguished from the other 'weed' grasses), white clover, dead matter, weed for all treatment forages; red clover for control pasture; chicory stem and leaf separately for chicory; plantain stem and leaf separately for plantain; chicory stem and leaf separately and red clover for TLR- and TSR-mix pastures. Each component was separately oven-dried (100 °C for 18 h). Botanical and chemical compositions are presented as means per treatment forage type.

3.3.4. Parasitology

Rectal faecal samples of 6-10g were taken from each animal for FLC and FEC. The modified Baermann (Hendriksen, 1965) and McMaster (Stafford *et al.*, 1994) techniques were used to recover lungworm larvae and GI nematode eggs from fresh faeces for FLC and FEC respectively as described in Section 2.3.3 (Chapter 2). All counts are expressed per gram of fresh faeces.

To measure FO, fresh faeces collected per animal for each 24-hour period were weighed each day during the collection period, mixed thoroughly by hand, and then triplicate samples of 200g were taken and oven dried (90^0C, 24 hrs). The dry samples were weighed and FO calculated on a DM basis per animal, per kg LW for 24 hours. Since forages have different dilution effects on FEC and FLC and thus able to influence the counts. Faecal output/kg LW was determined with two animals per group as described in Section 3.3.1 and these values were then applied to FEC and FLC from all the animals in the group, to correct the figures to a DM basis. Faecal DM, FEC and FLC were adjusted for LW and expressed as the number of eggs and larvae per gram of dried faeces (FECDM and FLCDM, respectively) as described in Heckendorn *et al.* (2006).

Faecal output (DM) g /24h/kg LW

$$= \frac{\text{Total fresh FO/deer/24h (g) x Total dry faecal weight/deer/24h (g)}}{\text{Animal's LW (kg)}}$$

(Equation 3.2)

The FLCDM were expressed using the following equation

$$\text{FLC per gram of dried faeces (FLCDM)} = \frac{\text{FLC lpg of wet faeces}}{\text{FO (g DM/day/kg LW)}} \quad \text{(Equation 3.3)}$$

The FECDM were expressed using the following equation

$$\text{FEC per gram of dried faeces (FECDM)} = \frac{\text{FEC epg of wet faeces}}{\text{FO (g DM/day/kg LW)}} \quad \text{(Equation 3.4)}$$

3.3.5. Blood sampling, serum biochemistry and haematology

Blood samples were taken fortnightly until May 15 using personal physical restraint on the calves for serum biochemistry, and haematological parameters analysis. Sampling procedure and laboratory analysis were done as described in Section 2.3.4 (Chapter 2).

3.3.6. Forage chemical analysis

Herbage samples were pooled per paddock, freeze dried and then ground to pass a 1 mm sieve (Wiley Mill, USA). Samples were analysed to estimate the levels of, organic matter (OM), *in-vitro* OM digestibility (OMD), dry matter (DM), nitrogen (N), hot water-soluble carbohydrates (HWSC), acid and neutral detergent fibre (ADF and NDF), and lignin, according to chemical methods described by McWilliam *et al.* (2004). Pectin was determined by quantitative determination of uronic acids (Blumenkrantz and Asboe-Hansen, 1973). Metabolisable energy (ME) concentration was determined by calculation using equation 3.5

$$\text{ME (MJ/kg DM)} = \text{OM} \times \text{OMD} \times 16.3 \quad \text{(Equation 3.5)}$$

Where OM = fraction of OM in the diet DM

OMD = predicted *in vivo* organic matter digestibility as a fraction of the OM

16.3 = a constant to convert DOMD (Digestible organic matter as a % of the dry matter) to ME allowing for methane and urine energy loss.

3.3.7. Data calculation and statistical analysis

Faecal output was calculated as stated in Equation 3.6 below, using organic matter digestibility (OMD) for each forage as estimated from *in-vitro* digestibility. The FO (g OM) and VFI (g OM/day/kg liveweight) were calculated from the mean of FO (g DM) and OMD.

$$\text{FO (g OM/day)} = \text{FO (g DM g /24h/kg LW)} \times \text{Forage OMD} \qquad \text{(Equation 3.6)}$$

Voluntary feed intake was calculated as;

$$\text{Voluntary feed intake (g OM/day/kg liveweight)} = \frac{\text{FO (g OM/day)}}{\text{1-OMD}} \qquad \text{(Equation 3.7)}$$

Data were analysed using SAS (Statistical Analysis System, version 9.1; SAS Institute Inc., Cary, NC, USA). Liveweight gain was calculated for each 14-day interval. Repeated measures of LW, serum biochemical and haematological parameters were analysed using the MIXED procedure with a linear model that considered the fixed effects of forage type, sampling date and sex, and their interactions, and the random effects of animal and residual error. Liveweight, serum biochemical and haematological parameters measured on 7 March were included in the model as covariates. A compound symmetry error structure was determined as the most appropriate residual covariance structure for repeated measures over time within animals.

Pasture botanical and chemical composition was analysed using MIXED procedure with a linear model that considered the repeated measure effect of forage replicates. Faecal larval count and FEC were analysed with the GENMOD procedure after natural logarithm transformation assuming a Poisson distribution, least squares means are presented as back-transformed means. Partial correlation relationships between traits such as FLC, FEC, FLCDM, FECDM, LWT, LWG serum biochemical and haematological parameters were obtained using multivariate analysis of variance (MANOVA) with a linear model of GLM procedure to produce univariate analyses for each of the dependent variables. Results for total counts of differential WBC as neutrophils, lymphocytes, eosinophils, basophils and monocytes were not presented in the results for failing to attain significance in contrast to results of differential WBC as a proportion of total WBC. Accumulated trigger treatment curves by forage treatment group were estimated using survival analysis with the PHREG procedure. Significance was declared at $P<0.05$.

3.4. RESULTS

3.4.1. Forage botanical composition and herbage mass

The botanical composition (%DM ± SEM) pre-and post-grazing herbage mass (kg DM/ha ± SEM) of all the forages grazed by deer are presented in Table 3.1 and Tables 3.6 and 3.7 in the appendices. The pre-grazed control pasture comprised ryegrass (55%), dead matter (42%), weed (2%), red (0.2%) and white clover (0.8%). The pre-grazed herbage mass of chicory constituted chicory leaf (53%), chicory stem (16%), dead matter (29%), white clover (1%), weed (0.8) and ryegrass (0.5%) while that of plantain constituted plantain leaf (39%), plantain stem (3.2%), white clover (15%), dead matter (33%), weed (7.4) and ryegrass (2%). The constituents of the pre-grazed TLR-mix were ryegrass (21%), chicory leaf (29%), chicory stem (4%) white clover (3%), red clover (6%), dead matter (31%) and weed (6%) while TSR-mix comprised ryegrass (17%), chicory (25%), chicory stem (3%), white clover (4%), red clover (10%), dead matter (33%) and weed (8%).

The pre- and post-grazing herbage mass was different between forages ($P<0.03$; Table 3.1); Control pasture had the higher pre-grazed herbage mass while chicory had the lower post-grazed herbage mass but the interaction between forage type and pre-and post-grazing herbage mass was not significant. Differences between pre- and post-grazing herbage mass were significant for TSR- and TLR-mixes ($P<0.03$ and $P<0.04$ respectively) but not for control pasture, chicory and plantain forages, probably due to differences in herbage growth during the grazing period which was not measured in this trial. There were differences in herbage yield between the forages ($P<0.0001$). Control pasture had a higher herbage yield than all the other forages while the TSR-mix yielded more herbage than chicory ($P<0.0001$), plantain ($P<0.03$) and TLR-mix ($P<0.04$) all of which had similar herbage yields-pre-grazing. However, changes in botanical composition from pre- to post-grazing can indicate diet selection in mixed swards, an increase indicates selection against and a decrease indicates selection for a component of the sward.

Table 3.1: Pre- and post-grazing mean herbage mass (kgDM/ha ± SEM) and mean botanical composition (%DM ± SEM)) of permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (Control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (Chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (Plantain) grazed by the young deer.

Forage type	**Control (n=4)**		**Chicory (n=4)**		**TLR-mix (n=7)**		**TSR-mix (n=7)**		**Plantain (n=4)**	
Grazing	Pre	Post	Pre	Post	Pre	Post	Pre	Post	Pre	Post
Herbage mass	3899 ± 316	3811 ± 297	2247 ± 157	1784 ± 173	2752 ± 178	2105 ± 186	3019 ± 150	2553 ± 147	2323 ± 309	2186 ± 271
Grass	54.8	50.0	0.5	1.0	20.5	26.4	16.7	21.4	2.0	2.6
Chicory leaf	-	-	53.0	42.5	28.7	18.3	24.7	13.7	-	-
Chicory stem	-	-	15.5	23.8	4.3	7.9	3.3	6.6	-	-
Red clover	0.2	0.0	0.0	0.0	6.1	2.7	9.7	3.3	0.0	0.0
White clover	0.8	0.0	1.0	0.0	3.4	3.3	4.3	4.6	15.2	18.0
Plantain leaf	-	-	-	-	-	-	-	-	39.2	27.8
Plantain stem	-	-	-	-	-	-	-	-	3.2	2.6
Weed	2.3	8.0	0.8	2.0	6.3	5.3	7.9	6.6	7.4	12.4
Dead	42.0	42.0	29.3	31.0	30.7	36.1	33.4	43.9	33.0	36.8
SEM	**5.7**	**5.7**	**5.9**	**5.9**	**5.7**	**5.7**	**4.5**	**4.5**	**4.9**	**4.9**

3.4.2. Forage chemical composition

There were significant differences in chemical composition between forages (Table 3.2 below and Table 3.8 in the appendices) except for OM, ME, ADF and HWSC concentrations which were similar. The NDF content of the control pasture was higher than that of the TLR-mix, chicory and plantain ($P<0.001$), but similar to that of the TSR-mix pasture. Control pasture N did not differ significantly from N content of any other pasture. However, plantain N was higher than that of both TLR- and TSR-mix pastures ($P<0.0001$).

Control pasture had lower pectin ($P<0.001$) and higher hemicellulose ($P<0.0001$) concentrations than the other forages. The cellulose content of control pasture was higher than that of plantain, but similar to that of chicory, TLR- and TSR-mixes ($P<0.001$). The ratio of readily fermentable to structural carbohydrates (RFC:SC) was lowest for control pasture, intermediate for both the TLR- and TSR-mixes and highest for chicory and plantain ($P<0.05$). The lignin content of control pasture was lower than that of TLR- and TSR-mixes < chicory < plantain ($P<0.0001$).

Table 3.2: Mean chemical composition (feed on offer; g/kg DM±SEM) of perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain) fed to young deer.

Forage	**Control n=4**	**Chicory n=4**	**TLR-mix n=7**	**TSR-mix n=7**	**Plantain n=4**	**SEM**
Organic matter	82.8	80.8	83.0	84.6	79.4	1.77
ADF	318.5	299.7	313.9	314.9	320.8	19.4
NDF	587.8 ^{a}	387.6 ^{b}	492.4 ^{b}	508.6 $^{a, b}$	462.4 ^{b}	30.4
Nitrogen	24.1 $^{a, b}$	22.2 $^{a, b}$	19.8 ^{b}	21.3 ^{b}	31.7 ^{a}	2.5
HWSC (***a***)	91.4	72.9	95.7	83.1	89.9	11.9
Pectin (***a***)	9.1 ^{c}	75.9 ^{a}	40.9 ^{b}	32.7 ^{b}	47.6 ^{b}	5.0
Hemicellulose (***b***)	269.3 ^{a}	87.9 ^{d}	178.5 $^{b, c}$	193.8 ^{b}	141.6 ^{c}	14.3
Cellulose (***b***)	279.6 ^{a}	230.7 $^{a, b}$	268.9 ^{a}	270.9 ^{a}	215.4 ^{b}	18.6
RFC:SC (***a:b***)	0.2 ^{b}	0.5 ^{a}	0.3 $^{a, b}$	0.3 $^{a, b}$	0.4 ^{a}	0.1
Lignin	38.9 ^{d}	69.0 ^{b}	45.0 ^{c}	43.9 ^{c}	105.4 ^{a}	3.8
ME MJ/kg DM	10.2	10.2	10.4	10.6	10.0	0.2

$^{a, b, c, d}$ Designates significant differences between treatments (P<0.05 or better) within rows

ADF- Acid detergent fibre; **NDF**- Neutral detergent fibre;

HWSC- Water soluble carbohydrates; **ME**- Metabolisable energy (calculated as ME)

RFC:SC – Readily fermentable carbohydrates to structural carbohydrates ratio

3.4.3. Voluntary feed intake and faecal output

Deer mean VFI (kgDM/deer/day) calculated from pre-and post-grazing herbage mass by forage grazed presented as a mean (number of grazing periods in parentheses) of the grazing periods is as follows; control pasture 3.5 (4), chicory 2.5 (4), TLR-mix 4.1 (7), TSR-mix 4.6 (7) and plantain 5.5 (6). Faecal output and VFI (calculated from the FO of the forages grazed by the young deer) are presented in Table 3.3 except for the TLR-mix. Control pasture had lower in-vitro OMD compared with the other forages ($P<0.001$) which had similar *in-vitro* OMD. However, there were no significant differences in FO and VFI (g OM/kg LW) for deer grazing any of the forages.

Table 3.3: Mean *in-vitro* organic matter digestibility (OMD,%DM±SEM), faecal output (FO) and VFI±SEM of the deer grazing perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), including the chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain)

Forage type		**Control n=2**	**Chicory n=2**	**TSR-mix n=2**	**Plantain n=2**
In-vitro OMD		75.8 ± 0.1	78.1 ± 0.7	76.6 ± 0.2	77.3 ± 0.1
FO (g WM/24h/kg LW)	Session 1	30.1	33.0	51.2	38.1
	Session 2	26.6	21.7	27.6	24.7
	Mean	28.4	27.4	39.4	31.4
FO (g DM/24h/kg LW)	Session 1	5.7	6.9	11.4	9.1
	Session 2	6.3	4.7	6	6.3
	Mean	6.0	5.8	8.7	7.7
FO (DM/day/kg LW)	Session 1	18.9	20.9	22.3	23.9
	Session 2	23.7	21.7	21.7	25.5
	Mean	21.3	21.3	22.0	24.7
FO (g OM/day)		4.6 ± 0.3	4.6 ± 0.9	6.7 ± 2.1	6.0 ± 1.1
VFI (g OM/day/kg LW)		18.8 ± 1.0	20.8 ± 3.9	28.6 ± 8.8	26.0 ± 4.6

OMD- Organic matter digestibility; **WM**- Wet matter; **OM**- Organic matter; **FO**- faecal out put; **VFI**- voluntary feed intake; **Session** – Period of faecal collection; Session 1- March 31 to April 3; Session 2- May 1st to 4th

3.4.4. Trigger treatment with anthelmintic

The cumulative proportions of deer being trigger treated from each forage are presented in Figure 3.1. Clinical parasitism was first observed on 11 April in six of 19 deer grazing control pasture and one of 19 deer grazing chicory. On April 24, post-mortem examination of a deer from the chicory group indicated complications related to lungworm infection after trigger treatment was the cause of death. On May 15, one calf from the control group with secondary pneumonia associated with lungworm infections was euthanized.

