EFFECTS OF PHYSICAL AND BIOCHEMICAL CHARACTERISTICS OF CONTRASTING LEGUME SWARDS ON SELECTIVE BEHAVIOUR OF GRAZING CATTLE

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Palmerston North, New Zealand

CESAR H. E. C. POLI

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ABSTRACT

In order to assess the effects of sward physical characteristics and secondary compound concentration on cattle ingestive behaviour and diet selection, two sets of experiments were carried out using two legumes [birdsfoot trefoil (*Lotus corniculatus* L.); red clover (*Trifolium pratense* L.)] with approximately neutral partial preference. The first set investigated the ingestive behaviour and diet selection response to manipulation of sward area, maturity and height using alternating sward strips. The second set tested the effects of plant morphology and secondary compound concentration on preference using sequences of spaced plants.

The first set of four experiments was conducted at Agresearch Flock House, near Bulls. Yearling heifers in groups of three grazed a sward formed by alternate 2.4 m wide strips of a mixture of birdsfoot trefoil cv. Goldie and white clover (*Trifolium repens* L.) cv. Pitau, and strips of red clover cv. Colenso. The experiments were formed by combinations of four treatments and five groups of animals over four successive three-day periods in a Row-Column Design balanced for previous treatment. This design was used to estimate the difference between periods, the difference between groups of heifers and the effect of previous treatments. Observations of the distribution of grazing activity and biting rate were made over 3-hour periods each evening. The distribution of grazing activity assessed the changes during three days of grazing (55 hours). The effects of contrasting areas, maturity and height of the alternate swards were examined in Experiments 1, 2 and 3, respectively. In the first experiment four treatments were imposed, the area ratio in percentage of each sward per treatment being: 20:80; 33:67; 67:33; 80:20. In Experiment 2 the treatments provided four combinations of maturity (immature/mature) of the two swards. In Experiment 3 the treatments were arranged in order to compare contrasts in height at the same vegetative stage of growth for the two swards. Experiment 4 was a small trial that showed that the proximity of a particular sward to the perimeter fence did not influence the proportion of grazing time spent on that sward.

The results of Experiments 1, 2 and 3 demonstrated that the physical contrasts between swards imposed by the treatments, and the variation in herbage mass and sward surface height between the first and third day of grazing, had important effects on selective behaviour. The effect of relative sward area was demonstrated to be important mainly when herbage mass and sward height were high, when the animals showed preferential selection for the sward of smaller area irrespective of which species was present with smaller relative area. The sward maturity effect was closely related to the preference for leaves and rejection of stems, though as the herbage mass and height decreased, the selection for leaf was offset by a selection of greater sward height and bulk density. The animals showed selection for taller and greater herbage mass swards, however, at high levels of herbage mass and height selectivity was reduced by the preference for a mixed diet. An overall analysis of the three experiments showed that there was a general partial preference for the two swards close to 50:50, though preference for birdsfoot trefoil was lower in Experiment 2 (40:60) than in either Experiment 1 or 3 (close to 50:50). This effect was mainly related to sward maturity and also indicated a need for further research on the effect of secondary compounds on animal preference.

The second set of experiments, Experiments 5 and 6, were conducted at Massey University and Agresearch, Palmerston North. In these experiments the response of grazing animals to contrasts in plant morphology and specific plant secondary compounds were examined in trials in which trained dairy cows grazed spaced plants of two "genotypes" (one accession and one cultivar) of birdsfoot trefoil with high or low concentration of extractable condensed tannins (ECT) (PI273938 and Goldie, respectively) and two "genotypes" (cultivars) of red clover with high or low formononetin concentration (Pawera and G-27, respectively). Plants were established in 4 linear sequences of 26, each providing three blocks (replicates) of balanced sets of 2 plant species, 2 genotypes (within each species), and plants either not trimmed or trimmed to minimise physical differences between genotypes within species. The plant sequences in Experiment 5 were grazed by four lactating cows and in Experiment 6 by two rumen-fistulated dry Friesian cows. In Experiment 6 the effects of rumen manipulation on preference were also tested by inserting minced material into the cow's rumen through the fistula to provide contrasts of low [birdsfoot trefoil (*Lotus corniculatus* L.) cv. Goldie] and high [lotus maku (*Lotus pedunculatus* L.)] concentrations of condensed tannin, and low [red clover (*Trifolium pratense*) cv. Astred] and high [red clover cv. Pawera] concentrations of formononetin.

The results of Experiments 5 and 6 demonstrated that the animals showed an immediate preference for large, dense and leafy plants. High concentrations of ECT also had an important negative effect on preference for birdsfoot trefoil, but this effect was confounded with a positive effect of plant morphology, mainly proportion of leaf. Formononetin did not have an important effect on preference of cattle.

The overall analysis of the six experiments showed that there was a relatively stable partial preference between birdsfoot trefoil and red clover demonstrating neutrality in preference between these two species. However this stability was sensitive to changes in sward area, plant morphology, sward structure (height and herbage mass) and secondary compound concentration. Observations showed that the animals did not graze randomly, but with the objective of obtaining a mixed diet. In tall, high mass and similar stage of maturity swards, the animals grazed preferentially the sward offered in smaller area or lower mass offered. In this context, the importance of leaf/stem ratio and high ECT concentration in affecting selection showed scope for manipulation of preferential behaviour through manipulation of the plant attributes. Improvement of leaf/stem ratio of birdsfoot trefoil and red clover, and reduction of ECT concentration in birdsfoot trefoil could therefore have a practical effect on animal preference. The preference for a mixed diet and the adjustment of this behaviour as sward conditions changed can be explained by interactions between three possible hypotheses: (i) animals tried to obtain a balanced diet; (ii) animals selected swards that provided the potentially higher rate of intake; (iii) animals sampled to constantly reinforce awareness of sward conditions.

This thesis is dedicated to my wife Beatriz

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CHAPTER 1

INTRODUCTION AND OBJECTIVES

To meet their requirements for maintenance, growth and reproduction, ruminants are faced with a complex of decisions that reflect the heterogeneity of the environment in which they are foraging (Gordon and Lascano, 1993). In contrast to carnivorous predators, herbivores usually need to deal with an environment where the food components are more evenly distributed in space and time and at low density (Illius and Gordon, 1990). In this environment the animal could take its entire daily intake of dry matter grazing unselectively from a few square metres of pasture. However, ruminants very seldom graze in a completely non-selective manner (Parsons et al., 1994a). It has been widely recognised that the diet of grazing animals is affected by the physical (e.g. Illius and Gordon, 1990; Gordon and Lascano, 1993; Hodgson et al., 1994; O'Reagain and Schwartz, 1995; Laca and Demment, 1996; Hodgson et al., 1997) and biochemical (e.g. Barry, 1989; Provenza and Balph, 1990; Van Soest, 1994; Provenza, 1995; Launchbaugh, 1996) characteristics of the sward, and understanding of these effects requires knowledge of the concomitant changes in ingestive behaviour (Hodgson, 1985).

Pasture characteristics vary spatially and temporally (O'Reagain and Schwartz, 1995) forming heterogeneous swards that need to be explored by the animals (Gordon and Lascano, 1993). In this heterogeneity, the effects (as demonstrated in several studies) of sward structural characteristics such as height, bulk density and herbage mass, and plant morphology, interact with the effect of sward biochemical characteristics to determine diet selection and intake (Hodgson et al. 1994). Several studies have been carried out to determine the effects of either physical sward characteristics (e.g. Milne et al, 1982; Burlison et al., 1991; Mitchell et al., 1991, 1993a; Ungar et al.,1991; Illius et al., 1992; Laca et al., 1992; Demment et al., 1993) or biochemical characteristics (e.g. Francis

1973, Whittaker and Feeny, 1971; Rhoades and Cates, 1976; Barry and Manley, 1984; Provenza and Malachek, 1984; Provenza et al. 1990, 1994; Kyriazakis et al. 1998) separately on ingestive behaviour. However, due to the complexity involved at the plant-animal interface, many of the interactions between grazing animals and sward characteristics are not well understood (Gong, 1993). As a consequence there is limited information on the influence of spatial and temporal heterogeneity in swards upon animal behaviour (Taylor, 1993). There is a major need for studies (Illius and Hodgson, 1996; Hodgson et al., 1997) where the effect of interactions between sward physical and biochemical characteristic are assessed.

Studies have shown that when animals are offered two different species, they do not demonstrate an absolute preference for one species, but a partial preference, resulting in a mixed diet (Newman et al., 1992; Parsons et al., 1994a; Cosgrove et al., 1996). So far, studies on partial preference have been restricted largely to either pen feeding or large blocks of monocultures. Newman et al. (1992) and Parsons et al. (1994a) demonstrated that this preferential behaviour has an important influence on dietary balance, and was itself influenced by the relative area of the alternative species, by the animals' previous experience, and by the time of the day. These findings indicate a new dimension for further research (Hodgson et al., 1997).

Torres-Rodriguez (1997) showed little contrast in the preference of grazing cattle between relatively large blocks of monocultures of birdsfoot trefoil and red clover. Thus, these species provide the opportunity to study the effects of manipulation of plant physical and biochemical contrasts on dietary preference and selective behaviour by grazing cattle against the background of relative neutrality in partial preference. The present study was designed to investigate these effects using experimental structures that minimise the requirement for animal movement to demonstrate partial preference. In addition, birdsfoot trefoil and red clover contain important secondary compounds (condensed tannin and formononetin). Very little information is available in relation to how their concentrations might influence animal appraisal and how their effects interact with sward physical characteristics in influencing selective behaviour.

The first studies involved alternate strips of two simple swards where the effects of sward area, maturity and height were investigated. The variation in discriminatory behaviour between and within experiments led to two further experiments with spaced plants where the effects of secondary compound concentration and morphological characteristics on partial preference were investigated.

The main objectives of the six experiments reported in this thesis were:

1) Evaluate the impact of the relative areas, maturity and height of alternate simple swards, formed basically by either birdsfoot trefoil and white clover or red clover, on diet selection by cattle in circumstances where the physical distribution of the alternative swards provided maximum opportunity for selection.

2) Investigate the specific effects of secondary compounds (condensed tannin in birdsfoot trefoil and formononetin in red clover) and plant morphology on cattle preference.

3) Assess seasonal variation in selective behaviour in relation to variations in sward physical characteristics and in concentrations of secondary compounds.

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION

Cattle production is a function of the ability of the animal to harvest nutrients in an effective and efficient manner (Stuth, 1991). Selection exerts an important influence on the ability of the animal to harvest its daily nutrient requirement where the variation in sward structure and the distribution of components within the sward allow the opportunity for selection (Hodgson et al., 1994).

Several pasture factors affect animal preference. This preference allows the grazing animal to select a diet that differs from the overall composition of the feed available (Leigh and Mulham, 1966ab; Dudzinski and Arnold, 1973; L'Huillier et al 1986). In this complex relationship Provenza and Balph (1990) suggest that forage environments present at least five problems or challenges to ruminants selecting dietary items: (1) variation among dietary items in kind and nutrient content (2) variation among potential dietary items in kind and amount of chemical defence, (3) variation in plant morphological defences, (4) temporal and spatial variation in the quantity and quality of forage, and (5) exposure of ruminants to unfamiliar foraging environments.

In this thesis attention is concentrated on a study of the preferences exhibited by grazing cattle between swards of red clover (*Trifolium pratense* L.) and birdsfoot trefoil (*Lotus corniculatus* L.)/white clover (*Trifolium repens* L.), and the influence of some physical and biochemical sward characteristics on these preferences. These species provide a useful model for investigation since Torres-Rodriguez (1997) has shown only limited discrimination between them by cattle when offered monocultures of these species in adjacent areas, in contrast to stronger preference shown by animals offered monocultures of these legumes in relation to perennial ryegrass.

The main objectives in this literature review are: (i) to describe and discuss the main models of animal grazing decisions in relation to the effects of physical and biochemical sward characteristics on diet selection; (ii) to investigate the role of animal senses in diet selection; (iii) to discuss the effects of sward spatial and temporal variability, and plant biochemical characteristics on grazing behaviour and diet selection. Particular emphasis is concentrated on tannins and formononetin as potential biochemical factors influencing selection.

2.2. DIET SELECTION BY GRAZING ANIMALS

2.2.1. Models of diet selection

Modelling diet selection is to a certain extent frustrating. Several attempts have been made to explain and predict diet selection. However the complexity of the subject has so far prevented the development of comprehensive models. Many different constraints affect the diet selected by free-ranging animals (Parsons et al., 1994a). The variation in diet selection happens not only due to differences among animal species (eg. Hofmann, 1989; Van Soest, 1994) but also due to differences in pasture composition and structure (e.g. Stephens and Krebs, 1986; Senft et al., 1987; Gordon and Lascano, 1993; Demment and Laca, 1995), environmental conditions (eg. Gordon 1989abc; Coughenour, 1991), animal physiological state and experience (eg. Newman et al., 1992, 1994b; Parson et al., 1994a; Provenza, 1995), time of the day (Parsons et al. 1994a), period of the year (Arnold 1987), plant biochemical characteristics (e.g. Barry and Blaney, 1987; Provenza and Malechek, 1984; Launchbaugh, 1996), and relationships with other animals (e.g. Penning et al., 1993).

Not only are there several factors that affect diet selection but also there are several possible approaches for modelling their effects. The approaches can be described as empirical, teleonomic or mechanistic (Thornley et al., 1994), but none are perfect in describing diet selection. Several models describe intake in functional terms, or empirically (Spalinger and Hobbs, 1992). However, this approach seems to be inappropriate for describing choice of food on most occasions since animals can modify intake according to changes in the relative availability of alternatives (Parsons et al., 1994a). A purely mechanistic approach is very difficult for describing diet selection since this can not consider aspects of behaviour that are not well enough understood to describe mechanistically (Parsons et al., 1994b). On the other hand, Thornley et al. (1994), taking a purely teleonomic approach, provided only a simple view of what is in fact a very complex problem.

Because of the complexity, no experimental program could expect to cover all aspects of the system at one time (Gordon and Hutchings, 1993). It seems that most of the models are additive rather than exclusive. The more models are created the more different situations can be understood. A group of researchers in the UK, for example, developed different models to explain diet selection. Thornley et al. (1994) using a cost-benefit model found that the animal maximises the benefits minus the costs when grazing. This agrees with what was empirically found by Kenney and Black (1986) and Black and Kenney (1984), who showed that animals prefer the forage that provides higher intake rate. This knowledge is expanded in the model of Parsons et al. (1994b), which demonstrates that the way plant species are distributed in the sward can have different effects on diet selection. This point is clarified by Newman et al. (1995), who developed a stochastic dynamic programming model of grazing behaviour. This demonstrates that diet preference may depend on the relative intake rates of the alternative plant species.

Diet selection models are summarised by Provenza & Balph (1990), who describe five main group of models to explain how ruminants select their diet. In the first case it is assumed that animals have the innate ability to sense, through taste and smell, specific nutrients and toxins in plants (euphagia). In the second, animals select vegetation that is immediately "pleasing" to olfactory, gustatory, and tactile senses and avoid that which is not (hedyphagia). The third assumes that diet selection is a function of animals' morphophysiology and size. The fourth assumes that the animals learn through foraging consequences, including pre- and post-ingestive consequences of foraging experience and social relationship. The last argues that animals forage to maximise nutritional gain per unit cost.

The first and second models were created to explain how animals could obtain a more nutritious diet than the forage on offer. These models, in fact, were not supported by research (Arnold and Hill 1972; Marten and Andersen, 1975). They are also criticised for not including the learning process (post-ingestive consequences) in selecting a diet (Provenza and Balph, 1990). However there are cases where animals show "nutritional wisdom". Bell and Sly (1983), for example, demonstrate that sodium deficient cattle can detect a few millimoles of salt by smell, and retain a "memory" of the locations. Although the third model does not include differences among different individuals within the same species, it complements the learning models. Provenza and Balph (1990) state that morphophysiology models provide a broad explanation for diet selection, while learning models fine tune to the level of individual animals. The last model, optimal foraging theory (Stephens and Krebs, 1986) gives a different explanation for animal diet selection. This theory states that evolutionary pressure selectively formed animals that hunt or graze for their food efficiently. Laca and Demment (1996) argue that this theory offers the strongest theoretical basis and framework to study foraging strategy of grazing animals. In fact, researchers (eg. Laca et al., 1993; Kenney and Black, 1984; and Black and Kenney, 1984) have shown that a choice between alternative forage or patches is strongly affected by the potential intake rate. However Griffiths et al. (1997) found that, contrary to expectations from optimal foraging strategy, grazing behaviour of cows at a current sward patch was unaffected by sward conditions at adjacent patches. Provenza and Balph (1990) criticised the optimal foraging theory because of the need for grazing animals to explore a varied diet where several other characteristics (eg. biochemical plant characteristics, plant morphology etc.) can influence the grazing decision. Crawley (1983) states that this model is more suitable for carnivores than herbivores, because of the limited variability of nutritional quality of prey, relative to the diet of herbivores.

The models outlined by Provenza and Balph (1990) do not accommodate more complex phenomena of selection that have been more recently observed, such as the effects on preference of recent dietary experience (Newman et al., 1992; Parsons et al., 1994a), animal state (Newman et al. 1994; Parsons et al., 1994a), time of the day (Parsons et al. 1994a), and vigilance requirements (Illius and Fitzgibbon, 1994). Newman et al. (1992), offering turfs of perennial ryegrass and turfs of white clover, observed that sheep preferred the opposite species to the one they had previously grazed. Parsons et al. (1994a), testing this hypothesis in swards, found that although sheep showed a preference for a high proportion of clover (the sheep did not graze at random), they sustain a mixed diet even in situations where a monospecific diet was readily possible. They suggest that sheep have a partial and changing preference for white clover in contrast to perennial ryegrass. Forbes (1995) argued that animals show a sensory-specific satiety. Animals choose to eat a variety of foods when none of them is aversive. These results advise caution when short term results are extrapolated to a long-term test of preference (Parsons et al., 1994a). In fact, it is wrong to suggest that sheep always show preference for white clover in relation to perennial ryegrass. Before the publication of Parsons et al. (1994a), only few studies (see Illius and Gordon, 1990) on animal preference comment on whether the preference for clover is total or is partial. Many studies have come to the conclusion that sheep prefer clover (van Dyne and Head, 1965; Hodge and Doyle, 1967; Bedell, 1973; Leigh and Holgate, 1978; Curll and Wilkins, 1980; Frame and Newbould, 1986; Curll and Gleeson, 1987; Lascano and Thomas, 1988; Vallentine, 1990; Ridout and Robson, 1991), although some suggest a lack of selectivity (eg. Clark et al., 1982; L'Huillier et al., 1984; 1986; Arnold, 1987). On the other hand other studies reported that selection is a response to variation in vertical distribution between species (Hodgson, 1981b; Milne et al., 1982; Illius et al., 1992) (see section 2.3.1).

More recently, Provenza (1996b) offers a new explanation for diet preference, based on avoidance of toxins and acquisition of nutrients. A key concept in this theory is food aversion. The decrease in preference for a food is a function of the sensory and post-ingestive feedback of that food. In this way animals also prefer the familiar to the novel and regard anything new with caution (Provenza, 1996a). However, they can become averse to what is too familiar, eaten too frequently or in excess, and search for a novel food and varied diets (Provenza, 1996a)

Nevertheless, diet selection also varies according to intrinsic differences among different animals. Although some studies (eg. Hoffman, 1989; Provenza and Balph, 1990; Van Soest, 1994) agree in modelling diet selection as a function of animal morphophysiology and size, they do not consider the differences between different animals of the same species. Hodgson (1985) mentions that increased nutrient demand will usually increase forage intake, but the effects on the individual components of intake appear to be variable and difficult to predict. Good examples of contrasting nutrient demand are lactating and non-lactating animals, shorn and unshorn sheep, fasted and non-fasted animals. Parsons et al. (1994a) comparing the diet preference of dry vs. lactating ewes found that, despite major differences in energy requirement and intake behaviour, no significant effects of physiological state on preference were detected. On the other hand, Newman et al. (1994) and Edwards et al. (1994), using different methods, have observed evidence of state dependent changes in preference when comparing fasted vs. unfasted sheep. Despite what was expected, fasted animals spent a significantly lower proportion of their time grazing clover (the higher intake rate component). These studies demonstrate that animal state should be considered in modelling diet preference. However more studies need to be done in this area (Parsons et al. 1994a).

Diet selection modelling also needs to consider the vigilant behaviour of ruminants. In evolutionary terms the best defence of ruminants against predators is to be vigilant. Ruminants are constantly vigilant, with a great capacity of the rumen to accumulate food to be processed later. Evidence of a trade-off between energy gain and scanning for predators while foraging suggests that vigilance is costly (Barnard, 1980; Underwood, 1982; Lendrem, 1983; Metcalfe and Furness, 1984). Illius and Fitzgibbon (1994), calculating the costs of vigilance in foraging ungulates, pointed out that an animal feeding selectively from the vegetation voluntarily accepts a reduced density of bites. This behaviour requires a trade-off between the advantage of being selective and the reduced opportunity to scan for predators. Since small animals incur lower vigilance costs than large animals, they can afford to be more selective (Illius and Fitzgibbon, 1994). This fact can be connected with the social behaviour of the animals. Penning et al. (1993) showed that sheep kept individually, at pasture, may not behave in the same way as when they graze as members of a flock. Although there is no clear evidence to explain this, it could be related to the fact that the animals do not feel so threatened in large groups, when they are better protected by more vigilant companions (Pulliam and Caraco, 1984).

2.2.2. Role of senses in diet selection

Several studies have been carried out on the function of the senses in dietary preference (e.g. Arnold, 1966a, 1966b; Jackson et al., 1968; Goatcher and Church, 1970ab; Bell and Sly, 1983; Warden and Dyk, 1971; Arave et al., 1989; Bazeley and Ensor, 1989). All the senses, in some way, influence diet selection. Preference, in fact is the result of a complex of sensing behaviours where the final choice is determined by the responses elicited to stimuli from the food (Arnold, 1966a). Edwards (1994) found that sheep have the ability to form associations between cues and rewards to direct their foraging, and increase their encounter rate with preferred patches in the environment. In addition, Edwards (1994) demonstrated that sheep are able to distinguish between ryegrass and white clover without sampling the two species, and can remember and associate different food types with their cues. More recently Provenza (1996a) argued that animals acquire preferences for the flavours of familiar foods that have been associated with the positive post-ingestive effects of nutrients. Provenza (1996b) explained that the reason why animals have varied diets is due to the decrease in preference for food as a result of sensory (taste, smell, texture) and post-ingestive feedback unique to each food. How the senses (sight, taste, smell and touch) affect animal preference and diet selection is discussed below.

2.2.2.1. Sight

Although sheep eyes possess cones (C.V. Ensor, in Forbes, 1996), they are thought to be colour-blind (Tribe and Gordon, 1949). Bazeley (1988) suggests that sheep may use sight to distinguish different shades of green. However Bazely and Ensor (1989) found that while sheep learned to discriminate between visual cues which varied in brightness, they failed to do so when the cues varied only in hue. This does not negate the possibility of sheep having a colour vision, but brightness might be important to distinguish grass, for example, with high and low protein content (Bazeley and Ensor, 1989).

Sight is seldom thought to be the primary sense acting in grazing preference. However it is known that cattle, like sheep and goats, can make quite complex discrimination between shapes (Forbes, 1996). Electrical activity of single neurones in regions of the brain was thought by Kendrick (1992) to be involved in feeding control. Mainly when food was moved towards the mouth, cells in the lateral hypothalamus and zona incerta respond to the sight but not the smell of food. Arnold (1966a) working with blinkered sheep under pasture conditions found that diet composition differed for control and blinkered sheep. These differences were attributed to the use of sight by sheep to orientate themselves while grazing. The blinkered sheep tended to graze all the strains of a species to the same height. Edwards (1994) also demonstrated the importance of sight on diet selection. Using identical bowls whose contents could not be seen, except from directly above, he removed the possibility of sheep identifying patch type. In fact, the sense of sight is very important for orientating animals in space.

Sight also affects fine selection. Arnold (1966a) found that species preference was unaltered by sight impairment, but the habit of grazing was modified. On short swards, sheep with blinkers took more taller components, those that they could feel first as the head was bent to graze (Arnold, 1966a). In fact, sheep can distinguish patterns of the pasture at quite a fine level. Cahn and Harper (1976) suggested that sheep select clover on the basis of leaf mark polymorphism. They found that unmarked leaves were initially preferred to marked ones. Edwards (1994) observed, in fact that sheep used visual cues and/or olfactory cues to determine patch type when directly above a patch, as patches were often rejected without sampling the patch.

2.2.2.2. Taste

Taste is one of the most powerful effects on animal preference. The ability of calves, for example, to discriminate among various sugar solutions and to show a preference for a specific sugar has been amply demonstrated (Stubbs and Kare, 1958; Bernard, 1964; Waldern and vanDy, 1971). Taste is believed to be involved in at least two psychological processes: food seeking behaviour and reinforcing value of a food (Goatcher and Church, 1970a). Many mammals readily accept sweet and reject bitter tastes (Provenza and Balph, 1990). This led several authors (Bate-Smith, 1972; Garcia and Hankins, 1975, 1977; Grill et al. 1984; Beauchamp and Cowart, 1987) to relate sweet taste with nutrients and bitter with toxins. In fact, Provenza et al. (1990) argued that odour and taste may be more important for enabling ruminants to identify and discriminate among subtle differences in diet items, than for innately recognising plants that are nutritious or toxic items based on pleasing or adverse gustatory and olfactory sensations.

Ruminant species differ among themselves in relation to preference (Arnold and Hill, 1972; Church, 1979; Hofmann, 1989). Goatcher and Church (1970b) comparing the taste responses of goats, sheep and cattle, using different concentrations of acetic acid and quinine hydrochloride, found that cattle were usually the first to make a discrimination, goats were generally second and sheep were normally last. These authors confirm what was found previously only with sheep (Goatcher and Church, 1970a) that stimulating effectiveness was greatest for bitter, followed by sour, salty and sweet tastes.

Taste (as well as smell and sight) helps the animal to discriminate according to what is pleasing. The taste-feedback interaction is noncognitive and is not related to a feedback event memory (Provenza, 1996b). Animals can change preference despite the knowledge of the cause of the feedback event. According to Provenza (1995a, 1996a, 1996b), animals acquire preferences for the flavours of familiar foods that have been associated with the positive post-ingestive effects of nutrients. The preference of animals increases if the food is adequate in nutrients (Provenza, 1996a). Preferences for flavours paired with energy (starch), for example, persist for at least 2 months following conditioning, which suggests animals acquire a liking for flavours paired with energy (Provenza, 1996a).

2.2.2.3. Smell

Together with taste, smell forms the flavour of a food. Although Tribe (1949) thought that odour had little effect on selection of plant species by grazing animals, Arnold (1981,1970) argued that the chemical signals, which mainly influence food selection, are those received at receptor sites for taste and smell. Arnold (1966) comments that marked changes in the relative acceptability of species, or strains of a species, occur when the sense of smell is impaired. McLaughlin et al. (1974) observed that sheep that had olfactory bulbectomy had less intense feeding, though with more re-entry into the feeder during meals, although they did not find any difference in daily feed intake.

The sense of smell is thought to be not very precise in ruminants (Arnold, 1981). However it is enough to make precise decisions about where to graze. Arave et al. (1989) demonstrates that flavour agents significantly increased preference for concentrates in dairy cows.

In addition, the importance of sense of smell in grazing animals is very clear when they avoid grazing close to patches of dung. Arnold (1981) observed in a sward with many dung patches that animals avoided the patches but ate very close to them. Norman (in Garner, 1963), comparing dung with urine, demonstrated that dung had the greater and more lasting influence, and the effect of urine disappeared in a relatively short period. The negative effect of the undesirable smell of dung on pasture palatability is enhanced by the fact that the ungrazed patches will become fibrous and coarse (Garner, 1963).

The olfactory effect in ruminant grazing decisions is also clear in relation to salt preference. Bell and Sly (1983) show that olfactory and gustatory receptors are able to detect very small amounts of sodium salt. Ruminants do not ingest salt as such unless they are in the metabolic state of sodium deficiency. Salt appetite increases in proportion to the bodily depletion of sodium (Bell and Sly, 1976, 1977). With the development of sodium depletion in cattle the ability to detect a very low concentration of salt increases.

Olfactory senses play an important role in the process of animal learning. Animals learn initially about the flavour of foods in utero and from mother's milk (Provenza and Balph, 1990). When young, learning from other animals is very important. Animals can smell where others have passed and how long ago others were there (Hart, 1985), influencing diet selection directly. In this process the animal learns the ability to discriminate between subtle chemical differences in different plants through odour and taste, and they associate the food flavour with post-ingestive consequences (Launchbaugh and Provenza, 1993).

2.2.2.4. Touch

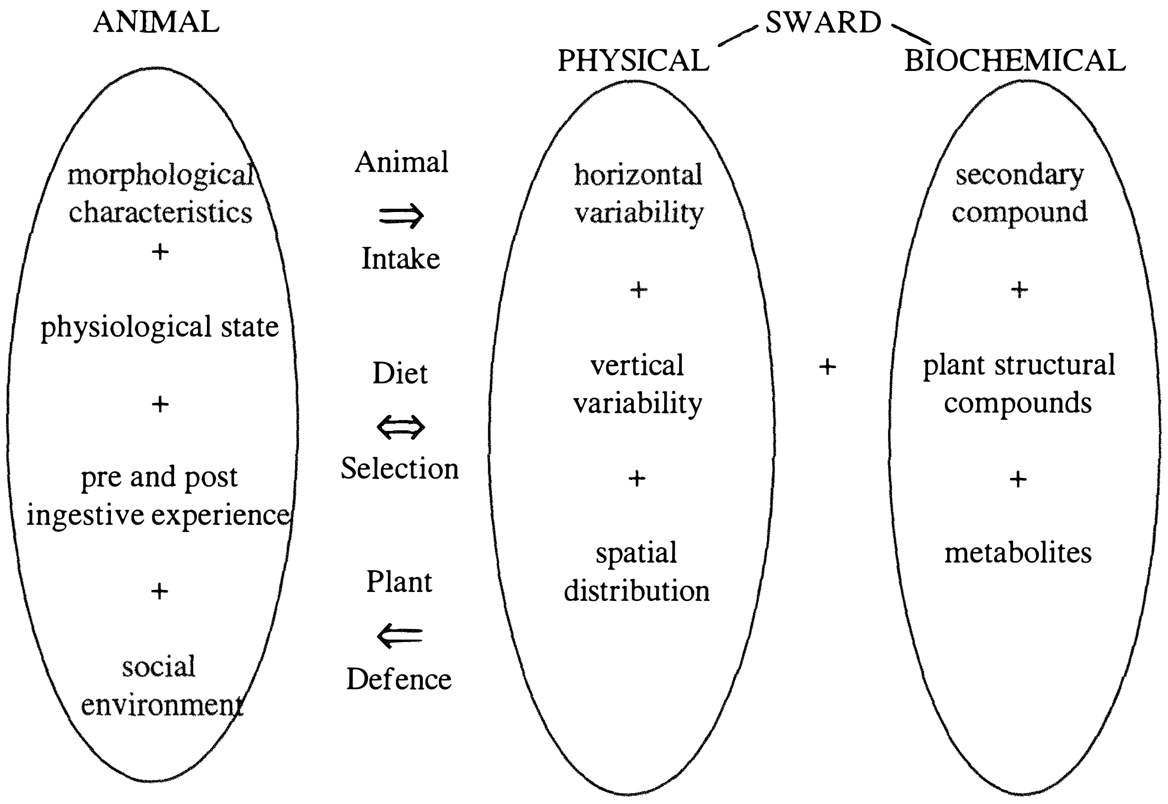
Hyde and Witherly (1993) and Garner (1963) claim that changes in texture have a great impact in the palatability of food. Animals usually select against rough, harsh, and spiny material (Vallentine, 1990). Van Niekerk et al. (1973) argue that the greater quantities eaten of pelleted forages are in part attributed to textural effect. According to Garner (1963), leaves that are very harsh to the human touch are harsh in the animal's mouth. This author mentions that the unpalatability of Yorkshire fog and cocksfoot is due to the hairiness and the silicious teeth on the leaves, respectively. In fact, leaf toughness is regarded as the most important mechanical attribute influencing grazing (Theron and Booysen, 1966; Coley, 1983). However Briske (1996) points out that there is not enough experimental evidence that mechanical attributes of plants are deterrents to grazing by vertebrate herbivores.

2.3. EFFECT OF SWARD CHARACTERISTICS ON GRAZING BEHAVIOUR, DIET SELECTION AND HERBAGE INTAKE

Diet selection and intake are a function of plant and animal characteristics. To ingest an adequate level of nutrients animals need to deal with plant physical variability and biochemical defence. The way they explore the physical and biochemical variability depends also on their own characteristics. The interactions between effects of sward physical and biochemical characteristics on intake and diet selection are illustrated in Figure 2.1., and are discussed in the following sections.

Although herbivores spend little time searching for food and face a relatively abundant and conspicuous food resource compared to carnivores (Stephens and Krebs,1986), searching time is an important component of grazing time (Laca and Demment, 1996) that varies with grazing conditions. To graze a diet, which has an adequate level of nutrients to meet requirements for maintenance, growth and reproduction, a herbivore is faced with a series of short-term tactical decision about what diet to select, how long to search between bites and the resulting rate of food intake. In the longer term, strategic decisions concern the length of time to spend feeding and where to feed (Gordon and Lascano, 1993). The complexity of the decision depends on the heterogeneity of the environment (Gordon and Lascano, 1993). One of the most important decisions the animals need to make is the trade-off between quality and quantity (Senft et al., 1987).

Figure 2.1. Schematic presentation of interactions between effects of sward physical and biochemical characteristic on intake and diet selection.



Although studies of how spatial variation of vegetation influences diet selection are very useful, they are relatively recent. The influence of spatial and temporal variation in food availability on diet selection is discussed below.

2.3.1. Spatial variability affecting grazing behaviour and diet selection

Diet selection is sensitive to variation in the horizontal and vertical distribution of dietary components (Edwards, 1994), and in sward component distribution (Arditi and

D'Acorogna, 1984; Parsons et al., 1994a; Thorney et al., 1994; Newman et al., 1995). Edwards (1994) suggests that the diet selected by sheep from two different swards, with the same proportions of the component plant species, may be quite different if spatial distributions of the plant species in the two swards are different. In fact, diet selection happens at different levels of scale: plant part level, plant level, patch level and landscape level (O'Reagain and Schwartz, 1995).

2.3.1.1. Plant part level

Selection for different parts of the plant is demonstrated in several studies. Animals are faced with differences in the nutrient content of different plant parts (Arnold, 1960), in potential bite dimensions due to size and or specific mass (O'Reagain and Schwartz, 1995), potential ingestion rate due to tensile strength or location and spatial arrangement within the canopy (O'Reagain and Schwartz, 1995). Animals usually respond to this variation by selecting leaf from stem (Arnold, 1960; Juko and Bredo, 1961; Arnold, 1964; van Dyne and Heady, 1965; Guy et al., 1981; Arnold, 1981; L'Huiller et al. 1984; L'Hiuller, 1986; Edwards, 1994), young components in relation to old (Arnold, 1960), and green in preference to dead material (Arnold, 1960; Juko and Bredon, 1961).

The factors that determine selection of plant parts are not well understood (Hodgson, 1990). Selection between plant components at a fine spatial scale is in several cases only the reflection of the easy prehension of one component in relation to another (Hendricksen and Minson 1981; Hodgson, 1990). However selection is also an animal ability. Edwards (1994), using homogeneously mixed pellets, found that sheep have the ability to select on a small scale. In reality, sheep tend to be more selective than cattle, and goats shows a greater preference for fibrous components than sheep and cattle (Hodgson, 1990).

2.3.1.2. Plant level

Herbivores select one plant in relation to others using several strategies. Plants affect animal selection according to their nutritive content, mainly leaf quality, and intake rate due to plant structure (O'Reagain, 1993). Considering the same availability and height, animals select different species according to the amount of stem and accessibility of leaves of high nutrient content and low tensile strength (O'Reagain and Schwartz, 1995). Plants with a high proportion of stems and leaves of high tensile strength are avoided (Theron and Booysen, 1966, Field, 1976; O'Reagain and Mentis, 1989, O'Reagain, 1993). The importance of each factor depends on the animal and environmental conditions. Nutritive content and tensile strength are also important factors in terms of selection of different plants of the same species. Gammon and Roberts (1978), for example, showed that animals tended to select plants that were defoliated previously mainly because grazed tufts remain green and leafy. Animals avoid tufts which are rank and contain senescent material (Mott, 1985; Ganskopp et al, 1993). In addition to selection for plants of high nutritive value and intake rate, animals also avoid some secondary compounds (O'Reagain and Schwartz, 1995 -see section 2.3.6.1).

2.3.1.3. Patch level

The variation in a sward is to some extent related to the grouping of plant species. Patches can vary from a single plant to landscape scale. However, beside plant species variability, the variability can be caused by several biotic processes, with the most important being grazing (O'Reagain and Schwartz, 1995). Grazing forms a mosaic of patches of varying size in a sward (Mott, 1985; Willms et al., 1988). The process of grazing increases the difference between the preferred and non-preferred species. Because animals tend to select previously defoliated plants (Gammon and Roberts, 1978), once the mosaic is formed, it tends to be maintained. The preferred patches are usually short and the ungrazed, rank and stemmy (Mott, 1985). This difference is also enhanced through the variability promoted by urination, defecation and trampling

(Thorhallsdotir, 1990; Ledgard et al., 1982; Jaramillo and Detling, 1992). Animals, particularly cattle, avoid grazing close to their dung (Hodgson, 1990)

2.3.1.4. Landscape level

The variation explored by free-ranging animals can also be described in relation to the landscape. A landscape is described by O'Reagain and Schwartz (1995) as an association of areas which differ markedly in species composition, vegetation structure and/or some physical characteristic such as slope, rockiness or soil fertility. Selection within the landscape is a complex process, involving several factors which are beyond the scope of this review. The reader is referred to recent reviews in this topic by Stuth (1991) and O'Reagain and Schwartz (1995).

2.3.2. Spatial heterogeneity and the process of diet selection

The animal may select in a horizontal dimension, from which patch to graze, and vertically from which plant part to graze and for how long (Gordon and Lascano, 1993). Animals graze certain patches where the density of a particular species exceeds some threshold (Arditi and Dacoragna, 1988). In this process, when the density is below the threshold, it is energetically more profitable for the animal to move on and continue searching for other patches (Kacelnik and Bernstein, 1988). Intake rate increases in areas where forage items are dense or most abundant (Dudzinski and Arnold, 1973; Trudell and White, 1981; Wickstrom et al. 1984). Kenney and Black(1984) and Black and Kenney (1984) found in intensive studies that the animal was strongly influenced by the potential intake rate. These results were then confirmed in large scale studies with sheep and cattle in swards with a range of variation in canopy height and bulk density (Illius et al., 1992; Demment et al., 1993). Bazely (1990) illustrated this point, showing that sheep graze tall patches more intensively than shorter ones, and Arnold (1987) showed that sheep consume more from high biomass than the low biomass areas. Clark et al. (in Gordon and Lascano, 1990) observed that sheep and goats moved from patch to patch but never stayed long on the shorter patch, and this contributed to the incomplete selection of the taller sward.

Preferential grazing between the different species in a sward is also influenced by spatial availability. Ridout and Robson (1991), for example, re-analysing the data of Clark and Harris (1985) who worked with different horizontal distributions of white clover and perennial ryegrass, showed that the percentage of clover in a diet was, in general, higher when white clover and perennial ryegrass were in strips of monocultures than intermixed. On the other hand, Armstrong et al. (1993) found that the size and distance between clover patches had no specific effect on the proportion of clover in the diet of weaned lambs in addition to that attributable to the overall proportion of grass and clover in the sward.

Animals also respond to the vertical variation between species. Hodgson (1981b), Milne et al. (1982) and Illius et al. (1992) found that for temperate pastures there is little difference between the composition of the diet and that of the upper strata of the canopy within which animals are known to be grazing. The variations in the frequency or severity of defoliation are likely to be directly related to the size of individual plants and their proximity to the surface of the vegetation canopy (Gammon and Roberts, 1978; Bircham and Hodgson, 1983; Briske, 1986). However this variation can be influenced by several factors such as plant biochemical characteristics (e.g. Malechek and Balph, 1987; Launchbaugh, 1996) and plant maturity (eg. Gardener, 1980; L'Huillier et al., 1984). In mature pasture, for example, animals prefer to graze shorter and younger plants (Hodgson and Ollerenshaw, 1969; Gibb and Ridout, 1988).