All deer grazing control pasture and chicory were trigger treated by day 43 (April 19) and 48 (April 24), respectively. By day 48, 17 (89%) of the deer grazing TSR-mix were trigger treated compared to 14 (74%) of those grazing TLR-mix pasture. By day 56 (May 2) 19 (5 repeated; 126%) each of both control- and chicory-grazed deer were trigger treated compared with 17 (89%), 15 (84%) and 5 (28%) of deer grazing TSR-and TLR-mix and plantain, respectively. By day 65 (May 11) 19 (8 repeated; 142%) each of both control- and chicory-grazed deer were trigger treated compared to 18 (95%) and 17 (90%) of the TLR-and TSR-mix-grazed deer, respectively.

Repeat treatments were required in 8 (42%) of animals grazing control pasture and chicory but for animals grazing plantain, TLR- and TSR-mix none required repeat anthelmintic treatment. At the trial conclusion only 9 (50%) of the plantain-grazed deer had been anthelmintic treated.

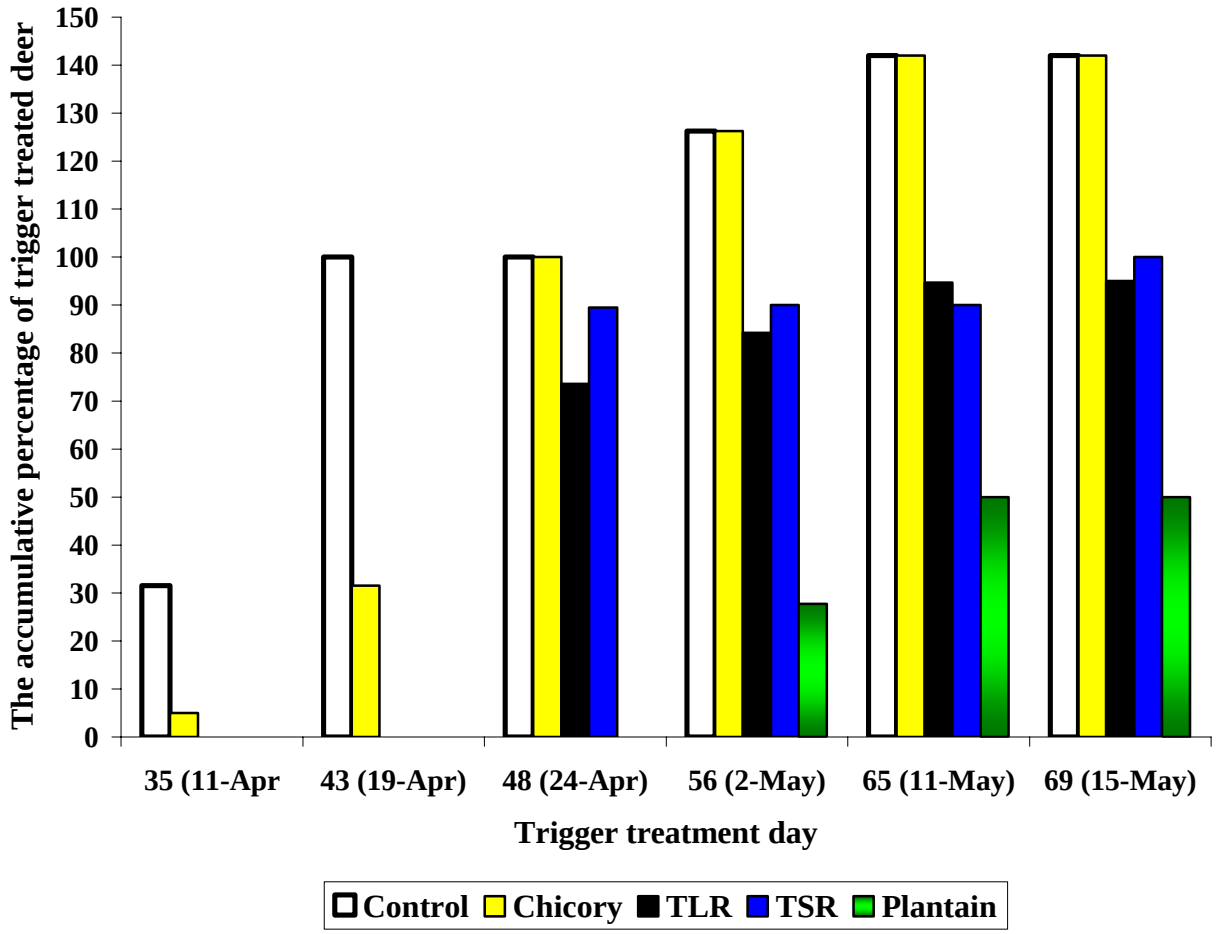

Figure 3.1: The accumulative percentage at each sampling date of young deer receiving trigger anthelmintic treatment and grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Grasslands Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

3.4.5. Deer liveweight gain

Mean (± SEM) LW are shown in Figure 3.2. The mean LWG (g/d) was significantly influenced by forage treatment ($P<0.0001$) and the interaction between forage type and time ($P<0.001$). There was no significant effect of sex or interaction between sex and forage type on LWG. Correlations between LWG or LW and FEC, FLC, FLCDM, FECDM were generally low and non-significant. However, some correlations were significant, despite being low. These include the correlation between LW and FECDM ($r = 0.11$, $P<0.02$) and between LWG and FLC ($r = 0.16$, $P<0.001$); LWG and FEC ($r = 0.12$, $P<0.01$). There was also a significant but low correlation between LWG and FECDM ($r = 0.09$, $P<0.05$) but not between LWG and FLCDM.

Repeated measures analysis showed LWG was similar between pasture groups to day 14 (March 21). Between day 14 and 29 (April 4) mean LWG was lower ($P<0.0001$) for calves grazing control pasture (-173g/d), intermediate for calves grazing plantain (103g/d) and chicory (43g/d) but higher ($P<0.001$) for deer grazing the TSR-mix (216g/d) and TLR-mix pastures (165g/d). However, between day 29 and 43 (April 19), calves grazing plantain were growing at 80g/d while calves grazing the other pastures lost weight (TLR-mix -42, control pasture -95, chicory -117 and TSR-mix pasture 144g/d) ($P<0.05$).

During the final fortnight, LWG of calves grazing control pasture (64g/d) was lowest, intermediate for calves grazing plantain (177g/d) and chicory (173g/d) and highest for those grazing TLR-mix (274g/d) and TSR-mix pastures (244g/d) ($P<0.001$). The mean LWG over the entire trial period was 130g/d on the TLR = 112g/d on the TSR = 109g/d on plantain > 69g/d on chicory > 45g/d on control pasture ($P<0.0001$). The mean final LWT on day 71 (15 May) was 51kg in deer on the TLR = 49kg on the TSR = 49kg on plantain > 45kg on chicory > 42kg on control pasture (Fig 3.2) ($P<0.0001$).

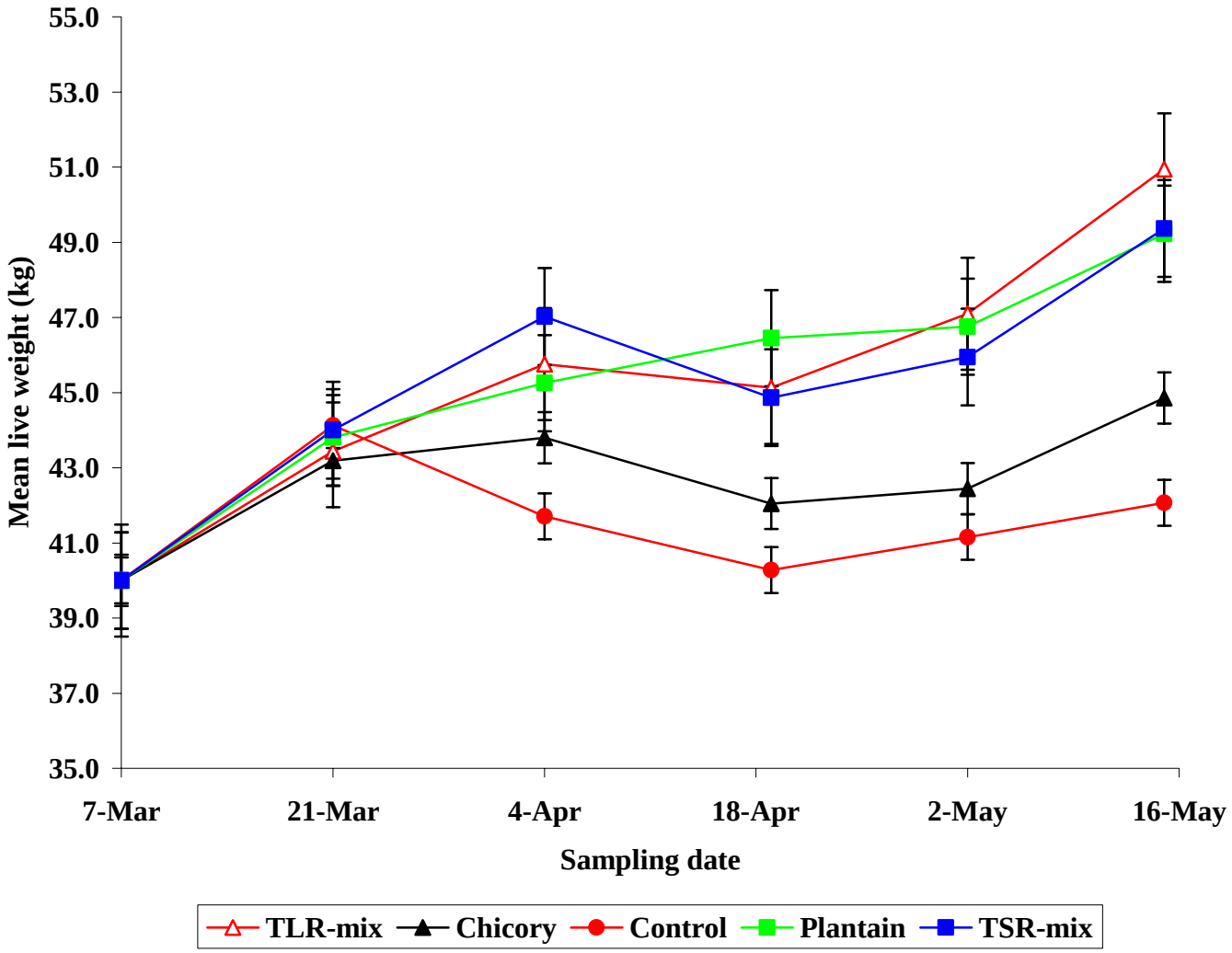

Figure 3.2: Mean (±SEM) liveweight (kg) of young deer trigger treated with anthelmintic and grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

3.4.6. Faecal egg and larval counts

Anthelmintic treatment with albendazole at weaning (Feb 20) reduced both FEC and FLC to zero by the start of the trial (Mar 7) and therefore this data is presented from March 21. Faecal larval and egg count data are presented in Table 3.4 while FLCDM and FECDM data are presented in Table 3.5 of the appendices. Trigger treatment contributed to the changes in mean and range of FLC, FEC, FLCDM and FECDM.

Mean FEC and FECDM peaked in early April for deer grazing all the forages; however, there was another peak on May 15 for deer grazing control pasture. Mean FEC and FECDM differed between forage types and with time ($P<0.0001$), and the interaction between forages type and time was significant ($P<0.05$). The mean and range of FEC and FECDM between 4 and 19 April were higher for calves grazing control pasture, the TSR-mix and chicory, than for calves grazing the TLR-mix and plantain ($P<0.04$). Mean FLC and FLCDM differed significantly between sampling dates ($P<0.0001$) and forage types ($P<0.03$) and there was a significant interaction between pasture type and time ($P<0.01$). The maximum values for FLC and FLCDM were high on April 19 for deer grazing all the forages but after trigger treatment (Section 3.4.4) the reduction was significant. The correlation between LWG or LWT and FLC, FEC, FLCDM, and FECDM is presented in Section 3.4.5. Therefore, FLCDM/FECDM results did not statistically differ from those of FLC/FEC between forage type and time or interaction of both.

Table 3.4: Mean (±SEM) and range of faecal larval counts (FLC, epg) and faecal egg counts (FEC, lpg) per gram of fresh faeces by sampling date of young deer grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

	Sample	**Control**			**Chicory**			**TLR-mix**			**TSR-mix**			**Plantain**		
	date	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**
FLC	**21-Mar**	130 [a, b]	0-305	0.2	90 [b, c]	0-368	0.2	54 [c]	0-425	0.4	231 [a]	0-2123	0.5	87 [b, c]	0-266	0.2
	4-Apr	208 [a]	0-663	0.2	135 [a]	0-477	0.2	127 [a]	0-278	0.2	224 [a]	0-1625	0.4	131 [a]	0-540	0.3
	19-Apr	1097 [a]	0-5400	0.4	662 [a, b]	1-2375	0.3	452 [b, c]	50-1018	0.2	1081[a]	0-5125	0.4	285 [c]	0-978	0.3
	2-May	1 [c]	0-7	0.5	11 [b, c]	0-158	0.9	91 [a, b]	0-1153	0.7	3 [c]	0-11	0.3	292 [a]	3-1035	0.3
	15-May	57 [a]	0-475	0.5	4 [b]	0-24	0.5	4 [b]	0-28	0.5	1 [b]	0-11	0.5	28 [a]	0-255	0.6
FEC	**21-Mar**	176 [a, b]	0-900	0.3	95 [b]	0-300	0.3	94 [b]	0-250	0.2	253 [a]	0-1000	0.3	203 [a]	0-600	0.2
	4-Apr	418 [a]	50-1700	0.3	145 [c]	0-450	0.3	233 [b, c]	0-550	0.1	345 [a, b]	100-1050	0.2	242 [a, b, c]	0-750	0.2
	19-Apr	219 [b]	0-1000	0.4	713 [a]	0-2532	0.3	270 [b]	50-700	0.2	729 [a]	0-4800	0.4	370 [a, b]	50-600	0.1
	2-May	42 [b]	0-300	0.5	35 [b]	0-200	0.5	10 [b]	0-110	0.8	17 [b]	0-100	0.4	284 [a]	0-550	0.2
	15-May	208 [a]	0-2500	0.6	23 [b, c]	0-50	0.3	14 [b, c]	0-150	0.7	12 [c]	0-50	0.4	50 [a, b]	0-333	0.6

[a, b, c, d] Designates significant differences of the mean between treatments (P<0.05 or better) within rows

3.4.7. Serum biochemistry and haematology

3.4.7.1. Serum biochemistry

Mean (±SEM) serum total protein, albumin and globulin are presented in Figures 3.3 to 3.5, respectively. More data (mean and range) on serum total protein albumin, globulin and the AGR by forage treatment and sampling date is presented in Table 3.9 of the appendices.

Serum total protein ranged from 54-100g/litre with a mean of 70.4g/l across forage treatments and measurement occasions. The overall ranges for serum albumin and globulin were 21-39g/l and 22-72g/l with a mean of 29.6 and 40.8g/l, respectively. The mean serum AGR was 0.8 with a range of 0.3-1.6. There was significant correlation between albumin concentrations and LWG ($r = 0.14$, $P<0.04$) and between albumin concentrations and LW ($r = 0.19$, $P<0.005$). Also the correlation between globulin concentrations and LW, but not LWG, was significant ($r = -0.16$, $P<0.02$). However, there were no significant relationships between either FLC or FEC and deer serum total protein, albumin or globulin concentrations.

Serum total protein concentration increased with time ($P<0.0001$). There were no significant main effects of forage type on serum total protein concentration. However, there was an interaction ($P<0.001$) between forage type and time driven largely by fluctuations in total serum protein concentrations of the deer on control pasture and TSR-mix relative to other forages. Serum total protein concentration was similar for all treatment forages on March 7, March 21, April 4 and May 2. However, on April 19, control pasture-grazed deer had lower serum total protein concentrations compared to chicory-($P<0.001$), TLR-mix-($P<0.01$) and plantain-($P<0.05$) grazed deer, but similar to the serum total protein concentration for TSR-mix-grazed deer. On May 15, TSR-mix-grazed deer had the highest serum total protein concentration compared to TLR-mix-($P<0.001$), chicory-($P<0.001$), control pasture-($P<0.01$) and plantain-($P<0.01$) grazed deer, all of which had similar concentrations of serum total protein.

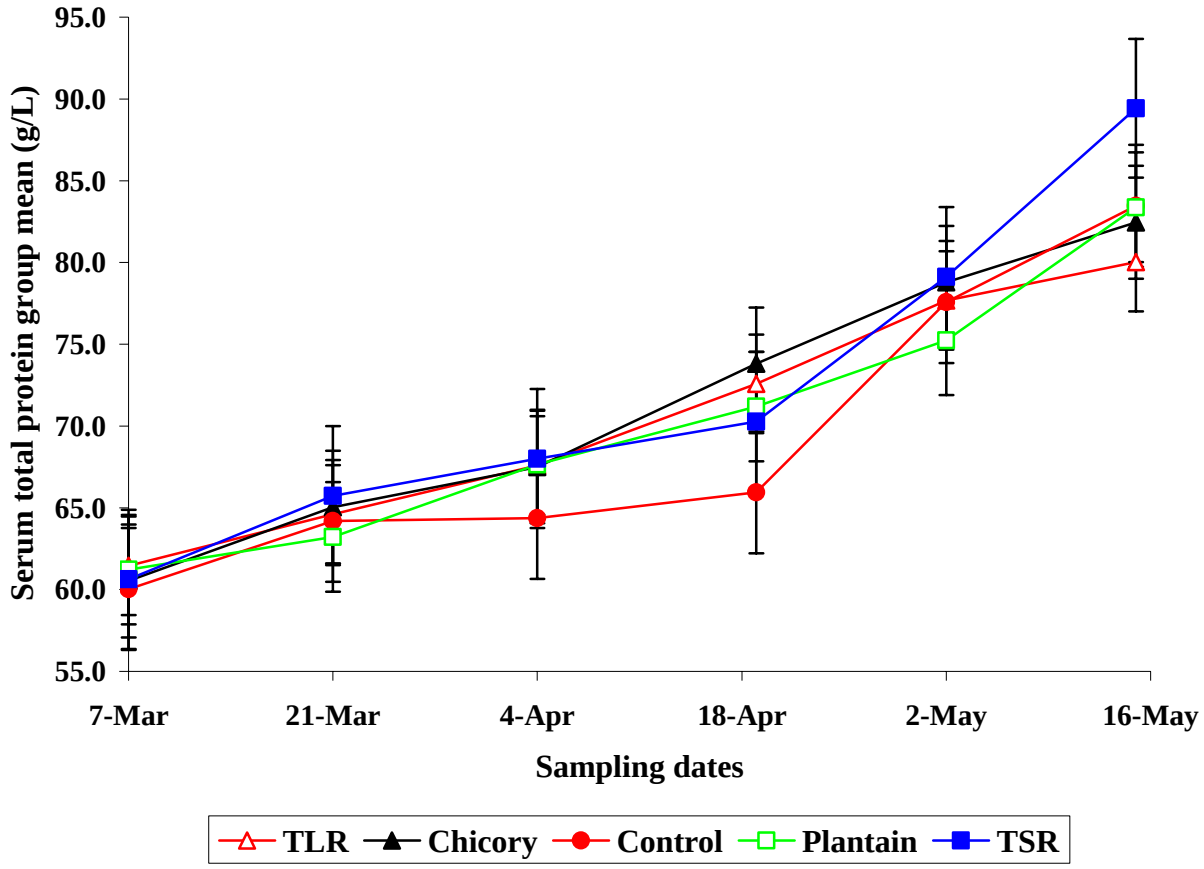

Figure 3.3: Mean (g/l±SEM) serum total protein concentration by sampling dates of stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

Mean serum albumin and globulin concentrations by sampling dates of the stags are presented in Figure 3.4. Mean serum albumin concentrations differed between stags grazed on different forage types ($P<0.001$) and reduced with time ($P<0.0001$) being lower on May 2, compared with March 7, with the converse true for globulin concentrations. The stags grazed on control pasture had low overall serum albumin concentrations compared with stags grazed on plantain ($P<0.0001$), TSR-mix ($P<0.001$) and TLR-mix ($P<0.001$) but was similar to the albumin concentrations of the stags grazed on chicory.

The interaction between forage type and sampling date was significant ($P<0.0001$) for serum albumin concentrations driven by fluctuations in albumin concentrations of stags grazing control pasture, TLR-mix and chicory relative to the other forages. The mean serum albumin concentrations was similar for stags grazing control pasture ($P<0.02$) and TLR-mix ($P<0.01$) but lower compared with stags grazing plantain, chicory was intermediate and the mean serum albumin concentrations of the stags grazing TSR-mix pasture was higher ($P<0.05$) than the mean concentration of the deer grazing TLR-mix pasture.

On April 4 the mean serum albumin concentrations was lower for stags grazing control pasture compared with stags grazing plantain ($P<0.0001$), TSR-mix ($P<0.001$) and TLR-mix ($P<0.01$) but similar to that of stags grazing chicory. Stags grazing plantain had higher mean serum albumin concentrations compared with stags grazing TLR-mix ($P<0.05$) and chicory ($P<0.003$), but similar to those grazing TSR-mix pasture. On April 19, control pasture-grazed stags still had the lowest mean serum albumin concentrations compared with stags grazing other forages ($P<0.0001$) while stags grazing plantain had the highest mean serum albumin concentration compared to stags grazing chicory ($P<0.001$), TSR-mix ($P<0.05$) and TLR-mix pastures ($P<0.05$) which were similar. On May 2, stags grazing control pasture and chicory had similar, but lower mean serum albumin concentrations compared with that of the stags grazing TSR-mix ($P<0.001$), TLR-mix ($P<0.01$) and plantain ($P<0.01$) which were similar. On May 15, the mean serum albumin concentrations were lower for the stags grazing control pasture compared with those of the stags grazing the other forages ($P<0.001$). Stags grazing TLR-mix, plantain and TSR-mix pasture had similar mean serum albumin concentrations, but higher than those of the stags grazing chicory ($P<0.001$).

Mean serum globulin concentrations did not differ significantly between deer grazing different forage types (Fig 3.4). However, the mean globulin concentration increased with time ($P<0.0001$) and there was a significant interaction between forage type and time ($P<0.001$), driven mainly by fluctuations in serum globulin concentrations of stags grazing control pasture, TLR-mix and chicory relative to the other forages. On April 19, stags grazing all forages had similar mean serum globulin concentrations, except for stags grazing chicory who had higher mean serum globulin concentrations compared with stags grazing plantain ($P<0.02$). On May 2, stags grazing control pasture and chicory had higher mean serum globulin concentrations compared with stags grazing plantain ($P<0.05$), which had similar concentrations to stags grazing both pasture-mixes.

On May 15, the mean serum globulin concentrations of the stags grazing control pasture (P<0.001), chicory (P<0.02) and TSR-mix (P<0.0001) were higher than those of the stags grazing TLR-mix pasture, while the mean serum globulin concentrations of the stags grazing TSR-mix was higher than that of the stags grazing chicory (P<0.02) and plantain (P<0.01). The mean serum globulin concentrations of the stags grazing control pasture, chicory or plantain were similar on May 15.

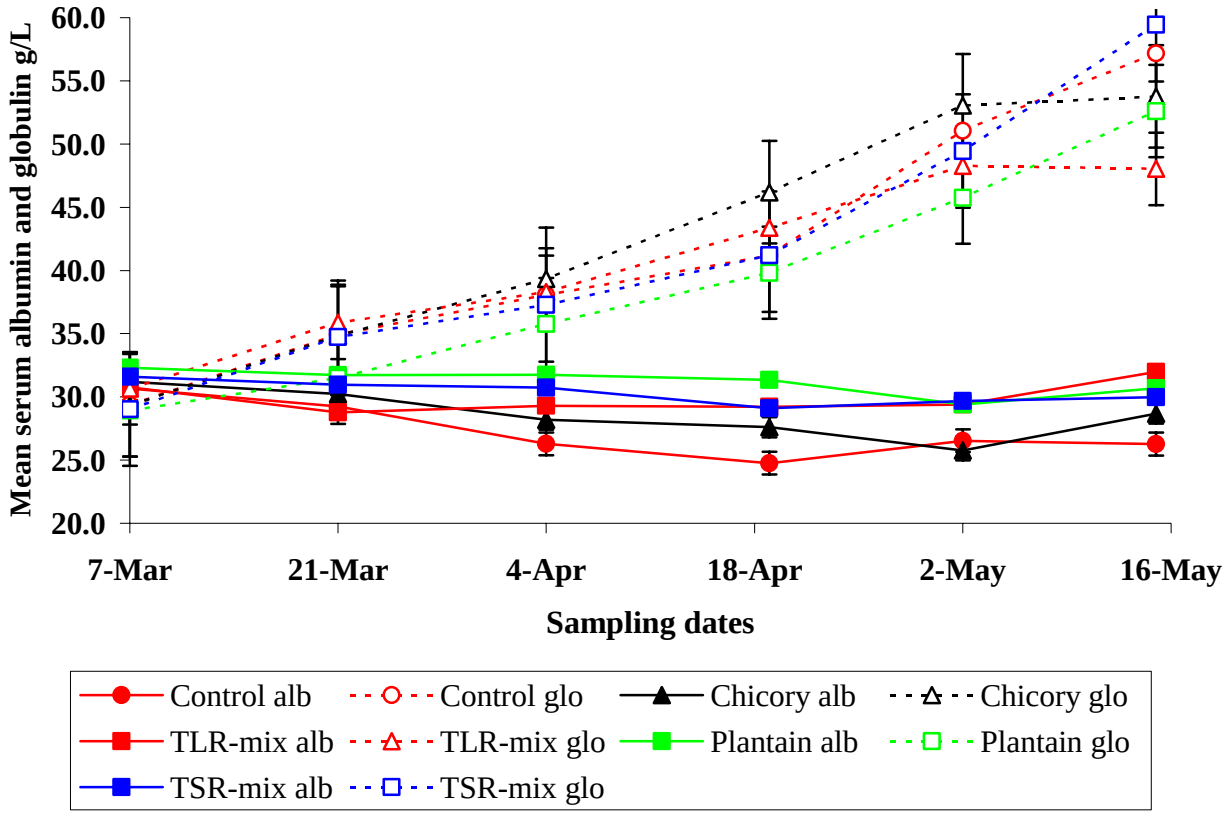

Figure 3.4: Mean (g/l±SEM) serum albumin (alb) and globulin (glo) concentrations by sampling dates of stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

Figure 3.5 presents the mean serum AGR from the stags grazing control pasture, TSR-mix, TLR-mix, chicory or plantain, with stags gazing control pasture or chicory having a lower overall AGR than that of the stags grazing plantain (P<0.01). The serum AGR of stags significantly differed between forage types (P<0.05) and decreased with time (P<0.0001) and the interaction between time and forage type grazed was also significant (P<0.001).