Spatial availability has different effects according to the animal species. Cattle are relatively indiscriminate surface grazers compared to sheep. Sheep tend to be more selective and to penetrate to a greater depth within the vegetation canopy (Grant et al.,1985; Collins,1989). Goats, on the other hand, concentrate their attention on the vegetation at intermediate levels, shallower than sheep (Collins, 1989). In a tropical environment, goats consume a diet with a higher legume content than sheep (Norton et al., 1990), reflecting the vertical distribution of the plants. Combinations of animal species in range swards can allow higher biomass of herbivores per hectare by complementary grazing (Cumming, 1982; Gordon, 1988). However this does not happen on sown pastures (Nolan and Connolly, 1977; Brelin, 1979; Nicol et al. 1993) because of the lack of vertical and horizontal variability for the different species to explore. In this case, cattle, sheep and goats have similar preference for green leaf components. Although Hughes et al. (1984) found more clover in sheep diet than in cattle or goat diets, this difference was negligible. In fact, diet selection is not only due to the animals' preference but also due to the combination of the distribution of the plant in the sward (vertical and horizontal) and the grazing depth.

In this complex of sward spatial variability, animal "memory" plays an important role. The ability to remember spatial distribution helps the animals to increase its encounter rate with preferred species (Olton et al., 1981; Bell, 1991). Certainly, animals can learn about spatial distribution (Brown and Gass, 1993). Bailey et al. (1989), using food patches in parallel and radial arm mazes, showed that cattle can remember the location of food. However Illius and Gordon (1990) and Illius et al. (1987) argue that animals need to sample to learn about the alternative foods on offer. In fact, it is very difficult for the animals to learn about plants and the location of different patches in a complex community, particularly if it is changeable (Edwards, 1994). However, Edwards (1994) demonstrated that when food patches remained in the same location, sheep learned to visit them. The ability of animals to return to the same preferred and known patches can lead to plants being changed or even grazed to extinction (Edwards, 1994), making the memory of limited use. However this behaviour shows the advantage to the animal of the partial preference described by Parsons et al (1994a) (see section 2.2.1). Edwards (1994) also demonstrates that sheep rapidly abandon discredited information and quickly learn the new distributions of food that they experience, making it easy for sheep to explore new environments. Sheep remember the spatial location of food for as long as three days (Edwards, 1994). Although cattle can remember for as long as 15 days (Laca et al.(unpublished data - in Edwards, 1994), memory declines after 8 hours

(Bailey et al., 1989) . In fact, animals use a combination of skills (eg. Spatial memory, sensory cues - see section 2.2.2) to choose their diet.

2.3.3. Temporal variability affecting grazing behaviour and diet selection

Animals have to cope not only with variability in spatial distribution in a sward, but also with variation over time. The temporal variation in a sward is a function of the normal changes in plant physiology, phenology and growth associated with seasonal or diurnal changes in environmental conditions (O'Reagain and Schwartz, 1995). The grazing action also induces temporal variability. An animal, for example, can deplete in seconds one plant and leave other plants intact. Grazing is a continuous process that alters sward component availability in time. Animals graze different parts of plants, and different patches, at different times. This asynchrony of defoliation affects the community structure (Edwards, 1994). Some plants may be disadvantaged in terms of plant competition (Lubcheno, 1978; Crawley and Pacala, 1991). Selective grazing, rather than random grazing, increases the disadvantage of the preferred species.

Plants at various developmental stages may possess various degrees of grazing protection (grazing avoidance) resulting from what Briske (1996) called developmental resistance. These mechanisms can vary throughout the growing season and with time following plant defoliation. Briske reviewed mechanical (production of thorns, silica, etc.) and biochemical plant defences that affect animal preference. Cyanide is a good example of short term modification. This increases within 18 hours of defoliation in potted plants of *C. plectostachyus* (Georgiadis and MacNaughton, 1988). Cyanide in this case acted as a deterrent to grazers. In the same way, Furstenburg and Hoven (1994) monitoring giraffe feeding behaviour realised that condensed tannin negatively affected acceptability and nutritional value of dietary browse. Tannin content increased due to browse disturbance and its level also changed daily due to temperature, light intensity and phenological status of the foliage (see section 2.3.6.2).

The temporal modifications that affect grazing behaviour and diet selection vary from a very short term (occurring over a few seconds to a few hours) to the very long term (years) (O'Reagain and Schwartz, 1995). Short term variability is influenced by the diurnal variation in plant chemical composition due to the normal plant physiological processes like photosynthesis, transpiration etc. This variation influences the grazing behaviour of ruminants. Sheep, on perennial ryegrass and on white clover swards, concentrated their grazing in the 4 hour before sunset when there is higher concentration of starch and sucrose in clover leaves and sucrose in grass (Penning et al., 1991). In this way the animals probably try to maximise their net intake of energy (O'Reagain and Schwartz, 1995). Animals are also affected, in the short term, by reduction in herbage quantity and quality. The grazing process, for example, promotes the reduction of plant size, reducing the bite size and intake rate, which in turn may reduce the preference for the grazed plant (Laca et al., 1994).

In the medium and long term, animal grazing behaviour and diet selection respond to the environment (eg. soil nitrogen, soil moisture, seasonal environment variations) and plant (eg. seasonal cycles of vegetation growth) variation (O'Reagain and Schwartz, 1995). Phenological development is known as one of the processes plants use to avoid grazing (L'Huiller et al., 1984; Briske, 1996). Reproductive culm development and accumulation of dead leaves, for example, are known to reduce animal preference for grasses (Willms et al., 1988; Ganskopp et al., 1992). The avoidance of mature seed heads and stem is explained by Hodgson et al.(1994) as reflection of differences in structural strength and shear strength rather than any direct perception by the animal of nutritional difference. In fact, Gammon and Roberts (1978) found that chances of defoliation of individual tillers in range grassland increased with height until culm development, after which defoliation declined sharply.

Herbivores demonstrate a range of strategies to cope with plant temporal variation. As the plant matures, pasture structure and quality change, and leaf accessibility may also be modified (O'Reagain, 1993). The first strategy that animals use is often to simply move to a different place in search for better grazing (O'Reagain and Schwartz, 1995).

However, if animals are restricted to a specific area they modify their diet selection and grazing behaviour according to the circumstance. One of the strategies used is to increase dietary breadth by including other less palatable species (Owen-Smith, 1994) or utilising other less palatable components (e.g. seed pods, leaf litter) of the same preferred species (Skinner et al., 1984 in O'Reagain and Schwartz, 1995). Herbivores also increase grazing time to compensate for the decrease in intake rate (Joblin, 1960) and modify their grazing time to increase selectivity. In order to increase the encounter rate with a preferred diet animals can increase travel speed (Collins et al., 1978) or look more thoroughly for the preferred species (Owen-Smith, 1994). These strategies may be followed by metabolic changes. Sheep, for example, accumulate body fat when sward conditions are good, for using in time of deficit (O'Reagain and Schwartz, 1995). On the other hand, some animals modify their digestive strategy to cope with the poor quality of the forage, increasing their digestive capacity or particle retention time (Holland, 1994; Lechner-Doll et al., 1990).

2.3.4. Effect of physical sward characteristics on herbage intake and diet selection

Several authors (Holmes, 1987; Nicol and Nicoll, 1987; Poppi et al, 1987; Rattray et al, 1987) agree that pasture allowance has the major effect on the quantity of feed consumed by a ruminant. However pasture allowance can not be used by itself to represent the effect of sward characteristics on intake. In some cases, for example, pasture allowance can be high due to low stocking rate, but intake is restricted by sward height or density. Pasture allowance has to be accompanied by the pasture characteristic like height or density to define the animal effect on herbage intake.

Sward height is one of the most important pasture characteristics that affect intake of grazing animals. Grazing cattle, for example, uses their tongues to pull a bunch of grass. It is very difficult for cattle to graze shorter than about 10 mm (Forbes, 1995). As herbage declines in height, bite mass decreases, and grazing time and number of bites increase (Chacon and Stobbs,1976). However as herbage becomes further defoliated (for cattle around 1000 kg ha^{-1}), there is reduction in grazing time, number of bites and biomass due to low leaf density (Forbes, 1995). Biting rate and grazing time are often regarded as the primary compensating responses of the animal to limitations in intake per bite (Hodgson,1981b). Nevertheless, increases in grazing time are seldom great enough to compensate for reductions in intake rate (Hodgson, 1981b; Penning, 1986). Intake per bite is probably the primary animal response to variations in sward conditions (Gordon and Lascano, 1993).

Like sward height, herbage bulk density is also an important sward component that influences intake. Burlison et al (1991) and Mitchell et al (1991, 1993) show a continuous pattern of response in intake per bite over a wide range of sward height and density. According to Burlison et al (1991) and Laca et al (1992) height and density effects are independent and additive. Estimates of both sward density and height are necessary to predict bite weight. Laca et al (1992) found that animals obtain heavier bites in tall sparse swards than on short dense ones of equal mass. However in short swards, less than 100 mm, bulk density does not seems to have a great effect (Ungar et al,1991). Bulk density, in fact, seems to be more important for tropical (tall and sparse) than for temperate (short and dense) pasture (Stobbs, 1973a).

The general rate of jaw movement (prehension, biting and chewing) in grazing animals is remarkably constant (Penning, 1986). Variations in biting rate (bites min^{-1}) reflect variations in the relative proportions of the three jaw activities, and they are therefore largely influenced by the manipulation necessary to graze effectively in swards of different structure (Penning, 1986; Laca et al, 1993). Animals taking small bites in a short sward, for example, graze uninterruptedly. They swallow faster than they can eat. As the bite weight increases, the animals are forced to perform exclusive chewing jaw movements between set of bites. When animals obtain a mouthful, they lift their heads during the longer period of chewing which is necessary before more bites can be taken (Laca et al 1993).

A better understanding of how sward characteristics influence intake and their interactions with animal variables is given by Burlison et al (1991). They defined intake per bite as a product of bite volume (BV) and bulk density of herbage in grazed strata (BD). They also defined bite volume as a product of bite depth and bite area. In fact, animal intake represented by bite weight varies less than bite dimensions because of compensatory effects between bite depth, bite area and sward bulk density (Black and Kenney, 1984; Laca et al, 1992). Laca et al (1992), for example, found little variation in bite weight amongst their cattle. Animals with small bite areas took deep bites. Nevertheless, because of the distribution of biomass between strata within the sward, bite depth has a major influence on variation in bite weight (Mursan et al, 1989). Because of this variation, herbage mass, by itself, is not a good predictor of intake (Laca et al, 1992).

Burlison et al (1991), Mitchell et al (1991, 1993), Laca et al (1992) and Gong et al (1993) found that bite depth is much more responsive than bite area to variation in sward conditions. In most circumstances, it is the major determinant of both bite volume and intake per bite. In temperate swards bite depth increases as sward height increases. Recent research has shown that the relationship, rather than being linear (Milne et al, 1982; Burlison et al, 1991), is asymptotic (Mitchell et al, 1991; Laca et al, 1992). In this case, the effort required to detach plant material near the ground may restrict bite depth. Barthram and Grant (1994), working with perennial ryegrass, and Dougherty et al (1992) with tall fescue, found that pseudostem may act as a deterrent to deep grazing penetration within the sward canopy. In this case, plant maturity would be an important factor. However some studies show that this is not necessarily the case. Burlison et al (1991) and Gong et al (1993) did not find any marked difference in the relationship between height and bite depth for vegetative and reproductive swards.

Several researchers (Burlison, 1991; Hughes et al., 1991, Mitchell et al., 1991, 1993) agree that bite area is less sensitive to sward change than bite depth. However, in more controlled conditions, with hand constructed swards, bite area decreased linearly with bulk density and increased quadratically with sward height (Black and Kenney, 1984; Laca et al., 1992). In this case a positive relationship between sward height and bite area occurs mainly on sparse rather than on dense swards (Mitchell et al., 1991). This relationship is better explained by Illius and Gordon (1987), Hughes et al. (1991) and Laca et al. (1993) where on short swards the bite area is limited by the difficulty of clamping plants between incisors and dental pad.

2.3.5. Effect of sward nutritional characteristics on herbage intake

The biochemical characteristics of a sward have a great influence in determining herbage intake. However factors that regulate dry matter intake (DMI) by ruminants are complex and not understood fully (NRC, 1996).

One of the main sward nutritional characteristics that affects intake is related to energy and fibre content. Animals compensate for changes in the concentration of available energy in the food, unless the physical capacity of the rumen restricts intake (Forbes, 1995). This effect is very well described by Conrad et al. (1964). They worked with dairy cows and concluded that intake of forage is controlled primarily by physical means, and the intake of more concentrated diets is controlled mainly by the cows' energy requirement. Later on, Bines (1979) working with lactating cows summarised the effects of the proportion of forage in the diet on the voluntary intake. The general trend is that there is a reduction in intake both above and below approximately 50% of forage in the diet. Below 50%, the reduction in intake is probably due to metabolic control, whereas above 50% it is due to physical limitation (Forbes, 1995).

Nevertheless this trend is not always true for ruminants. Infusions of glucose into the blood (Manning et al., 1959) and more recently duodenal infusions in dairy cows (Farvedin et al., 1992) failed to decrease dry matter intake (DMI). This shows that metabolic control of ruminants is different from monogastrics. Hodgson (1982a), observing a simple rectilinear relationship between DMI and forage digestibility (up to 80% of digestibility) from several trials, concluded that under grazing conditions the herbage intake of productive animals is seldom, if ever, likely to be affected by metabolic limits. Farvedin et al. (1995) explain that the metabolic limit observed in some studies can be more related to rumen activity than to metabolic action.

On the other hand, the reduction in intake due to physical limitation is determined by the rumen capacity. The faster the rate of disappearance of food from the digestive tract the less the physical limit of intake. Hovell et al. (1986) shows a very close linear relationship between the potential degradability of the DM and voluntary intake in sheep. Although intake and digestibility are somewhat interdependent, they are separate parameters of forage quality. Intake depends on the structural volume, and therefore the cell wall content, and its availability to digestion is determined by lignification and other factors (Van Soest, 1994).

The relationship between various forage constituents and animal intake depends on their association with plant structure. Cellulose, for example is more closely related with intake than digestibility. On the other hand, lignin is more closely associated with digestibility than with intake (Van Soest, 1994). The total structural matter - the plant cell wall, represented by NDF (Neutral-Detergent Fibre) - is the most consistent fraction related to intake because the cell wall contains the entire structural substance of the plant within which all other components are contained (Van Soest, 1994).

Intake is also controlled by the energy consumed. Donefer et al (1963) showed that intake in ruminants was not only controlled by physical limitation, but also by metabolic factors. Using pellets of alfalfa hay and concentrates, they found that sheep controlled their intake to a constant intake of digestible energy. At the same time, several experiments showed that infusion of short chain fatty acids depressed intake, confirming the possible mechanisms of metabolic control (Forbes, 1995).

Beside energy, there are other specific nutrients that affect intake in ruminants. As in other animals, low protein content of the food depresses voluntary intake, but the critical level in ruminants is lower than in monogastric species. In ruminants, the microorganisms can be supplied with urea from the saliva (Forbes,1995). In part the lower voluntary intake due to the deficiency of protein can be explained because protein deficiency reduces the activity of the rumen microflora and thus the rate of digestion of cellulose (Forbes,1995). Minerals are also important in relation to intake. A deficiency of essential minerals results in reduced food intake, and an excess of many of the minerals causes toxic effects (Forbes, 1995). Forbes (1995) comments that depression of voluntary intake in ruminants can be caused by: excess of arsenic, fluorine, molybdenum and selenium; deficiency of cobalt, magnesium, manganese and potassium; and excess and deficiency of calcium, copper, sodium, and zinc. He also mentions that deficiencies of vitamins A or D cause inappetence, and Riboflavin deficiency causes depressed intake in calves.

2.3.6. Effect of biochemical characteristics on grazing behaviour, diet selection and herbage intake

Plants produce a relatively distinct set of defensive chemicals and these chemical defences affect different animals in different ways (Freeland and Janzen, 1974). The chemical defence originates from the differentiation of the cells. The cellular level of development can be classified as growth (cell division and enlargement) or differentiation (chemical and morphological changes leading to cell maturation and specialisation) (Herms and Mattson, 1992). There is then a trade-off between growth and differentiation. A plant needs to grow fast enough to compete with other plants and at the same time to differentiate some cells for defence against pathogens and herbivores (Herms and Mattson, 1992).

There are several ways that ruminants can protect themselves against the toxic effect of plant chemicals. One way is through grazing a variety of species (Freeland and Janzen, 1974; Laycock et al., 1988). In this way, animals avoid toxic effects by eating plants or plant parts that do not contain large amounts of these chemicals and use several different detoxification pathways (Freeland and Janzen, 1974). To a certain extent this behaviour agrees with the partial preference postulated by Newman et al. (1992) and Parsons et al. (1994a) (see section 2.2.1). The preference for mixed diet might indicate an evolutionary adaptation to reduce the possibility of toxicity.

In fact, free-grazing animals are faced with complex decisions, including where and for how long to graze (Gordon and Lascano, 1993). Ruminants seem to avoid plants with strong odour or taste (Provenza et al., 1988), and acquire aversion to the food that causes illness (Burrit and Provenza, 1989a, 1991; Provenza, 1993). Animals that become ill after a meal of novel foods, avoid the foods whose flavours are most novel (Kalat, 1974; Burritt and Provenza, 1989a; Launchbaugh et al., 1993; Provenza et al., 1994). According to Provenza (1995) there is increasing evidence that neurally mediated interactions between the senses (taste and smell) and the viscera enable ruminants to sense the consequences of food ingestion, and these interactions operate in subtle, but profound ways to affect food selection and intake, as well as the hedonic value of food. Animals are also able to identify plant toxins by associating food flavour with post-ingestive feedback (Garcia, 1989; Provenza et al., 1990). According to Provenza and Balph (1990) any physiochemical agent that causes nausea can cause aversion. However ruminants have difficulties in learning response to toxic compounds that do not affect the emetic system of the midbrain and brainstem (Provenza et al. 1988, 1990). Bloating, allergies, lower intestinal discomfort and drugs that do not affect the emetic system of the midbrain and brainstem are examples that animals do not learn to avoid (Garcia, 1989).

On the other hand, animals show preference for the flavours of familiar foods that have been associated with the positive post-ingestive effects of nutrients (Provenza, 1995a, 1996b). Ruminants, for example, acquire preference that is paired with energy (Provenza, 1996a). However, flavour may not always be a good indicator of toxicity (Launchbaugh et al., 1993). Often chemical changes can not be detected by animals through taste and smell (Bryant et al., 1992; Provenza et al., 1992a). This makes it more difficult for mammalian herbivores to avoid phytotoxic plants.

Animals prefer the familiar food and regard anything novel with caution. However, preference decreases when familiar foods are eaten too frequently or in excess, which encourages the consumption of novel foods and varied diets (Provenza, 1996a). In fact, mammalian herbivores must sample food because the nutrient content and toxicity of the familiar food change frequently (Freeland and Janzen, 1974; Westoby 1974, 1978).

In this grazing process, ruminants demonstrate ability to remember the food location and discriminate among subtle chemical differences within plant species based on flavour and quickly associate with the post-ingestive consequences (Provenza and Balph, 1990). Provenza (1996a) offered an explanation of how ruminants select diets from an array of plant species that vary in nutrients and toxins. Provenza argued that animals show aversion to the food (rather than preference) as a result of sensory (flavour) and post-ingestive feedback unique to each food. In this way aversions cause animals to sample novel foods and eat varied diets (Provenza, 1996a).

Another adaptation enabling ruminants to cope with plant chemicals is through the presence in the rumen of a diversity of bacterial and protozoal flora that can degrade a wide variety of secondary compounds (Freeland and Janzen, 1974, Launchbaugh, 1996). The presence of micro-organisms helps ruminants generally suffer fewer negative effects from poisonous plants than non-ruminants (Smith, 1992). However there are interactions between micro-organisms and plant chemicals that are not beneficial. Examples are nitrate (Allison, 1978); cyanogenic glycosides (Conn, 1979) and formononetin (Keogh et al., 1996). Formononetin (an oestrogenic compound in some leguminous species - see section 2.3.6.3), for example, has only a very weak oestrogenicity effect, but it is converted to equol in the rumen, which is oestrogenically active (Shutt and Braden, 1968), and readily absorbed (Shutt et al., 1970).

2.3.6.1. Effect of plant secondary compounds on grazing behaviour and diet selection

Plants produce chemicals that initially were thought not to be involved in metabolic processes supporting growth, development or reproduction. They were named as secondary compounds (Launchbaugh, 1996). However nowadays these chemicals are known to be involved as regulators of plant growth or biosynthetic activities, transport facilitators and nutrient or waste storage compounds (Rosenthal and Bell, 1979). Ecologically, secondary compounds are very important, acting as defence substances against herbivory (Whittaker and Feeny, 1971; Rhoades and Cates, 1976; Lindroth, 1989, Van Soest, 1994) and they also enhance the ability of plants to survive stress conditions (Harborne, 1993). This review will deal with two specific secondary compounds - tannin and formononetin - of importance in this study.

2.3.6.2. Effect of condensed tannins on grazing behaviour and diet selection

Tannin historically was classified as the substance that converted hide into leather (Van Soest, 1994 ; Bernans et al., 1989; McLeod, 1974). Nowadays it is known that tannin is any phenolic compound that contains enough phenolic hydroxyls to form strong complexes with protein and other macromolecules (Van Soest, 1994). The classification of tannins into two groups, hydrolysable and condensed, by Frendenberg (1920) (quoted in McLeod, 1974), was until now accepted. However Van Soest (1994) argued that this division is an oversimplification because some tannins contain functional properties characteristic of both groups, and other polyphenolics (with tannin-like properties) do not fit into either category.

Condensed tannins (CT) are the most widely distributed tannins in vascular plants (McLeod, 1974; Swain, 1979), while hydrolysable tannins are restricted only to angiosperms (Swain, 1979). Tannin is of little importance in the lower orders of plants and most of the monocotyledons, like grasses (McLeod, 1974). In fact, the importance is greater in dicotyledonous plants, like the leguminosae (McLeod, 1974). Information on herbage and browse plants are included in this review, recognising differences in pattern of distribution and diet selection of these two kind of plants.

The tannins are a group of soluble phenolic compounds distributed in several plants which provide defence against pathogens (bacteria and fungi) (Swain, 1979) and herbivores (Rhoades and Cates, 1976; Zucker, 1983; Freeland et al. 1985), and delay decomposition when plant tissue becomes litter (Zucker,1983). Tannin does not seem to have any role in plant physiological processes (McKey, 1979). In addition it is energetically expensive to the plant to produce CT. This probably explains why some plants only produce CT in reproductive tissue (Barry, 1989).

CT production fluctuates in relation to genetic and environmental variables. Roberts et al. (1993), quantifying the amount of CT in ninety-seven accessions of birdsfoot trefoil, concluded that tannin concentration decreased from summer to autumn, but it was also related to geographic origin. Seasonal tannin changes were also verified with other species (Donnelly, 1959; Cope and Burns, 1971; Cope et al. 1977; Windham et al, 1988; Furstenburg and Hoven, 1994; Iason et al., 1995). Several of these studies (Clark et al., 1939; Stitt and Clarke, 1941; Donnelly, 1959; Cope et al., 1971; Windham et al., 1988; Iason et al., 1995) showed an increase in plant tannin concentration in summer, and some (Clark et al., 1939; Stit and Clark, 1941; Cope et al., 1971; Windham et al., 1988), like Roberts et al. (1993), reported a decline from summer to autumn. It is not very clear how temperature and moisture affect tannin concentration. Donnelly (1959) explained that tannin content increased with increase in temperature and decreased with rainfall. However, Cope et al. (1971) were not able to associate rainfall and temperature with tannin content. On the contrary, Furstenburg and Hoven (1994), working with 25 tree and shrub species verified that tannin levels decreased with increasing temperature during the day and increased with descending temperature through the night, and that tannin content was found to be higher in the shade than in direct sunlight. They also noticed that tannin content increased due to browse disturbance.

Tannin concentration is also variable according to the proportion and age of leaves. Leaves have higher concentration of tannin than stem (Iason et al.,1995 and Douglas et al., 1993). Douglas et al. (1993), comparing 12 herbaceous species found that CT in lamina was 2-5 times that of stem. On the other hand, Donnelly (1959) showed that tannin content increased with maturity. Iason et al. (1995), working with Yorkshire fog, reported significantly higher concentration of CT in dead versus living leaf. However, Coley (1983) and Furstenburg and Hoven (1994) working with tropical trees and shrubs reported the opposite behaviour. Furstenburg and Hoven (1994) found in African species that young leaves contained twice as much condensed tannin as old and mature leaves.

CT levels are also related to forage quality and soil fertility. High concentrations of CT are in general related to high levels of lignin (Barry, 1989). Low fertility soils are associated with increases in both lignin and tannin content (Barry, 1989). CT and lignin are both produced in plants from the shikimic acid biochemical pathway (Swain, 1979). Barry and Manley (1986) argue that the most probable explanation for an increase in CT and lignin concentration when nutrient stress increases, is that environmental stress stimulates the shikimic acid biochemical pathway.

Tannins protect plants, acting in the process of diet selection and voluntary intake. There are different processes that make large herbivores avoid or reduce the intake of high tannin content plant species. Traditionally, condensed tannins have been thought to decrease plant preference by digestion inhibition (Fenny, 1969; Rhoades and Cates, 1976; Swain, 1979). It is known that tannins defend plants against grazing by reducing protein, cell wall, and sodium digestion and retention (Rhodes and Cates, 1973; Zucker, 1983; Robbins et al., 1987ab). Condensed tannins can form complexes by bonding with both carbohydrates and proteins (Barry, 1989). At neutral pH, CT form a stronger bond with protein (McLeod, 1974). CT can then complex and render digestive enzymes inactive (Swain, 1979), and precipitate dietary proteins (Feeny,1969) making them less easily degraded. This may suppress microbial activity in the rumen, decreasing fibre digestibility and consequently intake (Barry and Blaney, 1987; Barry, 1989). Barry and Manley (1984) found that binding tannin with PEG 3350 increased forage intake and digestibility by sheep. Barry and Blaney (1987) explain that this fact might be the result of blocking the effects of tannins (by PEG 3350) on the rumen.

Tannin is also known as a defence compound due to its astringent properties (Bate-smith, 1973). According to Van Soest (1994), astringency is caused by the precipitation of salivary mucoproteins. He describes the tannin astringency flavour through the taste of beer, wine, tea and some fruit juice. In relation to ruminants Provenza and Malechek

(1984) argue that plants that contain high levels of tannin may also contain high levels of energy and nutrients, but the astringent sensation animals probably experience when consuming these plants may lead to their rejection. In this case, they worked with goats grazing two kinds of shrubs and concluded that goat nutrition was affected more by the adverse effects that tannins apparently had on palatability, than by the negative effects they had on digestibility.

Mammalian herbivores eat nutritive plants that contains toxins, but they generally limit intake in accordance with the concentration of the toxin (Provenza, 1995). Animals, therefore, must either instinctively recognise or learn to avoid the biochemical compound (Chapman and Blaney, 1979; Provenza and Balph, 1990). Instinctively animals would associate the flavour of the plant tissue with aversive post-ingestive consequences (Provenza et al., 1990). However, there are plants high in CT that are highly palatable (e.g. plants of blackbrush twig studied by Provenza and Malechek, 1984). In addition, the large variation of plants in a sward make it very difficult for animals to recognise and avoid plants that contain tannin (Provenza et al., 1990). Provenza et al. (1990) argue that animals learn to avoid plants high in CT because of the internal malaise promoted by CT and not because of its flavour. This post-ingestive feedback of malaise is a quick process (Provenza, 1995). Goats for example learn to limit intake of twigs containing tannin within one hour (Provenza et al., 1994).

The kind of animal also affects the degree of selection for tannin. The greater amount of tannin consumed by browser ruminants and wild animals is probably associated with active defences against plant tannins (Robbins et al., 1987a). Browsers, for example, show increased secretion of salivary proteins that bind and neutralise tannins (Mehansho et al.,1987). This binding factor seems to be absent or reduced in sheep and cattle saliva (Austin et al., 1989). Long-term ingestion of tannins induces enlargement of the salivary glands, although it is restricted to species capable of the adaptation (Van Soest, 1994). Wild animals also may have increased detoxification capabilities (Harborne, 1993). The faecal losses, for example, of metabolic nitrogen (probably represented by the indigestible tannin-mucoprotein complex) are higher in white-tailed deer than sheep and cattle (Austin et al., 1989). However wild animals must still balance the rate of intake with the rate of detoxification (Robbin et al., 1987a).

2.3.6.3. Effect of formononetin on grazing behaviour and diet selection

In the 1930's reports related severe abnormalities in conception by ewes with the consumption of subterranean clover. These abnormalities was then called "clover disease" (Marshall, 1973). In fact, Clover disease is promoted by isoflavonoid compounds, known as phytoestrogens, in *fabacea* (Bush and Burton 1994). High intake of phytoestrogens by ewes can promote oestrogenic activities, causing several reproductive effects: reduced fertility, dystocia, prolapse of the reproductive tract, high tail, increased death rate, lactation in virgin ewes and wethers, enlarged bulbo-urethral glands and urinary obstructions in wethers (Marshall, 1973).

The most important oestrogenic compounds known to be present in pasture include the Isoflavones and coumestans, produced by legume species; and zearalenone, produced by Fusaruim species and wide spread over New Zealand pasture (Keogh, 1995). Coumestans and Isoflavones are acetate-derived fragments and phenylpropanoids (see Wong, 1973; Bush and Burton, 1994). Coumestan (main compounds coumestrol, and methyl coumestrol) are important phytoestrogens in lucerne and other medics (McDonald, 1995). However they are generally not present in sufficient quantity, except in foliage that has been affected by pests and/or diseases, to cause reproductive problems in livestock (Keogh, 1995). The Isoflavones are the main compounds responsible for the "clover disease". The best known Isoflavone phytoestrogens are genistein, biochain A, daidzein and formononetin (Bush and Burton 1994). They are present in many *Trifolium* species including white clover (*Trifolium repens*) red clover (*Trifolium pratense*) and subterranean clover (*Trifolium subterraneum*) (Keogh, 1995). However subterranean and red clovers are the most commonly reported species with moderate to high oestrogenic potency (e.g. Keogh, 1995; Keogh et al., 1996; Marshall, 1973; Kelly et al., 1979). This is mainly due to their high concentration of formononetin.

Formononetin is the main Isoflavone phytoestrogen responsible for reproductive problems in sheep (Davies et al., 1970; Keogh, 1995; Keogh et al., 1996). In fact, Millington et al. (1964) reported that the oestrogenic effects in sheep were linked to the formononetin level of different subterranean clover strains, but not biochain A or genisten. This was later explained by Shutt and Braden (1968) who showed that biochain A and genistein are degraded to non-oestrogenic phenols in the rumen while formononetin is converted to equol. Equol is oestrogenically active and readily absorbed in the rumen (Shutt et al., 1970). The presence of free equol in the blood indicates the oestrogenic effect in sheep.

The concentration of formononetin varies within a plant. According to Keogh (1995) in both red clover and subterranean clover the highest concentrations occur in the youngest leaves, declining progressively as the leaves get older. Stems show the lowest concentration, with increasing concentrations in petioles, expanded laminae and expanding laminae (Bush and Burton, 1994). However Francis and Millington (1965) found for subterranean clovers that formononetin concentrations are usually higher in leaf laminae and in stems than in petioles. In the same way as leaves, the younger the stem, the higher the formononetin concentration (Keogh, 1995). The distribution of formononetin within the plant shows that a high proportion of the formononetin is situated in a readily accessible position for animals to graze (Keogh, 1995). However the formononetin concentration varies also according to the season. Kelly et al. (1979) working with the red clover cultivar Pawera in New Zealand, showed the highest concentration (1.38%) and oestrogenic activity (32.5 μg equivalents of oestradiol - 17β) in March and found the lowest concentration (0.64%) and oestrogenic activity (15.5 μg) in January. Plant formononetin concentration can also increase with marked phosphate deficiency (Marshall, 1973), waterlogging, low temperature, and defoliation (Neil and Marshall, 1970; Rossiter, 1970).

Genetic variation has been an important tool to overcome the problem of reproductive problems associated with "clover disease" (Nicollier and Thompson, 1982; Anwar, 1994). In the same way as subterranean clover (Smith et al., 1986), new cultivars of red clover have been successfully selected for low formononetin concentration. In New Zealand the low formononetin red clover cultivar, G27, was selected from the late-flowering, tetraploid cultivar Pawera (New Zealand, 1995).

The effect of formononetin on diet selection is still not very clear. Rossiter and Ozanne (1970) observed that when a choice is given to sheep, they show preference for particular cultivars of subterranean clover. However most of these cultivars have a large concentration of Isoflavone glycosides. Francis (1973) clarified this difference in palatability using chemically induced mutations of the Geraldton variety of subterranean clover. He showed that the mutant that lacks β-glucosidase enzyme was significantly less palatable than the other clovers. This means that the flavonoid glycosides will remain intact during mastication whereas in the other cultivars they are almost instantaneously hydrolysed. Francis (1973), therefore, concluded that in strains of clover high in Isoflavones, it is likely that larger amounts of unhydrolyzed glycosides will remain after the initial mastication and these could contribute to unpalatability. However, Harborne (1993) argues that there is no evidence of preferences for clover lines deficient in Isoflavone, reporting that tests show that sheep can not discriminate between a high Isoflavone strain of *Trifolium subterraneum* and a strain essentially lacking these compounds. Harborne (1993) concluded that presumably Isoflavones are not sufficiently repellent in taste to deter feeding. In fact, some secondary compounds like formononetin, rather than affecting diet selection or appetite, reduce the fitness of herbivores to avoid or limit future grazing (Rhodes and Cates, 1976; Launchbaugh, 1996). Phytotoxins that affect the reproductive system of the ingesting herbivores could selectively remove traits from the gene pool that allow animals to detoxify or tolerate a particular phytotoxin (Launchbaugh, 1996).

2.4. CONCLUSIONS

This literature review covered the progress over the last three decades on the influence of sward characteristics on animal ingestive behaviour. Much research carried out elucidated and quantified the effect of sward physical and biochemical characteristics on animal ingestive behaviour. In the effects of physical sward characteristics, herbage accessibility, sward surface height, bulk density and plant botanical composition are the most important sward characteristics studied. The empirical observations of their effects on animal ingestive behaviour generated important models, but because of the complexity involving the plant-animal interface, they can not be generalised for all grazing situations. Some of the models, for example, postulated that the animal prefers plants or components which maximise the intake rate, agreeing with optimal foraging theory. However several studies show that this is a rather simplistic way to explain a complex relationship. More recently research has demonstrated, for example, that animals constantly move between alternative swards, in most of the cases they do not have a unique preference, but they show a partial preference for dietary components. In fact, a unique explanation for the effect of physical sward characteristics on ingestive behaviour is apparently not practical because of the complex interactions among the factors concerned. Although the effects of sward physical characteristics on animal ingestive behaviour are becoming better understood nowadays, there is still need for research, involving more complex conditions.

Together with physical sward characteristics, ruminants also respond to sward biochemical characteristics. More recent studies have shown that ruminants respond not only to a direct sensory perception but also to a post-ingestive feedback where they avoid grazing the component which caused malaise. In this plant-animal interface, secondary compounds are important plant products where some have the function of protecting the plants against grazing. However their effect on animal ingestive behaviour is variable mainly because their concentration has been shown to vary according to several factors such as plant species, plant morphology, environmental condition, and season of the year.

The literature shows that condensed tannin is one of the most important secondary compounds that apparently protect the plants against grazing. The information about condensed tannin has been growing mainly in relation to its effect on nutritional value, providing an understanding of the mechanisms of ruminant digestion and absorption affected by condensed tannin in forages. Studies also have shown that avoidance of condensed tannin may reflect either astringent taste or post-ingestive feedback. However knowledge of the effect of condensed tannins is still limited in most of the cases to either penned animals or controlled conditions where physical sward characteristics are not relevant. There is scope for studies that involve variation in both physical and biochemical sward characteristics in grazing situation.

On the other hand, very little is known about the effects of several other secondary compounds on ingestive behaviour. In this context formononetin has been related to reproduction problems since 1930's, but little research has been carried out on the effect of formononetin on ingestive behaviour. In addition, most of the research carried out in this area involved sheep grazing subterranean clover. No report was found in the literature of a study of the influence of formononetin concentration in red clover on cattle diet selection.

Although several studies in the literature have demonstrated that in nature the effects of sward physical and biochemical characteristics on diet selection have spatial (e.g. plant part level, plant level, patch level and landscape level) and temporal (e.g. seasonal variation, plant maturity, secondary compounds concentration) variation, few studies have incorporated both variations. Because of the complexity of the animal-plant interface, most of the studies have restricted to either sward physical or biochemical characteristics, using either penned animals or uniform swards. There is a need for research combining these effects, involving both spatial and temporal variation, on ingestive behaviour of grazing animals.

CHAPTER 3

EXPERIMENTS 1, 2, 3 AND 4

3.1. INTRODUCTION

Selective grazing behaviour has an important effect on influencing the dietary balance of animals (Parsons et al., 1994a). Recent studies on partial preference (Newman et al., 1992; Parsons et al., 1994a; Cosgrove et al., 1996) are restricted to either pen feeding, or large adjacent blocks of monocultures. The literature also demonstrates that there is a lack of studies assessing grazing behaviour involving more complex conditions (Taylor, 1993; Illius and Hodgson, 1996; Hodgson et al., 1997). These experiments were therefore set up to investigate the effects of physical sward characteristics on diet selection where the physical distribution of the alternative swards minimised the requirement for animal movement, and where the physical and nutritional characteristics of the alternative sward were manipulated independently. In this context, previous studies have shown little contrast in preference between birdsfoot trefoil and red clover (Torres-Rodriguez, 1997). The small contrast observed provided an opportunity to use preferentially neutral species for determining the influence of sward physical characteristics on diet selection by grazing animals. This project also had the objective to assess the seasonal variation in diet selection in relation to variation in sward physical characteristics and in concentrations of secondary compounds.

To achieve these objectives four experiments were carried out using swards formed by alternate strips of birdsfoot trefoil mixed with white clover, and strips of red clover, with individual objectives as follows:

Experiment 1: to evaluate the impact of the relative areas of alternative simple swards on the demonstration of grazing behaviour and diet selection by cattle.

Experiment 2: to assess the effect of sward maturity on grazing behaviour and diet selection.

Experiment 3: to assess the effect of sward height at the similar vegetative stage of growth on grazing behaviour and diet selection.

Experiment 4: to give support to previous experiments in relation to the effect of the perimeter electric fence on animal grazing distribution on the plot.

3.2. MATERIAL AND METHODS

Four experiments were carried out in order to understand how the contrasting characteristics of two swards (birdsfoot trefoil with white clover, and red clover) affect diet selection, grazing behaviour and intake:

Experiment 1: contrasting area ratio of the two swards offered, to assess the effects of horizontal sward structure on selective grazing.

Experiment 2: contrasting periods of plant regrowth imposed, to assess the influence of plant maturity on selective grazing .

Experiment 3: contrasting sward height offered at similar stages of vegetative development, to assess the effect of vertical sward structure on selective grazing.

Experiment 4: assessment of the effects of spatial distribution of each sward in the plots upon selective grazing. This was a limited trial, the main objective being evaluation of proximity to fences on animal behaviour.

General details of site and experimental techniques are outlined here. Specific procedures applicable to individual experiments are considered later in the chapter.

3.2.1. Experimental site

The experiments were carried out between 30 October 1995 and 20 May 1996 at AgResearch Flock House in the Manawatu/Rangitikei region (40° 16'S, 175° 17'E). The site is a sandy soil classified as Rangitikei loamy sand (Cowie et al., 1972) on low-lying alluvial flats bordering the Rangitikei River, about 9 m above sea level. Average annual precipitation in this area is 875 mm with a dry period from January to March and strong westerly winds during October to November (spring). The weather conditions, determined approximately 300m from the experiment site, show an average monthly temperature ranging from 9°C (July) to 20°C (January). The daily rainfall and mean soil temperature (10 cm depth) during Experiments 1, 2 and 3 are presented in Appendix 3.2.

3.2.2. Swards

The trials were set up on a sward formed by alternate 2.4 m wide strips (see Plate 3.1.) of a mixture of birdsfoot trefoil (*Lotus corniculatus* L.) cv. Goldie and white clover (*Trifolium repens* L.) cv. Pitau, and strips of red clover (*Trifolium pratense* L.) cv. Colenso. The area was sown in November 1993 with 8 kg of coated seed/ha of birdsfoot trefoil and red clover. The white clover originated from the seed bank formed by the previous sward of a mixture of white clover, perennial ryegrass (*Lolium perenne* L.) and cocksfoot (*Dactylis glomerata* L.). In contrast to a substantial content of white clover in birdsfoot trefoil, very small amounts of volunteer white clover were found with red clover.

In the two years preceding the trial, the sward formed from alternate strips was rotationally grazed by steers. The previous sward had been grazed by cattle, ewes with lambs at foot, and weaned lambs.

Prior to sowing birdsfoot trefoil and red clover, 250 kg/ha of superphophate was applied. In addition, 200 kg/ha of DAP 13S was applied to the area annually.

Plate 3. 1. General view of experimental swards formed by alternate 2.4 m wide strips of a mixture of birdsfoot trefoil (*Lotus corniculatus* L.) cv. Goldie and white clover (*Trifolium repens* L.) cv. Pitau, and strips of red clover (*Trifolium pratense* L.) cv. Colenso

Plate 3.2. General view of experimental swards with grazing animals

3.2.3. Design

The experiments were set out in a Row-Column Design balanced for previous treatment, using four treatments and five replicate groups of three heifers in each experiment (Table 3.1.). In each experiment, four treatments were randomised in each period and replicate groups were allocated to treatments over time. The fifth replication was allocated as one extra treatment in each period. This design was used to control the difference between periods, the difference between groups of heifers and the effect of previous treatments. In this case, five replications were used in order to provide enough degrees of freedom for the residual.

Table 3.1. Distribution of four treatments with five groups of three heifers over four periods.

Period Group of Heifers	1	2	3	4
1	B	D	A	C
2	D	B	C	A
3	C	A	D	B
4	A	B	C	D
5	D	C	B	A

In order to obtain similar groups, the animals were separated into three main classes according to weight: heavy, medium and light. One animal from each class was randomly chosen to form each experimental group of three animals. The animals grazed for fifty five hours on each replication from 1.00 p.m. on Day 1 to 8.00 p.m. on Day 3. Between replications there were four-day intervals for pasture assessment and animal acclimatisation for the next treatment. Each group of three animals stayed together, allocated to treatments over time in each experiment.

3.2.4. Animals

Three different yearling dairy-cross heifer groups were used in Experiments 1, 2, and 3, respectively. The same heifers were used in Experiments 3 and 4. The overall average weight before starting each trial was 264 kg (ranging from 237 to 290 kg), 288 kg (ranging from 222 to 365 kg), and 171 kg (ranging from 145 to 208 kg) for Experiments 1, 2 and 3, respectively. All heifers were drenched with anthelmintic to remove any internal parasites, and bloat capsules were administered prior to each experiment.

For at least two weeks prior to the trial and during four days between replications, all heifers grazed an area adjacent to the plots composed of the same species and strips as the experimental swards. These adjacent areas were used for acclimatisation and reduction of previous treatment effects.

3.2.5. Measurements

3.2.5.1. Sward measurements

Herbage mass

Six samples were cut to ground level before and after grazing in each sward type of each treatment (12 samples per plot), using an electric shearing handpiece and a square sampling frame 0.1 m^2 in area. After cutting, the samples were washed, dried to constant weight in a forced-draught oven at a temperature of 70-80^{0}C, and weighed.

Botanical composition

Six samples of each sward in each treatment, at least 50g each, were also cut to ground level (excluding litter), before and after grazing, from the area beside the sampling frame used in the dry matter assessment. They were sealed in polythene bags and taken to the laboratory in an icebox. The six samples were bulked within plots and from this two sub-samples were taken. The first was used for assessment of botanical composition and separated into species, and then into leaf, petiole (only in clovers), stem (or stolon in white clover), flower and dead material. All the samples were dried and weighed individually. The second sub-sample was separated, as for the botanical composition determination, and freeze-dried for chemical analysis. These samples were ground prior to analysis using a hammer mill fitted with a 1 mm screen.

Pasture height and bulk density

Forty random readings were recorded from each sward in each plot using a sward stick (Bircham, 1981; Barthram, 1986). Readings were taken during the pre-grazing assessment, before the second day of grazing observation and during the post-grazing assessment. Sward bulk density was calculated by dividing herbage mass (g DM/cm^2) by sward height (cm) for each plot.

Pasture structure

An inclined point quadrat (Rhodes and Collins 1993; Montossi et al., 1994) was used to assess the vertical distribution of plant tissue within the sward canopy. Forty contacts per sward per treatment were recorded. The point quadrat observation was taken randomly before and after grazing.

3.2.5.2. Grazing Behaviour

The animals were observed during each of the three days of grazing in each treatment for 3 hours/day, using the method of Jamieson and Hodgson (1979). The distribution of grazing by individual animals between swards was recorded each evening from 4.00 to 7.00 pm at intervals of 10 minutes. In addition, morning observations (from 6.40 am to 9.40 am.) were carried out in the morning of the second day in order to assess the importance of diurnal variation in selective behaviour.

Between each 10 minutes of recording, rates of biting were measured using the 20 bite method of Forbes and Hodgson (1985). The seconds spent for the animal to take 20 bites were recorded. However, if the animal lifted its head the watch was stopped until the animal started grazing again. If the animal did not resume grazing in less than a minute, the recording was not considered. At least two assessments for each animal on each sward were recorded in each observation period.

3.2.6. Chemical analysis

Separated samples (see section 3.2.5.1) were stored at -20^{0}C. The samples were then freeze dried and ground to pass through a 1 mm diameter screen.

Each plant part of birdsfoot trefoil, white clover and red clover was analysed for formononetin concentration. Analyses were carried out on pre-grazing samples. One sample of leaf, petiole, stem and flower (when available) of birdsfoot trefoil, white clover and red clover was randomly chosen from each replication for the analysis of formononetin. In Experiment 2, separate analyses were carried out on samples of immature and mature plants. The formononetin content was determined by a fluorimetric assay described by Gosden and Jones (1978) and modified by Anwar (1994).

The analysis of extractable condensed tannin concentration was done only on birdsfoot trefoil (leaves and stems) since preliminary analysis showed insignificant concentration of extractable condensed tannin in leaves, petioles and stems of red and white clover. In Experiment 1 three samples, of three different replications, were randomly chosen; in Experiments 2 and 3 one sample, of each replication, from immature and mature and from short and tall swards, respectively, were randomly chosen and analysed. The analysis of extractable condensed tannin was carried out by a modification of the DMACA-HCl Protocol described by Li et al. (1996). The modified methodology is described in Appendix 3.1.

Similar amounts of samples were bulked to perform the herbage quality analysis. In Experiment 1 the samples were bulked across treatments and replicates to get one sample of each plant part for birdsfoot trefoil, white clover and red clover. In Experiments 2 and 3 the samples were bulked as in Experiment 1, but contrasts in plant maturity, and plant height characteristics, respectively, were bulked separately. Conventional indices of forage quality (protein, neutral detergent fibre (NDF), acid detergent fibre (ADF), carbohydrates (soluble sugars plus starch), ash and lipid) were determined by Near Infrared Reflectance Spectroscopy (NIRS) (Shenk and Westerhaus, 1994). *In vitro* dry matter digestibility (IVDMD) were calculated using the following equation (Roberts and Packman, 1983):

$$IVDMD = (-0.896 \times ADF) + 95.85$$

3.2.7. Statistical analysis

The sward and animal data were analysed using the statistical package SAS (SAS Institute Inc., 1985 and 1990) and S-plus (Math Soft, 1995). Analyses of variance were carried out to obtain information about the differences between legume species in relation to grazing time and rate of biting, balanced for previous grazing experience. Analyses of variance were also performed for comparison of the sward characteristics. Least square means and standard error of the difference was used to quantify the contrasts in grazing behaviour and sward characteristics. Regression analyses were carried out in Experiment 1 to clarify the relationships between the proportion of area or herbage mass allocated to the birdsfoot trefoil/white clover sward and the proportion of either grazing time or intake on that sward. Contrasts of sward maturity (in Experiment 2) and sward surface height (in Experiment 3) effects were also performed to determine the effects of the treatments (within each sward type and of the alternative sward) on grazing behaviour and animal intake. The point quadrat data were summarised using a point quadrat package (Butler, 1991).

3.2.8. Experimental layouts

3.2.8.1. Experiment 1

The first experiment was carried-out from 30 October until 27 November 1995. Each replication was composed of four 405 m^2 plots fenced to provide distinct area ratios (treatments) of birdsfoot trefoil and white clover (BW) in relation to red clover (RC). Four treatments were imposed, the area ratio in percentage of each sward per treatment being: 20:80; 33:67; 67:33; 80:20 (see Figure 3.1.).

3.2.8.2. Experiment 2

Experiment 2 started on 5 February, and finished on 1 March 1996. The treatments were arranged in order to compare differences in maturity of BW in relation to RC.

Four treatments were imposed. Each treatment was formed by different regrowth periods of BW and RC to provide maturity differences. The swards with a short period of regrowth (immature) were mowed first to 5 cm, and then topped after 4 weeks of regrowth to remove the flowers, leaving an residual of approximately 12 cm. After topping, this area had 3 more weeks of regrowth. Swards with a long period of regrowth (mature) had 9 weeks of regrowth after been mowed to 5 cm. Treatments provided all four combinations of maturity (immature/mature) and sward type (see Figure 3.2.).

The plot sizes were calculated in order to provide similar total quantities of herbage per group of heifers. Therefore, the plot size was determined according to the treatment. The treatment with more mature plants, for example, was estimated to provide greater herbage mass and, consequently, less area was allocated than for treatments with less mature plants.

3.2.8.3. Experiment 3

Experiment 3 ran from 15 April to 10 May 1996. The treatments were arranged in order to compare contrasts in height at the same vegetative stage of growth for birdsfoot trefoil and white clover in relation to red clover. The short swards were formed by mowing to approximately 7 cm height, then allowing four weeks of regrowth until the experiment started. The tall swards were left without being mowed (approximately 8 weeks of regrowth).

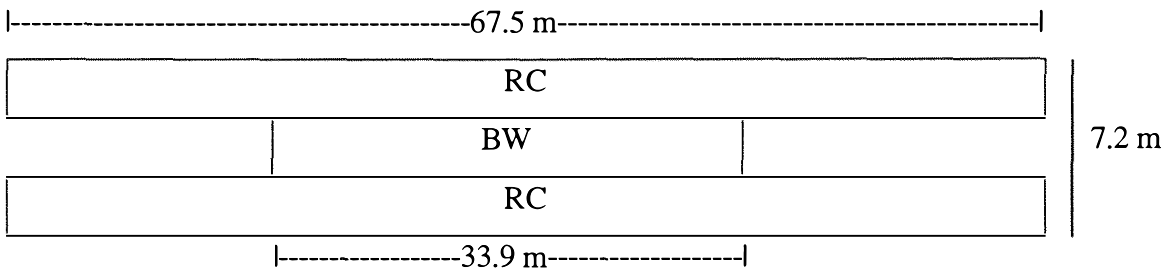
As in Experiment 2, plot sizes were calculated in order to provide a similar amount of herbage per group. Therefore, the plot size was determined according to the treatment. The treatment with taller plants, for example, was estimated to provide greater herbage mass and, consequently, less area than treatments with shorter plants (see Figure 3.3.).

3.2.8.4. Experiment 4

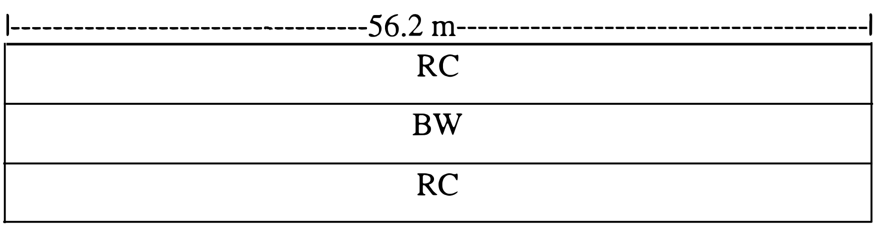
Experiment 4 was a small trial with the objective of assessing the effect of position in the plot on sward use. Each plot was fenced to incorporate four strips, one of which was mown to ground level to provide all combinations of one or two grazeble strips, adjacent to or separated from a fence, for the two sward types (see Figure 3.4.).

Figure 3. 1. Experiment 1 - treatment layout : area ratio birdsfoot trefoil and white clover (BW) : red clover (RC) (not to scale – strips of 2.4 cm width). Total area of each plot = 405 m^2

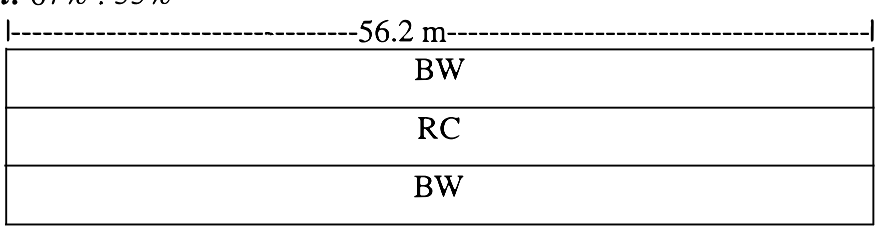
Treatment: 20% : 80%



Treatment: 33% : 67%



Treatment: 67% : 33%



Treatment: 80% : 20%

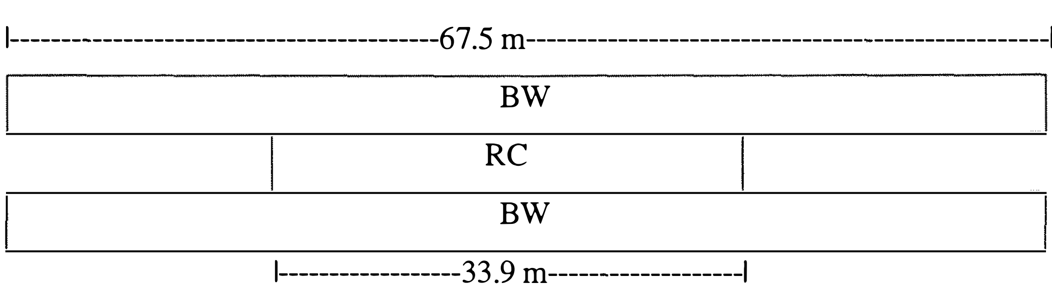


Figure 3. 2. Experiment 2 - treatment layout: plant maturity contrast [3 weeks ( ) and 9 weeks (/////) of regrowth] of birdsfoot trefoil and white clover (BW) : red clover (RC) (not to scale - strips of 2.4 cm width).

Treatment: 3 weeks : 3 weeks

|--------------------31.25 m--------------------|

BW
RC
BW
RC
BW
RC

14.4 m

Total. area = 450 m^2

Treatment: 3 weeks : 9 weeks

|--------------22.5 m--------------|

BW
////////////////RC////////////////
BW
////////////////RC////////////////
BW
////////////////RC////////////////

Total area = 324 m^2

Treatment: 9 weeks : 3 weeks

|--------------22.5 m--------------|

////////////////BW////////////////
RC
////////////////BW////////////////
RC
////////////////BW////////////////
RC

Total area = 324 m^2

Treatment: 9 weeks : 9 weeks

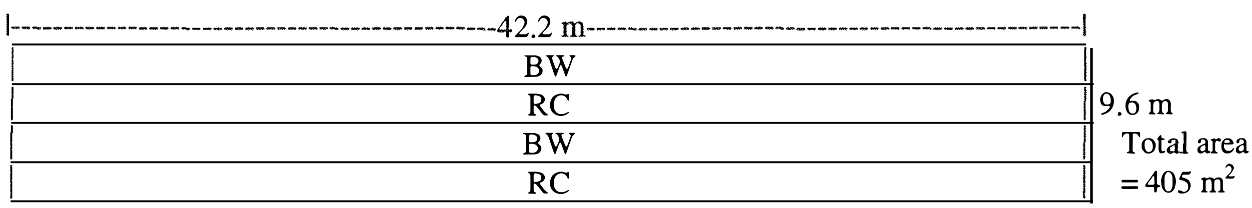
|----------17.57 m ----------| .

////////////BW////////////
////////////RC////////////
////////////BW////////////
////////////RC////////////
////////////BW////////////
////////////RC////////////

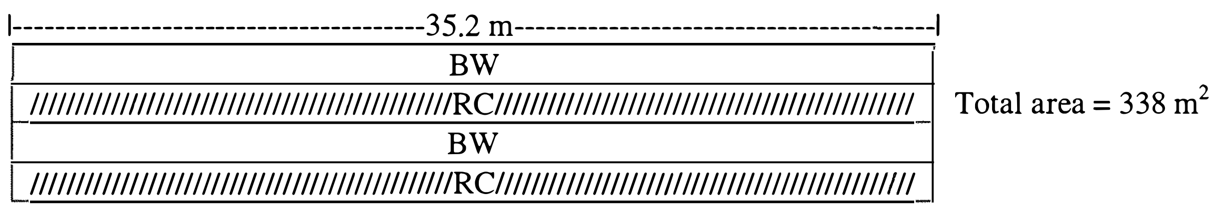
Total area = 253 m^2

Figure 3. 3. Experiment 3 - treatment layout : plant height contrast [short( ) and tall(///)] of birdsfoot trefoil and white clover (BW) : red clover (RC) (not to scale - strips of 2.4 cm width).

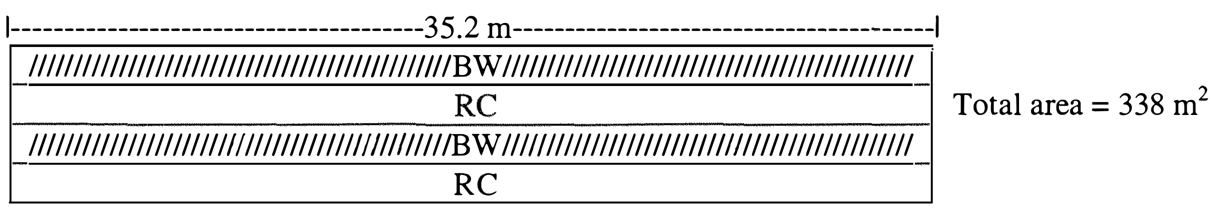
Treatment: short : short



Treatment: short : tall



Treatment: tall : short



Treatment: tall : tall

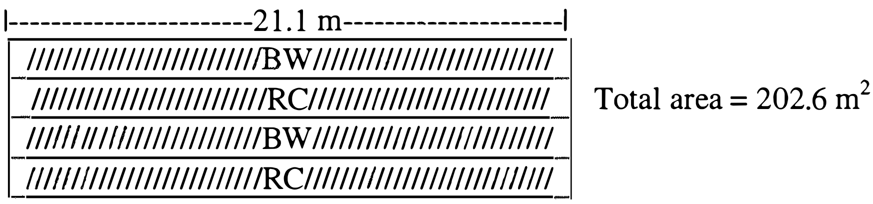


Figure 3. 4. Experiment 4 - treatment layout : spatial distribution contrast of birdsfoot trefoil and white clover (BW) : red clover (RC) (not to scale - strips of 2.4 cm width). Total area of each plot = 159.4 m^2

Treatment: BW beside 2 strips of RC

|---------------------16.6 m---------------------------|

RC	9.6 m
////////////////////////mowed////////////////////////	
RC	
BW	

Treatment: BW between 2 strips of RC

RC
BW
RC
////////////////////////mowed////////////////////////

Treatment: RC beside 2 strips of BW

BW
////////////////////////mowed////////////////////////
BW
RC

Treatment: RC between 2 strips of BW

BW
RC
BW
////////////////////////mowed////////////////////////

3.3. RESULTS

The results of the first four experiments are presented in this section. The results of each experiment are shown separately.

3.3.1. Experiment 1: *Effect of the proportion of area of birdsfoot trefoil (Lotus corniculatus* L.*) and white clover (Trifolium repens* L.*) sward in relation to red clover (Trifolium pratense* L.*) sward on grazing behaviour, diet selection and herbage intake.*

3.3.1.1. Sward measurements

Herbage mass, sward surface height and sward bulk density

Most of the interactions between treatments and sward type were not significant, so results are presented as main effects only. The results for herbage mass, surface sward height and sward bulk density are given in Table 3.2. The interaction means between treatments and sward type are presented in Appendix 3.3.

Both before and after grazing, red clover swards had significantly greater herbage mass and height, but significantly lower bulk density. This resulted in a similar amount of herbage removed per sward.

After grazing, sward height and bulk density showed a significant interaction between sward type and treatments (Appendix 3.3). In this case the difference in height between RC and BW swards within each treatment increased as the BW area decreased.

Table 3.2. Herbage mass, sward height and bulk density before and after grazing, and estimation of the herbage mass removed for birdsfoot trefoil and white clover (BW) and red clover (RC) swards in Experiment 1.

	BW	*RC*	*SED*[1]	*P-value*[2]
Herbage mass (kg DM/ha)				
Pre-grazing	3940	4570	164	0.0014
Post grazing	2380	3340	145	0.0000
DM removed	1560	1230	206	0.1329
Sward height (cm)				
Pre-grazing	19.1	27.7	1.33	0.0000
After 1 day grazing	12.3	21.9	0.87	0.0000
Post-grazing	7.5	13.3	0.35	0.0000
Bulk density (mg DM/cm³)				
Pre-grazing	2.06	1.67	0.172	0.0027
Post-grazing	3.17	2.51	0.216	0.0074

[1] SED — Standard error for differences of means when comparing BW with RC swards.
[2] P-value of the sward main effect.

There was also a significant interaction between sward type and treatments for bulk density post-grazing. The BW sward became denser, from pre to post grazing, more than the RC sward, except when 80 % of the area offered was BW.

<u>Sward composition</u>

The botanical composition of each sward before and after grazing is shown in Table 3.3. The interactions between treatments and sward types were not significant for most of plant components, so results are presented as main effects only. The interactions between treatments and sward types are given in Appendix 3.4.

Table 3. 3. Botanical composition of birdsfoot trefoil and white clover (BW) and red clover (RC) swards before and after grazing (DM basis): (a) percentage of components in live fraction, (b) percentage of live matter in total DM of each sward and (c) ratio of the total live matter of birdsfoot trefoil and white clover (B/W) in the BW sward, Experiment 1.

		BW	*RC*	*SED*[1]	*P-value*[2]
	Pre-grazing				
(a)	Leaf	44.0	41.1	2.89	0.3293
	Petiole	19.5	15.8	2.99	0.3264
	Stem	15.5	34.3	2.58	0.0000
	Flower	0.8	0.9	0.30	0.7000
	Grass	8.3	1.8	2.86	0.0350
	Other species	12.1	6.2	2.98	0.0680
(b)	Total live matter	95.7	91.5	1.97	0.0003
(c)	B/W ratio	0.68			
	Post-grazing				
(a)	Leaf	27.8	17.3	2.56	0.0008
	Petiole	29.1	16.2	1.53	0.0001
	Stem	27.7	48.1	3.36	0.0000
	Flower	0.7	0.8	0.52	0.8229
	Grass	5.3	5.5	3.81	0.9464
	Other species	9.1	11.9	2.90	0.3362
(b)	Total live matter	91.3	84.3	1.74	0.0003
(c)	B/W ratio	0.40			

[1] SED — Standard error for differences of means when comparing BW with RC swards.
[2] P-value of the sward type main effect.

Before grazing, the RC sward had significantly more stem material, more dead matter and less other broad leaf species than BW. Flowers were a minor component in both swards.

There were modifications in botanical composition as a consequence of grazing, though no statistical analyses were performed to compare pre with post grazing. The percentage of dead matter and stem increased from pre to post grazing in both swards. The decline in leaf content was greater in RC than in BW swards. After grazing, there were significantly more leaves and petioles in BW than in RC swards. White clover was the predominant species in BW before and after grazing, but there was greater difference after grazing.

3.3.1.2. Canopy structure within the sward

The point quadrat data from the two swards are summarised in Figures 3.5 and 3.6. Comparing the four treatments within each sward type, either before or after grazing, the structures and distributions of plant parts were similar. In all cases the dead material was distributed mainly in the bottom strata. In the BW sward before grazing, white clover was the main contributor, distributed mainly in the bottom and medium strata. Birdsfoot trefoil was more evenly distributed vertically in the canopy. In the RC sward before grazing, there was a major contribution of leaves and petioles mainly in the medium and upper strata. The proportion of stems was larger in the mid and bottom strata of the canopy. The relative contribution of red clover leaves, petioles and stems in the bottom strata increased after grazing.

3.3.1.3. Sward chemical composition

<u>*Extractable condensed tannin concentration*</u>

The concentration of extractable condensed tannin in leaves and stems of birdsfoot trefoil is presented in Table 3.4.

Table 3. 4. Extractable condensed tannin (ECT) concentration (%) of birdsfoot trefoil leaf and stem in Experiment 1 (DM basis).

Components	*ECT*	*SEM*[1]
Leaf	1.69	0.090
Stem	0.05	0.003

[1] SEM – Standard error of the means.
Number of observations contributing for each mean (n=3)

The data in Table 3.4. are the average of three replications for leaves and stems. The concentration of ECT was higher in leaves than in stems. The stems showed negligible amount of ECT.

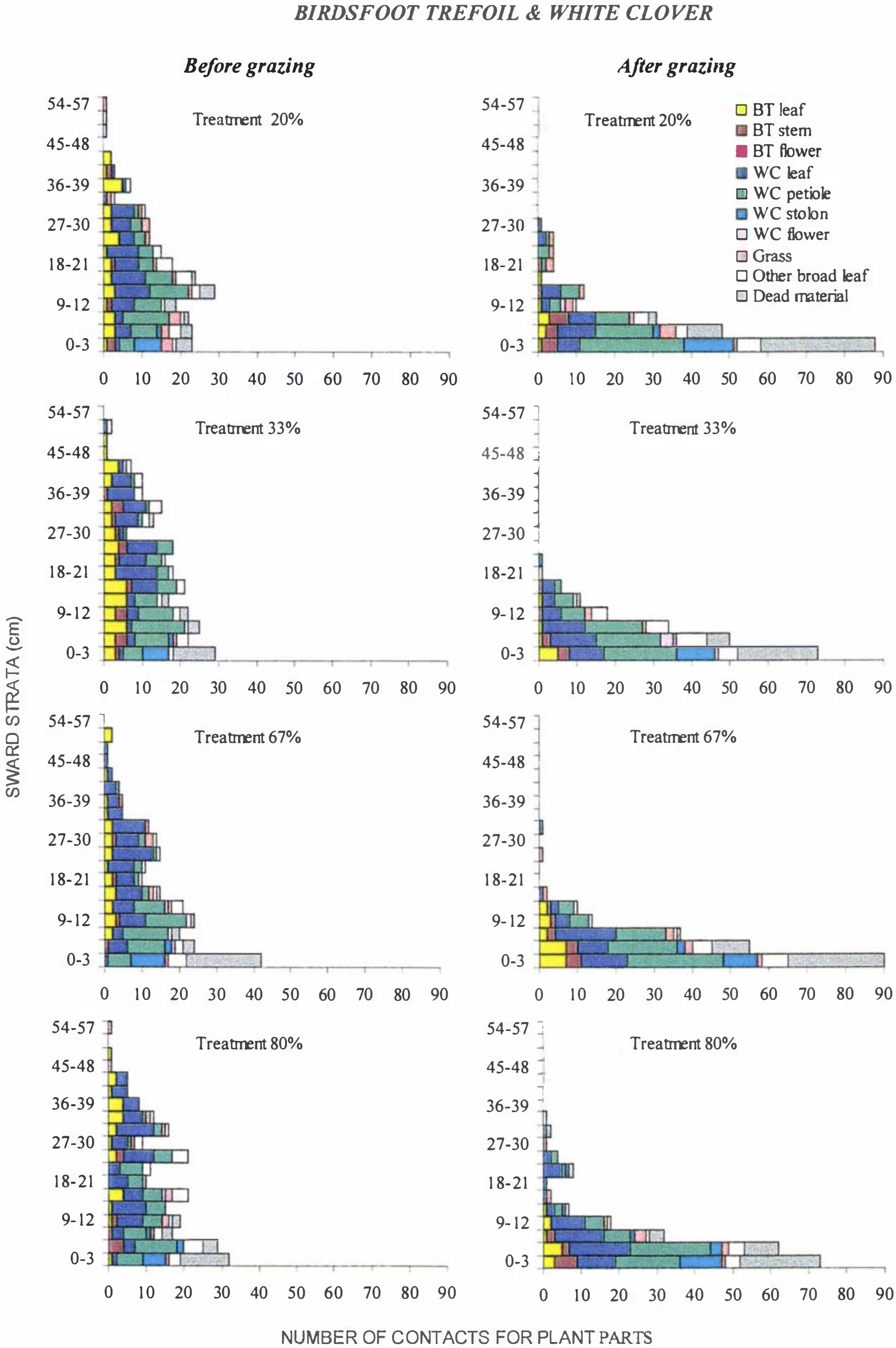


Figure 3.5. The stratum structure of plant parts within the sward canopy of birdsfoot trefoil and white clover before and after grazing in the four treatments (area ratio) in Experiment 1.

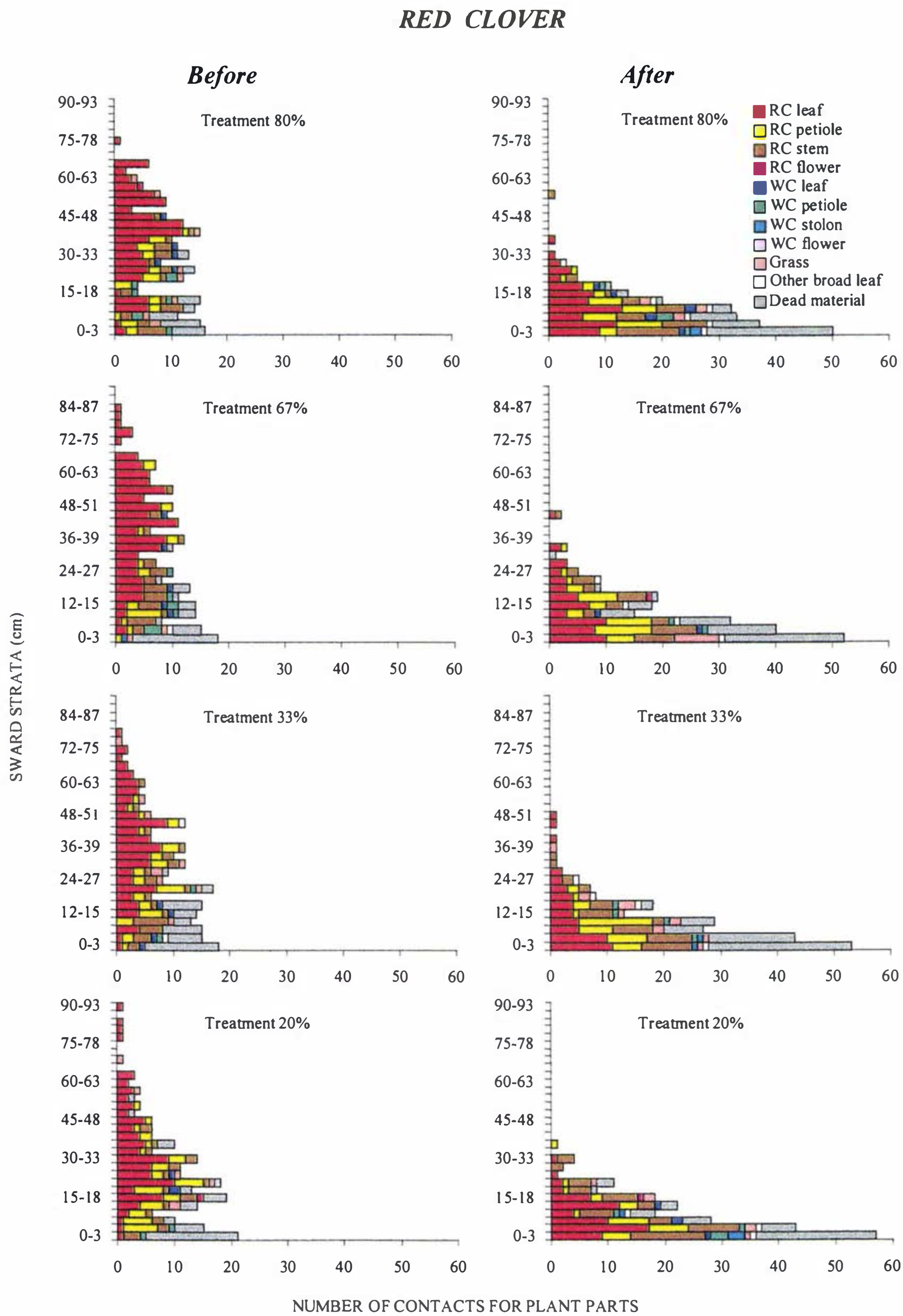


Figure 3.6. The stratum structure of plant parts within the sward canopy of red clover, before and after grazing in the four treatments (area ratio) in Experiments 1.

Formononetin concentration

The formononetin concentrations in BW and RC sward main species are presented in Table 3.5.

Red clover had significantly higher concentration of formononetin than birdsfoot trefoil and white clover in leaves, petioles and stems. Red clover had higher concentration of formononetin in leaves than in petioles or stems. The formononetin contents of red and white clover flowers were similar.

Table 3.5. Formononetin concentration (%) of leaf, petiole, stem and flower of birdsfoot trefoil (BT), white clover (WC) in birdsfoot trefoil and white clover sward (BW), and red clover (RC) in red clover sward (RC) in Experiment 1.

Plant components	*BW sward*		*RC sward*	*SED*[1]	P-value[2]
	BT	*WC*	*RC*		
Leaf				0.010[3]	0.0021[3]
	0.08	0.19	0.61	0.080[4]	0.0060[4]
				0.080[5]	0.0143[5]
Petiole	-[6]	0.16	0.50	0.056	0.0089
Stem	0.12	-	0.30	0.029	0.0116
Flower	-	0.16	0.17	0.045	0.9296

[1] SED — Standard error for differences of means
[2] P-value for the comparison of species.
[3] Standard error for differences of means and P-value for comparison between BT and WC.
[4] Standard error for differences of means and P-value for comparison between BT and RC.
[5] Standard error for differences of means and P-value for comparison between WC and RC.
[6] - no sample
Number of observations contributing to each mean (n=4)

General chemical composition

The general chemical composition of each plant part is presented in Table 3.6. Because the values represent a single sample derived by bulking across treatments and replications, statistical analysis was not possible. In general, the three species, white clover (WC), birdsfoot trefoil (BT) and red clover (RC) were similar in the percentage of crude protein (CP), acid and neutral detergent fibre (ADF, NDF) and *in vitro* dry matter digestibility (IVDMD). Although BT had a slightly lower percentage of CP, ADF and NDF in leaves than did red clover and white clover, the three legumes had low percentages of ADF and NDF and high percentages of all other chemical components, showing the high nutritive value of these species. BT had higher CHO in leaves, but lower percentage in stems, than WC and RC.

Table 3. 6. Crude protein (CP), lipid, acid and neutral detergent fibre (ADF, NDF), carbohydrates (soluble sugars plus starch)(CHO), ash and *in vitro* dry matter digestibility (IVDMD) determined by Near Infrared Reflectance Spectroscopy (NIRS) of the main components of birdsfoot trefoil and white clover, and red clover swards in Experiment 1 (percentage of DM basis).

	CP	*LIPID*	*ADF*	*NDF*	*CHO*	*ASH*	*IVDMD*
White clover							
leaf	34.0	2.9	19.2	20.6	5.3	12.1	78.6
petiole	14.9	1.1	31.7	33.6	8.1	10.7	67.4
stolon	17.9	0.9	25.3	21.5	12.4	9.0	73.2
flower	20.6	3.3	26.9	17.0	7.1	12.6	71.7
Birdsfoot trefoil							
leaf	27.9	3.9	17.0	15.7	13.6	10.4	80.6
stem	13.8	1.6	33.5	40.5	6.4	7.9	65.8
Red clover							
leaf	33.3	3.0	20.5	21.5	11.1	11.3	77.5
petiole	14.6	1.5	32.5	34.6	10.4	10.0	66.7
stem	12.4	0.8	32.6	34.5	13.0	8.0	66.6
flower	19.7	3.4	27.3	21.6	9.7	11.6	71.4

3.1.1.2. Animal measurements

Grazing time and intake

Measurements of grazing time and estimates of herbage intake per day are summarised in Table 3.7.

There was a significant interaction between treatment and sward type for grazing time in each of the three days of grazing. The magnitude of this interaction increased from the first to the last day. In absolute terms, as time passed the animals spent more time grazing the larger areas and consequently the difference in time spent grazing between different swards increased from Day 1 to Day 3. The total number of minutes spent grazing per animal also increased from Day1 to Day 3.

Results of the morning observation for Day 2 was consistently intermediate between evening observations of Day 1 and Day 2 in this and the following experiments (Appendix.3.6). Further evaluation of behaviour results is confined to evening observations.

Table 3. 7. The effect of treatments (area ratios) on grazing time (minutes) in the first, second and third days of grazing (Days 1, 2 and 3), and average DM intake per animal per day (kg DM/hd/day) during 55 hours of grazing in Experiment 1.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*			
	BW 20%	RC 80%	BW 33%	RC 67%	BW 67%	RC 33%	BW 80%	RC 20%	SED[1]	P-value[2]
Grazing time (min)										
Day 1	26.3	47.6	41.9	49.3	51.9	31.2	58.5	28.5	10.75	0.0129
Day 2	14.5	82.5	25.0	65.0	57.5	25.5	72.3	16.3	5.46	0.0000
Day 3	11.7	100.4	19.1	76.5	72.4	39.1	82.7	19.4	10.25	0.0000
Intake (kgDM/hd/day)	2.17	6.34	3.04	4.98	5.75	2.24	5.88	1.62	1.425	0.0004

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.

[2] P-value of the interaction: treatment*sward type effect.

There were significant effects of treatment on the proportion of grazing time ($Propn_{gt}$) activity on BW strips for all three days (Table 3.8), with no indication of a significant effect of the previous treatment between periods and no significant differences between groups of animals (P>0.05). Within days, variation in the distribution of grazing activity was linearly related to the proportional area of BW available ($Propn_{area}$, P<0.001), with no significant residual treatment effects (P>0.05) and no significant differences between groups of animals (P>0.05). On Day1 the animals allocated grazing activity preferentially to the minor sward component on each treatment, and the regression slope of $Propn_{gt}$ on $Propn_{area}$ was correspondingly low (Table 3.9, Figure 3.7). However, in Days 2 and 3 the regression did not differ significantly from a 1:1 relationship, implying neutrality of choice (Figure 3.9, Figure 3.7).

Regression based on the proportions of herbage DM on BW strips ($Propn_{dm}$) also showed similar linear trends between the proportion of DM offered and proportion of grazing activity with no significant residual treatment effects (P>0.05) (Table 3.9). This regression also differed significantly from 1:1 relationship (neutrality) on the first day but not on the last day of grazing (Figure 3.8). In these linear trends, there was no significant previous treatment and group of animals effect. In both regressions, $propn_{gt}$ on $propn_{area}$ and $propn_{gt}$ on $propn_{dm}$, the first day grazing activity was more variable.

There was a significant interaction between treatment and sward type effects on estimated forage intake per animal per day. On average an animal consumed between 7.5-8.5 kg DM/day. There was a linear relationship between the proportion of total intake from BW and proportion of area offered to the animals. The slope of the regression was not significantly different from 1:1 (slope = 0.76 ± 0.25), but the mean was significantly higher than neutrality (Figure 3.9). There was also no indication of a significant effect of previous treatment between periods, and no significant residual treatment effect and significant group of animals effect (P>0.05).

Table 3. 8. Treatment (20, 33, 67 and 80 % of the total area offered) effects on the proportion of grazing time (in relation to the total grazing time spent in plot) devoted to birdsfoot trefoil plus white clover swards (BW) in Experiment 1.

	Proportion of area of BW					
	20%	*33%*	*67%*	*80%*	SED[1]	P-value[2]
Day 1	0.35	0.46	0.60	0.69	0.069	0.0040
Day 2	0.15	0.26	0.70	0.80	0.035	0.0001
Day 3	0.09	0.20	0.66	0.79	0.074	0.0001

[1] SED – Standard error for differences of means when comparing different treatments.
[2] P-value of the treatment main effect.

Table 3. 9. Regression slopes of the proportion of grazing time ($propn_{gt}$) in relation to the proportion of area ($propn_{area}$) and dry matter ($propn_{dm}$) offered in the first, second and third days of grazing observation in Experiment 1 (slope significance in relation to neutrality value of 1.0).

Regression of	*Day 1*	*Day 2*	*Day 3*
Propn$_{gt}$ on propn$_{area}$	0.57 ± 0.1154 **	1.09 ± 0.0583 NS	1.17 ± 0.1235 NS
Propn$_{gt}$ on propn$_{dm}$	0.55 ± 0.0929 ***		1.20 ± 0.1093 NS

Significance of difference from 1.0: ** P≤0.01 and NS (not significant).
The slopes are originated from the analysis of variance, after period and group of animals had been added to the model.