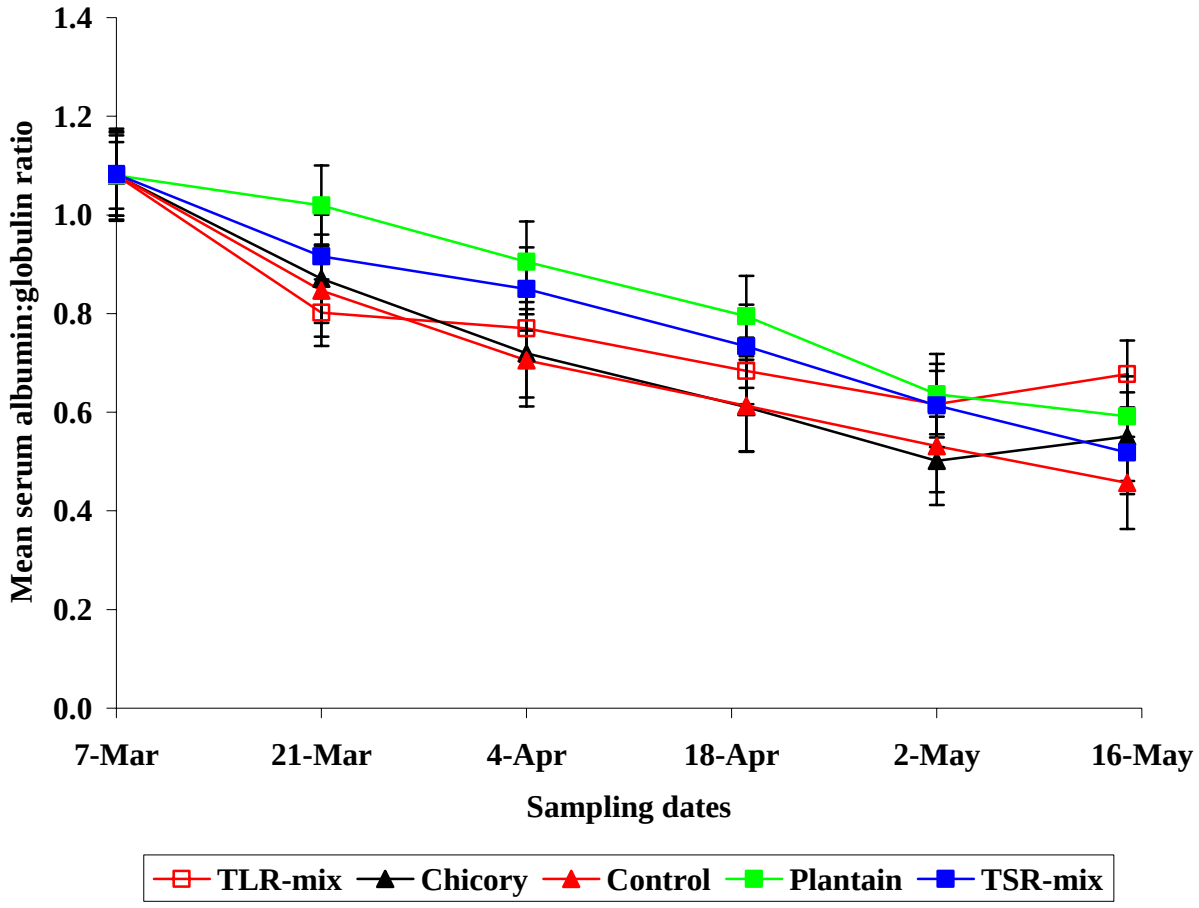

Figure 3.5: Mean (g/l±SEM) serum albumin to globulin ratio (AGR) by sampling dates of stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv. Ceres Tonic) (plantain).

On March 21, April 4 and 19 the AGR was similar and lower for the stags grazing control pasture ($P<0.01$), TLR-mix ($P<0.0001$) or chicory ($P<0.01$) compared with stags grazing plantain and TSR-mix pasture. However, on April 4 the AGR of the stags grazing the TSR-mix was higher compared with stags grazing chicory ($P<0.05$) and control pasture ($P<0.02$). On May 2, stags grazing all the forages had a similar AGR, except for stags grazing plantain which had higher values compared with stags grazing chicory ($P<0.05$). On May 15, the AGR was similar but lower for the stags grazing control ($P<0.0001$), chicory ($P<0.05$), and TSR-mix ($P<0.01$) compared with TLR-mix pasture, whilst stags grazing plantain had similar values to stags grazing chicory, TLR- and TSR-mix pastures, but higher values than stags grazing control pasture ($P<0.05$).

3.4.7.2. Haematology parameters of the stags

Mean red blood cell counts (RBC) were 11.1 x 10^{12}cells/l (range; 1.16-14.72 x 10^{12}cells/litre), mean corpuscular volume (MCV) 33.5fl (range; 28.9-39.5), mean corpuscular haemoglobin concentration (MCHC) 40.9g/dl (range; 30.0-45.7). The mean white blood cell counts (WBC) were 5.6 x 10^{9}cells/l (range; 2.59-11.33 x 10^{9}cells/litre). The mean percentage of neutrophils was 34.4% (range; 5.6-77.6%), lymphocytes 53.1% (range; 13.0-84.0%), eosinophils 2.7% (range; 0.0-10.6%), monocytes 7.2% (range; 0.0-31.6%) and basophils 2.7% (range; 0.0-23.0%). There were no significant relationships between either FLC or FEC and any haematological parameter investigated. Mean (±SEM) data for RBC counts, WBC counts, proportion of neutrophils and eosinophils are presented in Figures 3.6 to 3.9, respectively. More data on the mean of haematological parameters investigated by sampling dates is presented in Table 3.10 of the appendices.

Figure 3.6 presents changes in mean RBC counts of stags by sampling date and forage type grazed. RBC mean counts decreased with time ($P<0.0001$) and there was a significant interaction between time and forage type grazed ($P<0.0001$), with stags grazing control pasture having lower RBC counts on April 19 and May 15, compared with stags grazing the other forages ($P<0.0001$; $P<0.001$, respectively). This reduction in RBC of the stags grazing control pasture coincided with the peaks in the mean of both FEC and FLC (Table 3.4). However, overall there was no significant correlation between FEC, FLC or LWG and RBC

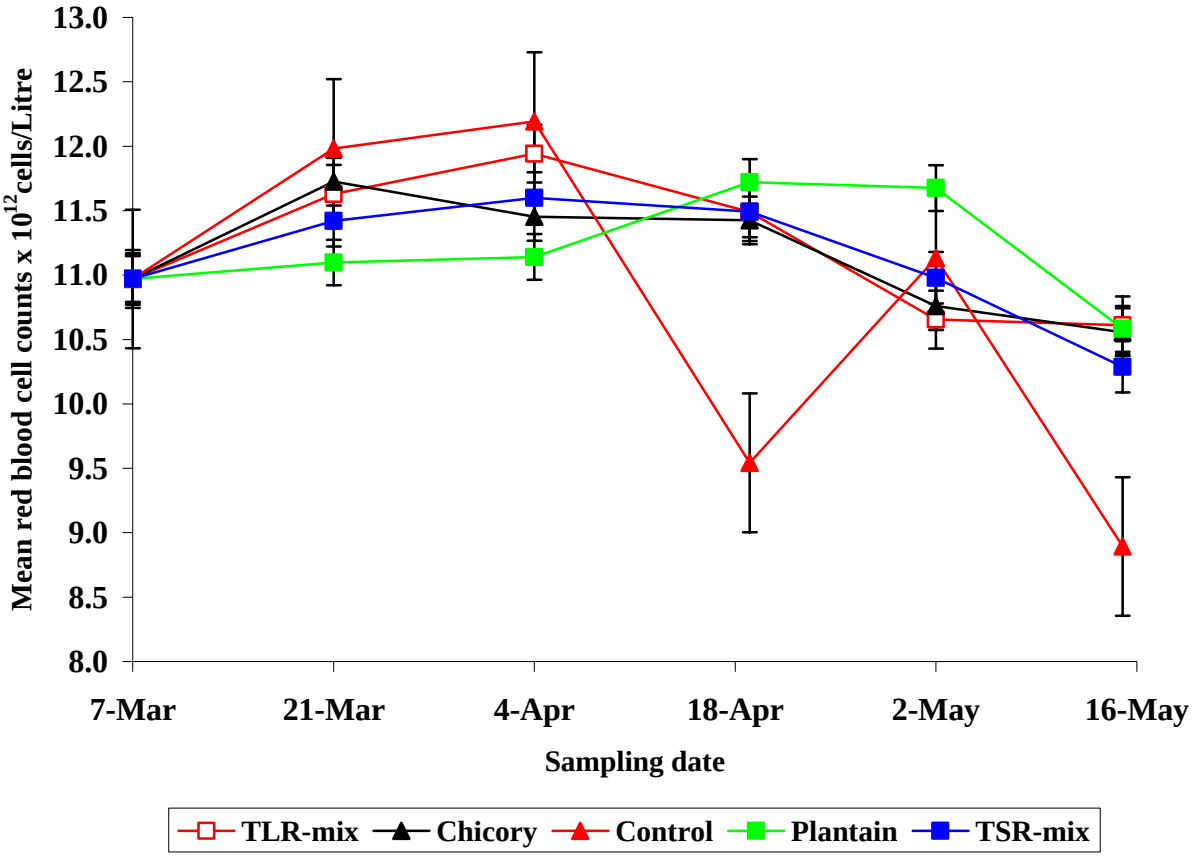

Figure 3.6: Mean (cells/L±SEM) red blood cell (RBC) counts of stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv. Ceres Tonic) (plantain).

Figure 3.7 presents changes in WBC of the stags by sampling date and forage type grazed. WBC counts tended to differ in stags grazing different forages ($P<0.06$) and significantly increased with time ($P<0.0001$). The interaction between forage type grazed and time was significant ($P<0.001$) driven mainly by fluctuations in WBC for stags grazing chicory and TSR-mix relative to the other forages.

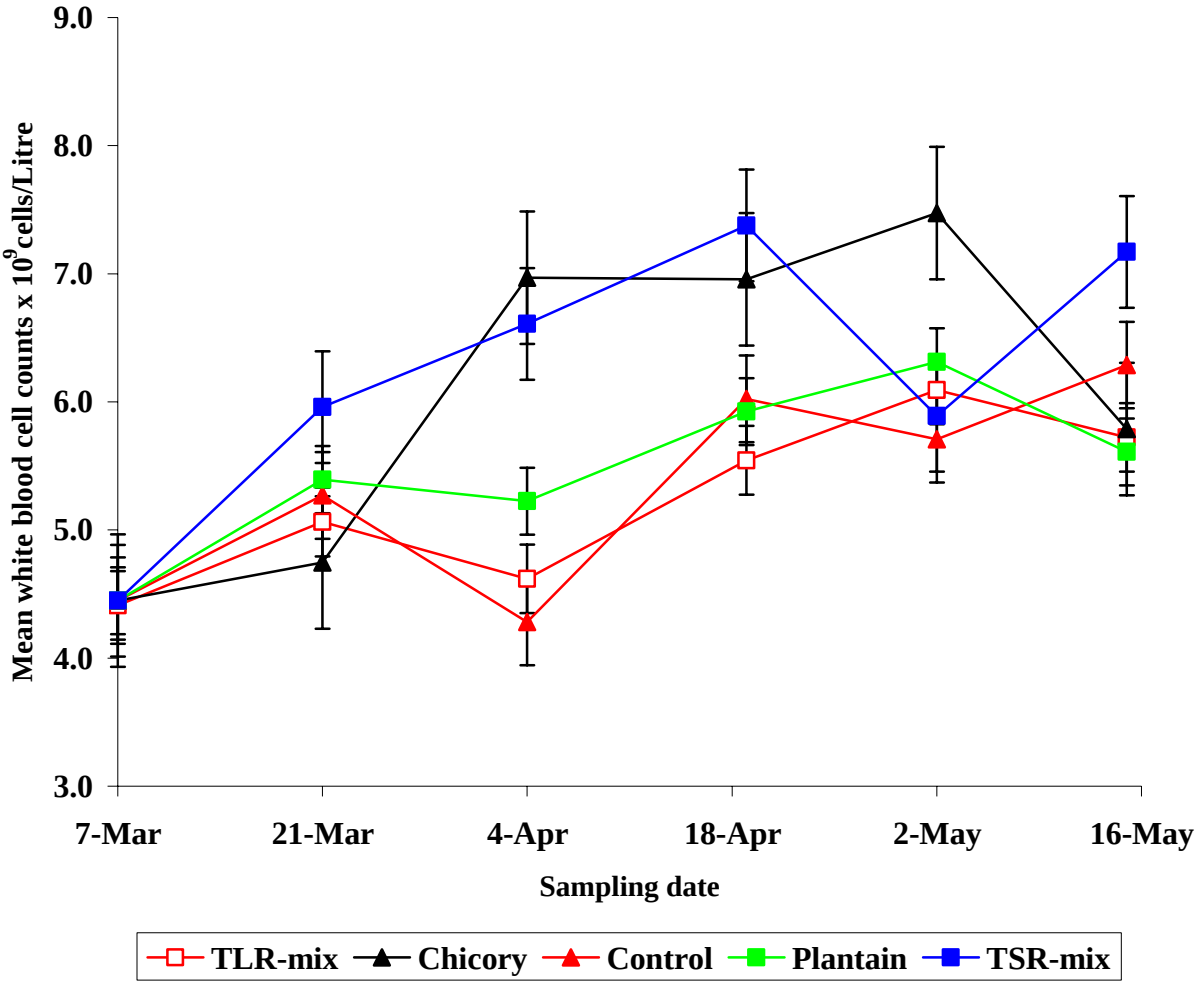

Figure 3.7: Mean (cells/L±SEM) white blood cell (WBC) counts of stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv. Ceres Tonic) (plantain).

Stags grazing control pasture, TLR-mix or plantain had lower WBC counts on April 4 compared with stags grazing chicory ($P<0.0001$), whilst stags grazing TSR-mix had higher mean WBC counts compared with stags grazing TLR-mix ($P<0.001$) or control pasture ($P<0.001$). On April 19, WBC counts for stags grazing TSR-mix were higher ($P<0.05$) than for stags grazing TLR-mix, control pasture and plantain. On May 2, stags grazing control or TSR-mix pasture had lower

WBC counts compared with stags grazing chicory ($P<0.01$), which were similar to stags grazing plantain and TLR-mix. On May 15, stags grazing TSR-mix pasture had the highest WBC counts compared with stags grazing TLR-mix ($P<0.02$), chicory ($P<0.04$) or plantain ($P<0.02$), but similar to those grazing control pasture.

There were no significant main effects of forage type or time for differential WBC counts except for the proportion of neutrophils and eosinophils. More detailed data on differential WBC counts as totals and proportions is presented in Table 3.9 of the appendices.

Figure 3.8 presents mean percentage neutrophils of the stags by forage type grazed and sampling dates. The proportion of neutrophils significantly differed between forage treatments ($P<0.02$), but no significant changes with time were found. The overall proportion of neutrophils in stags grazing chicory was higher compared with that of stags grazing plantain ($P<0.001$), TSR-mix ($P<0.01$) or TLR-mix ($P<0.02$) but similar to stags grazing control pasture.