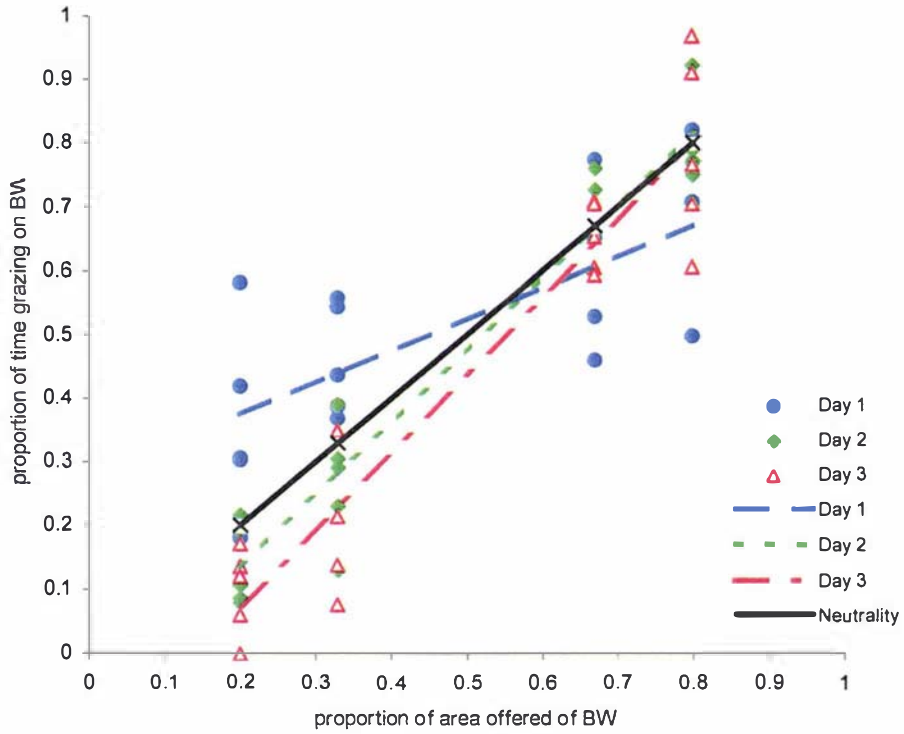


Figure 3. 7. Proportion of grazing time in relation to the proportion of area offered of birdsfoot trefoil plus white clover (BW)

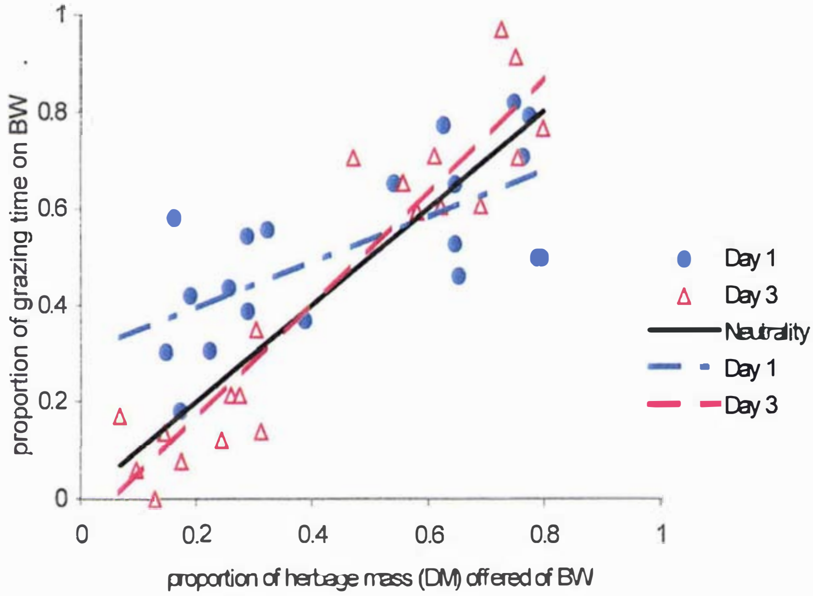


Figure 3. 8. Proportion of grazing time in relation to the proportion of herbage mass offered of birdsfoot trefoil plus white clover (BW)

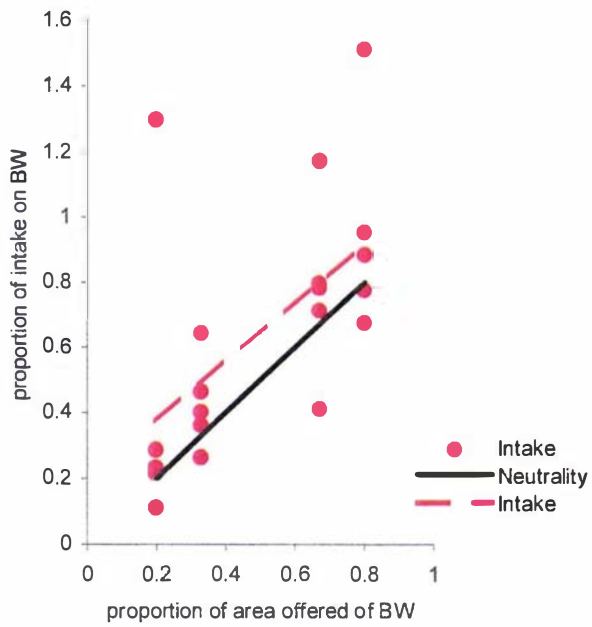


Figure 3. 9. Proportion of intake in relation to the proportion of area offered of birdsfoot trefoil plus white clover (BW)

Rate of biting

There was no significant interaction between treatment and sward type on the rate of biting on any of the three days of grazing. There was a significant main sward type effect (Table 3.10) with no significant difference between treatments. Bite rates were consistently higher on BW than on RC swards over all three days of measurements (52.3 vs 46.3 ± 0.59 bites/minute $P \leq 0.0001$). The rate of biting increased in both species and the statistical significance of the difference between BW and RC decreased from the first to the last day of grazing. The interaction means between treatments and sward type are presented in Appendix 3.3.

Table 3. 10. The effect of swards of birdsfoot trefoil and white clover (BW), and red clover (RC) on rate of biting (bites/minute) in the first, second and third days (total 55 hours) of grazing assessment in Experiment 1.

	BW	*RC*	*SED*[1]	*P-value*[2]
Rate of biting (bites/min)				
Day 1	49.5	45.1	0.72	0.0000
Day 2	52.7	46.2	1.36	0.0003
Day 3	54.3	47.5	1.63	0.0238

[1] SED — Standard error for differences of means when comparing BW with RC swards.
[2] P-value of the sward main effect.

3.3.2. Experiment 2: Effect of the maturity of birdsfoot trefoil (Lotus corniculatus L.*) and white clover (Trifolium repens* L.*) in relation to red clover (Trifolium pratense* L.*) on grazing behaviour, diet selection and herbage intake.*

3.3.2.1. Sward measurements

Herbage mass, sward surface height and sward bulk density

The results of herbage mass, sward surface height and sward bulk density are given in Table 3.11.

There was a significant interaction between treatment and sward type in relation to herbage mass. Before grazing, the sward with the longer period of regrowth (mature) had higher herbage mass. Considering only mature swards, herbage mass was greater in RC than BW. In contrast, for immature swards, herbage mass was greater in BW. Although there was more herbage mass removed from mature swards, there was no significant interaction between treatment and sward type in relation to the difference between pre and post grazing herbage mass. However there was also significant treatment main effect. The two treatments that had mature RC had the greatest amount of mass removed and the treatment with both swards (BW and RC) immature the smallest.

There was a significant interaction between treatment and sward type in relation to pre grazing height. Within each treatment, RC was always the tallest sward and mature plants were taller than immature. After the first day of grazing, there was no significant interaction between treatment and sward type in relation to height. RC swards were significantly taller (11.8 vs 15.2 cm, SED 0.700, $P<0.00004$) after one day of grazing, but not after three days (7.8 vs 8.4, SED 0.90, $P<0.5409$).

Table 3. 11. Herbage mass (kg DM/ha), sward height (cm) and bulk density (mg DM/cm^3) before and after grazing, and estimation of the herbage mass removed (kg DM/ha) of birdsfoot trefoil and white clover (BW) and red clover (RC) swards according to treatment in Experiment 2.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*			
	BW Immature	RC Immature	BW Immature	RC Mature	BW Mature	RC Immature	BW Mature	RC Mature	SED[1]	P-value[2]
Herbage mass (kgdm/ha)										
Pre-grazing	4270	3550	4240	6470	5450	3830	5660	5760	710	0.0044
Post grazing	2860	2730	2050	4080	3320	2550	3090	3290	393	0.0002
DM removed	1420	820	2190	2400	2130	1290	2570	2470	672	0.6859
Sward height (cm)										
Pre-grazing	18.5	27.6	19.0	39.3	24.3	26.9	22.2	38.6	1.92	0.0000
After 1 day grazing	12.3	15.4	10.8	15.4	12.7	15.5	11.5	14.5	1.40	0.7840
Post-grazing	8.5	9.0	7.6	8.4	7.7	8.3	7.6	7.9	1.80	0.9960
Bulk density(mgdm/cm^3)										
Pre-grazing	2.33	1.28	2.20	1.65	2.28	1.50	2.55	1.50	0.266	0.5190
Post-grazing	3.77	3.94	2.73	5.30	4.75	3.41	4.08	4.74	0.973	0.0608

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.

[2] P-value of the interaction: treatment*sward type effect.

There was also no significant interaction between treatment and sward type for bulk density. BW was consistently denser before grazing (main effect 2.34 vs 1.47 mgDM/cm^3, SED 0.133, P<0.0000). After the third day of grazing the swards did not differ significantly (main effect 3.83 vs 4.35 mgDM/cm^3, SED 0.486, P<0.2966). Both swards had much higher density after grazing than before.

Sward composition

Before grazing, there was a significant interaction between sward type and treatment effect for percentage of leaves and a marginal interaction for stems (Table 3.12). The difference in percentage of leaves was smaller when both swards were in the same stage of regrowth. Swards with long period of regrowth (mature) had a smaller proportion of leaves than with short period of regrowth (immature). Comparing within each treatment before grazing, immature swards had proportionally fewer stems and more leaves than mature. Although the interaction was not significant, there was much higher amount of flowers in mature than in immature swards. There was also a marginally higher amount of broad leaf weeds on RC than on BW (5.1 vs 9.7, SED 2.30, P<0.0579).

The sward type x treatment interaction for stem content was stronger after than before grazing, but that for leaf content declined. The percentage of stems increased from pre to post grazing and the difference between immature and mature within each treatment also increased. The percentage of flowers decreased but there was still a large contrast between immature and mature within each treatment. The percentage of dead matter increased in the sward from pre to post grazing.

Table 3. 12. Botanical composition of birdsfoot trefoil plus white clover (BW) and red clover (RC) swards before and after grazing, according to the treatments (plant maturity: Immature and Mature) (DM basis): (a) percentage of components in live fraction, (b) percentage of live matter in total DM of each sward and (c) ratio of the total live matter of birdsfoot trefoil and white clover (B/W) in the BW sward, Experiment 2.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*		SED[1]	P-value[2]
	BW Immature	RC Immature	BW Immature	RC Mature	BW Mature	RC Immature	BW Mature	RC Mature		
Pre-grazing										
(a) Leaf	30.2	31.1	34.7	24.9	22.3	31.8	23.7	25.5	3.70	0.0094
Petiole	13.8	14.7	13.4	8.6	14.1	14.5	10.5	9.8	4.25	0.7825
Stem	47.7	35.2	36.5	44.6	46.2	36.7	48.4	39.3	5.55	0.0535
Flower	5.1	6.3	4.9	12.6	12.4	6.4	13.5	19.2	3.75	0.0734
Grass	0.0	0.0	1.7	0.0	0.0	0.0	0.3	0.2	0.68	0.2279
Other species	3.2	12.8	8.7	9.3	5.0	10.5	3.5	6.0	4.60	0.5416
(b) Total live matter	90.3	88.7	90.3	91.3	90.2	91.0	93.6	93.0	2.92	0.9147
(c) B/W ratio	1.79		2.76		4.76		7.07		2.606	0.6467
Post-grazing										
(a) Leaf	15.2	13.6	15.0	8.6	8.6	9.8	9.2	5.4	2.74	0.2530
Petiole	13.1	14.5	15.0	8.3	8.6	10.6	6.2	6.8	2.46	0.0627
Stem	65.5	47.7	53.5	64.1	62.1	37.5	63.2	66.1	6.50	0.0015
Flower	2.3	3.3	1.1	9.8	8.6	2.9	10.1	5.4	3.10	0.0102
Grass	1.1	0.0	2.6	0.0	3.7	0.4	0.4	1.2	2.23	0.6003
Other species	2.7	20.8	12.8	9.1	8.5	38.6	10.9	15.2	7.10	0.0109
(b) Total live matter	79.1	71.3	79.2	81.1	81.3	68.8	89.3	69.2	5.99	0.0920
(c) B/W ratio	2.04		0.49		2.77		4.99		0.698	0.0123

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.

[2] P-value of the interaction: treatment*sward type effect.

Considering only the sward type main effect, BW had, on average, significantly higher percentage of live matter than RC (82.2 vs 72.6 %, SED 3.00, P<0.0032).

The amount of birdsfoot trefoil was higher than the amount of white clover in all treatments, except in the treatment where BW was immature in post-grazing. There were only significant differences between treatments after grazing. The treatment where both swards were mature had significantly higher amount of birdsfoot trefoil in relation to white clover.

3.3.2.2. Canopy structure within the sward

The vertical distributions of the plant parts of BW and RC swards within the sward canopy are shown in Figure 3.10 and 3.11. In BW swards birdsfoot trefoil had a proportionally larger contribution in upper strata than white clover. However the differences between treatments decreased after grazing. In RC swards there was a similar distribution of plant parts, comparing treatments within each period (before of after grazing), but mature swards had components in higher strata. In both swards the dead matter was distributed in the bottom strata and the leaf and petiole components were proportionally greater in the medium and high strata. The number of contacts in the lower strata increased after grazing.

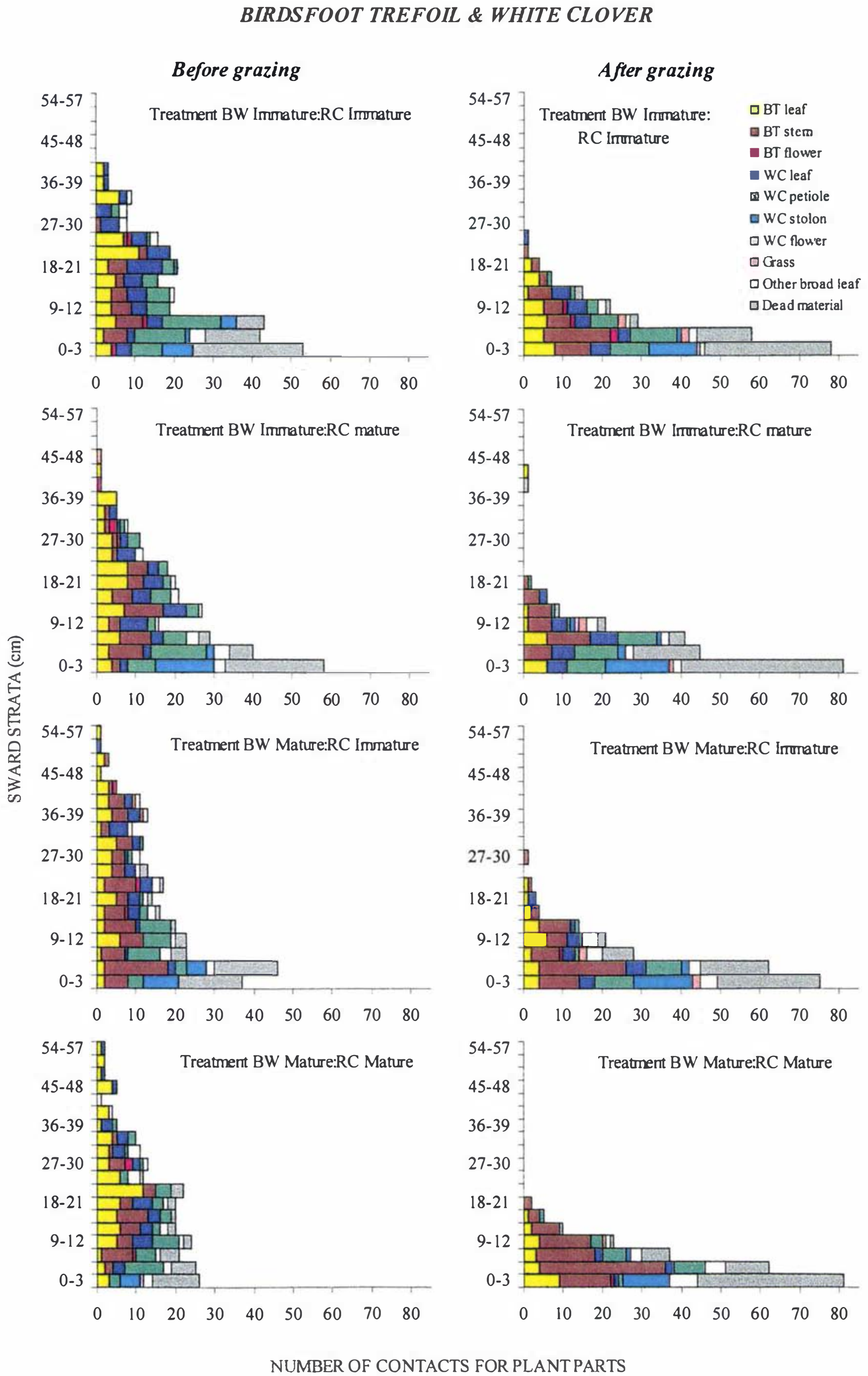


Figure 3.10.The stratum structure of plant parts within the sward canopy of birdsfoot trefoil and white clover (BW), before and after grazing in the four treatments (RC = red clover sward) in Experiment 2.

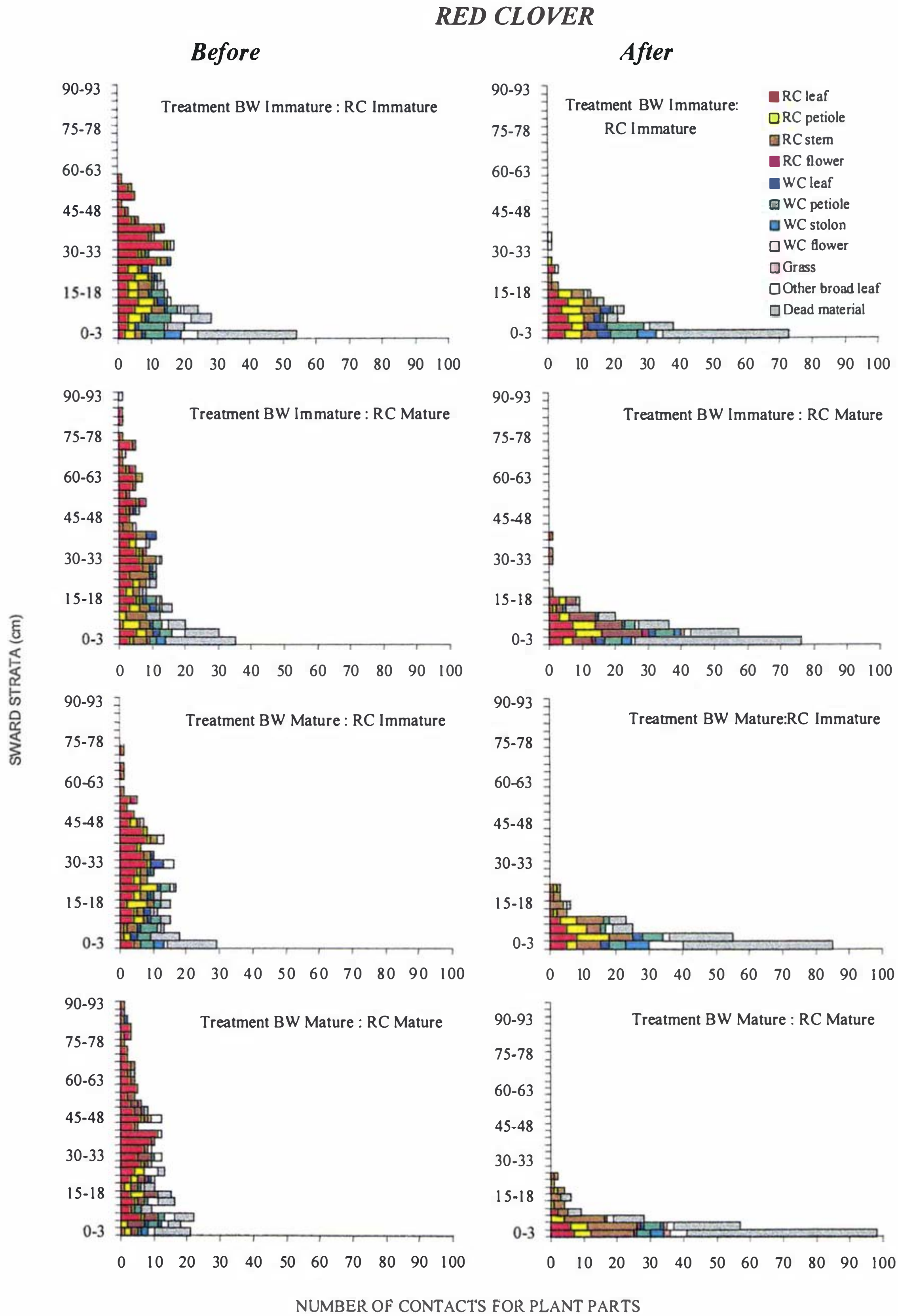


Figure 3.11.The stratum structure of plant parts within the sward canopy of red clover (RC), before and after grazing in the four treatments (BW = birdsfoot trefoil and white clover sward) in Experiment 2.

3.3.2.3. Sward chemical composition

Extractable condensed tannin concentration

The concentrations of extractable condensed tannin (ECT) in leaves and stems of birdsfoot trefoil plants for swards with long (Mature) and short (Immature) period of regrowth are given in Table 3.13. The concentration of ECT in leaves of immature plants was twice that in mature plants, a difference approaching significance at the 5 % level of probability over four replicates. The concentration of ECT in stems was insignificant at both maturities. The concentration of ECT in white clover and red clover was also negligible.

Table 3.13. The effect of extractable condensed tannin (ECT) concentrations of birdsfoot trefoil leaf and stem in Experiment 2 (%DM basis) according to the treatment.

	Mature	*Immature*	*SED*[1]	*P-value*[2]
Leaf	0.37	0.79	0.1478	0.0665
Stem	0.005	0.005	0.0200	1.0000

[1] SED – Standard error for differences of means when comparing Mature with Immature swards.
[2] P-value of the treatment main effect.
Number of observations contributing for each mean (n=4)

Formononetin concentration

There was no significant difference in formononetin concentration between immature and mature swards (Appendix 3.4). The formononetin concentrations in the main species of BW and RC swards are presented in Table 3.14. Compared with birdsfoot trefoil and white clover, red clover had a higher percentage of formononetin in all plant components, except flowers. The main concentrations of formononetin in red clover were in leaves and petioles. Birdsfoot trefoil did not differ from white clover in formononetin concentration in leaves. Although flowers were significantly different, they all had relatively low concentrations of formononetin.

Table 3.14. Formononetin concentration (%) of leaf, petiole, stem and flower of birdsfoot trefoil (BT), white clover (WC), in birdsfoot trefoil and white clover sward (BW), and red clover (RC), in red clover sward (RC) in Experiment 2.

	BW sward		*RC sward*		
	BT	*WC*	*RC*	*SED*[1]	P-value[2]
Leaf	0.12	0.17	0.50	0.0400[3]	0.0001[3]
Petiole	-[4]	0.21	0.42	0.0458	0.0034
Stem	0.10	-	0.33	0.0358	0.0007
Flower	0.07	0.13	0.10	0.0121(BT-RC) 0.0113(WC-RC)	0.0014

[1] SED – Standard error for differences of means when comparing means of each specie.
[2] P-value for the comparison of species.
[3] SED and P-value for the comparison of RC vs BW and RC vs WC.
[4]- no sample
Number of observations contributing to the mean of each specie (n=8), but n=5 for birdsfoot trefoil, n=6 for white clover and n=8 for red clover in flower assessment.

General chemical composition

The general chemical composition of each plant part is presented in Table 3.15. There was a similar chemical composition between the three main species, and only a small reduction in quality, comparing immature and mature swards. Birdsfoot trefoil had lower percentage of crude protein in the stems, compared with the other two species, and, as in Experiment 1, had higher proportion of soluble sugars plus starch in the leaves than in the stems.

Table 3. 15. Crude protein (CP), lipid, acid and neutral detergent fiber (ADF, NDF), carbohydrates (soluble sugars plus starch)(CHO), ash and in *vitro* dry matter digestibility (IVDMD) determined by Near Infrared Reflectance Spectroscopy (NIRS) of the main components of birdsfoot trefoil plus white clover, and red clover swards in Experiment 2 according to the period of regrowth (immature and mature) (percentage of DM basis).

		CP	*LIPID*	*ADF*	*NDF*	*CHO*	*ASH*	IVDMD
IMMATURE	***White clover***							
	Leaf	35.4	2.3	17.1	18.3	5.3	12.5	80.5
	Petiole	16.9	1.0	30.6	31.9	7.0	11.5	68.4
	Stolon	17.9	0.7	22.2	19.5	18.5	8.1	76.0
	Flower	21.0	3.3	30.5	30.2	5.1	13.8	68.5
	Birdsfoot trefoil							
	Leaf	32.8	3.4	16.2	17.7	9.9	11.7	81.3
	Stem	9.9	1.5	40.5	54.7	1.7	7.6	59.6
	Flower	28.0	7.6	18.0	6.5	8.2	11.9	79.7
	Red clover							
	Leaf	36.2	2.7	20.8	25.0	9.8	12.4	77.2
	Petiole	15.8	1.5	33.4	35.6	8.6	10.6	65.9
	Stem	11.4	0.9	36.7	43.1	9.0	8.3	63.0
	Flower	20.4	3.1	30.1	28.2	7.1	13.3	68.9
MATURE	***White clover***							
	Leaf	33.6	2.6	18.5	23.0	5.3	11.9	79.3
	Petiole	16.4	0.9	31.4	33.4	6.2	10.9	67.7
	Stolon	18.8	0.6	22.0	16.9	18.2	8.2	76.1
	Flower	20.5	3.3	30.4	30.2	5.5	13.6	68.6
	Birdsfoot trefoil							
	Leaf	31.1	3.2	16.7	16.5	9.9	11.8	80.9
	Stem	7.8	1.4	41.3	56.9	2.3	7.3	58.8
	Flower	11.7	4.3	24.2	22.9	11.9	8.2	74.2
	Red clover							
	Leaf	34.9	2.6	20.9	24.9	10.0	12.2	77.1
	Petiole	14.5	1.6	34.3	38.5	8.4	10.9	65.1
	Stem	8.3	0.8	39.3	49.7	9.8	7.3	60.6
	Flower	20.0	3.1	30.6	29.2	6.7	13.6	68.4

3.3.2.4. Animal measurements

Total grazing time and intake

Interactions between sward type and treatment were not significant for grazing time and intake variables, so results are presented as main effects only in Table 3.16 and Table 3.17. The interactions are given in Appendix 3.4.

Although estimates of intake from each sward did not differ, the animals consistently spent more time on red clover than on birdsfoot trefoil, independent of the sward maturity (Table 3.16). The total time spent grazing each treatment increased from the first to the third day, but there was no significant difference between treatments (Table 3.17.). Except when both swards where mature, the animals seemed to increase more the total grazing time per treatment from Day 1 to Day 2 than from Day 2 to Day 3.

Table 3. 16. The effect of swards of birdsfoot trefoil and white clover (BW), and red clover (RC) on grazing time (minute) during the three hours of grazing assessment in Days 1, 2 and 3 and on average intake per animal per day (kg DM/hd/day) in Experiment 2.

	BW	*RC*	*SED*[1]	*P-value*[2]
Grazing time (min)				
Day 1	31.8	44.9	4.97	0.0183
Day 2	33.6	52.3	4.40	0.0006
Day 3	32.3	55.6	5.11	0.0003
Intake (kg DM/hd/day)	4.86	3.95	0.718	0.2228

[1] SED -- Standard error for differences of means when comparing BW with RC swards.
[2] P-value of the sward main effect.

Table 3. 17. The effect of treatment on total grazing time (minute) during the three hours of grazing assessment in Days 1, 2 and 3 and on average intake per animal per day (kg DM/hd/day) on birdsfoot trefoil plus white clover (BW), and red clover (RC) sward in Experiment 2 (treatment A= BW and RC immature; treatment B=BW immature and RC mature; treatment C= BW mature and RC immature; D= BW and RC mature).

	Treatments					
	A	*B*	*C*	*D*	*SED*[1]	*P-value*[2]
Grazing time(min)						
Day 1	36.6	38.2	40.3	38.3	5.48	0.9214
Day 2	44.8	41.3	48.5	37.2	5.63	0.1998
Day 3	45.9	42.7	46.5	40.6	6.74	0.7670
Intake (kg DM/hd/day)	3.57	5.45	3.98	4.62	1.100	0.3105

[1] SED — Standard error for differences of means when comparing treatments.
[2] P-value of the treatment main effect

The effects of maturity contrast (immature – mature) within each sward type and within the alternative sward type on grazing time and intake within each type are shown in Table 3.18. The contrasts within each sward type were calculated as the differences in grazing time or intake/animal/day between immature and mature swards, at the same maturity for the alternative swards. The contrasts (where the alternative swards were either mature or immature) were then averaged. Contrasts within the alternative sward type was calculated as the differences in grazing time or intake/animal/day, within sward type, when the alternative sward was either immature or mature, and averaging to the results. Negative numbers represented greater effect of mature than immature sward. The Table 3.18 shows that there was no significant effect of BW or RC maturity on the time spent grazing in BW or RC, respectively. However in the third day the animals spent more time grazing BW when RC was immature than when it was mature.

Although there were no significant differences in intake/animal/day between swards or treatments (Tables 3.16 and 3.17), mature RC swards had on average higher intake than immature (Table 3.18). There was no apparent effect of BW maturity on intake/animal/day from BW, or of alternative sward maturity on intake from either sward.

The effect of treatment on the proportion of time spent grazing BW is shown in Table 3.19. There were no significant effects of treatment on the proportion of total grazing time on BW swards in Days 1 and 2 of grazing. However there was a significant treatment effect on Day 3 (Table 3.19.) when the animals spent proportionally less time grazing immature BW sward and more time grazing mature RC.

Table 3. 18. Sward maturity contrast effect on total grazing time and intake/animal/day contrasting the effect within each sward type (BW or RC) and the effect of alternative sward in the days 1, 2 and 3 of grazing assessment in Experiment 2.

	Grazing time			***Intake***
	Day 1	Day 2	*Day 3*	
Effect on behaviour on BW				
Maturity effect of BW sward	4.54[2]	-1.49	-3.34	-0.23
Maturity effect of RC sward	-5.59	1.71	15.35*	-0.14
Effect on behaviour on RC				
Maturity effect of RC sward	2.15	3.54	-6.73	-2.27*
Maturity effect of BW sward	5.37	-8.99	0.90	0.12
SED[1]	*5.730*	*5.426*	*5.902*	*0.991*

[1] SED — Standard error when comparing means with same levels of specie.
Contrast significance: * P≤0.05
[2] Contrast: immature – mature (negative numbers represent greater effect of mature than immature sward)

Table 3. 19.Treatment effects on the proportion of grazing time (in relation to the total grazing time spent in the plot) devoted to birdsfoot trefoil and white clover swards (BW) in Experiment 2 Treatment A= BW and RC immature; Treatment B=BW immature and RC mature; Treatment C= BW mature and RC immature; Treatment D= BW and RC mature).

	Treatments				*SED*[1]	*P-value*[2]
	A	B	C	D		
Day 1	0.39	0.51	0.39	0.41	0.0567	0.4334
Day 2	0.42	0.39	0.38	0.38	0.0614	0.9714
Day 3	0.42	0.27	0.46	0.34	0.0421	0.0464

[1]Standard error for differences of treatment means
[2] P-value for the comparison of treatment main effect.

Grazing time per kg of DM offered

The total time the animals spent grazing during the 3 hours of observation in Day 1 and Day 3 was divided by the amount of herbage mass available pre and post-grazing, respectively, in order to verify the effect of herbage mass on the distribution of grazing activity. The interaction between treatments and species for the number of minutes the animals spent grazing per kg of DM in each sward is given in Table 3.20. There was a significant difference between species (and marginal significant interaction between treatment and species) in Day 1 (0.41 vs 0.64 min/kgDM, SED: 0.079, P=0.0105, in BW and RC, respectively) and a significant interaction between treatment and species in Day 3. In both days, the animals spent more time, per kg of DM, grazing red clover swards, except where BW was immature and RC mature. The difference between swards (within each treatment) was greater when BW was mature. The largest difference, in both days, happened when RC was immature and BW was mature. The time the animals spent grazing per kg of DM increased from Day 1 to Day 3.

Table 3. 20. The effect of interaction between treatment (maturity: immature (Imm) and mature (Mat)) and sward type (birdsfoot trefoil and white clover (BW), and red clover (RC)) on grazing time per unit of DM (min/kg DM), in the first and third days of grazing in Experiment 2.

	Treatments									
	A		B		C		D		*SED*[1]	*P-value*[2]
	BW Imm	RC Imm	BW Imm	RC Mat	BW Mat	RC Imm	BW Mat	RC Mat		
Day 1	0.34	0.61	0.57	0.42	0.32	0.80	0.41	0.73	0.158	0.0701
Day 3	0.64	0.80	0.88	0.79	0.66	1.51	0.87	1.35	0.216	0.0403

[1] SED– Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

Rate of Biting

The results of rate of biting are presented in Table 3.21. There were significant interactions between sward type and treatment effects in the three days of grazing. Bite rate was consistently higher on BW than on RC in all four treatments on Day 1 (average 46.7 vs 40.8 bites/min, SED 1.57, P=0.0001), and the difference between BW and RC was larger when RC was mature and BW immature. In Days 2 and 3 there was also a higher rate of biting in BW swards, except for the treatment where RC was immature and BW mature (Treatment C). In these two days the largest difference in rate of biting between swards also happened when BW was immature and RC mature (Treatment B).

Maturity effect contrasts (immature – mature) of rate of biting within each sward type and effects of the adjacent sward are shown in Table 3.22. There were highly significant effects of maturity on rate of biting for both species in the three days of grazing assessment, but there was no significant effect of the adjacent sward on rate of biting. Immature swards in both sward types had significantly higher rate of biting then mature swards, and the contrasts were consistently greater in BW than in RC swards. This difference appeared to increase from Day 1 to Day 2 and decrease from Day 2 to Day 3 in both species.

Table 3. 21. The effect of swards of birdsfoot trefoil and white clover (BW), and red clover (RC) on rate of biting (bites/minute) in the first, second and third days of grazing assessment in Experiment 2 according to the treatment.

Rate of biting (bites/min)	*Treatment A*		*treatment B*		*treatment C*		*treatment D*		SED[1]	P-value[2]
	BW Immature	RC Immature	BW Immature	RC Mature	BW Mature	RC Immature	BW Mature	RC Mature		
Day 1	48.9	46.0	51.5	38.3	44.0	41.4	42.6	37.3	2.35	0.0190
Day 2	52.6	47.6	55.4	40.6	41.5	45.7	43.8	37.6	2.82	0.0022
Day 3	55.4	44.4	51.0	38.8	42.2	47.1	45.2	43.0	2.27	0.0002

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.
[2] P-value of the interaction: treatment*sward type effect.

Table 3. 22. Sward maturity effect [contrast: immature – mature (negative numbers represent greater effect of mature than immature sward)] on rate of biting contrasting the effect within each sward type [either birdsfoot trefoil and white clover (BW) or red clover (RC)] and the effect of adjacent sward in the first, second and third days of grazing assessment in Experiment 2.

	Rate of Biting		
	Day 1	Day 2	*Day 3*
Effect on behaviour on BW			
Maturity effect of BW sward	6.87*	11.36*	9.51*
Maturity effect of RC sward	-0.63	-2.54	0.71
Effect on behaviour on RC			
Maturity effect of RC sward	5.89*	7.61*	4.81*
Maturity effect of BW sward	2.82	2.46	-3.41
SED[1]	*1.664*	*1.737*	*1.717*

[1] SED — Standard error when comparing means with same levels of specie

Contrast significance: * P≤0.05

3.3.3. Experiment 3: Effect of height of birdsfoot trefoil (Lotus corniculatus L.*) and white clover (Trifolium repens* L.*) in relation to red clover (Trifolium pratense* L.*) on grazing behaviour, diet selection and herbage intake.*

3.3.3.1. Sward measurements

<u>Herbage mass, sward surface height and sward bulk density</u>

Interactions between treatments and sward type were significant, so results are presented as interaction effects of sward type and treatment in Table 3.23.

Before grazing, there was a significant interaction between treatments and sward type for herbage mass. Taller swards had greater herbage mass. However in the treatment where both swards were short, BW had higher herbage mass than RC, and in the treatment where both swards were tall, RC sward had higher herbage mass than BW. These differences disappeared after grazing. This modification can be visualised as a significant interaction of DM removed. Taller plants had higher DM reduction.

Short swards were on average 8 to 10 cm high, while the taller were between 14 to 16 cm. There was a significant interaction between sward type and treatment effects for the sward surface height before and after the first day of grazing. After 55 hours of grazing there was no residual significance, either in the interaction or in the sward main effect, but there were significant differences between treatments (main effect). After grazing, the treatment where both swards were initially tall (Treatment D) was on average the shortest, and the treatment where RC was tall and BW was short (Treatment B) was the tallest. Treatments where BW was tall and RC short (Treatment C), and where both swards were short (Treatment A) was intermediate in height (5.9, 6.6, 5.6 and 5.0 cm for Treatments A, B, C and D, respectively, SED 0.46, P=0.0407).

Table 3. 23. Herbage mass (kg DM/ha), sward height (cm) and bulk density (mg DM/cm^3) before and after grazing, and estimation of the herbage mass removed (kg DM/ha) of birdsfoot trefoil and white clover (BW) and red clover (RC) swards according to the treatment in Experiment 3.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*			
	BW Short	RC Short	BW Short	RC Tall	BW Tall	RC Short	BW Tall	RC Tall	SED[1]	P-value[2]
Herbage mass (kg DM/ha)										
Pre-grazing	2110	2040	2010	3060	2870	2120	2530	2920	234	0.00008
Post grazing	1290	1200	1610	1580	1410	1540	1670	1570	185	0.8062
DM removed	820	850	410	1480	1460	580	870	1350	224	0.00001
Sward height (cm)										
Pre-grazing	8.7	9.7	8.6	15.5	15.1	9.3	14.7	14.4	0.89	0.0000
After 1 day grazing	7.3	7.7	7.5	10.6	9.7	7.3	7.8	9.0	0.76	0.0002
Post-grazing	5.6	6.2	6.1	7.1	5.9	5.4	5.2	4.8	0.43	0.0629
Bulk density (mg DM/cm^3)										
Pre-grazing	2.41	2.20	2.36	2.00	1.89	2.27	1.72	2.03	0.1768	0.0120
Post-grazing	2.45	2.19	2.91	2.46	2.68	3.37	3.24	3.41	0.5541	0.4911

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.
[2] P-value of the interaction: treatment*sward type effect.

Bulk density also showed a significant interaction between sward types and treatment effects before grazing. Tall swards were on average less dense. After grazing this interaction disappeared. Bulk density of herbage remaining after grazing was greater than the herbage offered. This difference in bulk density was greater for taller swards.

Sward composition

Before grazing, BW had a significantly higher proportion of live matter than RC (91.6 vs 81.5 %, SED 1.63, $P<0.0000$). However there were no other significant differences between BW and RC swards, or between heights before grazing. The botanical composition differences were affected by the interaction between species and treatment (Table 3.24). There was a marginally significant interaction effect for the percentage of leaf and stem (Table 3.24), short swards had a higher proportion of leaves. Within each treatment, short swards also had less stem than tall swards.

After grazing, there was no significant interaction between treatments and sward type. There were significant main effects for the percentage of stems, grasses, other broad leaf species, and total live matter. BW had significantly more stems (43.2 vs 26 %, SED 8.42, $P=0.0498$), grasses (6.4 vs 1.7 %, SED 0.04, $P<0.0400$) and live matter (87 vs 73 %, SED 3.01, $P=0.00007$) than RC. On the other hand, RC swards had significantly more other broad leaf species (14.0 vs 26.8 %, SED 5.60, $P=0.0289$).

Before grazing, the taller swards had significantly more birdsfoot trefoil than the short ones (birdsfoot trefoil/white clover (B/W) ratio: 1.10 vs 0.34 , SED 0.3213, $P=0.0446$). After grazing, there was only a marginally significant contrast between short and tall. In both swards (tall and short) there was more white clover than birdsfoot trefoil (B/W ratio 0.7646 vs 0.3290, SED 0.2110, $P=0.0714$, for tall and short swards, respectively).

Table 3.24. Botanical composition of birdsfoot trefoil plus white clover (BW) and red clover (RC) swards before and after grazing, according to the treatments (plant height: Short and Tall) (DM basis): (a) percentage of components in live fraction, (b) percentage of live matter in total DM of each sward and (c) ratio of the total live matter of birdsfoot trefoil and white clover (B/W) in the BW sward, Experiment 3.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*			
	BW Short	RC Short	BW Short	RC Tall	BW Tall	RC Short	BW Tall	RC Tall	SED[1]	P-value[2]
Pre-grazing										
(a) Leaf	38.4	42.0	45.8	36.4	35.5	42.4	39.6	31.7	5.16	0.0794
Petiole	18.0	24.1	22.3	26.3	19.5	22.3	18.2	21.2	3.77	0.9269
Stem	32.6	14.6	19.7	24.5	20.4	19.6	20.3	18.6	5.88	0.0577
Flower	0.2	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.08	0.3288
Grass	1.5	2.4	0.6	0.7	4.9	0.8	1.8	0.7	2.76	0.5993
Other species	8.1	17.0	8.0	13.0	18.6	13.9	7.9	27.8	8.72	0.2763
(b) Total live matter	90.2	77.8	93.4	84.1	90.0	79.7	92.9	84.3	3.26	0.8551
(c) B/W ratio	0.40		0.29		1.19		1.02		0.457	0.9103
Post-grazing										
(a) Leaf	28.6	25.2	26.0	24.2	24.9	26.7	25.3	22.3	5.49	0.9078
Petiole	18.1	18.5	15.9	22.2	19.0	19.9	24.5	22.3	5.12	0.6974
Stem	37.7	21.5	35.8	28.8	67.5	27.9	31.9	25.8	16.85	0.4745
Flower	0.0	0.0	0.0	0.3	0.0	0.0	0.6	0.0	0.27	0.1881
Grass	6.6	2.3	6.6	1.3	5.4	2.4	7.0	0.6	4.35	0.9360
Other species	13.6	32.3	16.3	24.0	9.8	21.9	16.1	29.1	11.21	0.9183
(b) Total live matter	82.1	75.4	88.4	75.4	87.8	71.7	89.6	69.4	6.02	0.4546
(c) B/W ratio	0.28		0.38		1.06		0.46		0.300	0.1877

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.

[2] P-value of the interaction: treatment*sward type effect.

3.3.3.2. Canopy structure within the sward

The canopy structure determined from point quadrat data is summarised in Figures 3.12 and 3.13. The tall swards had greater variation in height. The leaf and petiole components were relatively more important in the medium and higher strata. In both swards the number of contacts of dead matter was greater in the bottom strata, and there was an increase of dead matter after grazing. In RC swards, the proportion of red clover leaves close to the ground increased from before to after grazing. In BW swards the proportion of birdsfoot trefoil in the bottom strata was larger in tall than in short swards. In contrast, the number of white clover contacts after grazing in the bottom strata increased more in short than in tall swards.

3.3.3.3. Sward chemical composition

<u>Extractable condensed tannin concentration</u>

There were no significant differences between short and tall birdsfoot trefoil in relation to concentration of extractable condensed tannin (ECT) (Table 3.25). Leaves had consistently higher concentration of ECT than stems. The concentrations of ECT in stems were negligible.

Table 3. 25. Extractable condensed tannin (ECT) concentration (%) of birdsfoot trefoil leaf and stem in Experiment 3, according to the sward characteristic (tall or short) imposed by the treatments (DM basis).

Components	*Short*	*Tall*	*SED*[1]	*P-value*[2]
Leaf	0.66	0.41	0.1257	0.1257
Stem	0.03	0.02	0.0131	0.3548

[1] SED – Standard error for differences of means when comparing Short with Tall swards.

[2] P-value of the treatment main effect.

Number of observations contributing to each mean (n=4).

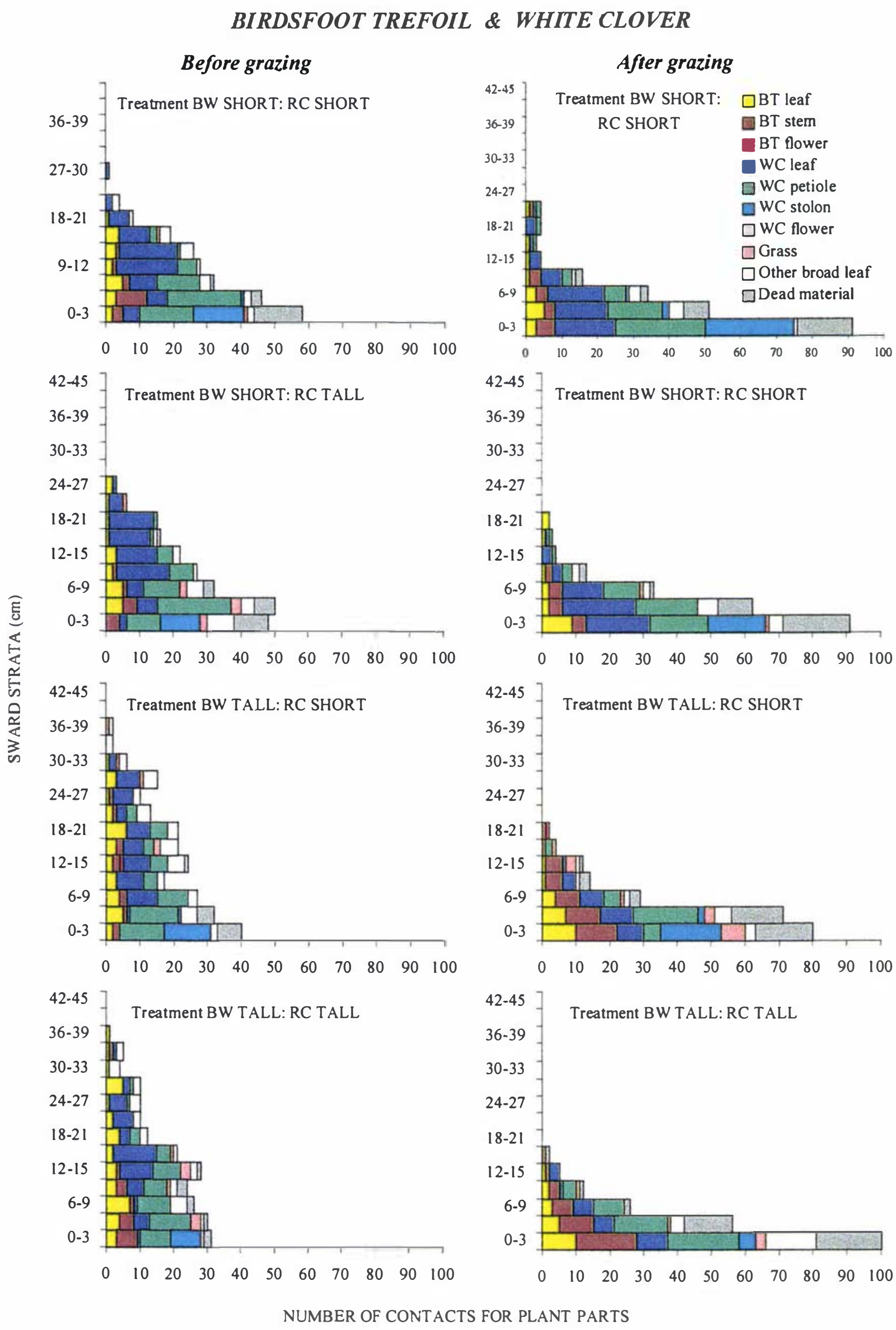


Figure 3.12.The stratum structure of plant parts within the sward canopy of birdsfoot trefoil and white clover (BW), before and after grazing in the four treatments in Experiment 3 (RC = red clover sward).

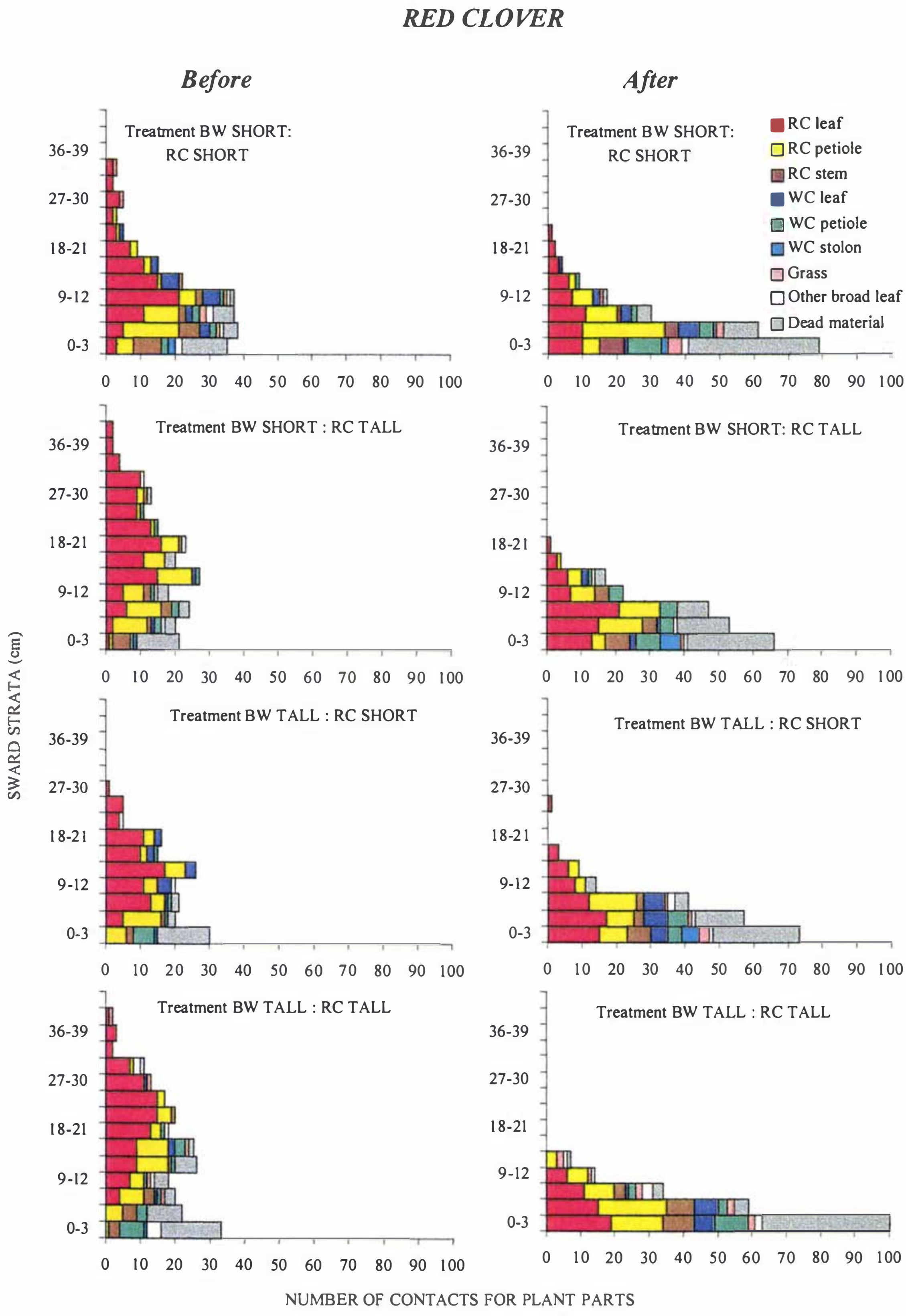


Figure 3.13.The stratum structure of plant parts within the sward canopy of red clover (RC), before and after grazing in the four treatments in Experiment 3 (BW = birdsfoot trefoil and white clover sward).

Formononetin concentration

Table 3.26 shows the concentration of formononetin in leaves, petioles and/or stems of birdsfoot trefoil, white clover and red clover. There was a significantly higher concentration of formononetin in red clover than in birdsfoot trefoil or white clover, and red clover leaves had the highest concentration. In the leaf component, white clover had a higher concentration than birdsfoot trefoil. In contrast to red clover, birdsfoot trefoil had a higher concentration in stems than in leaves.

Table 3.26. Formononetin concentration (%) of leaf, petiole and stem of birdsfoot trefoil (BT), white clover (WC) in birdsfoot trefoil and white clover sward (BW), and red clover (RC) in red clover sward (RC) in Experiment 3.

	BW sward		*RC sward*	*SED*[1]	*P-value*[2]
	BT	WC	RC		
Leaf	0.07	0.16	0.70	0.020[3] 0.040[4] 0.040[5]	0.020[3] 0.000[4] 0.000[5]
Petiole	-[6]	0.16	0.54	0.0204	0.0003
Stem	0.14	-	0.56	0.0850	0.0154

[1] SED — Standard error for differences of means
[2] P-value for the comparison of species.
[3] Standard error for differences of means and P-value for comparison between BT and WC.
[4] Standard error for differences of means and P-value for comparison between BT and RC.
5 Standard error for differences of means and P-value for comparison between WC and RC.
6 - no sample
Number of observations contributing to the mean of each specie (n=4).

General chemical composition

The general chemical composition of each species, when part of the short and tall swards, is given in Table 3.27. Although some general comparisons are reported, statistical analysis was not possible. The values represent single samples bulked across treatments and replications.

The three species had similar chemical composition. Within each species, leaves had the highest concentration of crude protein (CP), lipid, ash and digestibility (IVDMD), and the least of ADF and NDF. The distribution of sugar plus starch (CHO) varied according to the species. For birdsfoot trefoil, leaves had highest concentration of CHO, while for white clover, concentration was higher in stolons. Red clover had an even distribution between leaves, petioles and stems. There were no important differences between tall and short swards.

Table 3. 27.Crude protein (CP), lipid, acid and neutral detergent fibre (ADF, NDF), carbohydrates (soluble sugars plus starch) (CHO), ash and *in vitro* dry matter digestibility (IVDMD) determined by Near Infrared Reflectance Spectroscopy (NIRS) of the main components of birdsfoot trefoil and white clover, and red clover swards in Experiment 3 according to height treatment (percentage of DM basis).

		CP	LIPID	ADF	NDF	CHO	ASH	IVDMD
Short	***White clover***							
	leaf	34.8	3.0	16.3	17.6	5.8	12.1	81.2
	petiole	18.2	1.3	28.1	26.1	6.0	11.6	70.7
	stolon	16.8	2.2	22.3	26.2	21.2	8.3	75.9
	Birdsfoot trefoil							
	leaf	34.5	3.3	16.3	17.4	8.0	12.1	81.2
	stem	15.0	1.4	34.6	38.4	1.6	9.8	64.8
	Red clover							
	leaf	36.2	3.4	17.5	22.5	9.8	11.8	80.2
	petiole	18.0	1.7	29.3	26.6	8.6	11.5	64.9
	stem	13.7	0.8	34.5	36.1	8.9	9.1	69.6
Tall	***White clover***							
	leaf	35.0	2.4	16.3	12.5	5.7	12.5	81.2
	petiole	18.4	0.8	28.7	24.7	6.6	11.4	70.1
	stolon	19.7	2.4	21.0	25.8	23.7	8.0	77.0
	Birdsfoot trefoil							
	leaf	31.1	4.0	15.5	18.4	10.4	11.1	82.0
	stem	14.9	1.9	32.6	37.9	4.0	9.3	66.6
	Red clover							
	leaf	35.6	3.1	19.2	21.7	10.2	12.0	78.6
	petiole	18.1	1.2	30.4	26.1	9.2	11.3	68.6
	stem	16.1	0.8	32.7	30.3	9.6	9.3	66.6

3.3.3.4. Animal measurements

Total grazing time and intake

There were significant interactions between sward type and treatment for grazing time and intake (Table 3.28) during the three days of grazing measurement, though in the second day the significance for grazing time was marginal. The animals spent more time grazing taller swards on each of the three days, and also ate more herbage from tall swards. When both swards were offered at similar height, the animals spent similar time grazing each sward. The only exception was for Day 3 in the treatment with short BW and short RC, where animals spent more time grazing RC.

Although no statistical analyses were performed to compare days, the total grazing time per animal in each treatment increased from Day 1 to Day 3, except in treatment D (where both swards were tall). From the second to the third day the increase in grazing time happened mainly on red clover, except in the treatment where RC was short and BW was tall (Treatment C).

Animals ate more from tall swards when contrasts of tall and short swards were offered. However, considering the total intake/animal/day in each treatment, the lowest intake was found on plots where both species were tall. Within each plot, more was consumed from RC swards than from BW swards, except when BW was tall. However when both swards were short the difference in intake from each species was small.

Table 3.28. The effect of treatments (sward height) on grazing time (minutes) in Days 1, 2 and 3, and average DM intake (kg) per animal per day during 55 hours of grazing in Experiment 3, according to the treatments.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*			
	BW Short	RC Short	BW Short	RC Tall	BW Tall	RC Short	BW Tall	RC Tall	SED[1]	P-value[2]
Grazing time (min)										
Day 1	46.1	49.0	26.6	65.9	56.9	33.7	52.3	58.7	12.08	0.0236
Day 2	49.1	49.8	47.0	67.9	68.4	44.1	53.6	49.1	10.23	0.0600
Day 3	49.4	61.4	39.2	78.3	70.3	63.6	54.4	53.9	10.40	0.0397
Intake (kgDM/hd/day)	2.34	2.42	0.94	3.67	3.68	1.40	1.31	2.04	0.576	0.0003

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.

[2] P-value of the interaction: treatment*sward type effect

The effect of height contrasts (tall - short) within each sward type and of the alternative sward on grazing time and intake is presented in Table 3.29. As described in Experiment 2 results (section 3.3.2.2) the contrasts within each sward type were calculated subtracting the total grazing time (or intake/animal/day) the animals spent grazing on tall from short swards, considering that the alternative swards were the same height. The contrasts (where the alternative swards were either tall or short) were then averaged. On the other hand, the contrasts within the alternative sward type was calculated subtracting the averages of total grazing time (or intake/animal/day) the animals spent in a sward type that had as alternative sward either tall or short sward. In these cases negative numbers represented greater effect of short than tall sward. There was a significant BW height effect on time spent grazing BW in the first and last days of assessment. In these days the animals spent significantly more time grazing the tall than the short sward. In Day 3 the animals were also affected by the sward height of the alternative area. The animals spent significantly more time grazing BW when the RC alternative area was short. The grazing time in RC was only significantly affected by height on the first day. In this day the animals spent more time grazing taller than short swards. Behaviour on RC swards did not seem to be affected by the BW sward height.

The average intake/animal/day on BW and RC appeared to be significantly affected by sward height. Taller swards had greater intake than shorter. However the intake either in BW or RC was also affected by the alternative sward. The animals had greater intake from the swards when the alternative sward was short.

Table 3.29. Sward height contrast effect on total grazing time and intake/animal/day contrasting the effect within each sward type (BW or RC) and the effect of alternative sward in Days 1, 2 and 3 of grazing assessment in Experiment 3.

	Grazing time			*Intake*
	Day 1	Day 2	*Day 3*	
Effect on behaviour on BW				
Height effect of BW sward	18.25*[2]	12.95	18.05*	0.85*
Height effect of RC sward	12.06	-8.44	-13.06*†	-1.88*
Effect on behaviour on RC				
Height effect of RC sward	20.93*	11.52	3.60	0.94*
Height effect of BW sward	-11.26	12.22	-11.11	-1.32*
SED[1]	*6.572*	*6.491*	*5.901*	*0.349*

[1] SED — Standard error when comparing means with same levels of specie.
Contrast significance: * P≤0.05; *† P=0.05416 (marginal significance)
[2] Contrast: tall – short (negative numbers represent greater effect of short than tall sward)

The treatment effect on the proportion of grazing time on BW swards is shown in Table 3.30. There were significant effects of treatment on the proportion of grazing time on BW swards for Day 1, but there were no significant treatment effects for Day 2 and 3. In Day 1 the animals spent proportionally less time grazing short BW swards that had tall RC as alternative sward. They also grazed proportionally more time in BW swards that had short RC sward as alternative sward.

Table 3.30. Treatment effects on the proportion of grazing time devoted to birdsfoot trefoil and white clover swards (BW) in Experiment 3 Treatment A= BW and RC short; Treatment B=BW short and RC tall; Treatment C= BW tall and RC short; Treatment D= BW and RC tall).

	Treatments				*SED*[1]	*P-value*[2]
	A	B	C	D		
Day 1	0.46	0.29	0.63	0.46	0.0701	0.0493
Day 2	0.52	0.41	0.59	0.54	0.0555	0.2303
Day 3	0.44	0.34	0.51	0.50	0.0545	0.1667

[1]Standard error for differences of treatment means
[2] P-value for the comparison of treatment main effect.

Grazing time per kg of DM offered

The total time the animals spent grazing during the 3 hours of observation in Day 1 and Day 3 was divided by the amount of herbage mass available before and after grazing, respectively, in order to verify the effect of herbage mass on the distribution of grazing activity. The number of minutes per kg of DM the animals spent grazing each treatment is shown in Table 3.31. There were no significant differences between swards for Day 1 and for Day 3 of grazing. There was only a significant difference between treatments (main effect) in Days 1 and 3 of grazing. In both cases the animals grazed significantly longer per kg of DM when both swards were tall (Treatment D). The treatments with tall RC (C and D) had greater increase from Day 1 to Day 3 in grazing time per kg of dry matter in the plot than treatments with short RC (A and B), though no statistical analyses were performed to compare days.

Table 3.31. The effect of treatment on grazing time per kg of dry matter in the plot (min/kg DM) in the first and third day of Experiment 3 (Treatment A= BW and RC short; Treatment B=BW short and RC tall; Treatment C= BW tall and RC short; Treatment D= BW and RC tall).

	Treatments				*SED*[1]	*P-value*[2]
	A	B	C	D		
Day 1	1.18	1.06	1.08	2.07	0.152	0.0002
Day 3	2.32	2.23	2.77	3.62	0.329	0.0082

[1]Standard error for differences of treatment means
[2] P-value for the comparison of treatment main effect.

Rate of biting

There was a significant interaction between sward type and treatment for rate of biting in Day 1 and a marginal interaction in Day 3 (Table 3.32). In Day 2, there was a significant species main effect where the rate of biting was significantly higher on BW than on RC (55.8 vs 50.0 ± 2.014 bites/min, P= 0.0110). As in Day 2, the rate of biting was greater on BW than on RC for most of the treatments in Days 1 and 3. However in the treatment where BW was tall and RC was short there was higher rate of biting on RC than on BW. Comparing Days 1 and 3, there was a decrease in number of bites per minute in all treatments and both species.

Table 3.32. The effect of swards of birdsfoot trefoil and white clover (BW), and red clover (RC) on rate of biting (bites/minute) in the first, second and third days of grazing assessment in Experiment 3 according to the treatment.

	Treatments									
	A		B		C		D		*SED*[1]	*P-value*[2]
	BW short	RC short	BW short	RC tall	BW tall	RC short	BW tall	RC tall		
Day 1	58.2	54.1	59.6	47.7	56.6	58.2	54.2	48.0	1.882	0.0016
Day 2	57.5	47.7	55.6	48.5	57.5	53.2	52.6	50.7	4.028	0.5892
Day 3	56.5	52.0	51.9	44.2	51.5	53.6	46.5	44.5	2.391	0.0800

[1] SED-- Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

Height effects (contrasts: tall - short) on rate of biting within each sward type and of the adjacent sward are shown in Table 3.33. The rate of biting in BW swards was only marginally significantly affected by height in the third day of grazing. In this day, short BW swards had greater rate of biting than tall BW swards. In RC swards height had significant effects on rate of biting in the first and third days. In both days, short RC swards had significantly higher rate of biting. The rate of biting of both sward types was not affected by the adjacent sward treatments.

Table 3.33. Sward height effect [contrast: tall – short (negative numbers represent greater effect of short than tall sward)] on rate of biting contrasting the effect within each sward type [either birdsfoot trefoil and white clover (BW) or red clover (RC)] and the effect of adjacent sward in the first, second and third days of grazing assessment in Experiment 3.

	Rate of Biting		
	Day 1	Day 2	*Day 3*
Effect on behaviour on BW			
Height effect of BW sward	-3.49[2]	-1.52	-5.19*†
Height effect of RC sward	-0.52	-3.36	-4.83
Effect on behaviour on RC			
Height effect of RC sward	-8.32*	-0.89	-8.44*
Height effect of BW sward	2.22	3.83	0.90
SED[1]	*1.905*	*2.430*	*2.372*

[1] SED — Standard error when comparing means with same levels of specie

Contrast significance: * $P \leq 0.05$;

*† $P=0.05643$ (marginal significance)

[2] Contrast: tall – short (negative numbers represent greater effect of short than tall sward)

3.3.4. Experiment 4: Effect of position in the plot of birdsfoot trefoil (*Lotus corniculatus* L.) and white clover (*Trifolium repens* L.) sward in relation to red clover (*Trifolium pratense* L.) sward on grazing activity distribution.

3.3.3.1. Sward measurements

Herbage mass, surface sward height and sward bulk density

The interactions between treatments and sward type were not significant, so results are presented as main effects only. The results of measurements of herbage mass, surface sward height and sward bulk density are given in Table 3.34. The interaction between treatments and sward type is presented in Appendix 3.5.

Table 3.34. Herbage mass (kg DM/ha), and bulk density (mg DM/cm^3) before grazing and sward surface height (cm) before and after grazing birdsfoot trefoil and white clover (BW) and red clover (RC) swards in Experiment 4.

	BW	*RC*	*SED*[1]	*P-value*[2]
Herbage mass (kg DM/ha)				
pre-grazing	2050	2200	153	0.3237
Sward height (cm)				
pre-grazing	7.0	7.5	0.37	0.1683
post-grazing	4.3	4.5	0.27	0.5560
Bulk density(mg DM/cm^3)				
pre-grazing	2.93	2.98	0.2162	0.8019

[1] Standard error of the difference comparing means of different sward main effect
[2] P-value for sward main effect differences

There was no significant difference between BW and RC swards, which had similar herbage mass, height and density. They were relatively shorter and denser than the previous experiment (Experiment 3).

3.3.3.1. Animal measurements

Grazing time

There were no significant differences between treatments, comparing the proportion of grazing time the animals spent grazing the smaller sward in area of each plot. There was no influence of the minor sward location (close to the fence or in the middle of the plot) in the distribution of grazing activities. The proportion of grazing time the animal spent in the minor swards according to the position in the plot is given in Table 3.35. The average grazing time (in minutes) the animals spent grazing each sward in each plot is shown in Appendix 3.5.

Table 3.35. Proportion of time grazing (in relation to the total grazing time spent in plot) birdsfoot trefoil plus white clover (BW) and red clover (RC) swards according to the strip position (side: close to the fence; central: in the middle of the plot) in the plot in Experiment 4.

	Proportion of grazing time				SED[1]	P-value[2]
	BW side	BW central	RC side	RC central		
first day	0.32	0.29	0.34	0.33	0.0246	0.5277

[1] Standard error of the difference comparing means in the same species in different treatments
[2] P-value for treatment differences.

3.4. DISCUSSION

3.4.1. Evaluation of experimental procedures

The use of a pair of swards sown in strips provided a flexible and reliable condition to test animal preference. The use of two separate swards, rather than intermixed species avoided the inter-species competition and its effect on species-balance. The use of separate swards also made it easier to determine the animal selection, and for animals to find their preferred species (Parsons et al., 1994a), without needing to search the location (Newman et al., 1995). Swards formed by strips also provided a flexible approach for modification of each strip according to the treatment required in each experiment.

Before starting the experiment, and between each replicate run, the animals were continuously grazed on an adjacent area with the same species and design (alternate strips swards) of the experimental area to establish and maintain experience of experimental conditions. In this period all animals grazed together to avoid group effects. Previous studies with different animal species have shown that previous experience has a marked effect on grazing preference (Arnold and Maller, 1977; Provenza, 1996a). The previous experience in these experiments was clearly effective since the previous treatment effect and group effect were not significant in any of the three experiments.

Because of the relatively small size of the plots there was a concern about possible bias caused by the electric fences on animal preferential behaviour. In order to clarify this effect, Experiment 4 was carried out. This experiment showed that the proximity of a sward to the fence did not influence the proportion of grazing time spent on that sward. The comparisons in this experiment were very clear since both swards had similar physical characteristics (height, herbage mass and bulk density) (Tables 3.34, 3.35)

Five replications provided an accurate statistical assessment of the animal behaviour. Five replications, rather than four, were important to efficiently investigate the carry over effect, and showed clearly that selective behaviour was not affected by previous treatment.

There were several advantages for having white clover intermixed with birdsfoot trefoil. Although the use of pure birdsfoot trefoil swards would provide a clearer evaluation of the animal preferences between birdsfoot trefoil and red clover, the mixture with white clover was more realistic in practical farm terms. White clover also provided a better soil cover, not allowing weeds to invade the BW sward. In addition, white clover did not influence the secondary compound concentration effect, showing negligible concentration of ECT and low concentration of formononetin. Jones and Lyttleton (1971), Jones et al. (1973) and Li et al. (1996) reported that white clover is either free of, or has very low concentrations of condensed tannin. However there is evidence that there is tannin in flowers (Jones et al., 1976; Stockdale and Dellow, 1995). Small concentrations of formononetin were also found in other studies in white clover plants (Francis et al., 1967).

The decline in herbage available to the animals from the first to the second day and from the second to the third day was illustrated by the decline in surface height. The assessment of herbage mass on the second day by cutting quadrats was not possible because of the disturbance to animal behaviour. Although the use of a rising plate meter and sward surface height for predicting mass was tested before starting the experiment, they did not provide a reliable basis for prediction.

Point quadrat data provided a good illustration of the vertical distribution of sward components in the canopy, and provided estimates of botanical composition similar to hand separation. However there were variations between the two techniques. In Experiment 2, for example, the high proportion of stems in BW swards before grazing (Table 3.12) was not demonstrated in the point quadrat illustration (Figure 3.10). This effect may be related to the small stem surface area in relation to leaves to be touched by the point quadrat needle, relative to dry matter content in the hand separated samples. In addition, the percentage of dead matter was often underestimated by point quadrat. This agrees with results reported by Hodgson (1981a) and Grant (1985), who explain that the concentration of dead matter in the bottom strata is too dense to be recorded accurately. Although there were variations between techniques, the two techniques tended to complement one another.

Because concentrations of ECT in samples of white clover and red clover was negligible, the analysis of ECT was done mainly on samples of birdsfoot trefoil plants. Other studies also reported low concentration of ECT in white clover (Jones and Lyttleton, 1971; Jones et al., 1973; Li et al., 1996) and red clover (Jackson et al., 1996). ECT corresponds to the fraction of the total condensed tannin in the forage that is not bound to protein or fibre (Terrill et al., 1992). Douglas et al. (1993), Jackson et al. (1996) and Wang et al. (1995) found that the extractable fraction varied from 62.8 to 70.9% of the total condensed tannin in birdsfoot trefoil plants. The analysis of ECT concentration followed the DMACA-HCL protocol described by Li et al. (1996). Li et al. (1996) compared the DMACA-HCL with vanillin-acid procedure, and they found that DMACA-HCL was more sensitive in condensed tannin detection, particularly for material with low concentration of tannin. The DMACA-HCL procedure is also a more specific methodology to determine the condensed tannin concentration than butanol-HCL (W. C. McNabb, pers. comm.).

The ECT concentrations observed in these experiments for cultivar Goldie (Tables 3.4, 3.13, 3.25) were lower than values reported elsewhere (Douglas et al., 1995, and Douglas et al., 1993), though the concentration of ECT in leaves was within the limits found by previous studies for birdsfoot trefoil in New Zealand (Jackson et al., 1996; John and Lancashine, 1981; Li et al., 1996; Lowther et al., 1987; Terrill et al., 1992; Wang et al., 1995; Waghorn et al. 1987ab) (see comments in section 4.4.3 – Chapter 4).

Formononetin was determined by a modification (Anwar, 1994) of the fluorimetric assay described by Gosden and Jones (1978). According to Anwar (1994), the modification was necessary to ensure complete hydrolysis of formononetin glycoside, especially in plant components such as stems which contain relatively small amounts of ß-glucosidase. In Experiments 1, 2 and 3 the formononetin concentration of red clover was in agreement with published results of several cultivars of red clover (Francis et al., 1966; Kelly et al., 1979; McMurray et al., 1986 Anwar 1994)

Although variation of preference between morning and afternoon grazing periods has been found in grazing (Parsons et al., 1994a) and indoor feeding (Fisher et al., 1997) studies, the grazing behaviour observation in this study was restricted to late evening (4.00 to 7.00 p.m. in winter and 5.00 to 8.00 p.m. in summer). Thus, grazing preference measured in these experiments may not entirely reflect the preferences of animals during a full day. However grazing behaviour in the evening reflects the preference in one of the main grazing periods of the day (Arnold, 1981 and Hodgson, 1990) and consequently for a substantial portion of the total grazing activity. Morning observations were carried out between Day 1 and Day 2 in Experiments 1, 2 and 3 to clarify the difference between morning and afternoon animal diet selection (Appendix 3.6). The results showed that preference did not change according to time of the day. The proportion of time spent grazing one of the two sward types in the morning of Day 2 was on average intermediate between the evening observations of Day 1 and Day 2 in each experiment.

Based on previous studies (Cosgrove et al., 1996) ten minute intervals between observations were used to provide an estimate of grazing activity. Hull et al. (1960) did not find a significant difference between continuos recording and up to 30 minute intervals. Continuous recording is important mainly for minor behaviour patterns like drinking water, but not for a major behavioural activity such as grazing (Gary et al., 1970). Although continuous assessment is more precise, it is very difficult to carry out without automatic equipment (Hodgson, 1982b).

The rate of biting in BW and RC swards agrees with results summarised by Hodgson (1985): a range of 21 - 66 bites/minute for cattle. The 20 bites method described by Forbes and Hodgson (1985) usually over-estimates the long-term mean rate of biting because it does not consider the short-term interruptions to grazing activity. However the estimates of maximum rate of biting provided a standardised basis for comparison between swards.

3.4.2. Definition: Selection and Preference

The separate definition of diet selection and preference is important because it reflects the research context and clarifies the information obtained. This study follows the definition of Hodgson (1979) where "preference is a general term that describes the discrimination exerted by animals between areas of sward or the components of a sward canopy" and selection is the "preference modified by opportunity for selection". Therefore preference is demonstrated by the animals when free choice and equal opportunity is offered. In the four experiments described in this section, conditions most closely approached "free choice" in Day 1 when the herbage mass and height was relatively high. However, in most situations in these studies the animals demonstrated selection. In contrast, in Experiments 5 and 6 (see Chapter 4), where access to a range of spaced plants offered similar opportunity, preferential behaviour was mainly tested.

3.4.3. Components of ingestive behaviour and selection

Grazing time, rate of biting and intake per bite partly describe the ingestive behaviour and the multiplication of these three factors results in the estimation of herbage intake (Allden, 1962; Allden and Whittaker, 1970). Each factor of this equation is a component of selective and intake behaviour, and all could be used as indices of preference. However there is a reciprocal relation between intake per bite and rate of biting (Hodgson et al., 1994). Animals usually have greater rate of biting for small bites than for large bites (Laca et al., 1992). Thus, different rates of biting and intakes per bite can generate similar total herbage intake. Because of this compensation, it is difficult to determine selection using rate of biting without considering intake per bite. Therefore, the estimation of grazing time is one of the best parameters for determining animal selection when estimation of herbage intake is not possible

In these studies because the estimation of herbage intake was only possible by the difference in herbage mass before and after grazing, not considering the variation between days, grazing time, rather than herbage intake, was used to estimate diet selection. The assessment of grazing time was a good reflection of the herbage intake estimated per sward in E1 and E3 (Tables 3.7, 3.28). Both intake and grazing time (for Days 2 and 3) in E1 were highly affected by the amount of area offered of each sward (Figures 3.7,3.9). In E3 the greater intake of tall rather than short swards reflected the fact that the animals spent more time grazing tall than short swards (Table 3.29). However, in E2, although the animals spent more time grazing RC, on average over the three days of grazing, the animals had similar intakes between the two species (Table 3.16). In this case the difference between grazing time and the estimation of intake was probably related to the fact that the estimation of intake was a mean value for three days and did not take into account the important variation between days. Therefore, it can be concluded that estimation of grazing time provided a good estimation of the animals diet selection during the three days of grazing. Several other studies (e.g. Penning et al., 1991; Parsons et al., 1994a; Cosgrove et al., 1996) have also used grazing time as an important assessment of diet selection and animal preference.

Rate of biting reflected morphological characteristics of the sward. Although in all three experiments rate of biting on BW was on average higher than on RC, the rate of biting varied according to the sward conditions and from Day 1 to Day 3. While in E1 the rate of biting increased from Day 1 to Day 3 (Table 3.10), in E3 it decreased (Table 3.32). This difference was apparently related to the difference in sward height and physical characteristics. Because of the lower herbage mass in E3, the animals had much lower residual after two days of grazing in E3 than in E1. In E1 the increase in rate of biting with progressive defoliation probably was one of the compensating responses of the animal to limitations in intake per bite (Hodgson, 1981b). The animals apparently were getting smaller bites. Small bites with a high proportion of leaves required less manipulative movements (Hodgson, 1985), so the animals could swallow faster and graze uninterruptedly (Laca et al., 1993). On the other hand, in E3 on Day 3, the animals had a slower rate of biting than on Day 1, as a result of the fact that after two days of grazing, the sward had been trodden on the ground and the animals had greater difficulty in prehending. Allden and Whittaker (1970); Kenney and Black, (1984a,b) and Colebrook et al., (1987) explain that rate of biting is affected by ease of prehension. The animals had to spend time looking and trying to find where and how to graze. The animals probably also reduced their intake rate to avoid grazing close to their dung and urine (Garner, 1963; Arnold, 1981). In E2 there was no apparent change in rate of biting across the three days. The increase in sward density, promoting greater bite size and slower rate of biting was balanced by the reduction in proportion of leaves, generating reduction of bite size and increasing the rate of biting (Penning 1986; Laca et al., 1993).

The rate of biting apparently did not influence the diet selection between different swards, because, as shown in E2 and E3 (Tables 3.22, 3.33), the rate of biting on one sward type was not affected by the alternative sward condition. Rate of biting was more a consequence of sward physical characteristics than a cause of selection. The animals probably regulated the rate of biting according to the specific sward conditions in each bite (Penning, 1986; Laca et al., 1993).

The greater rate of biting observed in BW in all three experiments, mainly in Day 1 (Table 3.10, 3.21, 3.32), was probably the consequence of combined effects of a lower sward surface height and higher bulk density (Tables 3.2, 3.11, 3.23). Several studies (Chacon and Stobbs, 1976; Hodgson and Jamieson, 1981; Milne et. al., 1982; Philips and Leaver, 1985; Penning et al. 1991; Burlison et al., 1991; Laca et al., 1992; Mitchell et al., 1993) have observed a negative relationship between rate of biting and either sward height, herbage mass or bulk density. The animals apparently spent more time selecting leaf in RC swards than they did in BW swards. Although in E3 there were no important differences in height and bulk density between BW and RC within the same height treatment, the BW swards had a greater proportion of their mass closer to the ground than RC (as demonstrated by the point quadrat analysis). This greater density was related to the presence of white clover close to the ground in BW swards (Figures 3.12, 3.13).

The discussion of animal selective response to variation in sward characteristics will be concentrated on the most important behaviour response observed. The discussion will focus first on an overview of the pattern of selection across experiments and then will provide discussions based on effects of different experiments, of sward changes from Day 1 to Day 3 and of high herbage mass and height (Day 1). The discussion will then finish with more detailed comparisons within each sward. In this way the main effects of each swards characteristics on diet selection will be covered.

3.4.4. Pattern of selection across experiments

Indications of the seasonal variation in selective behaviour was obtained by averaging, within each experiment, the proportion of grazing time spent on BW and the physical swards characteristics during the three days of grazing observation (Table 3.36). The standard error of the mean (SEM) estimated for the proportion of grazing time in each experiment was calculated by averaging the variances obtained in the three days of observation within each experiment. This was a useful analysis for verifying patterns of selection across experiments, though interpretation was limited since there was no replication of experiments.