There was a significant interaction between time and forage type grazed ($P<0.0001$) for the proportion of neutrophils driven by fluctuations of neutrophils in stags grazing all the forages. Stags grazing control pasture had a lower proportion of neutrophils on April 4 compared with stags grazing TLR-mix ($P<0.04$) or chicory ($P<0.001$), whilst stags grazing chicory had a higher proportion of neutrophils than stags grazing TSR-mix or plantain ($P<0.01$). On April 19, the percentage of neutrophils from the blood of stags grazing TLR-mix was lower than that of stags grazing chicory ($P<0.001$), control ($P<0.04$) or TSR-mix ($P<0.02$), whilst stags grazing chicory had a higher proportion of neutrophils compared with stags grazing plantain ($P<0.01$). On May 2, stags grazing control pasture or chicory had higher proportions of neutrophils compared with stags grazing plantain ($P<0.01$), TLR-mix ($P<0.05$) or TSR-mix ($P<0.05$). On May 15, the proportion of neutrophils for stags grazing control pasture was higher than that of stags grazing plantain ($P<0.004$) or TSR-mix ($P<0.02$) and the proportion of neutrophils in stags grazing chicory was higher compared with that of stags grazing plantain ($P<0.05$).

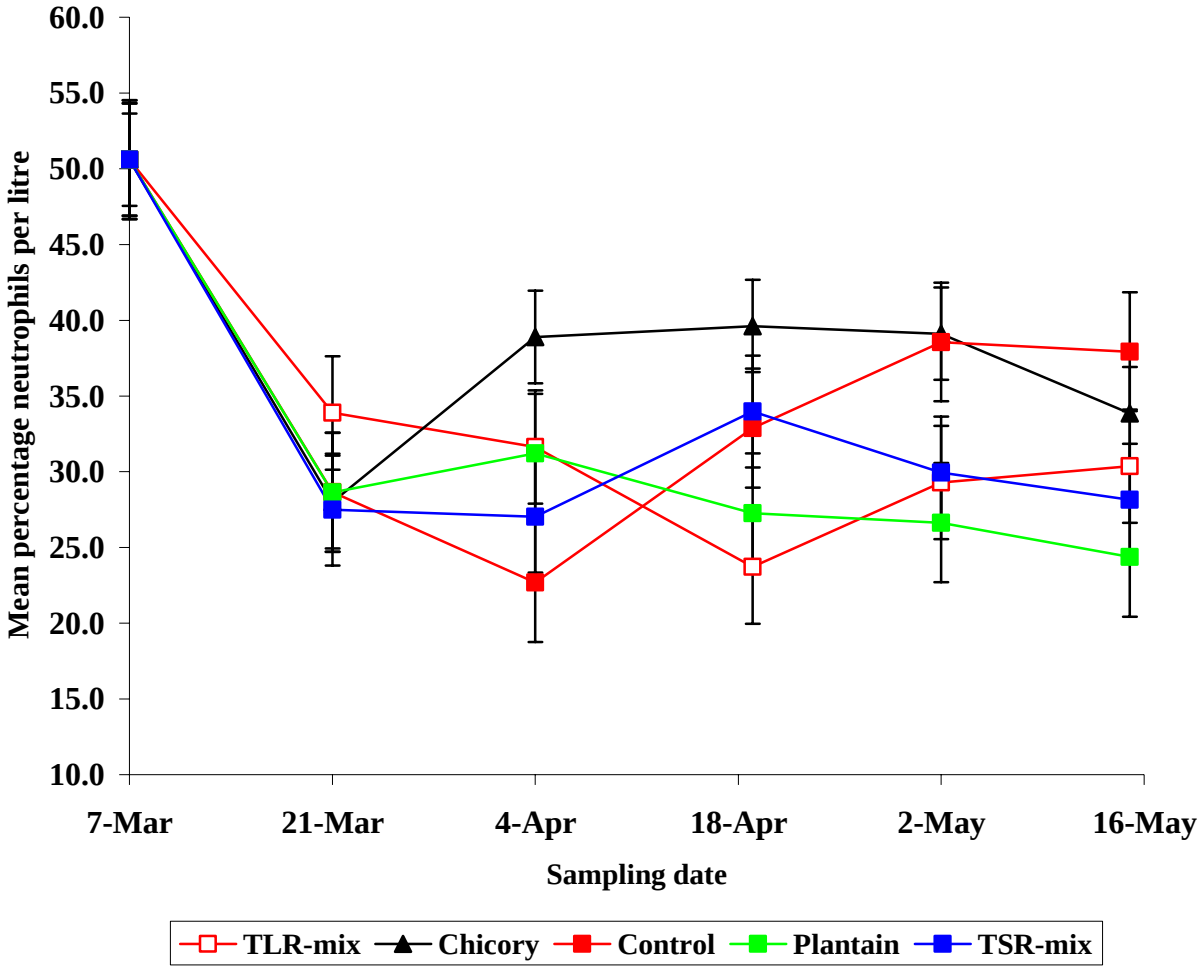

Figure 3.8: Mean (% cells/l±SEM) neutrophils stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv. Ceres Tonic) (plantain).

Figure 3.9 presents the mean proportion of eosinophils in blood of stags by forage type grazed and sampling date. The proportion of eosinophils in stags grazing all the forage types increased with time ($P<0.0001$) and the interaction between time and forage type grazed was significant ($P<0.001$), driven by fluctuations of eosinophils in stags grazing all the forages except for stags grazing TSR-mix.

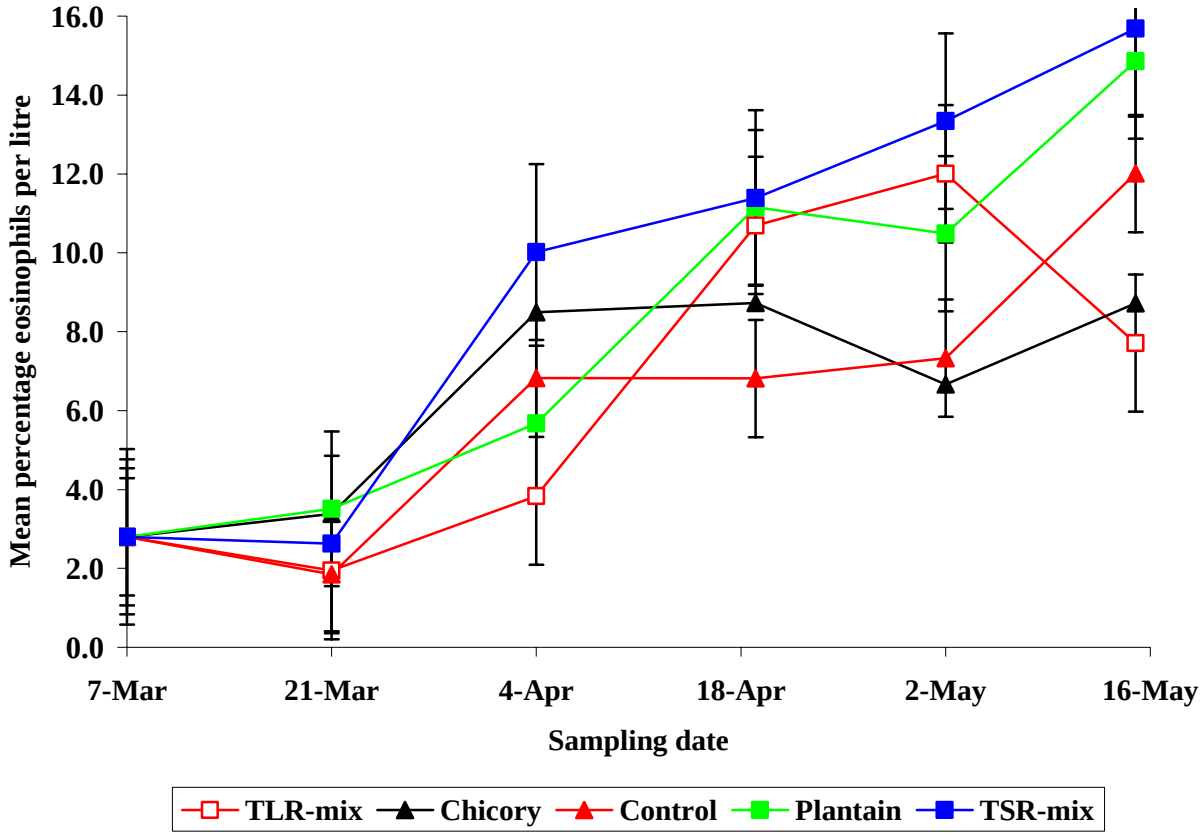

Figure 3.9: Mean (% cells/L±SEM) eosinophils of stags grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

Stags grazing TLR-mix pasture had a lower proportion of eosinophils on April 4 compared with stags grazing TSR-mix pasture ($P<0.003$) or chicory ($P<0.02$). On April 19, stags grazing control pasture had a lower proportion of eosinophils compared with stags grazing plantain or TSR-mix ($P<0.05$). On May 2, stags grazing either control pasture or chicory had lower proportions of eosinophils than stags grazing either TLR-mix or TSR-mix pasture ($P<0.01$). On May 15, the proportion of eosinophils was lower in stags grazing TLR-mix pasture compared with stags grazing control pasture ($P<0.04$), plantain ($P<0.001$) or TSR-mix ($P<0.0001$). Similarly, stags grazing chicory had a lower proportion of eosinophils compared with stags grazing either plantain ($P<0.01$) or TSR-mix pasture ($P<0.02$).

3.5. DISCUSSION

This is the first report of young deer grazed on either plantain or pasture mixes including different forage species under a regime of minimal anthelmintic input. Results suggest that forage species, either sown as a pure crop or in a pasture mix, may influence LWG and resilience to internal parasitism of young deer post-weaning during autumn. This in turn may reduce the requirement for anthelmintic use in young farmed deer grazing these forages. However, the design of this pilot study was limited by physical and financial resources. This prevented additional groups on each forage suppressively treated with anthelmintic as used in the study by Hoskin et *al.* (1999) for chicory and perennial ryegrass-based pasture. Therefore, effects of nutrition and endoparasitism on growth of weaner deer are potentially confounded. Further, for the same reasons, this study was not replicated. Thus, any potential effects of forage feeding value and anti-parasitic plant compounds of chicory (Hoste *et al*, 2006) and plantain (Knight *et al.,* 1996; Gustine *et al.,* 2001) on parasitism and growth also cannot be separated in a scientifically robust manner in this study. It is therefore acknowledged that this study was of an applied nature and these observations are preliminary and based on a design to establish whether further replicated studies are warranted, particularly with plantain and pasture mixes.

However, despite the limitations of the current study, these results are consistent with previous suggestions that narrow-leaved plantain may have anthelmintic properties (Knight *et al.,* 1996; Gustine *et al.,* 2001) and/or higher feeding value compared with diploid perennial ryegrass-based pasture, such as observed previously when fed to lambs in summer (Moorhead *et al.,* 2002) and young deer in autumn (Hoskin *et al.,* 2005). Results of this study suggest that further investigations of both the feeding value of plantain and anti-parasitic effects of secondary compounds found in plantain against internal parasites of ruminants are justified.

Clinical signs of lungworm infection and greater faecal egg counts were evident in deer grazing control pasture and chicory and therefore these animals required earlier and more anthelmintic treatments compared with other forages. In the current study, group mean peak faecal lungworm larval and GI nematode egg counts coincided with lower LWG for deer on control pasture and chicory. The effect of internal parasitism in deer grazing control pasture and chicory may have contributed to low LWG during the trial, culminating in lower overall LW at the end of the trial

in those groups. Whilst trigger anthelmintic treatment was completely effective at eliminating lungworm larvae and gastrointestinal nematode eggs from faeces, the shedding of infective larvae and eggs from the untreated deer in the same group may have contributed to pasture contamination and therefore increased the chances of rapid re-infection.

In two previous studies, young deer grazing chicory vs perennial ryegrass pasture during autumn required no anthelmintic treatment (Hoskin *et al.,* 1999; 2003b). In another trial, grazing chicory reduced the development of deer lungworm larvae to L3 (infective stage) larvae in weaner deer (Schreurs *et al.,* 2002). A recent trial found FEC of lambs from untreated ewes and lambs grazing chicory were significantly lower than in those grazing grass (Athanasiadou *et al.,* 2007). Those studies suggest that pure chicory swards may be a potential tool for increasing resilience and/or resistance to internal parasites in grazing ruminants with reduced anthelmintic input and may result in higher LWG compared with grass-based pastures. However, in the current study, although deer grazing control pasture had lower LWG, and higher FEC and FLC compared to those grazing chicory, deer grazing chicory required anthelmintic treatment and were trigger treated earlier than those grazing plantain, or TLR-and TSR-mixes, due to clinical parasitism and lower LWG.

The difference in requirement for anthelmintic input whilst deer grazed chicory during autumn between previous studies (Hoskin *et al.,* 1999; 2003b) and the current study may be due to a range of factors, either in isolation or in combination, including: seasonal differences in parasite epidemiology and forage botanical and chemical composition; pasture and chicory larval contamination at study commencement; herbage allowance (5 vs 3 kgDM/deer/day); chicory cultivar (Grasslands Puna vs Grasslands Choice) and trigger treatment criteria. There may also be subtle differences between chicory cultivars, since Grasslands Puna was the cultivar grazed in the previous studies (Hoskin *et al.,* 1999; 2003b; Schreurs *et al.,* 2002; Athanasiadou *et al.,* 2007) while Grasslands Choice was grazed in the present trial. But whether the two cultivars have any significant differences in morphology, chemical composition or anti-parasitic compound content affecting stages of the parasite life-cycle has not been investigated. Due to the herbage allowance in the current study being lower than that of the previous studies (Hoskin *et al.,* 1999; 2003b) deer could have grazed to a lower residual DM thus predisposing animals to higher risk of parasitic contamination (larval ingestion).

The level of pasture contamination with parasite larvae in the current study was not measured, but may have also been high relative to these previous studies probably due to constant shedding from the infected animals despite the design (individual trigger treatment) used being the same as that used by Hoskin *et al*. (2003b). It has been previously suggested that animals grazing on chicory may ingest lower numbers of larvae, due to the broad-leaved plant morphology and sward structure of chicory, which may provide a microclimate and physical environment which negatively affects larval development, migration and survival (Moss & Vlassoff, 1993; Marley *et al.,* 2006a, b). The short-acting activity of the anthelmintic used (albendazole) may also have contributed to a faster build up of parasites in the pasture since both FLC and FEC obtained in the present study greatly exceeded the counts observed in the previous studies (Hoskin *et al*. 1999; 2003b). Hoskin *et al.* (1999) used short-acting anthelmintic (ivermectin) with group trigger treatment and Hoskin *et al.* (2003b) used moxidectin with individual trigger treatment. Moxidectin 0.5% pour-on has a claim for minimum persistent activity of 35 to 42 days against re-infection with lungworms (mature and immature) and GI nematodes (Mackintosh *et al.,* 1997; Waldrup *et al.,* 1998).