Table 3.36. Overall averages of the proportion of grazing time on BW swards ($prop_{GT}$), physical sward characteristics, extractable condensed tannin concentration (ECT - leaves of birdsfoot trefoil) and formononetin concentration (leaves of red clover) in Experiments 1, 2 and 3 (E1, E2 and E3) carried out in November (Nov), February (Feb) and April-May (Apr-May) 1995/1996.

		E1 Nov	E2 Feb	E3 Apr-May
$Prop_{GT}$ on BW		0.48	0.39	0.47
SEM[1]		0.021	0.027	0.030
Sward height (cm)	BW	13.0	13.5	8.5
	RC	21.0	18.9	8.9
Herbage mass (KgDM/ha)	BW	3160	3870	1935
	RC	3955	4030	2000
Bulk density ($mgDM/cm^3$)	BW	2.61	3.08	2.45
	RC	2.09	2.91	2.49
Leafiness (%)	BW	35.9	19.8	33.0
	RC	29.2	18.8	31.4
Proportion of stems (%)	BW	21.6	52.9	33.2
	RC	41.2	46.4	22.6
ECT (%)	BW	1.69	0.58	0.54
Formononetin (%)	RC	0.61	0.50	0.70

[1]SEM – Standard error of the means

Comparing the three experiments (Table 3.36), E2 showed, based on SEM, a significantly different pattern from the other two in terms of the proportion of grazing time spent on BW. This result shows that there was a pattern of seasonal variation in selective behaviour between BW and RC. Although E3 had the least sward surface height and herbage mass, there was no indication that the animals selective behaviour was, on average, significantly different from E1. The difference in proportion of grazing time between E2 and the other two experiments was apparently related to differences in plant morphological characteristics. For both swards the highest proportion of stems, and the lowest proportion of leaves occurred in E2. The proportion of stem was particularly high in BW swards. This pattern of seasonal variation was apparently not related to the secondary compound concentrations. The greatest contrast found between Experiment 1 and the two later experiments in ECT concentration did not reflect the differences in proportion of grazing time, and the small contrast in formononetin concentration between experiments suggests limited opportunity for seasonal effect of formononetin on animal preference. There is, then, an indication that the animals grazed proportionally more RC to avoid the high concentration of stems in BW. Thus, it can be concluded that this pattern of grazing behaviour observed is mainly related to differences in plant morphological characteristics promoted by differences in plant maturity. Nevertheless, the possibility of this seasonal variation also being affected by the concentration of secondary compound needs to be examined (see Experiments 5 and 6 - Chapter 4). It can also be concluded that although the animals had different behaviour between days within each experiment, the overall selective behaviour was not affected by the difference in sward surface height and herbage mass.

As shown in Table.3.36, the ECT concentration of birdsfoot trefoil leaves decreased from Experiment 1 (in spring) to Experiment 3 (in autumn). This seasonal pattern was also observed in other studies (Cope et al., 1971; Windham et al., 1988; Roberts et al., 1993). However the decrease of condensed tannin concentration between late spring and late summer disagrees with reports for several plant species (Clark et al., 1939; Stitt and Clarke, 1941; Donnely, 1959; Cope et al., 1971; Windham et al., 1988 and Iason et al., 1995). These authors found that the concentration of condensed tannin increased during summer months. However the concentration of ECT can also vary for several other reasons such as site to site variation (Douglas et al., 1993), soil fertility (Barry, 1989) and differences in plant morphology (Douglas et al., 1993; Iason et al., 1995). A better understanding of this variation can be achieved by comparing the ECT concentration of these three experiments with Experiments 5 and 6 (see Chapter 4).

The variation in formononetin concentration in leaves of red clover (Table 3.36) between experiments was relatively small compared with studies of Anwar (1994), who found formononetin concentrations at various stages of vegetative leaf development for cultivar Pawera varied between 0.75 and 2.16%. This small variation is probably related to the genotype. Anwar (1994) also found smaller variation of formononetin concentration in cultivar G-27 than in the cultivar Pawera. According to Nicollier and Thompson (1982) a significant portion of the isoflavone variation in clover is related to the genetic variance. The use of two different genotypes in Experiments 5 and 6, helped to clarify the variation in formononetin in red clover between different periods of the year (see Chapter 4).

3.4.5. Comparison of diet selection in Experiments 1, 2 and 3

Comparing experiments, herbage mass had an important effect on selection. The animals showed selection for swards with greater herbage mass, except in Day 1 of E1 and E2. In E1 Day 1, when the herbage mass was on average greater than 3900 kg/ha, the animals spent proportionally more time grazing in the minority sward type than was justified by the proportion of area offered, independent of the herbage mass (Tables 3.8, 3.9; Figures 3.7, 3.8). In a similar way, in E2 Day 1 the animals did not graze in proportion to herbage mass, but grazed for longer per kg of DM available in RC than in BW, except when BW was immature and RC was mature (Table 3.20). This lower effect of herbage mass in Day 1 in E1 and E2 contrasts with the greater effect of herbage mass in E3, where the animals grazed in proportion to herbage mass offered (section 3.3.3.1 – *Grazing time per Kg of DM offered*). This difference can be explained by the fact that swards in Experiment 3 had the lowest herbage mass and the lowest height. Although the animals' intake should not have been constrained by the herbage mass available (Holmes, 1987; Hodgson, 1990; Gibb et al., 1997) selection behaviour did seem to be influenced by herbage mass levels found in E3. The increasing effect of declining levels of herbage mass on diet selection could also be observed over time in E1, when in Day 3 the animals grazed in proportion to herbage mass available (Table 3.9, Figure 3.8). However, as indicated above, in E2 the animals were also strongly affected by sward morphological composition. This observation suggests that above some limits (approaching 4000 kg DM/ha in these studies), the influence of herbage mass on cattle diet selection is reduced.

The greater grazing time on the sward with greater herbage mass (represented by sward height and bulk density) in Day 3 of E1 (Tables 3.9, Figure 3.8), and Day 1 and 3 of E3 (section 3.3.3.1 - *Grazing time per Kg DM offered*) also demonstrated animal preference for taller swards with greater bulk density. Gong (1993) also found greater effect of bulk density on ingestive behaviour when sheep grazed clover than when they grazed grass. According to Burlison et al. (1991) and Laca et al. (1992) height and density effects on intake are independent and additive.

As in other studies (Mitchell et al., 1991, Illius et al., 1992; Laca et al., 1992; Demment et al., 1993; Gong et al., 1996; Gibb et al., 1997; Torres-Rodriguez, 1997), sward surface height was one of the most important pasture characteristics that affected ingestive behaviour. Its importance was demonstrated mainly in Day 1 (when the contrast between the two swards was high) of E2 (except in the treatment: BW immature and RC mature) and E3. In these experiments the animals grazed proportionally more time in the tallest sward (Table 3.11, 3.19, 3.23, 3.30). However in E1 Day 1 the animals were strongly influenced by area, grazing proportionally more in the minor area (Table 3.8, Figure 3.7). Two possible hypothesis can be suggested to explain the fact that the height effect was stronger in E2 and E3 than in E1. Firstly, the height effect in E1 was reduced because it was above some limits of herbage mass (as discussed earlier), but sward maturity influenced the preference for red clover in E2. Secondly, selective behaviour that drives the animal to seek a mixed diet (see section 3.4.7) was stronger than the preference for taller swards in E1. These two hypothesis were clarified with E5 and E6 where cattle preference was tested using spaced plants of birdsfoot trefoil and red clover of different heights but similar maturity.

<u>Effect of alternative sward</u>

The greater the physical constraints on a sward, the more the animals were affected by the alternative sward. In E2 and E3 the total grazing time on one sward was affected by the alternative sward only on Day 3 (Tables 3.18, 3.29). On this day the herbage mass and height was relatively low and prehension was more difficult. This result suggests that the effect of the alternative sward and, by inference, of alternative patches within a sward on diet selection is probably more important when there are physical limitations to ingestion such as occurs when height is low. This result also reinforces the conclusion drawn above that the animals were strongly influenced by the herbage mass available. However, it does not agree with Griffiths et al. (1997), who did not find evidence that use of individual sward patches was influenced by conditions on adjacent patches. This fact may be explained by the fact that Griffith et al. (1997) did not test the situation where all swards had low sward surface height and herbage mass.

3.4.6. Diet selection changes over time (from Day 1 to Day 3)

The animals were able to adjust their diet selection as the sward characteristics changed. In E1, the animals progressively adjusted their selection so as to graze in proportion to area and herbage mass offered, having initially selected strongly for the minority sward (Tables 3.8, 3.9, Figures 3.7, 3.8). This result is confirmed by the results in E2 and E3. In this adjustment the selection for the sward with greater proportion of leaf was overridden by a stronger selection for greater sward height and bulk density. Although several studies have shown that animals prefer leaves and reject stems (O'Reagain and Mentis, 1989; O'Reagain, 1993), in Day 3 of E2 and E3 the animals selected taller swards with higher herbage mass and greater percentage of stems. As discussed earlier (section 3.4.5), in Day 3 of E2 and E3 the animals were affected by the alternative sward. In E2 Day 3 they grazed substantially less time in BW when they had mature RC as the alternative sward (Tables 3.18, 3.19). The attraction for mature RC is probably related to the fact that this was one of the swards with greater herbage mass (more height and bulk density), and more stems left after two days of intense grazing (Tables 3.11, 3.12). Similar behaviour happened in E3 Day 3, when the grazing time on BW was affected by both the sward height in BW and by the height of the alternative sward (Table 3.29). Animals spent more time grazing tall than short BW, but this grazing time increased when the alternative sward was short and decreased when the alternative sward was tall. In these cases the animals selected swards with higher herbage mass (taller and higher bulk density) (Table 3.23) and greater proportion of stems (Table 3.24), once most of the leaves were lost from the canopy. The selection of swards with higher proportion of stems in Day 3 contrasts with the behaviour observed when comparing the pattern of selection across experiments (see section 3.4.4) and in Day 1 of the three experiments (see section 3.4.7) where the animals showed selection for swards with higher leaf/stem ratio.

3.4.7. Diet selection in Day 1 (high herbage mass and height)

Results from Day 1 in each of the three experiments, when the herbage mass and height was high, suggests that selection was determined more by physical sward characteristics than by the preference of a specific species. In E1 the animals allocated grazing activity preferentially to the minor sward component on each treatment (Tables 3.8, 3.9, Figure 3.7). In E2 the animals allocated a greater proportion of time grazing RC probably because of its greater height and proportion of leaves (Tables 3.11, 3.12, 3.16). In E3 the animals grazed according to the sward height and in proportion of herbage mass available (Tables 3.23, 3.28, section 3.3.3.1 - *Grazing time per Kg of DM offered*). These results confirmed the findings of Torres-Rodriguez (1997) where heifers did not show preference between monocultures of red clover and birdsfoot trefoil, but their preference was affected by sward physical characteristic (in this case, sward surface height).

This behaviour in Day 1 also shows that the animals consistently grazed both swards on offer, even though they could have met intake requirements by grazing only one sward type. Parsons et al. (1994a) and Cosgrove et al. (1996) described this behaviour where animals do not have a unique preference for a species or sward as partial preference. Newman et al., (1992) suggested some explanation for this behaviour: (a) animals try to obtain a balanced diet; (b) animals try to maximise intake rate; (c) physiological responses to a novel diet. In E1 Day 1 the fact that the animals spent proportionally more time grazing on the minority sward than the proportion of area offered to them, demonstrated that the animals either needed to sample other species (Illius and Gordon, 1990) or preferred a mixed diet (Parson et al., 1994a Cosgrove et al., 1996; Torres-Rodriguez, 1997). Illius and Gordon (1990) and Illius et al., (1987) explain that animals need to sample to learn about alternative food on offer. Although Edwards (1994), working in controlled conditions demonstrated that sheep quickly learn about the location of food "patches" and do not need to continue the sampling strategy, he also pointed out that changeable sward conditions could make the memory of limited use. According to Freeland and Janzen (1974) the preference for mixed diet might indicate an evolutionary adaptation to reduce the possibility of toxicity. In this context, the effect of secondary compounds concentration might be important and a discussion in the context of E5 and E6 is important to clarify this behaviour (see General Discussion - Chapter 5).

Dwell time was calculated from the grazing time observations (each ten minutes) of Day 1 to clarify the behaviour observed where the animals had access to alternative swards (Table 3.37). The dwell time corresponds to the amount of time each animal spent grazing one sward before moving to the alternative one. It was calculated based on the group mean value. Although this analysis provided a good relative impression of the animal movement between swards within each plot, it is limited by the fact that the grazing observation was done only each 10 minutes and no other comparable evidence was found in the literature. The results show that in all three experiments the animals changed swards relatively frequently, and the dwell time of both swards were similar (each about 20 minutes). This result indicates that the animals were constantly moving from one sward to another and achieving a mixed diet. However, the proportion of grazing time changed as the sward conditions changed. In Day 1 of E1 and E3, for example, the proportion of grazing time spent on BW was significantly affected by the treatment (Tables 3.8 and 3.30). On the other hand, in Day 1 of E2 the proportion of grazing time was not affected by treatment, the animals grazed about 40-50% of the total grazing time on BW in all treatments.

Table 3.37. Amount of time (dwell time - minutes) the animal spent grazing in swards of BW or RC before moving to the alternative sward in Experiments 1, 2 and 3 (E1, E2 and E3, respectively), Day 1.

	BW	RC
E1	23.2	19.6
SEM[1]	2.21	1.94
E2	20.3	25.5
SEM	2.52	2.60
E3	19.1	22.8
SEM	1.93	2.21

[1]SEM – Standard error of the mean calculated from the variation among the five group of animals in four replications

As discussed earlier in relation to the pattern of selection across experiments (section 3.4.4), sward morphological characteristics also had important effects on allocation of grazing activity between swards in Day 1 of E2. The small difference in morphological characteristics between swards (Table 3.3, 3.24) in E1 and E3, reflected the small influence of this component on selection. However in E2, when the sward surface height and herbage mass was high, the animals spent more time grazing in RC because of it was taller and had greater proportion of leaves. In Day 1, the animals avoided grazing BW swards probably because of the lower percentage of leaves and higher percentage of stems (Table 3.12). In fact, the treatment that had the highest proportion of grazing time on BW was the treatment in which BW had the highest proportion of leaves and the lowest proportion of stems (Treatment B: BW immature/RC mature) (Table 3.20). This result agrees with other studies where animals showed preference for swards with a greater percentage of leaves (Theron and Booysen, 1966; O'Reagain and Mentis, 1989; O'Reagain, 1993).

The animal preference observed in each experiment was unlikely to be influenced by the ECT concentration. Although the highest ECT concentration was observed in E1 (Table 3.4), in that experiment the animals seemed to be more influenced by the area ratio than by the species composition (Tables 3.7, 3.8, 3.9, Figure 3.7). The greatest contrast in ECT concentration between treatments was found in E2. In this case the small effect of plant maturity on diet selection in Days 1 and 2 (Table 3.18), and the small variation between days in the proportion of grazing time devoted to graze BW (Table 3.19) demonstrated that ECT concentration probably had small effect on diet selection. The variation in ECT concentration observed between mature and immature birdsfoot trefoil leaves (ECT concentration of 0.37 and 0.79 %, respectively – Table 3.13) was probably not enough to affect selection. In addition, the difference in amount of leaf removed in E2 between mature and immature plants (Table 3.12) was not so clear as the difference in ECT concentration. The higher concentration of condensed tannin in immature plants than in mature is also found in other plant species. Coley (1983) and Furstenburg and van Hoven (1994), for example, found with tropical trees and shrubs, a higher percentage of tannin in young leaves than in old. However, Donnelly (1959) showed that tannin concentration increased with maturity in Serica lespedeza.

As expected, there was a significantly higher concentration of formononetin in red clover than in birdsfoot trefoil or white clover (Tables 3.5, 3.14, 3.26). There was also a consistently higher concentration of formononetin in leaves of red clover than in stems or petioles across the three experiments. This result agrees with the observations of McMurray et al. (1986) and Anwar (1994). However, because there were relatively small differences for the percentage of leaves and stems between RC swards within each experiment (Appendix 3.3, Tables 3.12, 3.24), it is very unlikely that the formononetin concentration had a significant effect on the variation of grazing preference among RC swards. On the other hand, the fact that the concentration of formononetin was not affected by the variation in red clover maturity in E2 disagrees with the observation of Keogh (1995) and Anwar (1994). These authors reported that in red clover there is higher concentration of formononetin in young leaves, declining progressively as the leaves age. However, Anwar (1994) also observed that the difference in concentration between old and young leaves changes according to genotype. The small effect of maturity in the currently studies probably reflected the characteristic of the cultivar Colenso. No previous study was found in the literature comparing the effect of maturity on formononetin concentration of this cultivar.

White clover, birdsfoot trefoil and red clover species showed a high nutritive value compared to standards described by NRC (1989, 1996), particularly in relation to leaves (Tables 3.6, 3.15, 3.27). Leaves had higher quality based on a lower percentage of fibre and a higher percentage of protein and digestibility, than the petioles and stems. However, grazing preference was unlikely to have been affected by the sward nutritive value, because there were only small differences in quality between species. The effect of nutritive value on diet selection was also examined in E5 and E6 (see Chapter 4).

3.4.8. Diet selection within each sward

Defoliation caused a substantial decrease in the proportion of leaves and an increase in the proportion of stem and dead matter of both swards in all treatments. The increased proportion of dead material and stems found in both manual morphological separation (Tables 3.3, 3.12, 3.24) and point quadrat analysis (Figures 3.5, 3.6, 3.10, 3.11, 3.12, 3.13) was explained probably by the selection of green leaf material by animals. Animals usually select leaf from stem (Arnold, 1960; Juko and Bredon, 1961; Arnold, 1964; van Dyne and Heady, 1965; Guy et al., 1981; Arnold, 1981; L'Huiller et al. 1984; L'Hiuller, 1986; Edwards, 1994), young components in relation to old (Arnold, 1960), and green in preference to dead material (Arnold, 1960; Juko and Bredon, 1961). However as discussed in section 3.4.6, in conditions of low herbage mass and sward surface height in Day 3, the animals demonstrated selection for swards with high proportion of stems.

In most of the BW swards in the three experiments, birdsfoot trefoil / white clover ratio (B/W ratio) was lower after defoliation than before (Tables 3.3, 3.12, 3.24), indicating a greater intake of birdsfoot trefoil than of white clover. This result together with the point quadrat data, showing larger contribution of birdsfoot trefoil in the upper strata (in relation to white clover – Figures 3.5, 3.10, 3.12), suggested that the animals grazed more birdsfoot trefoil because it was in the upper strata. This agrees with the results of Gammon and Roberts (1978), Hodgson (1981b), Milne et al. (1982), Bircham and Hodgson (1983) Briske (1986) and Illius et al (1992), all of which suggest that the variation in the frequency or severity of defoliation of individual sward component is likely to be directly related to the size of individual plants and their proximity to the surface of the vegetation canopy.

3.5. CONCLUSIONS

Sward physical characteristics described by contrasts (in E1, E2 and E3) of sward area, maturity and structure (represented mainly by height and herbage mass) were important determinants of selective behaviour. However their effects were also altered by the reduction in herbage mass and height from Day 1 to Day 3. Conclusions from these three experiments are summarised below:

1) The use of strips of specific swards provided a flexible condition to test animal diet selection. The small difference between groups of animals, the absence of any previous treatment effect and the agreement between intake and grazing time in E1 and E3 showed a good reliability of the results.

2) Rate of biting apparently did not influence diet selection between swards. Rate of biting was more a consequence of sward physical characteristics than a cause of selection.

3) The results suggest that there was a seasonal variation in diet selection between BW and RC and that this variation was influenced mainly by the effect of sward maturity. This seasonal variation and maturity effect could be mainly explained by the preference for leaves and rejection of stems of each species.

4) The effect of the alternative sward on cattle diet selection was more important as the sward physical limitations increased. This result may be extrapolated to contrasts between patches in a heterogeneous sward.

5) The animals had the capacity to adjust their diet selection as sward characteristics changed.

6) Results from Day 1 of E1, E2 and E3 suggest that selection between BW and RC swards was determined more by physical sward characteristics than by the preference for a specific species.

7) Even though the animals could meet intake requirements by grazing only one sward type, they grazed both swards, and frequently changed between swards, to achieve a mixed diet.

8) Sward area was an important determinant of selective behaviour between two alternative swards, but its importance varied according to the herbage mass and height.

9) Herbage mass and sward surface height had a strong effect on animal diet selection, however these experiments suggest that when both swards had high herbage mass and height their influence on diet selection was reduced.

10) Although there was variation between experiments, and between mature and immature swards in ECT and formononetin concentration, preference did not seem to be affected by the levels of ECT and formononetin concentration found in this experiment.

11) The animals showed greater intake of birdsfoot trefoil than white clover in BW swards. This behaviour can be explained by the fact that birdsfoot trefoil was distributed higher in the sward canopy.

CHAPTER 4

EXPERIMENTS 5 AND 6

4.1. INTRODUCTION

Experiments 5 and 6 were carried out in order to provide explanation for the selective behaviour pattern observed in Experiments 1, 2 and 3 in relation to how physical and biochemical characteristics of plants influence animal preference, and to what extent this influence affected seasonal variations in preference. The effects of plant morphological characteristics were related to the effects of concentrations of extractable condensed tannin in birdsfoot trefoil and to formononetin in red clover. Although condensed tannin (e.g. Barry and Blaney, 1987; Barry, 1989; Waghorn *et al.*, 1990) and formononetin (e.g. Marshall, 1973; Keogh, 1995) are recognised to be important secondary compounds in pastures, very little is known of their influence on animal appraisal and selective behaviour.

In these experiments dairy cows grazed spaced plants of two genotypes of birdsfoot trefoil with high and low concentrations of extractable condensed tannins, and two genotypes of red clover with high and low formononetin concentrations. For convenience, the different cultivars (birdsfoot trefoil: Goldie; red clover: Pawera and G-27) and accession (birdsfoot trefoil: PI273938) used in these experiments will be described as different "genotypes". Experiment 6, using a rumen content modification approach, also had the objective of determining the effect of post-ingestive feedback from secondary compounds on cattle diet selection. These experiments were based on recent reports and studies that show that the selective response to secondary compounds is related to post-ingestive feedback (e.g. Provenza, 1995) and that sheep can make rapid changes in their diet selection as a result of manipulation of rumen environment (Carter and Grovum, 1990; Hou, 1991 in Cooper et al., 1995).

4.2. MATERIAL AND METHODS

Two experiments (Experiments 5 and 6) were carried out to investigate the influence of condensed tannin in birdsfoot trefoil and formononetin in red clover on diet selection by dairy cows. In both experiments sequences of spaced plants of two genotypes of birdsfoot trefoil (*Lotus corniculatus* L.), providing low (cultivar Goldie) and high (accession PI 273938) concentration of condensed tannin (CT) and two of red clover (*Trifolium pratense* L.) providing low (cultivar G-27) and high (cultivar Pawera) concentration of formononetin, were offered to dairy cows. The same field design was used in each experiment to determine dietary preference, grazing behaviour and trade-off decisions of cows. In Experiment 6, rumen conditions in fistulated cows were modified by the addition of plant material to investigate the effect of rumen feed back on selective behaviour. In Experiment 5 treatments were run three times while in Experiment 6 they were run twice. The methodology used in each experiment is described below.

4.2.1. Experimental site

Both experiments were located in Palmerston North (40^0 23'S, 175^0 37'E), Manawatu, with an average annual rainfall of approximately 1000 mm. The weather conditions monitored at an adjacent site at AgResearch showed that the average monthly soil temperature in the year of the experiments (from July 1996 to June 1997) ranged from 8.0°C (July 1997) to 17.7°C (February 1997). The monthly rainfall and mean soil temperature (10 cm depth) from July 1996 to June 1997 compared with 60-year average values at the site are presented in Appendix 4.1.

4.2.1.1. Experiment 5

Experiment 5 was carried out at the Dairy Cattle Research Unit (Dairy 3), Massey University. The research area was a flat paddock with a soil classified as Tokomaru silt loam, a yellow-grey earth which has poor internal drainage (Cowie, 1972). A sward of perennial ryegrass with white clover formed this paddock before the experiment, and was rotationally grazed by dairy cows. The experiment was repeated in the same area three times (three periods): 17 January, 19 March and 24 April.

4.2.1.2. Experiment 6

Experiment 6 was carried out at the AgResearch Grasslands Research Centre. The soil is classified as a recent Manawatu silt loam (Cowie, 1972). Before this trial, the area was sown to a perennial ryegrass - white clover sward. This sward had been grazed by sheep, and occasionally by cattle. The trial was carried out in two periods on 22, 23, 28, 29 January and on 3, 4, 8, 9 April 1997.

4.2.2. Glasshouse sowing and management

A random sample of seeds of each genotype was sown in a glasshouse on 23 August 1996. Five hundred and sixty plastic pots (14 cm diameter and 15 cm height) were sown (140 pots per genotype). Four seeds per pot were sown in a medium composed of 60% peat/40% pumice, and a mixture of 100g agricultural lime+300g dolomite+60g of Micromax[1]+300g PG[2] mix per 100 l of medium. *Rhizobium trifolii* were applied to the pots with red clover and *Rhizobium loti* to the pots with birdsfoot trefoil. They were applied in slurry with water. Glasshouse temperature was regulated between 25^{o}C and 15^{o}C and the pots were watered daily.

Thinning started on 13 September with the appearance of the first complete leaf (leaf with 3 leaflets) to eliminate the weaker plants. When the plants had about 4-5 leaves (23 September 1996) they were thinned finally to one plant per pot. The plants stayed in the glasshouse until transplanted to the field.

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[1] The Micromax constituents were 12% Fe, 2.5% Mn, 1% Zn, 0.5% Cu, 0.1% B, 0.05% Mo and 15% of combined sulphur.

[2] The PG mix constituents were: 14%N, 16%P_2O_5, 18%K_2O, 0.03%B, 0.12% Cu, 0.02% Mo, 0.16% Mn, 0.04% Zn, 0.09%Fe.

4.2.3. Site preparation and management

Before planting the experiments, the existing perennial ryegrass/white clover sward was killed with a broad spectrum herbicide (Round-up+ Granstar). The areas were planted on 25 and 26 October (Experiment 6) and on 29 and 30 October 1996 (Experiment 5). Before the first grazing on both sites, the weeds were hand sprayed with Buster (broad spectrum herbicide) and 9 g (90 kg/ha) of a 15-10-10 (N-P-K) fertiliser were applied per plant. After each post-grazing assessment, all the plants were cut at 7 cm and the same amount of fertiliser was then applied per plant. Before the third grazing in Experiment 5 and the second grazing in Experiment 6, a combination of a broad spectrum herbicide (Round-up), a broad leaf weed control (Granstar), and a residual herbicide (Simazine) was used to control the weeds present between the plants. The information about Experiments 5 and 6 is summarised in Table 4.1.

Table 4. 1. Management schedule, agrochemical and fertiliser applied in Experiment 5 and 6.

Management	*Schedule*		*Agrochemical and Fertiliser applied*
	Experiment 5	*Experiment 6*	
Area preparation	16/10/96	15/10/96	3 l/ha Round-up[1] + 40 g/ha Granstar[2]
Planting	29-30/10/96	25-26/10/96	
Fertiliser	23/12/96 and after grazing	20/12/96 and after grazing	9 g of 15-10-10 (N-P-K)/plant
Weed control	23/12/96	20/12/96	Buster[3] 30 ml/l
	02/04/97	01/04/97	0.1 ml/l Round-up + 33 mg/l Granstar + 1 ml/l Simazine[4]
Experiment runs	17/01/97 19/03/97 24/04/96	22-23/01/97 28-29/01/96 03-04/04/96 08-09/04/96	

[1] 360 g/l Glyphosate
[2] 750 g/kg Tribenuron
[3] 200 g/l Glufosinateammonium
[4] 500 g/l Simazine

4.2.4. Design

The trials were formed by isolated plants spaced at one meter intervals in sequences of 26 plants, each providing blocks (replicates) of sequences of eight treatments in a balanced design. The experimental design was balanced with enough replication in order to allow plant morphological modification. Differences in plant morphology were anticipated between genotypes within species, and this effect was balanced by a trimming treatment. In each block one plant of each genotype was trimmed to make the two plants of the same species similar in height, area and leafiness. This resulted in 8 treatments (2 species x 2 genotypes x (± trimming)) allocated to individual position in balanced sequences (Table 4.2.).

Table 4. 2. Descriptions of the 8 treatments.

Treatm. Number	*Species*	*Genotype*	*Concentration of secondary compounds*	*Plant morphology modification*
1	Birdsfoot trefoil	Goldie	Low – Condensed Tannin	Not trimmed
2	Birdsfoot trefoil	Goldie	Low – Condensed Tannin	Trimmed
3	Birdsfoot trefoil	PI273938	High –Condensed Tannin	Not trimmed
4	Birdsfoot trefoil	PI273938	High –Condensed Tannin	Trimmed
5	Red clover	G-27	Low – Formononetin	Not trimmed
6	Red clover	G-27	Low – Formononetin	Trimmed
7	Red clover	Pawera	High –Formononetin	Not trimmed
8	Red clover	Pawera	High –Formononetin	Trimmed

The eight treatments were arranged at random in linear series with three replications (3 blocks), forming a sequence of 24 plants. The treatment order was balanced across sequences so that each treatment was followed by each other treatment at least once. All of the treatments were present with equal frequency down the sequence (Figure 4.1.). The first and last plants in each sequence were included to minimise end effects and were not included in the analysis. Before pre-grazing assessment any flower present was hand removed from all plants.

Figure 4.1. Distribution of the set of four sequences with the eight treatments (treatments: 1 to 8 - see the description of the 8 treatments in Table 4.2) arranged in 3 blocks.

SEQUENCE 1	SEQUENCE 2	SEQUENCE 3	SEQUENCE 4	
7	1	3	5	
4	2	4	6	
1	8	3	7	
7	5	1	3	
5	1	6	5	BLOCK 1
6	7	8	4	
2	4	7	2	
3	6	2	1	
8	3	5	8	
1	3	5	8	
4	6	2	1	
8	4	7	2	
3	7	8	4	BLOCK 2
2	1	6	5	
6	5	1	3	
5	8	3	7	
7	2	4	6	
7	4	3	5	
5	5	6	4	
6	2	4	2	
2	7	7	1	BLOCK 3
3	8	1	8	
8	6	5	6	
4	1	8	7	
1	3	2	3	
1	7	5	3	

4.2.5. Animals and sequence allocations

4.2.5.1. Experiment 5

Animals

Four mature lactating Friesian cows were used. The animals were trained for four weeks before starting the trial. Initially the cows were trained to graze down a narrow raceway and to be accustomed to fences and people. In order to get used to plant species, one week before starting the trial, the cows were trained in an adjacent area with similar plant sequences to the experimental area.

Because the cows in the first run (January/96) did not graze birdsfoot trefoil, two weeks before the cows entered the adjacent areas in the second run, they were offered every day pre-clipped birdsfoot trefoil to get used to this species. No extra pre-clipped red clover was offered to the cows because the animals had already experience of grazing swards containing red clover and they did not show any rejection of this species in the first run.

Sequences allocation

One set of four sequences (see Figure 4.1.) was used. Each sequence of 26 plants was randomly allocated and grazed by one cow. Four spare sequences were planted to be used for training responses. Two more sequences were used for probe and visual assessment calibration (see Section 4.2.8).

4.2.5.2. Experiment 6

<u>*Animals*</u>

Two rumen-fistulated dry Friesian cows were used. The cows were trained for 6 weeks before starting the trial. The training procedure was done as in Experiment 5: one week before starting the trial the cows were trained in adjacent plant sequences with the same plant species and genotypes.

The cows had previous experience in grazing birdsfoot trefoil and red clover and they did not show any rejection in grazing these species during the training period.

RUMEN MODIFICATION

The rumen contents of the cows were modified to provide contrasts between high and low concentrations of tannin, and high and low concentrations of formononetin. Plants of birdsfoot trefoil cv. Goldie (*Lotus corniculatus* L. – low condensed tannin content), lotus maku (*Lotus pedunculatus* L.- high tannin content), red clover cv. Pawera (*Trifolium pratense* – high formononetin content) and red clover cv. Astred (low formononetin content) were applied directly into the cow's rumen through a fistula of approximately 100 mm diameter.

Areas with birdsfoot trefoil, lotus maku and red clover were cut in the evening before the day of the experiment. After cutting, the material was kept in a chiller until it was used. Next morning the material was minced to provide a better distribution of secondary compounds. In order to get a good minced material, alfalfa pellets had to be mixed to increase the dry matter content. The botanical composition and chemical analyses of the minced materials are presented in Appendix 4.10.

After mincing, the material was then put into the cow's rumen through the fistula, about one hour before running the experiment. The cows were fasted overnight and rumen content was removed before the minced material was inserted. The objective was to remove 20-25 kg fresh rumen contents and replace with 15-20 kg minced material, though the amount of rumen content removed and the material inserted varied according to the cow's size, amount of minced material available and previous amount of rumen content (see Appendix 4.10). It was important not to create an impression of satiety in the cows by adding the minced forage to the filled rumen, and it was also important to achieve some mixing of mince material with the digesta in order to facilitate feedback response. Previous studies with these cows suggested that the minced material accounted for about 25% of rumen digesta wet weight (G. C. Waghorn, *pers. comm.*) (see Appendix 4.10).

Sequence allocation

Two sets of the four sequences (see Figure 4.1.) were used. Each sequence of 26 plants was randomly allocated and grazed in each period by one cow. Two spare sequences were planted to be used for training responses.

The rumen contents of the two cows were modified in balanced sequence according to Tables 4.3 and 4.4 in the first and second period, respectively. Each set of four sequences in each period corresponded to a run. The cows grazed the first set (in the first two days – first run) when they had rumen contents modified for formononetin concentration. The second set of sequences was grazed when the cows had their rumen content modified for tannin concentration. In the second period, the same approach was used, however in this case the first four sequences were grazed by cows that had their rumen content modified for tannin concentration, and the second four sequences for formononetin concentration. High and low rumen modifications for each secondary compound were balanced between cows, runs and periods as shown in Table 4.3. To avoid residual effects, there was at least 5 days interval between when the rumen was modified with formononetin and when it was modified with tannin.

Table 4. 3. Schedule of rumen modification in the first period.

		Period 1	
Run	Date	Cow 95	Cow 223
1	22/01/97	Low Formononetin	High Formononetin
	23/01/97	High Formononetin	Low Formononetin
2	28/01/97	High Tannin	Low Tannin
	29/01/97	Low Tannin	High Tannin

Table 4. 4. Schedule of rumen modification in the second period.

		Period 2	
Run	Date	Cow 95	Cow 223
3	03/04/97	High Tannin	Low Tannin
	04/04/97	Low Tannin	High Tannin
4	08/04/97	High Formononetin	Low Formononetin
	09/04/97	Low Formononetin	High Formononetin

4.2.6. Grazing behaviour assessment

In both experiments (Experiment 5 and 6), the cows during the trial were allowed to walk undisturbed down the sequences. The animals were trained for not reversing direction or going backwards. They were allocated to a holding area as soon as the sequence was completed. The time required to complete the sequence was on average 5 minutes (ranging from 1 to 9 minutes). During grazing the cow's behaviour was assessed in terms of the time (seconds) spent grazing and the number of bites taken from each grazed plant. The assessment was registered manually and by video camera.

General views of the plant sequences and animal grazing observation are shown in Plate 4.1 and 4.2, respectively.

Plate 4. 1. General view of the sequence of plants, before grazing

Plate 4. 2. Animal grazing observation.

4.2.7. Plant assessment

A series of descriptive measurements were made on individual plants before and after each grazing:

Plant height

Plant height was measured with a sward stick (Bircham, 1981; Barthram, 1986). The highest point of each plant was measured in half centimetre increments.

Plant diameter

Two diameters (perpendicular and parallel to the sequence) of each plant were measured with a ruler in centimetre units.

Plant density

Visual assessments of the number of stems were made for each plant before grazing, and given a score from 1 to 10 in 0.5 units, 10 being the densest plant available at the time of the leafiness and herbage mass calibration (Real-Ferreiro, 1997).

Leafiness

An eye estimation was made of the percentage of leaf per plant, in intervals of 5%, with respect to the total plant material (Real-Ferreiro, 1997). The eye estimation was firstly calibrated using plants on spare sequences in Experiment 5, offering contrasting ranges in size and leafiness. These plants had their leaves (leaf lamina + petioles) weighed separated from the other plant parts.

<u>*Habit*</u>

The plants were visually compared with angles drawn on paper (Real-Ferreiro, 1997):

Score 1 - angle between the main stems and the horizontal (parallel to the soil) in the interval of 0^0 -18^0 interval - plant completely prostrate.

Score 2 - angle between the main stem and the horizontal in the interval of 18^0-36^0.

Score 3 - angle between the main stem and the horizontal in the interval of 36^0-54^0.

Score 4 – angle between the main stem and the horizontal in the interval of 54^0-72^0

Score 5 – angle between the main stem and the horizontal in the interval of 72^0-90^0

<u>*Herbage mass*</u>

Herbage mass was assessed with a pasture probe GrassMaster (manufactured by Tru-Test®) pre and post-grazing. The Pasture probe was calibrated with spare plants in Experiment 5 to provide contrasts in morphology (size, leafiness, density and habit) for the calibration. Separate equations were used to estimate herbage mass per plant for each species. The two equations are presented in Appendix 4.2. The average of three random readings per plant was used for estimation of herbage mass. The herbage removed from each plant was assessed through the difference between pre and post grazing assessments.

4.2.8. Morphological and chemical analysis

Four intact stems were harvested from each plant (not considering the first and the last plant in each sequence) before grazing. The cuttings were done in a way to avoid modification of plant structure. Two stems were randomly chosen for morphological determination and two for chemical analysis. The samples were then bulked across blocks within each sequence according to genotype and trimming condition. In this way, there were 4 replicates per treatment (8 treatments) in each run.

4.2.8.1. Morphological analysis

The samples for morphological analysis were taken to the laboratory and separated into leaf, petiole (only in clovers), stem, flower and dead material. All the samples were dried to constant weight in a forced-draught oven, at a temperature of 70-80^{0}C. The samples then were weighed individually.

4.2.8.2. Chemical analysis

The samples for laboratory analysis were stored at -20^{0}C. The samples were then freeze dried and ground to pass through a 1 mm diameter screen. The red clover samples were analysed for formononetin content using the methodology described on Section 3.2.6.1 (Chapter 3) and the birdsfoot trefoil samples were analysed for extractable condensed tannin content using the methodology described on Section 3.2.6.1 (Chapter 3) and Appendix 3.1.

After taking sub-samples for extractable condensed tannin and formononetin analysis, similar amounts of the residual samples were bulked across sequences to obtain 8 samples (4 different cultivars x trimmed and not trimmed plants) for each run in Experiment 5, and 8 samples for each different rumen modification run in Experiment 6. These samples were analysed, using Near Infrared Reflectance Spectroscopy (NIRS) (Shenk and Westerhaus, 1994) for estimation of the content of crude protein, neutral detergent fibre (NDF), acid detergent fibre (ADF), carbohydrates (soluble sugars plus starch), ash and lipid content. *In vitro* dry matter digestibility (IVDMD) was calculated from ADF, according to equations presented in Section 3.2.6.2 (Chapter 3).

4.2.9. Statistical analysis

The plant and animal data were analysed using the statistical package SAS (SAS Institute Inc., 1985 and 1990). Analyses of variance were carried out to obtain information on treatment contrasts in the number of bites taken and grazing time per plant, balanced for previous treatment. Analyses of variance were also performed for comparison of the sward characteristics. Least square means and standard errors of differences were used to quantify the contrasts in grazing behaviour and sward characteristics. Analyses of variance were performed for Experiments 5 and 6 to verify specific effects in each experiment:

4.2.9.1. Experiment 5

A combined analysis between the second and third periods was first performed to clarify the effects of the interactions between period and treatment (species, secondary compound concentration and trimming). Analyses of variance were also carried out within periods to check animal preference between different legumes. Because the variation within each species in relation to number of bites was different, a separate analysis of each species was performed to clarify the trimming and secondary compound concentration effect on the number of bites taken from each plant.

4.2.9.2. Experiment 6

As for Experiment 5, combined analyses were used first to check the interaction between period and treatment (rumen chemical, species, plant secondary compound concentration and trimming) effects and the interaction between rumen chemical and species effects. Separate analyses, combining specific rumen chemical with specific species over periods were performed to obtain the rumen concentration and plant genotype effect on number of bites. Finally, particular analyses were carried out in each period, combining specific rumen chemical with specific legume species, to determine the trimming and genotype effects on number of bites taken in each plant.

4.2.9.3. Correlation Analyses

Correlation analyses were used to clarify the relationships among plant biochemistry, plant morphology and number of bites per plant. Correlation analysis was firstly carried out to investigate the importance of general plant chemical characteristics on number of bites. Correlation analyses were also performed to clarify the relationship of ECT and formononetin concentration with number of bites and morphological characteristics of birdsfoot trefoil and red clover plants, and the relationship between plant morphology and number of bites. In this analysis, logarithmic transformation of the data was used. The correlation analysis, followed by covariance analysis, was used to separate the effects of plant morphology and ECT concentration on number of bites.

4.2.9.4. Covariance analyses

Analysis of covariance was used to separate the plant morphological effect from the secondary compound effect on the number of bites taken per plant. Because the effect of secondary compounds on number of bites was significant only in plants of birdsfoot trefoil, the analysis of covariance was concentrated on number of bites taken in birdsfoot trefoil plants. These analyses were performed to partition differences between the two genotypes (cultivar Goldie and accession PI273938) in terms of the effects of volume (height and area), leafiness, or secondary compound concentration. As in the correlation analyses, logarithmic transformation of the data was used. The regression coefficient (R-square) of each analysis was then compared to quantify the morphological and biochemical effects.

Three different covariance analyses were carried out in each experiment. Covariance analysis was firstly carried out using individual data of all experimental plants. In this case plant morphological characteristics (leafiness and volume) were used as covariates while genotype effect was used as a class variable (described as high and low concentration of ECT). Genotype effect (class variable) and one covariate, either leafiness or volume, were added to a basic model to determine their effect on R-square changes. A second analysis was then performed considering only the untrimmed plants to test the natural plant morphological variation. A third analysis was carried out including both plant morphology and ECT concentration as covariates. In this analysis values of plant volume and leafiness were averaged according to sets of plants bulked for ECT chemical determination.

The basic model changed according to the experiment. In Experiment 5 the basic model used contained the effects of plant sequences (= effect of cow), blocks (within sequences) and trimming. In Experiment 6 the basic model contained the effects of day, cow, rumen chemical added to the rumen, rumen chemical concentration, plant sequence, block (within sequence) and trimming. However in the second covariance analysis of both experiments the effect of trimming was not included in the basic model, and in the third analysis, because values of plant volume and leafiness were averaged according to sets of plants bulked for ECT chemical determination, the basic model did not include the effect of blocks within sequence.

4.3. RESULTS

4.3.1. Experiment 5: Effect of condensed tannin in birdsfoot trefoil (Lotus corniculatus L.*) and formononetin in red clover (Trifolium pratense* L.*) on preference and grazing behaviour of dairy cows.*

4.3.1.1. Plant characteristics

The results are firstly presented for comparison between birdsfoot trefoil (BT) and red clover (RC) (Table 4.5). Because birdsfoot trefoil was not grazed in Period 1, only results of Periods 2 and 3 will be presented here. Attention will then be focused on the main trimming and genotype (secondary compound concentration) effects for comparisons within each species in Tables 4.6 and 4.7.

Comparisons between species

The plant characteristics of the two genotypes of birdsfoot trefoil (BT) and the two genotypes of red clover (RC), with and without trimming in Period 2 and 3 are presented in Table 4.5. There were significant interactions involving plant species, genotypes and trimming effects. Plants were generally bigger in Period 2 than in Period 3. BT plants were taller and with larger area than RC, but RC had a higher proportion of leaves and were more erect and denser than BT. On average, the untrimmed plants had more herbage mass than the trimmed ones. Comparing the eight treatments, untrimmed plants of cultivar Goldie had the greatest height, mass and area.

The estimation of the amount of area, height and leafiness removed by grazing demonstrated that RC had significantly greater reduction of these characteristics than BT. The estimation of herbage mass removed showed that in Period 2, accession PI273938 and cultivar Goldie had significantly the lowest and highest, respectively, amount of mass removed by the animals, and in Period 3 trimmed plants had significantly more mass removed than untrimmed.

More detailed analyses considering trimming and genotype differences within each species are given below.

Comparisons between cultivars of the same species

Attention in this section is focussed on effects of trimming and secondary compound concentration (differences between genotypes) within each species and their repeated measurements in different periods (Table 4.6 and 4.7).

BIRDSFOOT TREFOIL

Since birdsfoot trefoil was not grazed in Period 1, only Periods 2 and 3 are presented here (Table 4.6). In Period 2, the genotype with low extractable condensed tannin (ECT) concentration was significantly taller and had greater herbage mass, percentage of leaves and density, but did not differ in plant area and habit with the high ECT concentration genotype. The untrimmed plants were significantly taller with greater mass, area, leafiness and density, but did not differ in habit.

Table 4. 5.Characteristics of untrimmed (Ntrim) and trimmed (Trim) plants of birdsfoot trefoil cultivar Goldie (Low Tannin) and accession PI273938 (High Tannin), and red clover cultivars G27 [Low Form (formononetin)] and Pawera [High Form (formononetin)] in Periods 2 and 3 of Experiment 5.

		Birdsfoot trefoil(BT)				*Red clover(RC)*				
		Low Tannin		High Tannin		Low Form		High Form		*SED*[1]
		Ntrim	Trim	Ntrim	Trim	Ntrim	Trim	Ntrim	Trim	
PERIOD 2	**Height (cm)**									
	pre-grazing	18.9	15.1	13.7	13.7	15.6	12.9	15.0	13.5	1.27
	removed[2]	2.1	0.6	-0.2	-0.2	4.4	1.3	5.0	2.9	0.97
	Plant mass (gDM/m^2)									
	pre-grazing	494	417	389	330	393	369	351	369	20.4
	removed	130	88	61	16	86	69	77	79	23.7
	Plant area (cm^2)									
	pre-grazing	5540	3210	4510	3140	1790	1210	2260	1240	536.6
	removed	380	60	120	60	570	180	530	570	163.2
	Leafiness (%)									
	pre-grazing	50	42	40	37	75	72	75	74	2.4
	removed	8	5	2	1	21	12	29	12	3.8
	Habit									
	pre-grazing	2.6	2.5	2.2	2.3	2.7	3.0	2.3	3.1	0.267
	Density									
	pre-grazing	7.3	6.4	6.2	5.5	7.9	7.8	7.7	7.6	0.297
PERIOD 3	**Height (cm)**									
	pre-grazing	14.1	11.0	15.5	11.0	12.7	10.2	11.2	10.2	1.077
	removed	1.4	0.4	1.0	0.3	3.1	1.2	2.1	1.5	0.707
	Plant mass (gDM/m^2)									
	pre-grazing	430	394	400	361	363	340	356	325	24.8
	removed	92	99	87	55	93	77	103	55	22.1
	Plant area (cm^2)									
	pre-grazing	3160	1770	3440	1740	1440	970	1930	970	370.6
	removed	180	40	60	40	130	40	150	60	118.0
	Leafiness (%)									
	pre-grazing	47	43	42	40	75	69	73	69	2.3
	removed	5	2	4	2	18	15	24	17	3.3
	Habit									
	pre-grazing	2.4	2.2	2.2	2.4	3.0	2.8	2.2	3.0	0.206
	Density									
	pre-grazing	6.9	6.5	6.0	6.5	7.8	7.7	7.4	7.2	0.353

[1] SED – Standard error for differences of means.
[2] Removed = pre-grazing minus post-grazing assessment

Table 4.6. Characteristics of birdsfoot trefoil plants before grazing, and difference between before and after grazing (removed from height, mass, area and leafiness) according to trimming (Ntrim = untrimmed; Trim = trimmed) and genotype [secondary compound concentration (sec. comp. conc.): high (accession PI273938), low (cultivar Goldie)] effects in Period 2 and 3 of Experiment 5.

		Birdsfoot trefoil						
		Trimming			Sec. comp. conc			SED[1]
		Ntrim	Trim	P-value[2]	High	Low	P-value[2]	
PERIOD 2	**Height (cm)**							
	pre-grazing	16.3	14.4	0.0437	13.7	17.0	0.0006	0.87
	removed[3]	0.9	0.2	0.1444	-0.2	1.4	0.0034	0.50
	Plant mass (gDM/m²)							
	pre-grazing	441	373	0.0005	359	455	0.0001	17.8
	removed	95	52	0.0386	38	109	0.0015	20.3
	Plant area (cm²)							
	pre-grazing	5020	3180	0.0001	3820	4380	0.1764	402
	removed	250	60	0.1073	90	220	0.2736	115
	Leafiness (%)							
	pre-grazing	45	40	0.0002	39	46	0.0001	1.3
	removed	5	3	0.1619	1.5	6.5	0.0016	1.5
	Habit							
	pre-grazing	2.4	2.4	0.7998	2.2	2.5	0.0827	0.16
	Density							
	pre-grazing	6.8	6.0	0.0001	5.9	6.9	0.0001	0.17
PERIOD 3	**Height (cm)**							
	pre-grazing	14.8	11.0	0.0001	13.3	12.6	0.3388	0.73
	removed	1.2	0.4	0.0123	0.7	0.9	0.4547	0.33
	Plant mass (gDM/m²)							
	pre-grazing	415	377	0.0913	380	412	0.1533	21.7
	removed	89	77	0.4761	71	95	0.1685	17.4
	Plant area (cm²)							
	pre-grazing	3300	1760	0.0001	2590	2470	0.6575	286
	removed	120	40	0.3055	50	110	0.4373	78
	Leafiness (%)							
	pre-grazing	45	42	0.0032	41	45	0.0003	1.0
	removed	5	2	0.0053	2.7	3.7	0.2589	0.9
	Habit							
	pre-grazing	2.3	2.3	0.7042	2.3	2.3	0.7042	0.11
	Density							
	pre-grazing	6.5	6.2	0.1191	6.0	6.7	0.0001	0.16

[1] SED – Standard error for differences of means when comparing either trimmed with untrimmed plants or high and low sec. comp. conc. genotypes.

[2] P-value of the treatment main effect.

[3] Removed = pre-grazing minus post-grazing assessment

In Period 3 the genotype with low ECT concentration had a significantly ($P<0.05$) greater percentage of leaves and density than the high ECT concentration genotype. Similarly to Period 2, the untrimmed plants were significantly taller and had larger area and percentage of leaves, but they did not differ in herbage mass, habit and density from the trimmed plants.

The amount removed with grazing was also affected by trimming and genotype effects. In Period 2, the animals removed significantly ($P<0.05$) more herbage mass from untrimmed plants, however there was no significant trimming effect in the removal of height, area or leafiness. In the same period, the genotype with low ECT concentration had greater reduction in height, mass and leafiness than the genotype with high ECT concentration, but they did not differ significantly in area reduction. In Period 3, untrimmed plants had significantly greater reduction in height and leafiness, but there was no significant trimming effect on mass and area. In this period there were no significant differences between genotypes in relation to area, mass and percentage of leaves removed by grazing.

RED CLOVER

The trimming and genotype (secondary compound concentration) effects on RC plants are presented in Table 4.7. In Period 1, untrimmed plants differed from trimmed mainly because of the larger area. In Period 2, untrimmed plants were taller, had larger area and were more prostrate (had proportionally more stems close to the ground). In Period 3, untrimmed plants were significantly taller than trimmed plants, with more herbage mass, area and leafiness, but did not differ in habit and density. There were no significant differences between cultivars with high and low formononetin concentration in each of the three periods ($P>0.05$).

Table 4. 7. Characteristics of red clover plants before grazing and difference between before and after grazing (removed from height, mass, area and leafiness) according to trimming (Ntrim = untrimmed; Trim = trimmed) and genotype [secondary compound concentration (Sec. Comp. Conc.): High (accession PI273938), Low (cultivar Goldie)] effects in Period 1, 2 and 3 of Experiment 5.

		Red clover (RC)						
		Trimming			Sec comp conc.			
		Ntrim	Trim	P-value[2]	High	Low	P-value[2]	SED
PERIOD 1	**Height (cm)**							
	pre-grazing	23.6	22.4	0.4788	23.0	23.1	0.9328	1.72
	removed[3]	4.9	4.5	0.7695	4.4	5.1	0.6330	1.33
	Plant mass (gDM/m²)							
	pre-grazing	343	316	0.3038	310	350	0.1288	25.5
	removed	28	7	0.3743	30	320	0.2389	23.9
	Plant area (cm²)							
	pre-grazing	3720	2680	0.0062	3120	3280	0.6599	357
	removed	210	120	0.5831	120	210	0.5850	167
	Leafiness (%)							
	pre-grazing	76	74	0.2119	74	76	0.2119	1.5
	removed	31	31	0.9406	29	33	0.1245	2.8
	Habit							
	pre-grazing	3.2	3.0	0.3445	3.1	3.2	0.8491	0.21
	Density							
	pre-grazing	7.7	7.4	0.4690	7.9	7.2	0.2308	0.51
PERIOD 2	**Height (cm)**							
	pre-grazing	15.2	13.2	0.0096	14.2	14.2	0.9780	0.75
	removed	4.7	2.1	0.0015	4.0	2.8	0.1409	0.75
	Plant mass (gDM/m²)							
	pre-grazing	372	369	0.8011	360	381	0.0694	11.2
	removed	82	74	0.4948	78	78	0.9730	11.1
	Plant area (cm²)							
	pre-grazing	2030	1220	0.0004	1750	1500	0.2231	204
	removed	550	170	0.0011	350	370	0.8069	105
	Leafiness (%)							
	pre-grazing	75	73	0.3656	74	74	0.6967	1.6
	removed	25	16	0.0088	24	17	0.0228	3.1
	Habit							
	pre-grazing	2.5	3.0	0.0212	2.7	2.9	0.4258	0.21
	Density							
	pre-grazing	7.8	7.7	0.7108	7.6	7.9	0.2700	0.22
PERIOD 3	**Height (cm)**							
	pre-grazing	12.0	10.2	0.0045	10.7	11.5	0.2040	0.59
	removed	2.6	1.4	0.0432	1.8	2.1	0.5964	0.58
	Plant mass (gDM/m²)							
	pre-grazing	359	333	0.0187	340	350	0.2962	10.7
	removed	98	66	0.0116	79	85	0.6365	11.9
	Plant area (cm²)							
	pre-grazing	1680	970	0.0024	1450	1200	0.2684	217
	removed	140	50	0.3146	110	80	0.7997	91
	Leafiness (%)							
	pre-grazing	74	69	0.0299	71	72	0.5744	2.2
	removed	21	16	0.0808	20	17	0.2384	2.8
	Habit							
	pre-grazing	2.6	2.9	0.0990	2.6	2.9	0.0990	0.17
	Density							
	pre-grazing	7.6	7.5	0.6016	7.3	7.7	0.1966	0.32

[1]SED – Standard error for differences of means when comparing either trimmed with untrimmed plants or high and low sec. comp. conc. genotypes.

[2] P-value of the treatment main effect.

[3] Removed = pre-grazing minus post-grazing assessment

The amount removed by grazing from cultivars with high or low formononetin concentration did not differ significantly in the three periods of measurements, except in Period 2 where the cultivar with high formononetion concentration had greater reduction in percentage of leaves. In Period 1, there were no significant differences between trimmed and untrimmed plants in reduction of height, mass, area and leafiness. In Period 2, untrimmed plants had significantly greater height, area and leafiness removed by grazing. In Period 3 there was a significantly larger decrease only in height and mass of untrimmed plants, compared with trimmed plants.

4.3.1.2. Sward chemical composition

Extractable condensed tannin concentration

There was a significant interaction (in absolute terms) in concentration of extractable condensed tannin (ECT) between genotype and period in plants of birdsfoot trefoil (Table 4.8.). There was a larger difference between genotypes in Period 2 than in Period 3. In proportional terms, accession PI273938 had on average 4.2 times more tannin than cultivar Goldie. Comparing within each genotype, the concentration of ECT was greater in Period 2 than in Period 3. There was no significant interaction between trimming and genotype effect (Table 4.8) and no difference between trimmed and untrimmed (trimming main effect) birdsfoot trefoil plants in ECT content (P=0.3984).

Table 4. 8. Extractable condensed tannin (ECT) concentration (%DM) of birdsfoot trefoil genotypes considering the interactions with period and trimming effects (untrimmed: Ntrim; trimmed: Trim) of Experiment 5.

		Goldie	*PI273938*	*SED*[1]	*P-value*[2]
Period	2	0.72	3.28	0.214	0.0007
	3	0.47	1.81		
Trimming	Ntrim	0.48	2.47	0.214	0.8063
	Trim	0.72	2.62		

[1]SED – Standard error for differences of means
[2] P-value of the interactions : period *genotype or trimming*genotype.
Number of observation contributing for each mean (n=8)

<u>*Formononetin concentration*</u>

There was a significant interaction between trimming and genotype effect in formononetin content in red clover (Table 4.9). Pawera had higher concentration of formononetin when trimmed, though G-27 did not show difference between trimmed and untrimmed. There were no significant interactions with period (Table 4.9). In proportional terms, overall Pawera had 2.3 times more formononetin than G-27.

Table 4. 9. Formononetin concentration (%DM) of red clover genotypes considering the interactions with period and trimming (untrimmed: Ntrim; Trimmed: Trim) effects of Experiment 5.

		G-27	*Pawera*	*SED*[1]	*P-value*[2]
Period	1	0.28	0.67	0.031	0.1551
	2	0.30	0.72		
	3	0.30	0.65		
Trimming	Ntrim	0.29	0.63	0.025	0.0086
	Trim	0.26	0.73		

[1]SED – Standard error for differences of means
[2] P-value of the interactions : period *genotype or trimming*genotype.
Number of observation contributing for each mean of the interaction genotype*period (n=8) and genotype*trimming (n=12)

<u>*General chemical composition*</u>

The general chemical composition of each genotype is presented in Table 4.10. There were significant differences between genotypes in relation to the percentage of protein, lipids, ADF and *in vitro* DM digestibility (IVDMD). The accession PI273938 had significantly the lowest proportion of protein compared to the other genotypes, and the cultivar Goldie had significantly the highest percentage of lipid and IVDMD. In this case, the two cultivars of red clover had the lowest percentage of lipid and IVDMD.

Red clover had significantly higher NDF (27.0 vs 24.1, SED 0.922, P=0.0071) and ash (10.5 vs 8.7, SED 0.113, P=0.0001) than birdsfoot trefoil, independent of genotype.

Table 4. 10. Percentage of crude protein (CP), lipid, acid and neutral detergent fibre (ADF, NDF), carbohydrates (soluble sugars plus starch)(CHO), ash and *in vitro* dry matter digestibility (IVDMD) determined by Near Infrared Reflectance Spectroscopy (NIRS) of plants of birdsfoot trefoil and red clover of Experiment 5 (percentage of DM basis).

	Birdsfoot trefoil		*Red clover*		*SED*[1]	*SED*[2]	*SED*[3]	*P-value*[4]
	Goldie	PI273938	G-27	Pawera				
Protein	21.7	17.5	23.6	24.0	0.456	0.345	0.404	0.0001
Lipid	3.7	3.4	2.8	2.9	0.066	0.050	0.058	0.0010
ADF	20.8	23.9	25.2	25.5	0.729	0.551	0.646	0.0033
NDF	25.5	22.6	27.0	26.9	1.297	0.981	1.150	0.1002
CHO	16.0	16.6	14.2	14.4	0.912	0.689	0.808	0.7899
Ash	8.7	8.8	10.4	10.5	0.173	0.130	0.153	0.5696
IVDMD	77.2	74.4	73.3	73.0	0.658	0.497	0.583	0.0033

[1]SED – standard error for differences of means when comparing means between birdsfoot trefoil genotypes.
[2]SED – standard error for differences of means when comparing means between red clover genotypes.
[3]SED – standard error for differences of means when comparing means of species.
[4]P-value of genotypes within each species.
Number of observation contributing for each mean (n=6).

4.3.1.3. Number of bites per plant

There were no significant interactions between period and treatment (species, trimming and secondary compound effects) (P>0.05) in relation to number of bites per plant, so attention is focused within each period.

In Period 1 the animals grazed only red clover, therefore comparisons including the four genotypes are made only for Period 2 and 3. There were no significant interactions with trimming effect. However, there was a significant main effect of trimming in both periods (Period 2 and Period 3). Plants that had been trimmed had less bites (Period 2 – 7.6 vs 3.1, SED 1.00, P=0.0001; Period 3 – 8.4 vs 3.6, SED 1.21, P=0.0002). The number of bites per plant was marginally greater for RC than BT in Period 2 (6.3 vs 4.4, SED 1.00, P=0.0526), but there was no significant difference in Period 3 (P=0.6198). However there was a significant interaction between plant secondary compound concentration and species effects on the number of bites (Table 4.11). In Period 2, the BT genotype with high concentration of ECT had significantly the least number of bites (P=0.0046). In Period 3, there was similar selective behaviour, but the difference was only marginal (P=0.0616).

Table 4. 11. Average of number of bites per plant in birdsfoot trefoil (BT) and red clover (RC), in relation to plant secondary compound concentration (Sec.Comp.Conc.) and trimming (Trim = trimmed; Ntrim = untrimmed) characteristics in Period 1 and Period 2, Experiment 5.

		Sec. Comp. Conc.			*Trimming*			*SED[1]*
	Species	Low	High	P-value[2]	Trim.	Ntrim.	P-value[2]	
Period 1	RC	10.3	9.4	0.5138	8.0	11.7	0.0126	1.39
Period 2	BT	6.6	2.1	0.0068	1.9	6.8	0.0035	1.57
	RC	5.5	7.2	0.1305	4.3	8.3	0.0007	1.07
Period 3	BT	7.5	3.9	0.0693	3.0	8.4	0.0086	1.91
	RC	5.3	7.3	0.1912	4.2	8.4	0.0096	1.53
	SED[3]	1.422	P-value[3]	0.0046				
	SED[4]	1.715	P-value[4]	0.0616				

[1] SED – Standard error for differences of means when comparing means with the same level of treatment (sec. comp. conc. or trimming)
[2] P-value of the sec. comp. conc. or trimming main effect.
[3] SED - Standard error for differences of means and P-value when comparing means of the four genotypes (interaction sec. comp. conc.* species) in Period 2.
[4] SED - Standard error for differences of means and P-value when comparing means of the four genotypes (interaction sec. comp. conc. *species) in Period 3.

Individual analyses for comparisons of genotypes within each species were carried out to clarify genotype and trimming effects. The analyses are presented in Table 4.11. The interaction between secondary compound concentration and trimming effects was not significant in all three periods, so results are presented as main effects only.

The animals took significantly more bites from plants with low ECT concentration than with high ECT concentration, and from untrimmed than trimmed plants of birdsfoot trefoil. However the difference between high and low ECT concentration in Period 3 was only marginal.

In red clover, trimming had a major influence on bite number. There was no significant effect of formononetin concentration. There were significantly more bites in untrimmed plants than trimmed in all three periods of measurement.

Correlation and covariance analysis using number of bites per plant were carried out for better understanding of the animal preferential grazing. The analyses are presented in section 4.3.1.5 and 4.3.1.6.

4.3.1.4. Rate of Biting

The values of number of bites per minute in birdsfoot trefoil and red clover plants according to the secondary compound concentration and trimming effect are presented in Tables 4.12 and 4.13. Comparing only the grazed plants, there was consistently no significant difference between birdsfoot trefoil (BT) and red clover (RC) in Periods 2 (P2) and 3 (P3) (42.5 vs 36.9 bites/min, SED 7.47, P=0.5542 in P2; 38.2 vs 42.4 bites/min, SED 2.76, P=0.0856 in P3; for BT and RC, respectively). There was also no significant effect of secondary compound concentration and trimming (Tables 4.12, 4.13.), and no significant interactions of these effects with period.

Table 4.12. Average of number of bites per minute (biting rate) in birdsfoot trefoil in relation to secondary compound concentration (Sec.Comp.Conc.) and trimming (Ntrim = untrimmed; Trim = trimmed) characteristics in Period 2 and Period 3.

	Birdsfoot trefoil	
	Period 2	Period 3
Sec. comp. conc.		
Low	43.1	39.8
High	49.2	41.3
SED	4.050	3.872
P-value	0.1246	0.6022
Trimming		
Ntrim	46.9	38.4
Trim	45.7	42.7
SED[1]	4.117	4.001
P-value[2]	0.5527	0.1970

[1]SED – Standard error for differences of means when comparing means with the same level of treatment (sec. comp. conc. or trimming)
[2] P-value of the sec. comp. conc. or trimming main effect.

Table 4.13. Average of number of bites per minute in red clover, in relation to secondary compound concentration (Sec.Comp.Conc.) and trimming (Trim = trimmed; Ntrim = untrimmed) characteristics in Periods 1, 2 and 3, Experiment 5.

	Red clover		
	Period 1	Period 2	Period 3
Sec. comp. conc.			
Low	31.1	36.1	42.8
High	33.7	39.5	42.3
SED[1]	2.260	2.700	3.892
P-value[2]	0.2497	0.2032	0.7448
Trimming			
Ntrim	31.0	38.3	42.3
Trim	33.8	37.3	42.8
SED[1]	2.260	2.700	3.849
P-value[2]	0.1992	0.6511	0.8510

[1]SED – Standard error for differences of means when comparing means with the same level of treatment (sec. comp. conc. or trimming)
[2] P-value of the sec. comp. conc. or trimming main effect.

4.3.1.5. Correlation Analyses

Correlation analysis was firstly carried out to investigate the importance of general plant chemical characteristics on number of bites per plant. Correlation analyses were also performed to clarify the relationship of ECT and formononetin concentration with number of bites and morphological characteristics of birdsfoot trefoil and red clover plants, and the relationship between plant morphology and number of bites. The correlation analysis, followed by covariance analysis (section 4.3.1.6), was used to separate the effects of plant morphology and ECT concentration on number of bites. The full correlation coefficient matrices of each analysis is presented in Appendices 4.3, 4.4, 4.5, 4.6.

Number of bites vs General plant chemical characteristic

The correlation coefficients and the probability of significance between general plant chemical characteristic and number of bites are given in Table 4.14. The number of observations for this analysis was limited to the number of samples used in the chemical analysis: one sample of each treatment in each period. Because in Period 1 only red clover plants were chemically analysed, correlation analysis was based on 12 observations of red clover and 8 of birdsfoot trefoil. The number of bites was averaged according to the sets of samples bulked for chemical analysis. Because of the limited number of observations the differences between periods were not considered.

Lipid in birdsfoot trefoil was the only compound to have a significant ($P<0.05$) correlation with number of bites. Carbohydrates (soluble sugars plus starch) and ash in red clover showed marginal significance. The other plant chemical characteristics did not have significant ($P>0.05$) correlation with number of bites.

Table 4. 14. Pearson Correlation coefficients (*r*) from correlation analysis between number of bites per plant and percentage of protein, lipid, acid and neutral detergent fibre (ADF, NDF), carbohydrates (soluble sugars plus starch)(CHO), ash and *in vitro* dry matter digestibility (IVDMD) of plants of birdsfoot trefoil and red clover of Experiment 5 (percentage of DM basis).

Number of Bites	*Protein*	*Lipid*	*ADF*	*NDF*	*CHO*	*Ash*	*IVDMD*
Birdsfoot trefoil							
r	0.6287[1]	0.7905	-0.3072	0.5888	-0.5275	0.3839	0.3115
P-value	*0.0950*	*0.0195*	*0.4592*	*0.1246*	*0.1791*	*0.3477*	*0.4526*
Red clover							
r	-0.4650[2]	-0.1737	-0.1932	0.3327	0.5523	-0.5405	0.1955
P-value	*0.1277*	*0.5892*	*0.5474*	*0.2906*	*0.0626*	*0.0696*	*0.5426*

[1]Number of observation contributing for each correlation of birdsfoot trefoil (n=8)
[2]Number of observation contributing for each correlation of red clover (n=12)

ECT concentration vs Plant morphology and Number of bites in birdsfoot trefoil plants

The correlation analyses between ECT concentration and either birdsfoot trefoil morphological characteristics or number of bites taken from birdsfoot trefoil plants in Periods 2 and 3 are presented in Table 4.15. In these analyses values of plant morphology and number of bites were averaged according to sets of plants bulked for ECT chemical determination. There were significant negative correlations ($P<0.05$) between ECT concentrations and plant height, leafiness and number of bites in Period 2, but in Period 3 (Table 4.15) there was only a significant negative correlation between ECT concentration and leafiness.

Table 4.15. Pearson Correlation coefficients (r) from correlation analysis between extractable condensed tannin concentration and plant area, height, volume, leafiness and number of bites per plant (N. Bites) of birdsfoot trefoil in Periods 2 and 3 of Experiment 5.

		Area	*Height*	*Volume*	*Leafiness*	*N.Bites*
Period 2	r	-0.0973	-0.6054	-0.2683	-0.6728	-0.5031
	P-value	*0.7199*	*0.0130*	*0.3151*	*0.0043*	*0.0470*
Period 3	r	0.0399	0.1448	0.0926	-0.4860	-0.0138
	P-value	*0.8878*	*0.6065*	*0.7428*	*0.0662*	*0.9609*

Number of observation contributing for each correlation (n=16)

Formononetin concentration vs Pant morphology and Number of bites in red clover plants

The correlation analyses between formononetin concentration and red clover morphological characteristics, and between formononetin concentration and number of bites taken from red clover plants in Periods 1, 2 and 3 are presented in Table 4.16. In this analysis values of plant morphological characteristics and number of bites were averaged according to sets of plants bulked for formononetin chemical determination. There was no significant correlation ($P>0.05$) between formononetin concentration and plant height, area, volume and leafiness, or number of bites, in the three periods.

Table 4.16.Pearson Correlation coefficients (*r*) from correlation analysis between formononetin concentration and plant area, height, volume, leafiness and number of bites per plant (N. Bites) of red clover in Periods 1 and 2 of Experiment 5.

		Area	*Height*	*Volume*	*Leafiness*	*N.Bites*
Period 1	*r*	-0.1596	-0.1063	-0.1718	-0.1854	-0.1024
	P-value	*0.5548*	*0.6951*	*0.5245*	*0.4919*	*0.7058*
Period 2	*r*	0.1411	-0.0492	0.0962	0.0926	0.1725
	P-value	*0.6021*	*0.8565*	*0.7230*	*0.7330*	*0.5229*
Period 3	*r*	0.0119	-0.1795	-0.0522	-0.1048	0.2558
	P-value	*0.9650*	*0.5060*	*0.8477*	*0.6993*	*0.3389*

Number of observation contributing for each correlation (n=16)

Number of bites vs Plant morphology

BIRDSFOOT TREFOIL

The correlation analysis between number of bites per plant and morphological characteristics of birdsfoot trefoil plants in Periods 2 and 3 is shown in Table 4.17. In this analysis individual data of all experimental plants was used. In both periods there were significant and positive correlations between number of bites and plant area, height, volume and leafiness.

Table 4.17.Pearson Correlation coefficients (*r*) from correlation analysis between number of bites per plant and area, height, volume and leafiness of birdsfoot trefoil plants in Periods 2 and 3 of Experiment 5.

		Area	*Height*	*Volume*	*Leafiness*
Period 2	*r*	0.2847	0.4398	0.3945	0.5749
	P-value	*0.0499*	*0.0018*	*0.0055*	*0.0001*
Period 3	*r*	0.4381	0.5089	0.5049	0.4341
	P-value	*0.0018*	*0.0002*	*0.0003*	*0.0020*

Number of observation contributing for each correlation (n=48)

RED CLOVER

The correlation analysis between number of bites per plant and morphological characteristics of red clover plants in Periods 1, 2 and 3 is shown in Table 4.18. As in the previous analysis, this analysis was carried out using individual data of all experimental plants. In the three periods there were significant and positive correlations between number of bites and plant area, height and volume. The correlation between number of bites and leafiness was only significant in Period 3.

Table 4.18. Pearson Correlation coefficients (*r*) from correlation analysis between number of bites per plant and area, height, volume and leafiness of red clover plants in Periods 1, 2 and 3 of Experiment 5.

		Area	*Height*	*Volume*	*Leafiness*
Period 1	*r*	0.5595	0.4473	0.6525	0.1126
	P-value	0.0001	0.0014	0.0001	0.4462
Period 2	*r*	0.7837	0.4789	0.7842	0.0485
	P-value	0.0001	0.0006	0.0001	0.7431
Period 3	*r*	0.5676	0.5731	0.6379	0.3497
	P-value	0.0001	0.0001	0.0001	0.0148

Number of observation contributing for each correlation (n=48)

The correlation matrices showed that the coefficients derived from the smaller number of observations used in the analyses involving ECT and Formononetin concentration were not substantially different from the analyses carried out with individual plant data (more number of observations). There was only a notable greater correlation coefficient between number of bites and height of red clover in Period 2 (0.14 vs 0.48) when more observations were used. However the significance between number of bites and either area (P= 0.0499 vs 0.3651) or volume (P= 0.0055 vs 0.1654) of BT in Period 2 was also greater when more observations were used.

4.3.1.6. Use of Covariates

Covariance analyses were used to separate the plant morphological effect from the secondary compound concentration effect on bite number. Because the effect of secondary compounds on number of bites was significant only in plants of birdsfoot trefoil, the analysis of covariance is concentrated on number of bites taken from birdsfoot trefoil plants.

Covariance analysis was firstly carried out using individual data of all experimental plants (number of observations (n) = 48). Plant morphological characteristics (leafiness and volume) were used as covariates while genotype effect was used as a class variable (described as high and low concentration of ECT). Genotype effect (class variable) and one covariate, either leafiness or volume, were added to a basic model to determine their effect on R-square changes. The basic model of the analysis of variance considered as causes of variation the effects of sequences, blocks (within sequences) and trimming. A second analysis was then performed considering only the untrimmed plants (n=24) to test the natural plant morphological variation. In this case, the effect of trimming was not included in the basic model. A third analysis was carried out including both plant morphology and ECT concentration as covariates (n=16). In this analysis values of plant volume and leafiness were averaged according to sets of plants bulked for ECT chemical determination, and the basic model did not include the effect of blocks within each sequence. These three analyses are presented below.

Genotype effect (class variable) vs Plant morphological characteristics (covariate)

TRIMMED AND UNTRIMMED PLANTS

The R-square changes when adding genotype and either the percentage of leaves or plant volume to a basic model in the Periods 2 and 3 are given in Figure 4.2 and 4.3. The basic R-square (without the addition of genotype effect or any covariate) in Period 2 was lower than in Period 3 and the variation in R-square was greater in Period 2 than in Period 3. The basic model explained about 40% in Period 2, and 50% in Period 3 of the total variation. The improvement in R-square after fitting covariates and genotype effect varied from 12 to 27% (adding both leafiness and genotype effects in Periods 3 and 2, respectively). Leafiness explained more variation than volume in Period 2 (27 vs 14 %), but they had similar effect on R-square variation in Period 3 (15 vs 17 %). The genotype effect improved the magnitude of R-square more in Period 2 than in Period 3 (14 vs 3 %).

In both periods there was a significant effect of genotype (marginal in Period 3), leafiness and volume when added to the basic model. In Period 2, leafiness explained more variation in number of bites than genotype. There was only a small residual effect of genotype after leafiness had been added to the model. In this period plant volume explained a similar amount of variation as genotype. In Period 3, leafiness was much more important than the genotype effect to explain variation in number of bites. After adding leafiness to the model, the genotype effect was not significant (P=0.9525).

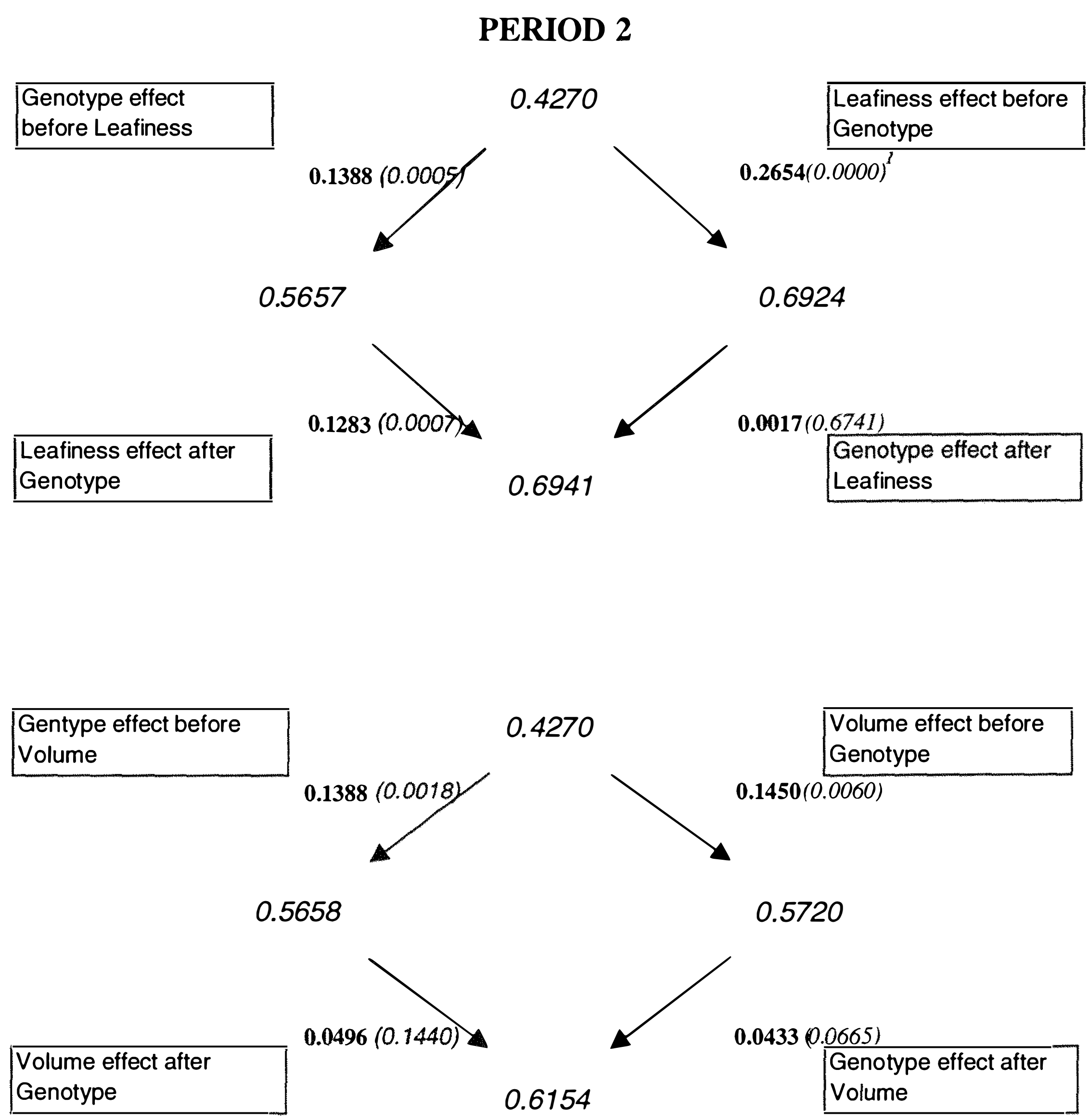

Figure 4.2. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or genotype effect had been added. Considering all experimental plants of birdsfoot trefoil in Period 2 of Experiment 5.

[1]P-value for the differences of R-squares.