Most previous studies on forage evaluations to determine feeding value and health (Barry *et al*., 2002) have investigated permanent perennial ryegrass, using diploid ryegrasses such as cultivar Nui that have high levels of aftermath heading, high tiller density, early flowering date and high endophyte and therefore low animal productivity relative to current tetraploid ryegrasses (Woodfield & Easton, 2004). Current tetraploid ryegrasses tend to be of higher nutritive value than diploids of equivalent parentage (Easton *et al.*, 2002). However, the low ratio of RFC:SC and low protein and estimated ME content of forages grazed in this study reflect dryer than normal autumn conditions for the Manawatu (NIWA, 2006) and the partly reproductive status of all forages grazed. In the current trial, differences in herbage growth during the grazing period were not measured, but differences between pre- and post-grazing herbage mass were significant for TLR- and TSR-mix pastures suggesting greater herbage intake compared to the other forages. The outcome of grazing deer on TLR- and TSR-mixes of pasture, although confounded by parasitic effects in the current study, shows the potential value of renewing pastures with modern tetraploid ryegrass species cultivars and/or a high legume/herb component to the mix. However, the relative contribution of the different tetraploid ryegrasses and the other components of the pasture-mix swards such chicory, to the outcomes of the present study is unknown and requires further investigation. In addition, further investigations are important to provide more

information on the performance of deer on pure forage crops compared with pasture-forage mixes especially chicory, which in previous studies as a pure crop, has increased LWG and reduced requirement of anthelmintics in farmed young deer.

The serum biochemistry and haematology values found in the current study are similar to those reported by Wilson and Pauli (1982) for farmed red deer of 3-8 months of age which had the following means; total plasma protein 65.0g/l, corpuscular haemoglobin concentration (MCHC) 36.8g/dl, packed cell volume 45.9% and WBC counts 6.2 x 10^9cells/l. The proportion of differential WBC counts (mean and range) as neutrophils 49.0% (18-90%), eosinophils 2.8% (0-24%), basophils 4.1% (0-21%), lymphocytes 42.9% (8-74%) and monocytes 0.7% (0-7%). In addition, serum total protein, albumin, globulin MCV and MCHC concentrations from the current study are also similar to those reported in other previous studies of red deer (Chapman, 1977; Kent, *et al.*, 1980; Hoskin *et al.*, 1998). Therefore, given the wide range in these parameters it may be difficult to evaluate and interpret changes related to internal parasitic infections from the data in this study.

In a trial with deer trickle infected with lungworm and GI nematode infective larvae, Hoskin et al. (1998) observed elevated pepsinogen and gastrin, reduced serum albumin and elevated serum globulin. Waldrup *et al.* (1994) also found decreased serum albumin with increased serum globulin concentrations in artificially parasitized red deer, and that serum albumin concentration increased with anthelmintic treatment. In the current study, the concentrations of serum albumin were significantly lower for deer grazing control pasture and chicory over time while globulin concentrations were high most of the time. Given that the proportion of deer receiving trigger treatment due to clinical parasitism was high in deer grazing these two forages, it may be worth suggesting that although statistically no relationship between FLC or FEC and serum total protein, albumin or globulin at the individual animal level was found, endoparasitism may have contributed to serum protein loss due to plasma leakage resulting in reduced albumin and a triggered immune response resulting in high concentrations of globulin (Coop & Kyriazakis, 1999). This requires further research. In addition, the relationship between FLC or FEC and serum total protein, albumin or globulin was analysed at the individual animal level. But given the variation between animals in serum proteins and parasitism it is suggested that these parameters be analysed at the group mean level rather than individual animal level.

In sheep it has been found that there is increased loss of N from the body of the host animal either as endogenous proteins (e.g. mucus, plasma leakage or sloughed cells) or excretion as N in urine and faeces (Sykes & Coop, 1976; Macrae, 1993) resulting in partitioning of nutrients to the repair of damaged tissues and the immune response (Coop & Kyriazakis, 1999). If these endogenous N losses are not replaced through commensurate synthesis, the losses are reflected in depression of serum albumin concentration (Steel *et al*., 1980; Vaughan *et al*., 2006). Recent reviews have suggested that many of the pathological changes in the gastrointestinal tract may be undesirable consequences of the immune response, rather than physical damage caused by the parasite *per se* (Hein *et al*., 2001; Colditz, 2002). Greer *et al*. (2005) and Vaughan *et al.* (2006) found corticosteroid-induced immuno-suppression prevented a reduction in serum albumin concentration that was observed in sheep not administered with corticosteroids during infection with *Trichostrongylus colubriformis* alone and/or with *Teladorsagia circumcincta*.

Such studies have not been done in deer and little information is available in literature on protein loss in deer associated with internal parasitism. In addition, data is lacking on what happens to plasma acute phase protein concentrations due to lung damage associated with lungworm infections alone (van Reenen, 1982). In young deer infected with both GI and lung nematodes, less nitrogen is retained (Hoskin, 1998), with a consequent reduction in LWG and carcass weight (Hoskin *et al.,* 1999, 2000). Changes in plasma proteins in the current study may suggest that leakage of plasma proteins does occur in deer due to lungworm and GI nematode infections and therefore further investigations are warranted in farmed deer.

The mean of WBC and RBC counts in the current study were similar to the mean reported by Wilson and Pauli (1982). However, the proportion of the differential WBC counts in the current study was lower for neutrophils (34.4 vs. 53.9%) but higher for monocytes (7.2 vs. 1.1%) and lymphocytes (53.1 vs. 37.2%). In red deer the ratio of neutrophils to lymphocytes increases with increasing age; up to 3 years the ratio is 0.5, whereas in older deer the ratio is 1.4 (Upcott & Herbart, 1965; Chapman, 1977). In the current study the proportion of lymphocytes was greater than that of neutrophils which is similar to the findings reported by Upcott and Herbert (1965) in young deer less than 3 years old, but Wilson and Pauli (1982) showed a reverse trend for young farmed red deer of 3-8 months old. This seems to concur with previous studies that differential WBC counts vary markedly between individuals and both within and between deer species (Perdesen & Pedesen, 1975; Chapman, 1977; English & Lepherd, 1981) and therefore further

investigation of the relevance of haematological parameters as indicators of internal parasitism in farmed deer is necessary.

Results from this study suggest that forage species, either sown as a pure crop or in a pasture mix, may influence LWG and resilience to internal parasitism of young deer post-weaning during autumn. This in turn may reduce the requirement for anthelmintic use in young farmed deer grazing these forages. However, the effects of nutrition and endoparasitism on growth of weaner deer are potentially confounded. Any potential effects of forage feeding value and anti-parasitic plant compounds of chicory and plantain on parasitism and growth also cannot be separated in a scientifically robust manner in this study. However, the study was of an applied nature and these observations are preliminary and based on a design to establish whether further replicated studies are warranted, particularly with plantain and pasture mixes. Therefore, the anthelmintic trigger treatment and LWG data in this study suggest that plantain, TLR- and TSR-mixes may all have a role in aiding control of endoparasitism in young growing deer in autumn with low anthelmintic input in deer production systems based on permanent perennial ryegrass-based pasture. More research into nutritional and parasitological aspects of chicory, plantain and forage mixes for deer and other young ruminants is warranted.

3.6. APPENDICES

Table 3.5: Mean (±SEM) and range of faecal larval counts (FLCDM, lpg) and faecal egg counts (FECDM, epg) per gram of dried faeces by sampling date of young deer grazing either permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv*. Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

	Sample	**Pasture**			**Chicory**			**TLR-mix**			**TSR-mix**			**Plantain**		
	date	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**	**Mean**	**Range**	**SEM**
FLCDM	**21-Mar**	22 [a, b]	0-51	0.2	15 [b, c]	0-63	0.2	6 [c]	0-49	0.4	27 [a]	0-244	0.5	11 [b, c]	0-35	0.2
	4-Apr	35 [a]	0-111	0.2	23 [a]	0-82	0.2	15 [a]	0-32	0.2	26 [a]	0-187	0.4	17 [a]	0-70	0.3
	19-Apr	183 [a]	0-900	0.4	114 [a, b]	0-409	0.3	52 [b, c]	0-117	0.2	124 [a]	0-589	0.3	37 [c]	0-127	0.3
	2-May	0 [c]	0-1	0.4	2 [b, c]	0-26	0.9	10 [a, b]	0-132	0.7	0 [c]	0-1	0.3	38 [a]	0-134	0.4
	15-May	9 [a]	0- 79	0.5	1 [b]	0-4	0.8	0 [b]	0-3	0.4	0 [b]	0-1	0.5	4 [a]	0-33	0.6
FECDM	**21-Mar**	29 [a, b]	0-150	0.3	16 [b]	0-52	0.3	11 [b]	0-29	0.3	29 [a]	0-115	0.3	26 [a]	0-78	0.3
	4-Apr	70 [a]	5-283	0.3	25 [c]	0-78	0.3	27 [b, c]	0-63	0.2	40 [a, b]	11-121	0.2	31 [a, b, c]	0-97	0.2
	19-Apr	36 [b]	0-167	0.4	123 [a]	0-437	0.3	31 [b]	6-80	0.2	84 [a]	0-551	0.4	48 [a, b]	6-78	0.2
	2-May	7 [b]	0-50	0.7	6 [b]	0-34	0.6	1 [b]	0-13	0.7	2 [b]	0-12	0.4	37 [a]	0-71	0.2
	15-May	35 [a]	0-416	0.6	4 [b, c]	0-9)	0.5	2 [b, c]	0-17	0.6	1 [c]	0-6	0.4	6 [a, b]	0-43	0.6

[a, b, c, d] Designates significant differences between treatments (P<0.05 or better) within rows

Table 3.6: Pre-grazing mean herbage mass (kg DM/ha±SEM) and mean botanical composition (%DM±SEM) of permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain) according to paddocks grazed by the young deer.

Forage	Paddock	Herbage mass kg DM/ha	Grass %	Chicory leaf %	Chicory stem %	White clover %	Red clover %	Weed %	Dead %	Plantain leaf %	Plantain stem %
Control	8	5051	46	-	-	2	1	0	52	-	-
	9	3468	62	-	-	0	0	3	35	-	-
	7	2908	65	-	-	0	0	6	29	-	-
Chicory	36A	2295	0	51	18	1	0	1	30	-	-
	36B	2447	0	35	26	3	0	0	36	-	-
	30	2099	2	76	0	0	0	1	21	-	-
TLR-mix	5	2989	8	46	4	3	9	4	27	-	-
	15	2890	46	7	0	3	3	9	34	-	-
	14	3292	19	1	0	6	9	12	53	-	-
	3	2276	6	57	11	1	1	0	24	-	-
TSR-mix	16	3163	17	42	2	5	14	11	10	-	-
	4	3031	8	31	10	6	14	3	29	-	-
	17	3100	20	11	0	5	6	14	46	-	-
	26	2806	19	23	6	4	5	4	42	-	-
Plantain	25	2164	2	-	-	10	0	9	28	50	3
	19	2747	3	-	-	24	0	5	31	35	4
	20	2830	1	-	-	9	0	11	49	26	4

Table 3.7: Post-grazing mean herbage mass (kg DM/ha±SEM) and mean botanical composition (%DM±SEM) of permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain) according to paddocks grazed by the young deer.

Forage	Paddock	Herbage mass kg DM/ha	Grass %	Chicory leaf %	Chicory stem %	White clover %	Red clover %	Weed %	Dead %	Plantain leaf %	Plantain stem %
Pasture	8	3938	48	-	-	0	0	0	52	-	-
	9	3521	36	-	-	0	0	16	48	-	-
	7	2695	68	-	-	0	0	16	16	-	-
Chicory	36A	1846	0	32	33	0	0	2	35	-	-
	36B	2068	0	27	30	0	0	1	42	-	-
	30	1877	4	80	0	0	0	3	13	-	-
TLR-mix	5	1803	22	14	15	4	5	2	40	-	-
	15	2376	53	3	0	2	3	11	30	-	-
	14	3220	21	0	5	7	0	11	56	-	-
	3	841	3	50	8	0	0	0	39	-	-
TSR-mix	16	2295	28	10	2	7	6	4	44	-	-
	4	2198	11	7	1	7	3	8	65	-	-
	17	2800	25	1	6	3	0	17	49	-	-
	26	2442	18	33	12	3	3	3	30	-	-
Plantain	25	1872	0	-	-	9	0	16	42	31	3
	19	2092	6	-	-	25	0	7	29	31	3
	20	2962	2	-	-	22	0	17	43	16	1

Table 3.8: Mean chemical composition (g/kgDM) of permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain) according to paddocks grazed by deer.

Forage	Paddock	Organic matter	Ash	ADF	NDF	CP	HWSC	Pectin	Hemicellulose	Cellulose	RFC:SC	Lignin	Gross energy (kJ/g DM)
Control	8	76.9	15.3	33.1	61.3	15.1	72	8	28.2	29.2	0.1	4.0	17.5
	7	86.3	17.4	32.2	57.7	19.3	68	11	25.5	28.2	0.2	4.0	16.9
	9	85.1	11.6	30.2	57.3	10.8	134	9	27.1	26.5	0.3	3.8	17.7
Chicory	30	79.2	20.8	21.8	28.3	20.6	74	77	6.5	14.7	0.7	7.2	16.7
	36B	79.2	17.3	31.0	40.3	9.3	73	84	9.3	24.0	0.5	7.0	16.7
	36A	84.0	15.0	37.1	47.6	11.6	72	67	10.6	30.6	0.3	6.5	16.7
TLR-mix	5	82.6	16.0	29.8	44.9	12.4	147	57	15.1	25.5	0.5	4.3	17.0
	3	78.7	23.3	33.5	46.0	13.3	66	51	12.4	28.6	0.3	4.9	15.3
	15	86.1	14.7	29.5	49.5	12.6	103	30	20.0	25.5	0.3	4.0	17.1
	14	84.8	12.7	32.7	56.6	11.1	67	25	23.8	27.9	0.2	4.8	17.5
TSR-mix	26	82.7	21.4	38.2	62.2	13.8	62	13	24.0	34.0	0.1	4.2	15.4
	16	86.1	15.3	29.4	49.1	12.8	93	36	19.8	25.4	0.3	4.0	16.9
	17	86.0	14.5	30.3	51.0	14.0	89	28	20.7	25.7	0.3	4.6	17.4
	4	83.8	16.3	28.1	41.1	12.7	89	54	12.9	23.3	0.4	4.8	16.8
Plantain	25	78.6	13.9	33.0	46.8	18.3	74	42	13.8	20.4	0.3	12.6	17.9
	19	83.0	13.9	33.5	48.2	18.8	83	50	14.8	23.0	0.4	10.5	17.9
	20	76.5	16.3	29.8	43.7	22.3	113	51	13.9	21.3	0.5	8.5	17.4

Table 3.9: Mean and range (g/l) of serum total protein, albumin, globulin and albumin to globulin ratio of the male deer grazing permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain).

Forage	**Sampling**	**Total protein g/l**		**Albumin g/l**		**Globulin g/l**		**A:G ratio**	
	date	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**	**Mean**	**Range**
Control	7-Mar	60	55-65	31	28-33	29	26-32	1.1	0.9-1.1
	21-Mar	64	61-72	29	25-31	35	31-42	0.9	0.6-1.0
	4-Apr	63	56-68	26	21-29	37	32-43	0.7	0.5-0.9
	19-Apr	67	63-72	25	23-27	42	37-47	0.6	0.5-0.7
	2-May	77	69-83	27	24-29	50	41-58	0.5	0.4-0.7
	15-May	83	71-98	26	24-30	56	45-72	0.5	0.4-0.7
Chicory	7-Mar	60	57-67	31	29-35	29	23-36	1.1	0.8-1.5
	21-Mar	65	61-73	30	27-27	35	27-42	0.9	0.7-1.3
	4-Apr	67	63-73	28	26-29	39	34-44	0.7	0.6-0.9
	19-Apr	74	62-81	28	24-31	46	38-50	0.6	0.6-0.7
	2-May	77	66-91	26	21-30	51	41-70	0.5	0.3-0.7
	15-May	81	72-95	29	26-31	53	42-68	0.6	0.4-0.7
TLR-mix	7-Mar	62	58-67	31	29-34	31	26-35	1.0	0.8-1.3
	21-Mar	65	61-70	29	26-32	36	33-43	0.8	0.6-0.9
	4-Apr	68	63-74	29	26-33	38	35-44	0.8	0.6-0.9
	19-Apr	73	69-79	29	25-33	44	39-50	0.7	0.5-0.8
	2-May	78	70-91	29	25-33	49	39-59	0.6	0.5-0.9
	15-May	80	69-88	32	28-34	48	37-56	0.7	0.5-0.9
TSR-mix	7-Mar	60	54-65	32	29-35	29	22-33	1.1	0.9-1.5
	21-Mar	65	61-71	31	27-34	34	28-42	0.9	0.7-1.1
	4-Apr	68	59-73	31	27-35	37	27-43	0.9	0.7-1.2
	19-Apr	72	62-78	30	25-33	42	31-48	0.7	0.5-1.0
	2-May	79	66-86	30	25-34	49	36-61	0.6	0.4-0.8
	15-May	89	69-100	30	27-34	59	37-70	0.5	0.4-0.9
Plantain	7-Mar	61	57-64	32	27-37	29	23-33	1.1	0.9-1.6
	21-Mar	63	58-67	32	27-38	31	26-34	1.0	0.9-1.5
	4-Apr	68	61-73	32	28-37	36	27-41	0.9	0.8-1.4
	19-Apr	71	62-75	31	27-39	39	35-44	0.8	0.7-1.1
	2-May	75	69-85	30	27-36	46	42-55	0.7	0.6-0.8
	15-May	82	73-100	31	25-37	51	40-72	0.6	0.4-0.9
All Deer		**71**	**54-100**	**30**	**21-39**	**41**	**22-72**	**0.8**	**0.3-1.6**

Table 3.10: Mean of hematological parameters of the male deer grazing permanent perennial ryegrass (*Lolium perenne* cv. Grasslands Nui) pasture (control), a long-rotation tetraploid ryegrass (cv. Banquet) pasture mix (TLR-mix) and short-rotation tetraploid ryegrass (cv. Delish) pasture mix (TSR-mix), with both pasture mixes including the same chicory (*Cichorium intybus* cv. Choice), red (*Trifolium pratense cv.* Grasslands Pawera) and white clover (*Trifolium repens cv.* Bounty and Kopu II), pure chicory (cv Choice) (chicory) and narrow-leaved plantain (*Plantago lanceolata* cv Ceres Tonic) (plantain) according to sampling dates.

	Sample date	**WBC x 10^9 cells/l**	**RBC x 10^{12} cells/l**	**HGB g/l**	**HCT l/l**	**MCV fl**	**MCHC g/l**	**PLT** x 10^9 cells/l	**MPV fl**	**Neut %**	**Neut** ($x10^9$/l)	**Lymp %**	**Lymp** ($x10^9$/l)	**Mono %**	**Mono** ($x10^9$/l)	**Eos %**	**Eos** ($x10^9$/l)	**Baso %**	**Baso** ($x10^9$/l)
Control	7-Mar	4.1	10.9	137	0.4	34.7	402	420	4.9	47.9	2.0	46.7	1.9	2.5	0.1	1.7	0.1	1.1	0.0
	21-Mar	5.2	12.0	166	0.4	33.3	417	428	5.2	28.7	1.5	64.1	3.3	2.8	0.1	2.0	0.1	2.3	0.1
	4-Apr	4.2	12.4	165	0.4	32.4	411	475	5.3	23.4	1.0	63.6	2.7	3.7	0.2	6.6	0.3	2.5	0.1
	19-Apr	5.9	10.1	133	0.3	33.0	402	532	5.3	35.7	2.1	52.8	3.1	2.2	0.1	7.2	0.4	3.8	0.2
	2-May	5.5	11.1	154	0.4	34.3	403	395	4.5	36.6	2.1	48.9	2.6	4.2	0.2	8.0	0.4	3.0	0.2
	15-May	6.3	8.9	133	0.3	34.7	433	430	4.8	37.4	2.4	45.8	2.9	2.4	0.2	11.7	0.7	3.1	0.2
Chicory	7-Mar	4.3	11.3	156	0.4	34.1	405	330	4.9	52.2	2.3	41.3	1.7	2.2	0.1	3.4	0.3	0.6	0.0
	21-Mar	4.8	11.6	159	0.4	33.1	414	393	5.1	25.3	1.3	66.3	3.1	2.4	0.7	3.1	0.2	2.8	0.1
	4-Apr	6.6	11.4	154	0.4	32.7	415	358	5.4	37.7	2.6	48.8	3.1	2.2	0.1	8.4	0.6	4.7	0.2
	19-Apr	6.8	11.7	154	0.4	32.7	404	393	5.3	45.3	3.3	41.5	2.7	3.0	0.2	6.6	0.4	3.4	0.2
	2-May	6.9	10.4	136	0.3	32.4	404	350	5.9	35.2	2.5	51.8	3.5	2.4	0.2	7.9	0.5	2.5	0.2
	15-May	5.4	10.3	149	0.4	34.0	426	296	5.0	32.2	1.8	52.2	2.8	2.2	0.1	10.7	0.6	2.5	0.1
TLR-mix	7-Mar	4.6	10.9	146	0.4	33.7	400	404	5.1	53.7	2.6	39.4	1.7	2.5	0.1	3.2	0.2	1.1	0.1
	21-Mar	5.2	11.6	155	0.4	32.6	410	473	5.1	34.8	1.8	58.1	3.0	2.3	0.1	2.0	0.1	2.6	0.1
	4-Apr	4.6	11.9	157	0.4	32.4	406	410	4.9	31.8	1.5	58.2	2.2	3.3	0.2	3.7	0.2	3.3	0.2
	19-Apr	5.5	11.6	150	0.7	32.5	402	365	5.4	26.4	1.5	58.2	3.2	2.7	0.1	10.1	0.6	3.7	0.2
	2-May	6.2	10.5	137	0.3	32.4	403	443	5.1	30.9	1.9	51.8	3.2	3.2	0.2	11.5	0.8	4.1	0.3
	15-May	5.7	10.5	156	0.4	35.2	408	351	5.2	29.7	1.7	56.5	3.2	2.9	0.2	8.0	0.5	2.8	0.2
TSR-mix	7-Mar	5.1	9.9	149	0.4	30.7	363	401	5.0	48.2	2.5	45.8	2.4	2.1	0.1	2.8	0.1	1.1	0.1
	21-Mar	5.9	11.3	154	0.4	33.3	411	409	5.3	27.6	1.7	64.8	3.8	3.0	0.2	2.2	0.1	2.8	0.2
	4-Apr	6.6	11.7	159	0.4	33.0	411	363	4.8	27.3	1.8	58.4	3.9	2.7	0.2	9.2	0.6	3.3	0.2
	19-Apr	7.2	12.1	159	0.4	33.0	400	417	5.2	36.4	2.6	48.1	3.5	2.6	0.2	10.3	0.8	3.3	0.2
	2-May	5.9	10.9	145	0.4	32.8	407	455	5.8	29.3	1.7	51.8	3.1	2.9	0.2	13.6	0.8	2.8	0.2
	15-May	7.2	10.2	150	0.4	34.9	422	384	6.0	27.4	1.8	52.0	3.8	2.3	0.2	16.0	1.2	3.1	0.2
Plantain	7-Mar	4.3	11.4	158	0.4	34.6	402	427	4.8	51.4	2.3	39.7	1.7	2.2	3.1	2.7	0.1	0.9	0.0
	21-Mar	5.4	11.1	152	0.4	33.6	411	527	5.5	28.6	1.6	61.8	3.3	2.9	0.2	3.5	0.2	3.1	0.2
	4-Apr	5.4	11.5	158	0.4	33.2	417	470	6.0	30.5	1.7	56.4	3.2	2.9	0.2	7.2	0.4	2.7	0.1
	19-Apr	5.7	11.8	161	0.4	33.7	403	515	5.6	28.0	1.6	55.6	3.2	2.8	0.2	10.5	0.6	3.0	0.2
	2-May	6.3	11.8	163	0.4	33.9	408	426	5.7	27.1	1.7	55.9	3.5	3.5	0.2	10.3	0.7	3.0	0.2
	15-May	5.5	10.6	161	0.4	35.8	423	448	5.1	23.2	1.2	56.0	3.0	2.8	0.1	15.5	0.9	2.5	0.2

CHAPTER 4:

GENERAL DISCUSSION

4.1. Introduction

This thesis presents two studies of internal parasitism in farmed deer. The first study (Chapter 2) investigated the influence of some management practices including weaning date and anthelmintic treatment on internal parasitism and growth of weaners and reproduction in hinds. The second study (Chapter 3) was a preliminary comparative evaluation of the effect of forage species and combinations on internal parasitism and growth in young farmed deer.

Data in Chapter 2 has shown that pre-rut weaning date and sub-clinical parasitism during summer and early autumn can influence LWG in young farmed deer. The trigger treatment and LWG data in Chapter 3 suggest that plantain, TLR-and TSR-mixes and chicory may have a role in aiding control of internal parasitism in young deer in autumn. The outcome in Chapter 3 has application to low anthelmintic input in deer production systems. Serum protein and haematological parameters investigated in both studies (Chapter 2 and 3) have demonstrated the need for further research to establish diagnostic markers for both sub-clinical and clinical internal parasitism in farmed young deer.

This discussion focuses on the main findings from both trials; namely, the impact of weaning date on deer production including growth of deer calves and reproduction in hinds, effects of internal parasitism, indicators of internal parasitism and the, effect of forage species and combinations on internal parasitism and growth in young farmed deer. It also contains recommendations for future research.

4.2. Indicators of internal parasitism

In both trials, there were significant fluctuations in serum biochemical and haematological parameters over time in parasitized deer according to anthelmintic treatment offered (Chapter 2) and forage type grazed by deer (Chapter 3). The data in this study has also shown that LWG of the young deer calves was reduced by both sub-clinical (Chapter 2) and clinical parasitism (Chapter 3). Therefore reduced LWG may have been indicative of sub-clinical parasite infection for Chapter 2 and associated with sub-clinical parasitism on a group basis for Chapter 3 (due to the confounding effects of forage feeding value and parasitism on the growth of the deer calves). This highlights the importance of regular monitoring of weaner deer LWG to increase the probability of early detection of growth limiting factors such as sub-clinical parasitism.

4.2.1. Diagnostic markers

The use of serum proteins and haematological parameters as diagnostic markers for sub-clinical (Chapter 2) and clinical parasitism (Chapter 3) was investigated. There were no strong correlations or statistically significant relationships between serum biochemistry and haematological parameters (i.e., RBC, WBC, neutrophils, and eosinophils) and FLC or FEC found in both studies. However, serum albumin concentrations were high in anthelmintic-treated calves while serum globulin levels were lower in the same calves and the converse was observed for the untreated controls (Chapter 2), which is in agreement with previous studies. In a trickle infection trial (Hoskin, 1998), deer were artificially infected with lungworm and GI nematode infective larvae, and elevated pepsinogen and gastrin, reduced serum albumin and elevated serum globulin were observed. Waldrup *et al.* (1994) also reported decreased serum albumin and increased serum total protein in parasitized red deer, and that serum albumin increased with anthelmintic treatment.

Chapter 3 data also shows significant reductions in serum albumin and elevated serum globulin and fluctuations and differences in haematological parameters with time and between parasitized deer grazing different forage treatments. In deer grazing control pasture and chicory these changes coincided with the peak FLC and FEC. Therefore the reduced serum albumin and elevated globulin in the parasitized deer may be used as markers to aid in the diagnosis of sub-clinical and clinical endoparasitism in young deer. However, given the wide range in these parameters it may be difficult to evaluate and interpret changes related to internal parasitic

infections from the data in this study. Therefore, trickle infection trials under controlled conditions are required to determine reference mean and range of serum albumin and globulin concentrations as well as for haematological parameters. In addition, the relationship between FLC or FEC and serum total protein, albumin or globulin was analysed at the individual animal level. But given the variation between animals in serum proteins, haematological parameters and parasitism it is suggested that these parameters also be analysed at the group mean level rather than individual animal level (Audige *et al.*, 1998a).

These results suggest reduction of serum albumin in the control calves may be occurring due to nematode infection. The reduction in serum globulin may be explained at least in part by a reduction in immunoglobulins, although these were not directly measured in this study. In Chapter 3, changes with time in serum proteins and haematological parameters were more significant in the deer grazing control pasture and chicory which received most trigger treatment (142%) due to clinical parasitism. The relationship between FEC and FLC and actual worm count is not well researched. In addition, the relationships between serum biochemical and haematological parameters and FEC may not be significant because the pathogenic stage tends to be the immature stage of the GI nematodes (Mason, 1994) which do not lay eggs.

This study has yielded some information about the potential for using serum biochemistry such as the reduced serum albumin and elevated serum globulin in parasitized deer and haematological parameters such as fluctuations and differences in WBC counts, neutrophils and eosinophils to aid in diagnosing internal parasitism in young deer. However, only little information on serum biochemistry and/or haematological parameters relationship with internal parasitism of young farmed deer appear in literature. These parameters are also subject to large variation between individual animals. Therefore, trickle infection trials, under controlled conditions, using lungworm and GI nematode infective larvae to infect deer may be required to validate any relationship between serum proteins or haematological parameters with FLC, FEC and actual worm count. However, due to large animal to animal variation in these parameters, large numbers of animals would be required for such validation experiments.

4.2.2. Effects of internal parasitism on deer growth and reproduction

The FLC and FEC in both trials from calves are indicative of the susceptibility of young deer calves to internal parasitism in summer (Chapter 2) and autumn (Chapter 3) when both lungworm and GI parasite larval availability is maximised on pasture due to favourable conditions (Mason, 1994; Charleston, 2001). These studies have demonstrated that sub-clinical and clinical parasitism can result in LWG depression in young deer (Chapter 2 & 3, respectively). The interaction between anthelmintic treatment and weaning date may have significantly influenced the higher LWG for deer calves treated and weaned in March (Chapter 2). Clinical parasitism occurring in young deer calves grazing permanent ryegrass, chicory, plantain, TLR- and TSR-mixes during the autumn (Chapter 3) resulted in reduced LWG. In addition, the timing of elevation in FLC and FEC (with low but significant correlations) corresponded with the reduction in LWG for the deer grazing control pasture and chicory, suggesting an effect of internal parasitism on LWG. These findings are consistent with previous studies that demonstrated that internal parasitism reduces VFI and LWG (Hoskin *et al.,* 1999; 2000; 2003b). Therefore, reduction in LWG associated with endoparasitism has a potential marked effect on the ability of young deer to reach slaughter weight by one year of age (Hoskin *et al.,* 1999), which economically is not viable in a venison production system.

Further, the findings suggest that internal parasitism in young farmed deer can be managed through regular anthelmintic treatment (Chapter 2) and grazing specialised forages and/or pasture-forage mixes (Chapter 3). However, the former would be contingent upon anthelmintic efficacy. The failure of moxidectin to reduce FEC to zero (Chapter 2) could be interpreted as sub-optimal efficacy and/or anthelmintic resistance (Charleston & McKenna, 2002; Hoskin *et al*., 2005b). Given that a recent survey has shown that moxidectin pour-on was the most commonly used anthelmintic for weaner deer (Castillo-Alcala *et al.*, 2007) based on the belief that it was the most effective anthelmintic, apparent inefficacy or emergence of nematode anthelmintic resistance in deer GI nematodes should be a concern since this may result in major loss of production in young farmed deer in future.

4.3. Impact of weaning date on deer growth and reproduction

The study objective in Chapter 2 was to examine the impact of early or late pre-rut weaning with and without anthelmintic treatment on growth of calves, and weaning date effects on hind reproductive parameters (i.e., conception date and rate).

4.3.1. Growth of deer calves

This is the first research into the impact of pre-rut weaning date on parasitism and LWG of deer calves (Chapter 2). Deer calves weaned in March had higher LWG than the February-weaned calves. Previous studies have demonstrated that calves left with their dams to suckle longer (post –rut) had higher LWG (Pollard *et al.,* 2002; Gomez *et al.,* 2002). This was attributed to mother's milk (Arman, 1974; Loudon *et al.,* 1983) as the major source of nutrition and/or with age-related stress associated with weaning (Pollard *et al.,* 2002; Bao *et al.,* 2004). However, there is a lack of information on the effect of pre-rut weaning date on LWG of young farmed deer.

The experimental design of this study (Chapter 2), which did not consider possible effects of animal behaviour and stress associated with weaning management, may have influenced weaner LWG post-weaning differently between the early and late-weaned groups. The calves were also weaned to permanent ryegrass-based pasture of low to moderate feeding value under summer dry conditions. This may have reduced VFI although this was not measured in this trial. Differences in LWG here should therefore be interpreted with caution. It is suggested that in further work of this type, groups weaned at different times should be weaned into separate paddocks under the same conditions, whilst considering the quality of feed.

However, the study has shown that pre-rut weaning date, (although, confounded by weaning process management) and sub-clinical parasitism during summer and early autumn may influence LWG in young farmed deer. More research is needed to fully understand the production implications of weaning date in farmed deer, and any such research must be multi-variable to address the potentially confounding effects of management procedures, nutrition, parasitism and other health issues and the range of growth outcomes, both long, and short-term that may be influenced by weaning date.

4.3.2. Hind reproduction

The study (Chapter 2) compared reproduction of two groups of pre-rut weaned hinds. The current study has shown no statistically significant differences in reproductive parameters resulting from the two pre-rut weaning dates (Chapter 2). However, there is a trend that may suggest that the closer the weaning date is to the rut the more likely fertility of the hinds may be reduced. Therefore, perhaps in different circumstances, or with more animals, a significant difference would be observed as this is a low number of deer for this type of study and as a result had low statistical power (63% at $P<0.05$, $\acute{\alpha}=5\%$). However, this trend is consistent with previous suggestion that the later the hinds are weaned, the less likely they are to have a BCS >2.0 (Hamilton & Blaxter, 1980) and that lactating hinds come into oestrous some days later than non-lactating hinds (Wilson, 1984b), therefore conceiving later.

In addition, weaning of calves before mating of hinds has been reported to result in reduced spread of calving without any effect on fertility (Hamilton & Blaxter, 1980). Weaning before mating resulted in a more compact calving because suckling can delay calving through later mating, which is above any effects of LW (Loudon *et al.,* 1983). Pollard *et al* (2002) reported an earlier median conception date in hinds weaned prior to the rut compared with hinds weaned post-rut. However, weaning date did not have a significant effect on BCS in this study and thus, there was no significant effect of BCS on conception rate of the hinds. This probably was due to the small range in BCS and the small number of deer hinds used in this study (Chapter 2). Pre-rut weaning in NZ is often practiced due to feed shortage in late summer and early autumn (Nicol & Barry, 2002) to optimise nutrition and BCS of the hinds, otherwise delayed calving dates in the following season (Adam, 1988) may occur due to late conception. Therefore, more research is required to validate the advantages of pre-rut weaning on hind reproduction in farmed deer using hind numbers which meet statistical power criteria. Power analysis has indicated that n=48, 51 and 59 will give a power of 78, 80 and 85% respectively $P<0.05$, $\acute{\alpha}=5\%$ (Faul *et al.,* 2007) for conception date.

4.4. Forages for deer production

The specialised forages such as chicory, sulla, red clover, lotus and lucerne potentially contribute to a higher feed intake, LWG (Kusmartono *et al.,* 1996; Barry *et al.,* 2000) and have a higher feeding value, providing the opportunity to improve deer calf growth in summer and autumn (Nicol & Barry, 2003). Beneficial effects on host physiology and performance under parasitic challenge have been found with the consumption of some of these forage plants when compared to control forages such as ryegrass, white clover or lucerne in sheep (Niezen *et al.,* 1998; 2002; Ramìrez-Restrepo *et al.,* 2004; 2005) goats (Paolini *et al.,* 2003) and deer; (Hoskin *et al.,* 1999; 2000; 2003b). These benefits have been attributed to secondary compounds such as CT and SL in chicory (Hoste *et al.,* 2006).

This study has presented the first report of young deer grazed on either plantain or pasture-forage mixes containing chicory and red clover under a regime of minimal anthelmintic input. Chapter 3 showed that the forage species or pasture-forage mix grazed affected the timing of onset of sub-clinical and clinical parasitism (trigger treatment), the severity of subsequent sub-clinical or clinical signs and LWG. The anthelmintic trigger treatment and LWG data suggest beneficial effects on host physiology and performance under parasitic challenge for deer grazing plantain, TLR-and TSR-mixes and chicory compared with deer grazing permanent perennial ryegrass. Although, in the current study deer grazing pure chicory did not perform as expected from the previous studies on the same property (Hoskin *et al.,* 1999, 2003b), the most likely reasons are discussed in Section 3.5.

The differences in parasitism and LWG observed between deer grazing permanent perennial ryegrass, chicory, plantain, TLR- and TSR-mixes in Chapter 3 could have been due to differences in dynamics of the larvae of deer nematode species present in the sward possibly associated with different plant morphology and sward structure, therefore influencing larval development to infective stage, migration or survival (Niezen et al., 1998; Marley *et al.,* 2006a, b). In addition, differences in plant chemical composition and/or differences in nutritional status of the animal caused by different forage chemical composition may be other contributing factors.

All these factors were not investigated due to the design of the study being limited by resources preventing additional treatments for comparisons such as additional groups on each forage

suppressively treated with anthelmintic as used in the study by Hoskin et *al.* (1999) for chicory and perennial ryegrass-based pasture. Therefore, any effects of nutrition and internal parasitism on LWG of weaner deer were potentially confounded in this study (Chapter 3) as would occur in the practical farming situation. Also any potential effects of forage feeding value and anti-parasitic plant compounds of chicory (Hoste *et al.,* 2006) and plantain ((Knight *et al.,* 1996; Gustine *et al.,* 2001) on parasitism and growth also cannot be separated in a scientifically robust manner in this study. However, results provide sufficient data to suggest that more research into nutritional and parasitological aspects of these forages and pasture-forage mix in farmed deer is warranted.

It is desirable to minimise anthelmintic usage, to lower costs and to reduce the risk of development of anthelmintic resistance and risk of carcass chemical residues (Hoskin *et al.,* 1999) in deer production systems. Furthermore, markets for venison are increasingly demanding reduced chemical input into deer production systems (Loza, 2001; Parker & Loza, 2002). Grazing systems that include legumes and herbs with CT such as sulla, lotus and chicory can be used to substantially increase the growth of weaner deer for venison production whilst maintaining deer health and reducing requirements for chemical inputs (Barry et *al.,* 2002; Section 1.4.5.7; 1.5.4.2). The results presented in Chapter 3 add evidence that there is potential for forage crops especially those with high concentrations of secondary compounds (i.e., plantain, chicory and red clover; Section 1.4.5.7), to be used on deer farms to aid in control of internal parasites, reducing anthelmintic use, whilst simultaneously increasing deer production through direct nutritional effects of the forage. While these preliminary results are promising, field research applying direct vs indirect mechanisms is required to evaluate the use of plantain, chicory and forage-pasture mixes for growth of farmed young deer under systems of minimal anthelmintic input.

4.5. Conclusion

This is the first report (Chapter 2) of the relationship between pre-rut weaning date and internal parasitism in young deer pre-weaning and up to 6 (February 17- March 31) weeks post-weaning in summer and early autumn. The study has shown that pre-rut weaning date, (although, confounded by weaning process management) and sub-clinical parasitism during summer and early autumn may influence LWG in young farmed deer. The failure of moxidectin to reduce FEC to zero raises the question of the efficacy of this macrocyclic lactone anthelmintic against GI

nematodes in farmed deer and/or emergence of farmed deer GI nematode resistance. The study has also highlighted the need for further research to demonstrate the advantages or disadvantages of pre-rut weaning on growth of deer calves and hind reproduction.

The anthelmintic trigger treatment and LWG data in this study (Chapter 3) suggest that plantain, TLR- and TSR-mixes and chicory may all have a role in aiding control of endoparasitism in young growing deer in autumn in deer production systems based on permanent perennial ryegrass-based pasture with low anthelmintic input. But any potential effects of forage feeding value and anti-parasitic plant compounds of chicory, plantain and pasture-forage mixes on parasitism and growth could not be separated in this study. It is therefore, acknowledged that these observations are preliminary and based on a design intended only to establish whether further replicated studies are warranted, particularly with plantain and pasture mixes.

The fluctuations and differences in serum protein and haematological parameters with time and between anthelmintic treated and untreated controls (Chapter 2) and between deer grazing different forage types (Chapter 3), have suggested possible effects of sub-clinical and clinical internal parasitism in farmed young deer. Although, there were no significant relationships between serum albumin, globulin, total protein and haematological parameters with FLC and FEC in both studies, there were low and significant correlations between serum biochemistry and LWG (Chapter 3). Also anthelmintic treatment decreased serum globulin concentrations and increased serum albumin concentrations (Chapter 2). These findings highlight the need for further investigations on these parameters as possible indicators of sub-clinical and clinical internal parasitism in farmed deer.

4.6. Recommendations for future research

4.6.1: Little is known about the epidemiology and pathology of, and diagnostic markers for lungworm and GI nematode infections in young deer. The fluctuations and differences in plasma proteins in the current study may suggest that leakage of plasma proteins do occur in deer due to GI nematode infections as well as fluctuations in haematological parameters investigated. Therefore further investigations under controlled conditions are warranted on serum proteins and haematological parameters as indicators of internal parasitism in farmed deer.

4.6.2: This is the first study on the impact of pre-rut weaning date on internal parasitism and LWG of young deer calves. However, upon analysis of the result, it was apparent the study could have been confounded by the weaning management. Further research is recommended to investigate the impact of pre-rut weaning date and weaning process management on internal parasitism and LWG in young deer.

4.6.3: The current study has shown no difference in reproductive parameters of hinds resulting from pre-rut weaning in February or March. Therefore, more research is required to validate any potential advantages and disadvantages of pre-rut weaning on hind reproduction.

4.6.4: This is the first report of young deer grazed on plantain or pasture-forage mixes under a regime of minimal anthelmintic input. Results of this study suggest that plantain might have anthelmintic properties and the potential to increase LWG. Therefore, further investigations of both the feeding value of plantain, the dynamics of larval development and survival on plantain swards and anti-parasitic effects of secondary compounds found in plantain against internal parasites of ruminants are justified.

4.6.5. The factors which might have contributed to the difference in requirement for anthelmintic input whilst deer grazed chicory during autumn between previous studies and the current study require further investigation. Whether chicory cultivars have any significant differences in morphology, chemical composition or anti-parasitic compound content affecting stages of the parasite life-cycle need to be investigated for efficient use of different chicory cultivars in deer production systems.

4.6.6. The relative contribution of the different tetraploid ryegrasses and the other components of the pasture-forage mix swards such as chicory, to the outcomes of the present study is unknown and requires further investigation. Therefore more research into nutritional and parasitological aspects of these forages and pasture-forage mixes for deer and other young ruminants is warranted.

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