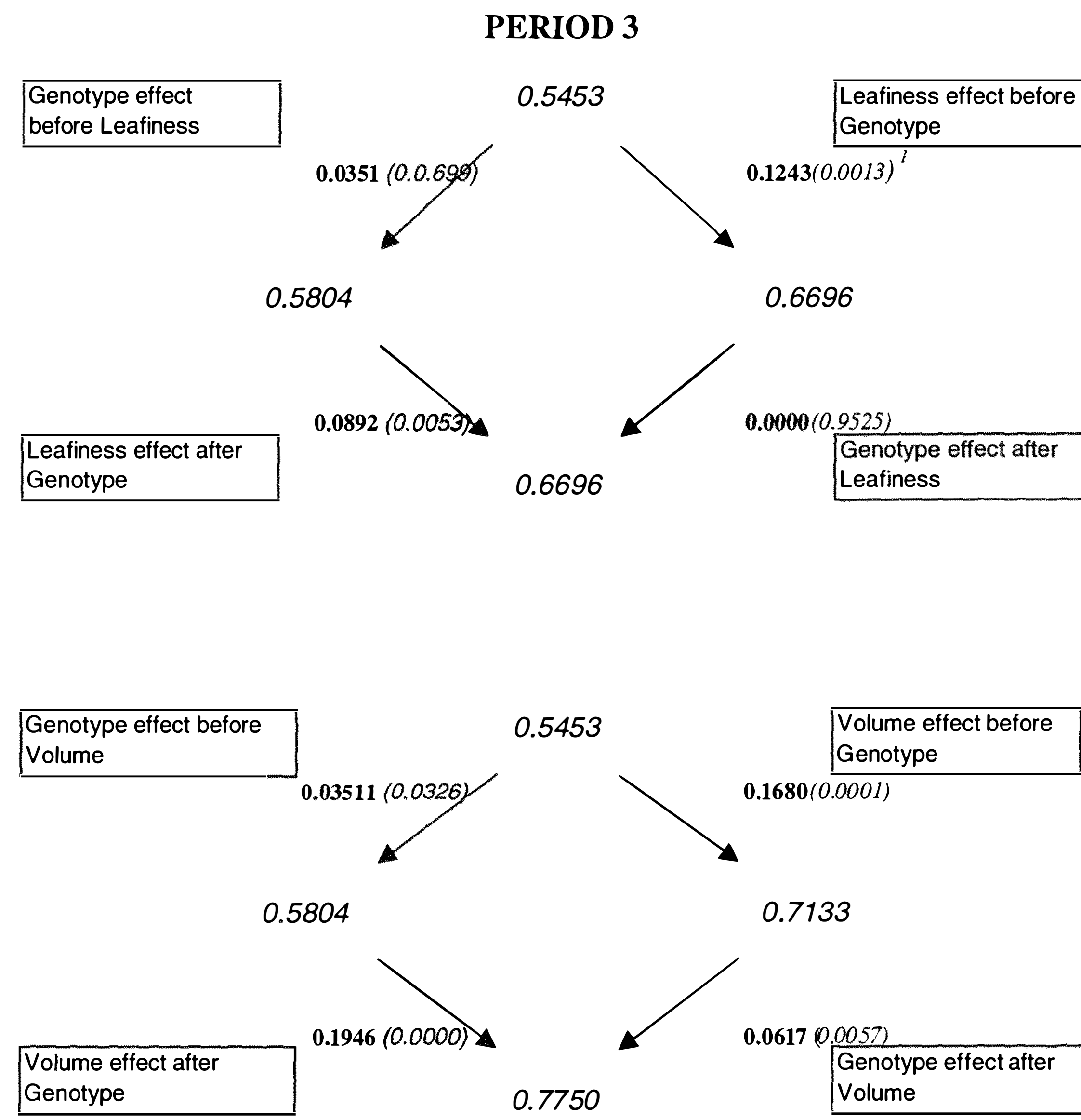

Figure 4.3. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or genotype effect had been added. Considering all experimental plants of birdsfoot trefoil in Period 3 of Experiment 5.

[1] P-value for the differences of R-squares.

UNTRIMMED PLANTS

The R-square changes when adding genotype effect and either the plant leafiness or volume in Period 2 and 3, considering only untrimmed plants of birdsfoot trefoil are presented in Figure 4.4. and 4.5. In both periods the basic model, without the covariate and genotype effects, explained between 45 and 47 % of the total variation. Leafiness and genotype effects together explained around 35 % of the total number of bites variation in both periods. Plant volume together with genotype effect explained much more variation in number of bites in Period 3 than Period 2. The improvement in R-square in Period 2 was about 25%, but in Period 3 was about 43%. In Period 3, the basic model together with genotype and volume effects explained most of the variation (R-square = 0.9055) in number of bites.

In Period 2, leafiness had an important effect on the variation in number of bites, but it did not have a significant effect after genotype had been added to the model. In this period leafiness was more important than volume to explain the changes of R-square. Volume had a marginal effect (P=0.0969) on changes of R-square, but also did not have significant effect after genotype had been added to the model. In both cases, after adding leafiness or volume to the model, genotype effect was not significant. The effect of genotype was lower than leafiness and greater than volume, though the differences between genotype effect and the effect of the covariates were small.

In Period 3, leafiness and volume had similar effects on the variation of number of bites. The variation in number of bites explained by differences in genotypes seems to be independent of plant volume, but related to plant leafiness. The genotype effect after volume had been added to the model, and the volume effect after genotype had been added to the model were highly significant. On the other hand, the genotype effect was not significant after leafiness had been added to the model.

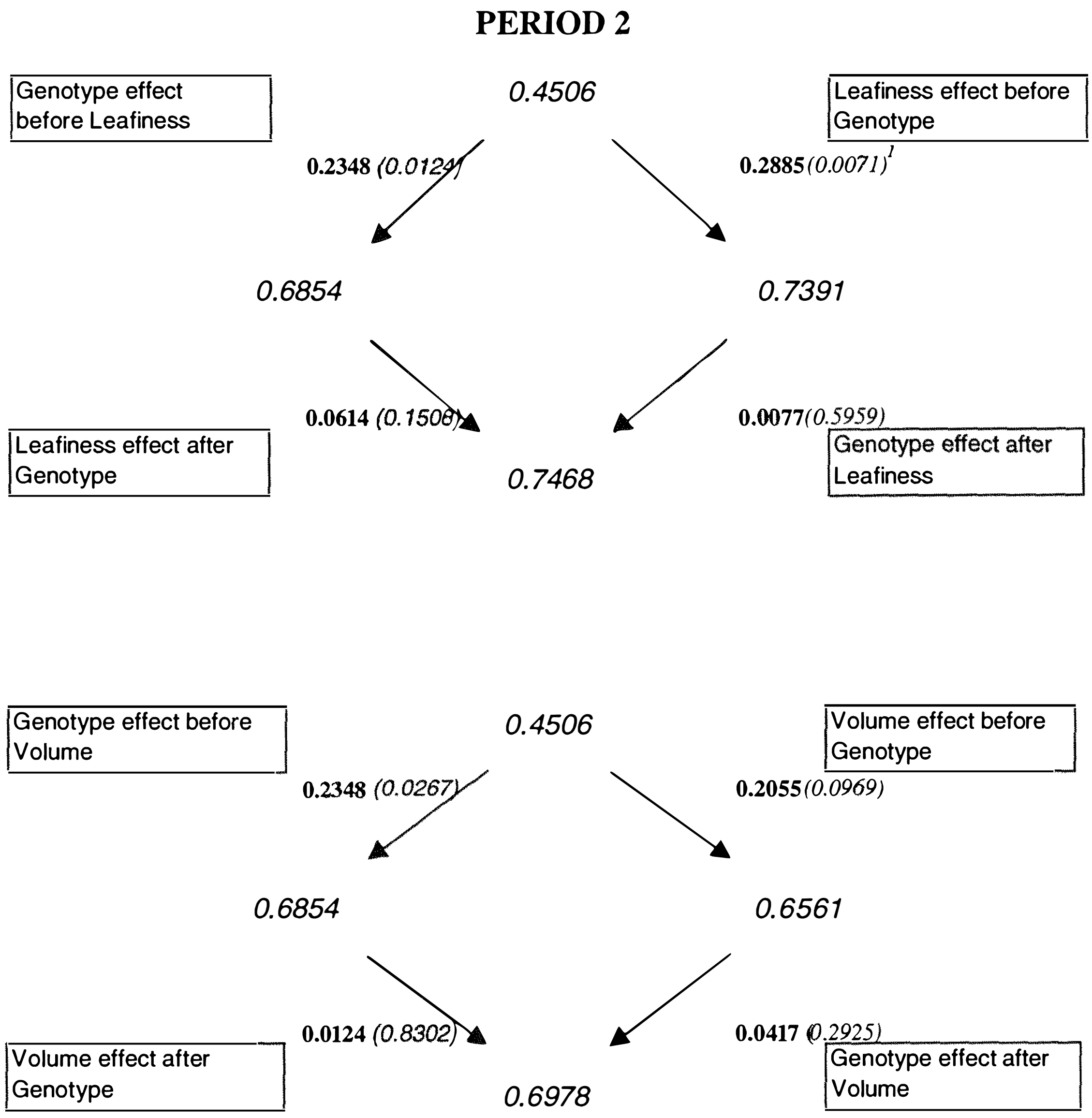

Figure 4.4. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology (leafiness or volume) or genotype effect had been added. Considering only untrimmed birdsfoot trefoil plants in Period 2 of Experiment 5.

[1]P-value for the differences of R-squares.

PERIOD 3

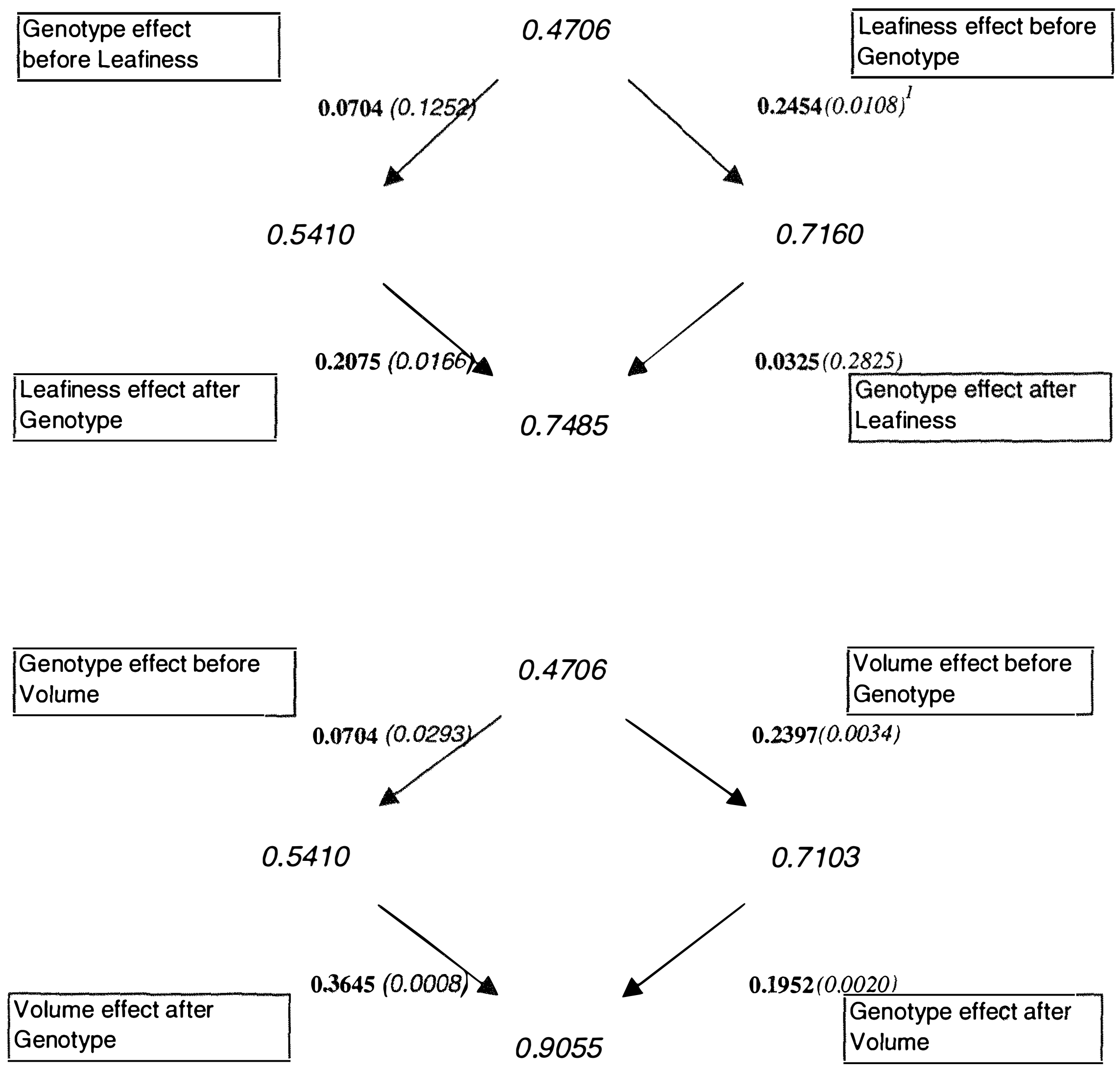

Figure 4.5. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology (leafiness or volume) or genotype effect had been added. Considering only untrimmed birdsfoot trefoil plants in Period 3 of Experiment 5.

[1]P-value for the differences of R-squares.

ECT concentration (covariate) vs Plant morphological characteristics (covariate)

The R-squares and the differences of R-squares when plant leafiness or volume and ECT concentration were added to the model in the analysis of variance, considering either Period 2 or Period 3, are given in Figures 4.6 and 4.7. The basic model in Period 2 explained less variation (59%) in number of bites than in Period 3 (80%). Because a large amount of the variation was explained by the basic model, the improvement in R-square after fitting the covariates was much smaller in Period 3 (from 2 to 12%) than in Period 2 (about 20%).

In Period 2, there were significant effects of both ECT concentration and leafiness on R-square, but there was no significant effect ($P>0.05$) of volume. However ECT concentration and leafiness were not independent. After adding one covariate, the effect of the other became non significant. In Period 3, ECT concentration and plant morphological characteristics were not important in explaining the number of bites. In Period 3 there were no significant effects of ECT concentration and plant morphology (leafiness and volume) on R-square changes.

The effect of the covariate ECT concentration increased relatively to the differences between genotypes. Comparing the three different covariate analyses, the ECT concentration (covariate) and genotypes (class variable) had stronger effects in Period 2 than Period 3. In all analyses leafiness had the strongest effect on changes of R-square. The effects of genotype (class) or ECT concentration (covariate) were never significant after leafiness had been added to the model.

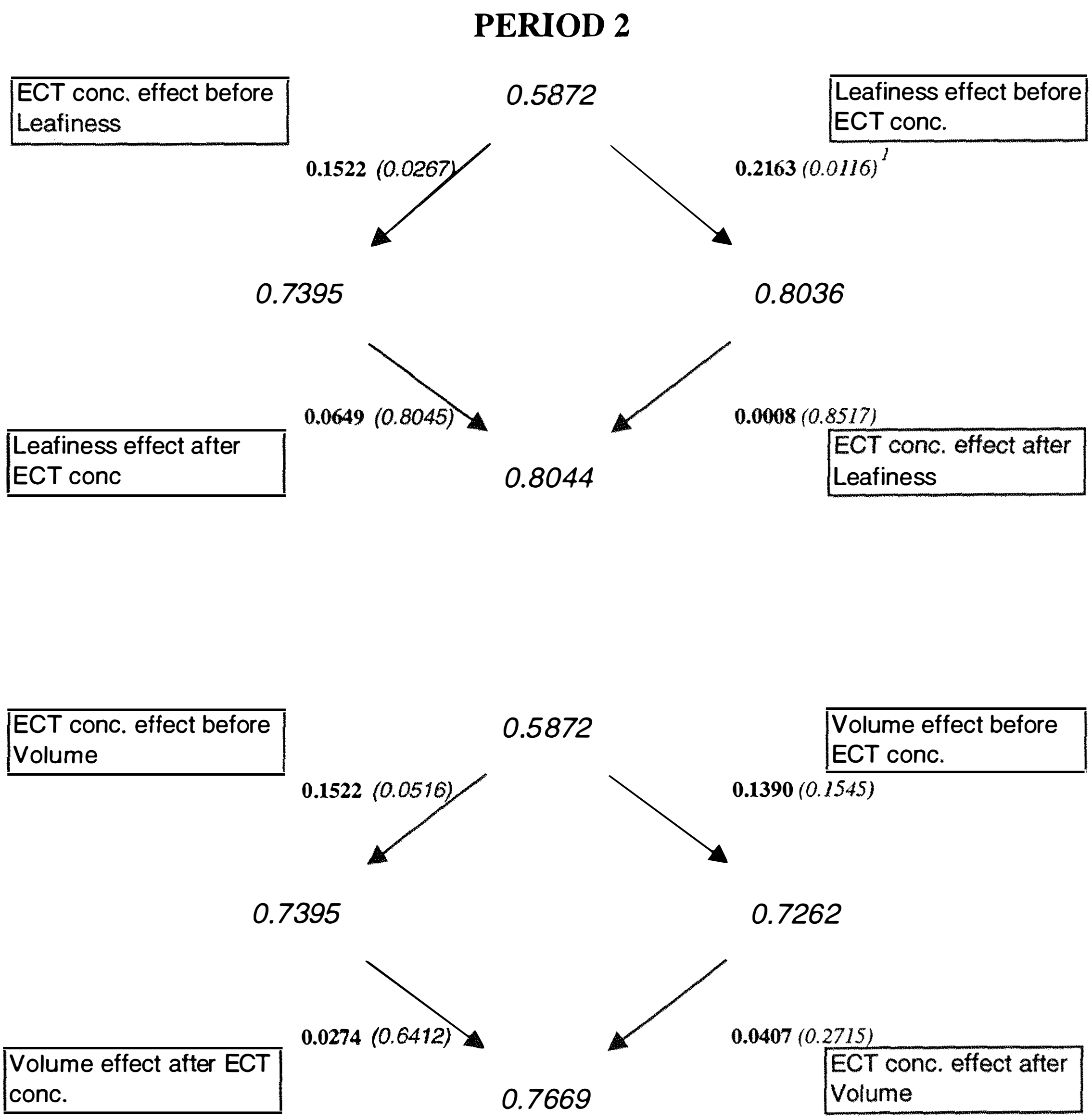

Figure 4.6. Effect of extractable condensed tannin concentration (covariate) (ECT conc.) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or ECT conc. had been added. Considering plants of birdsfoot trefoil in Period 2 of Experiment 5.

[1]P-value for the differences of R-squares.

PERIOD 3

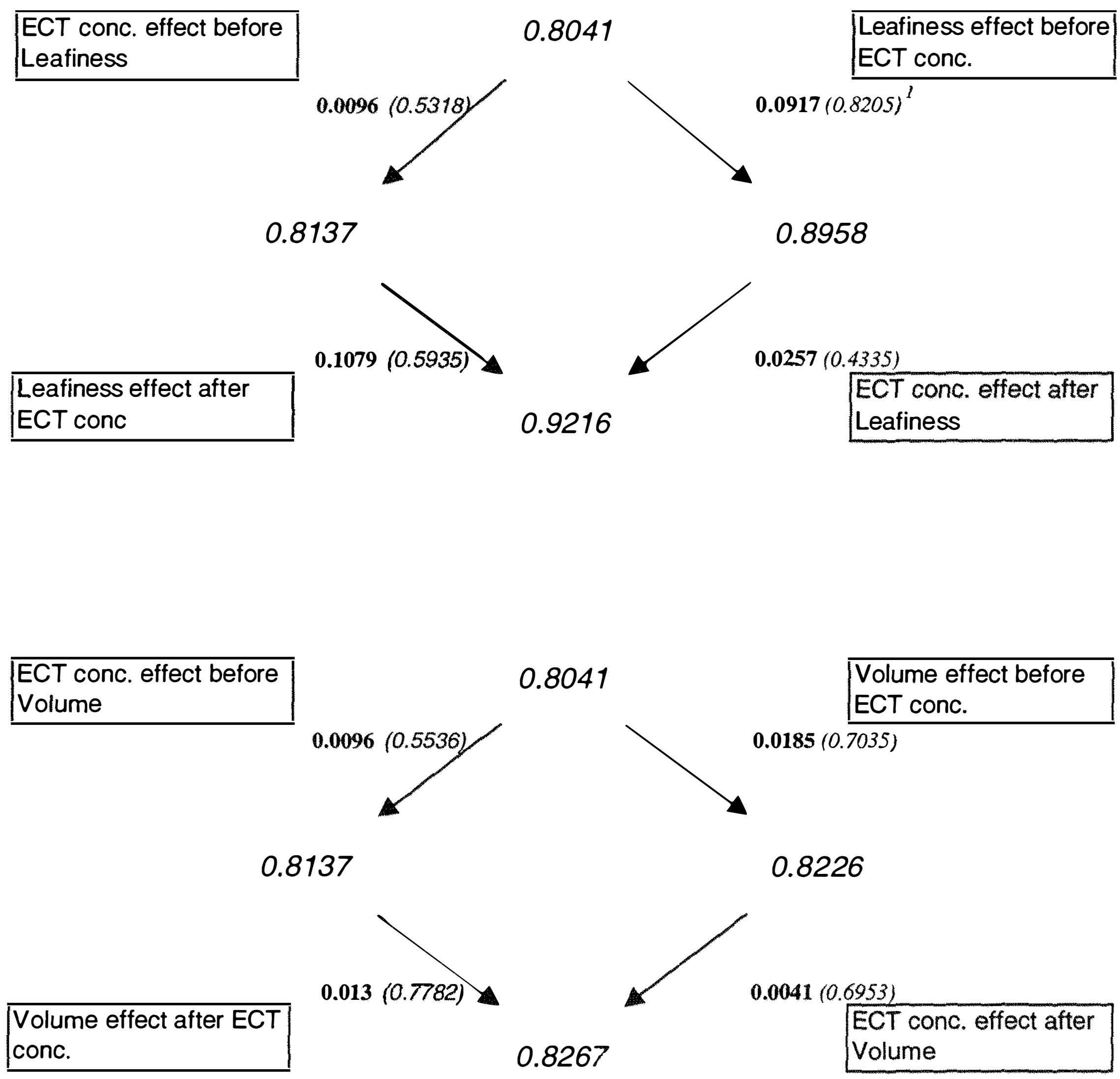

Figure 4.7. Effect of extractable condensed tannin concentration (covariate) (ECT conc.) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or ECT conc. had been added. Considering plants of birdsfoot trefoil in Period 3 of Experiment 5.

[1]P-value for the differences of R-squares.

4.3.2. Experiment 6: Effect of condensed tannin in birdsfoot trefoil (Lotus corniculatus L.*) and formononetin in red clover (Trifolium pratense* L.*) on diet selection and grazing behaviour of dairy cows: rumen content modification approach*

4.3.2.1. Plant characteristics

Comparisons between species

Plant characteristics of the different genotypes of birdsfoot trefoil (BT) and red clover (RC), trimmed and not trimmed are presented in Table 4.19 and Table 4.20. Table 4.19 corresponds to the first period of assessment, and Table 4.20 to the second period. Each table is subdivided into runs according to sets of four plant sequences (each sequence was formed by 26 plants). In each run, one rumen chemical modification was tested: Run 1 in Period 1 and Run 2 in Period 2 - formononetin was tested; Run 2 in Period 1 and Run 1 in Period 2 – tannin was tested (see rumen modification procedure section 4.2.6.2 and Appendix 4.10).

Species, genotype and trimming effects influenced the difference in plant morphological characteristics. There were significant interactions involving plant species, genotypes and trimming effects. On average RC plants had greater proportion of leaves and density than BT, and untrimmed had greater size than trimmed plants in most plant characteristics. Comparing all experimental plants, BT had the greatest variation between genotypes and between trimmed and untrimmed plants. Untrimmed plants of cultivar Goldie generally had the greatest height, area and herbage mass and were the most erect. On the other hand, in most runs accession PI273938 had the smallest height, herbage mass, proportion of leaves, and density.

The amount of height, herbage mass, area and leafiness removed also varied according to species, genotype and trimming effects. Red clover had, in most runs, greater reduction in leafiness than BT. Untrimmed cultivar Goldie had, in most runs, the largest reduction of height, herbage mass and area, and accession PI273938 had one of the lowest reductions in height. More detailed analyses comparing genotypes within each species are presented in the next section.

Table 4.19. Characteristics of untrimmed (Ntrim) and trimmed (Trim) plants of birdsfoot trefoil Goldie (Low Tannin) and accession PI273938 (High Tannin), and red clover cultivars G27 (Low Form) and Pawera (High Form) in Period 1, Runs 1 and 2 (rumen content modified with formononetin and tannin, respectively).

		Birdsfoot trefoil(BT)				*Red clover(RC)*				*SED*[1]
		Low Tannin		High Tannin		Low Form		High Form		
		Ntrim	Trim	Ntrim	Trim	Ntrim	Trim	Ntrim	Trim	
RUN 1	**Height (cm)**									
	pre-grazing	26.2	16.5	14.9	14.4	21.4	17.7	23.3	21.0	1.885
	removed[2]	5.2	1.9	1.2	0.8	2.6	2.8	4.0	4.2	1.618
	Plant mass (gDM/m^2)									
	pre-grazing	419	297	248	274	324	295	333	311	21.2
	removed	77	11	-2	28	35	16	14	29	29.0
	Plant area (cm^2)									
	pre-grazing	3110	1570	2430	1610	3640	3030	3780	2850	499.6
	removed	710	190	410	140	830	310	990	830	331.5
	Leafiness (%)									
	pre-grazing	40	36	32	32	74	74	74	72	1.83
	removed	7	2	2	1	25	22	23	26	2.53
	Habit									
	pre-grazing	3.5	2.8	2.3	2.6	2.8	2.6	2.7	2.8	0.242
	Density									
	pre-grazing	5.9	5.1	4.4	4.5	7.3	7.2	7.3	7.1	0.334
RUN 2	**Height (cm)**									
	pre-grazing	30.4	17.9	15.7	15.2	25.0	21.9	25.9	21.0	2.558
	removed	7.5	3.0	1.8	2.2	3.2	2.2	1.7	1.3	1.479
	Plant mass (gDM/m^2)									
	pre-grazing	400	284	232	230	383	352	376	343	25.2
	removed	106	34	23	32	54	27	29	21	19.9
	Plant area (cm^2)									
	pre-grazing	5010	2750	3340	2620	5500	4810	6880	4640	681.2
	removed	940	270	520	210	-30	180	-160	-410	384.1
	Leafiness (%)									
	pre-grazing	38	34	32	31	74	71	73	72	1.94
	removed	3	0	3	2	7	6	5	5	2.30
	Habit									
	pre-grazing	3.4	2.5	2.2	2.2	2.5	2.4	2.7	2.2	0.367
	Density									
	pre-grazing	6.2	5.1	4.7	4.4	7.9	7.6	7.9	7.7	0.352

[1] SED – Standard error for differences of means

[2] Removed = pre-grazing minus post-grazing assessment

Table 4.20. Characteristics of untrimmed (Ntrim) and trimmed (Trim) plants of birdsfoot trefoil Goldie (Low Tannin) and accession PI273938 (High Tannin), and red clover cultivars G27 (Low Form) and Pawera (High Form) in Experiment 6 Period 2, Runs 1 and 2 (rumen content modified with tannin and formononetin, respectively).

		Birdsfoot trefoil(BT)				*Red clover(RC)*				
		Low Tannin		High Tannin		Low Form		High Form		*SED*[1]
		Ntrim	Trim	Ntrim	Trim	Ntrim	Trim	Ntrim	Trim	
RUN 1	**Height (cm)**									
	pre-grazing	15.5	9.7	12	9.7	11.3	10.5	14.12	10.5	1.466
	removed[2]	4.0	1.0	0.3	0.2	0.9	1.3	1.6	0.4	0.761
	Plant mass (gDM/m²)									
	pre-grazing	392	284	228	232	321	338	322	306	30.4
	removed	113	17	1	31	30	41	40	25	25.9
	Plant area (cm²)									
	pre-grazing	2060	1070	2710	1070	1040	980	1630	980	359.0
	removed	560	200	320	100	10	30	90	0	177.4
	Leafiness (%)									
	pre-grazing	42	39	32	31	74	69	67	67	2.92
	removed	8	5	2	2	10	7	7	5	3.06
	Habit									
	pre-grazing	3.1	2.7	2.1	2.6	2.7	2.7	2.8	2.7	0.259
	Density									
	pre-grazing	5.8	5.6	4.5	3.9	7.2	7.0	6.6	6.7	0.566
RUN 2	**Height (cm)**									
	pre-grazing	16.4	11.4	11.5	11.4	17.0	15.0	18.4	15.0	1.377
	removed	4.5	1.3	0.9	0.4	3.5	1.8	2.7	2.3	0.624
	Plant mass (gDM/m²)									
	pre-grazing	386	328	255	242	417	379	387	367	24.1
	removed	110	57	29	24	115	49	62	67	22.2
	Plant area (cm²)									
	pre-grazing	4930	1390	2050	1380	2930	1920	3250	1920	569.0
	removed	1770	250	280	160	230	120	370	100	262.3
	Leafiness (%)									
	pre-grazing	45	38	31	31	75	70	67	67	2.69
	removed	13	5	2	2	22	13	13	13	3.41
	Habit									
	pre-grazing	2.6	2.8	2.4	2.7	2.7	2.9	2.7	2.8	0.278
	Density									
	pre-grazing	6.4	5.6	4.0	3.8	7.7	7.3	7.0	6.9	0.311

[1] SED – Standard error for differences of means
[2] Removed = pre-grazing minus post-grazing assessment

<u>*Comparisons between genotypes within each species*</u>

BIRDSFOOT TREFOIL

The characteristics of birdsfoot trefoil plants in the different periods and runs, according to trimming and genotype (secondary compound concentration) effects are presented in

Table 4.21 and 4.22. Comparing the genotypes of birdsfoot trefoil (BT) in Runs 1 and 2 of Period 1, there was a significant interaction between trimming and genotype effects for height, mass and habit. Untrimmed Goldie was significantly taller with more mass and a more erect habit than the other plants in both runs. In Run 1 untrimmed plants had a significantly greater area and accession PI273938 a significantly lower percentage of leaf and density. In Run 2, untrimmed plants had more area, leafiness and were denser, and cultivar Goldie had significantly more area, leafiness and density than accession PI273938.

In Run 1 of Period 1 there was a significant interaction between trimming and genotype effects in relation to reduction of area and mass, and a significant trimming effect for reduction of leafiness. Untrimmed cultivar Goldie had significantly the greatest area and mass removed, and trimmed plants the least leafiness reduction. In Run 2 there were significant interactions between trimming and genotype effects in relation to reduction of height and mass, and a significant trimming effect for area removed. Untrimmed cultivar Goldie had significantly the greatest height and mass removed, and untrimmed plants had greater reduction of area than trimmed plants.

In Period 2 there was, in Run 1, a significant trimming effect for height and area; a significant genotype effect for percentage of leaves, habit and density; and a significant interaction between trimming and genotype for mass. In this case untrimmed plants were taller and had more area. Cultivar Goldie had greater percentage of leaves, density and were more erect than accession PI273938, and untrimmed cultivar Goldie had significantly more mass. In the last run there were significant interactions between trimming and genotype effects in relation to height, area and leafiness. There was also a significant main effect of trimming for mass, and a main effect of genotype for mass and density. Untrimmed cultivar Goldie had ($P<0.05$) greater height, area and percentage of leaves. Untrimmed plants had significantly ($P<0.05$) more mass, and cultivar Goldie had more mass and were denser than accession PI273938.

Table 4.21. Characteristics of birdsfoot trefoil plants before grazing and difference between before and after grazing (removed from height, mass, area and leafiness) according to trimming (Ntrim = untrimmed; Trim = trimmed) and genotype [secondary compound concentration (Sec. Comp. Conc.): high (accession PI273938), low (cultivar Goldie)] effects in Period 1, Runs 1 and 2 of Experiment 6.

		Birdsfoot trefoil (BT)						
		Trimming			Sec comp conc.			
		Ntrim	Trim	P-value[2]	High	Low	P-value[2]	SED[1]
RUN 1	**Height (cm)**							
	pre-grazing	20.6	15.4	0.0001	14.6	21.3	0.0001	1.056
	removed[3]	3.2	1.3	0.1942	1.0	3.6	0.0769	1.415
	Plant mass (gDM/m^2)							
	pre-grazing	333	286	0.0205	261	358	0.0001	19.6
	removed	38	19	0.3690	13	44	0.1325	20.1
	Plant area (cm^2)							
	pre-grazing	2770	1590	0.0001	2020	2340	0.1800	233.4
	removed	560	170	0.1433	280	450	0.5037	261.7
	Leafiness (%)							
	pre-grazing	36	34	0.1193	32	38	0.0002	1.43
	removed	5	1	0.0533	2	4	0.0918	1.56
	Habit							
	pre-grazing	2.9	2.7	0.2756	2.5	3.2	0.0010	0.151
	Density							
	pre-grazing	5.1	4.8	0.1390	4.5	5.5	0.0001	0.282
RUN 2	**Height (cm)**							
	pre-grazing	23.0	16.6	0.0012	15.4	24.1	0.0001	1.828
	removed	4.6	2.6	0.0582	2.0	5.3	0.0032	1.105
	Plant mass (gDM/m^2)							
	pre-grazing	316	257	0.0029	231	342	0.0001	18.3
	removed	65	33	0.0503	28	70	0.0103	15.5
	Plant area (cm^2)							
	pre-grazing	4180	2690	0.004	2980	3880	0.0241	380.9
	removed	730	240	0.219	370	610	0.2531	205.9
	Leafiness (%)							
	pre-grazing	35	32	0.0337	31	36	0.0001	1.13
	removed	3	1	0.0932	2	2	0.7317	1.20
	Habit							
	pre-grazing	2.8	2.4	0.0807	2.2	3.0	0.0027	0.231
	Density							
	pre-grazing	5.4	4.8	0.0140	4.5	5.7	0.0001	0.257

[1] SED – Standard error for differences of means when comparing means with the same level of treatment (trimming or sec. comp. conc.)

[2] P-value of the treatment (trimming or sec.comp. conc.) main effect.

[3] Removed = pre-grazing minus post-grazing assessment

Table 4.22. Characteristics of birdsfoot trefoil plants before grazing and difference between before and after grazing estimates (removed) of height, mass, area and leafiness according to trimming (Ntrim = untrimmed; Trim = trimmed) and genotype [secondary compound concentration (Sec. Comp. Conc.): High (accession PI273938), Low (cultivar Goldie)] effects in Period 2, Runs 1 and 2 of Experiment 6.

		Birdsfoot trefoil (BT)						
		Trimming			Sec comp conc.			
		Ntrim	Trim	P-value[2]	High	Low	P-value[2]	SED[1]
RUN 1	**Height (cm)**							
	pre-grazing	13.8	9.7	0.0011	10.9	12.6	0.1241	1.122
	removed[3]	2.1	0.6	0.0224	0.2	2.5	0.0018	0.661
	Plant mass(gDM/m²)							
	pre-grazing	310	258	0.0399	230	338	0.0001	24.2
	removed	57	24	0.1183	16	65	0.0232	20.7
	Plant area (cm²)							
	pre-grazing	2380	1070	0.0001	1890	1560	0.2845	300.8
	removed	440	150	0.1085	210	380	0.3454	177.2
	Leafiness (%)							
	pre-grazing	37	35	0.2268	32	41	0.0001	1.69
	removed	5	4	0.3390	2	6	0.0209	1.72
	Habit							
	pre-grazing	2.7	2.6	0.8208	2.3	2.9	0.0055	0.182
	Density							
	pre-grazing	5.2	4.7	0.2984	4.2	5.7	0.0006	0.394
RUN 2	**Height (cm)**							
	pre-grazing	14.0	11.4	0.0031	11.5	13.9	0.0053	0.809
	removed	2.7	0.9	0.0030	0.7	2.9	0.0004	0.571
	Plant mass (gDM/m²)							
	pre-grazing	321	285	0.0327	249	357	0.0001	16.0
	removed	70	40	0.0549	27	83	0.0006	14.8
	Plant area (cm²)							
	pre-grazing	3490	1380	0.0001	1710	3160	0.0054	484.6
	removed	1020	200	0.0020	220	1010	0.0027	243.1
	Leafiness (%)							
	pre-grazing	38	35	0.0482	31	41	0.0001	1.73
	removed	7	4	0.0062	2	9	0.0001	1.35
	Habit							
	pre-grazing	2.5	2.7	0.1504	2.54	2.70	0.3334	0.17
	Density							
	pre-grazing	5.2	4.7	0.1649	3.92	6.00	0.0001	0.35

[1] SED – Standard error for differences of means when comparing means with the same level of treatment (trimming or sec. comp. conc.).
[2]P-value of the treatment (trimming or sec. comp. conc.) main effect.
[3] Removed = pre-grazing minus post-grazing assessment

In Run 1 of Period 2, there were significant interactions between trimming and genotype for the amount of height and mass removed by grazing. Cultivar Goldie untrimmed had the greatest reduction in height and mass. There was also a significantly larger reduction in leafiness, independent of trimming effect, of cultivar Goldie. In Run

2 of Period 2 there were significant interactions between trimming and genotype effect in relation to reduction in height, area and leafiness. There was also a significant main effect of genotype for mass. Untrimmed cultivar Goldie had significantly ($P<0.05$) greater reduction of height, area and percentage of leaves. Cultivar Goldie had, independent of trimming effect, significantly ($P<0.05$) larger reduction of mass than accession PI273938.

RED CLOVER

The red clover plant characteristics in different periods and runs, according to trimming and genotype (secondary compound concentration) effects, are presented in Table 4.23 and Table 4.24. In Run 1 of Period 1, there were no significant differences in plant characteristics and in the amount removed of each plant characteristic by grazing. In Run 2 there was a significant trimming effect only on height and area. Untrimmed plants had larger areas and were taller than trimmed plants. In this run there were no significant effects of either trimming or genotype for the amount removed of any plant characteristic.

In the Period 2 there were more significant differences between trimmed and untrimmed plants and between cultivars G-27 and Pawera. In Run 1 there was a significant interaction between trimming and genotype effects for area. Untrimmed plants of cultivar Pawera had the largest area. Untrimmed plants also were significantly taller than the trimmed ones. In the last run, untrimmed plants were significantly taller and with more area, and cultivar G-27 had a higher percentage of leaves than cultivar Pawera, independent of the trimming effect.

In Run 1 of Period 2, there were significant interactions between trimming and genotype for the amount removed by grazing of height and area. Trimmed plants of Pawera had the lowest reduction of area and untrimmed plants of Pawera had the least reduction of height. The amount of mass removed by grazing in Run 2 was also affected by an interaction between trimming and genotype effects. Untrimmed G-27 had the greatest reduction in mass and G-27 trimmed had the lowest reduction.

Table 4. 23. Characteristics of red clover plants before grazing and difference between before and after grazing estimates (removed from height, mass, area and leafiness) according to trimming (Ntrim = untrimmed; Trim = trimmed) and genotype [secondary compound concentration (Sec. Comp. Conc.): High (cultivar Pawera) and Low formononetin (cultivar G-27)] effects in Period 1, Runs 1 and 2 of Experiment 6.

		Red clover (RC)						
		Trimming			Sec comp conc.			
		Ntrim	Trim	P-value[2]	High	Low	P-value[2]	SED[1]
RUN 1	**Height (cm)**							
	pre-grazing	22.3	19.3	0.0579	22.1	19.5	0.1001	1.527
	removed[3]	3.3	3.5	0.8121	4.1	2.7	0.1539	0.956
	Plant mass (gDM/m²)							
	pre-grazing	352	326	0.1075	343	335	0.6090	16.1
	removed	24	22	0.8999	21	25	0.7846	14.0
	Plant area (cm²)							
	pre-grazing	3710	2940	0.0741	3320	3330	0.9697	417.0
	removed	910	570	0.1143	910	570	0.1144	210.4
	Leafiness (%)							
	pre-grazing	74	73	0.3148	73	74	0.3148	1.224
	removed	24	24	1.0000	25	24	0.4895	1.788
	Habit							
	pre-grazing	7.3	7.2	0.5800	2.8	2.7	0.7395	0.186
	Density							
	pre-grazing	2.8	2.7	0.5333	7.2	7.3	0.7080	0.165
RUN 2	**Height (cm)**							
	pre-grazing	25.5	21.5	0.0340	23.4	23.5	0.9818	1.809
	removed	2.5	1.8	0.5128	1.5	2.7	0.2453	1.039
	Plant mass (gDM/m²)							
	pre-grazing	379	348	0.0679	360	367	0.1426	16.8
	removed	42	24	0.6440	250	400	0.2024	11.9
	Plant area (cm²)							
	pre-grazing	6190	4720	0.0116	5760	5160	0.2799	550.1
	removed	-100	-110	0.9534	-290	80	0.2155	284.3
	Leafiness (%)							
	pre-grazing	74	72	0.2551	73	72	0.8984	1.61
	removed	6	5	0.6200	5	6	0.1426	1.25
	Habit							
	pre-grazing	2.6	2.3	0.2594	2.5	2.5	1.0000	0.29
	Density							
	pre-grazing	7.9	7.7	0.3394	7.8	7.8	0.9303	0.24

[1]SED – Standard error for differences of means when comparing means with the same level of treatment (trimming or sec. comp. conc.).

[2]P-value of the treatment (trimming or sec. comp. conc.) main effect.

[3]Removed = pre-grazing minus post-grazing assessment

Table 4.24. Characteristics of red clover plants before grazing and difference between before and after grazing (removed from height, mass, area and leafiness) according to trimming (Ntrim = untrimmed; Trim = trimmed) and genotype [secondary compound concentration (Sec. Comp. Conc.): High (cultivar Pawera), Low formononetin (cultivar G-27)] effects in Period 2, Runs 1 and 2 of Experiment 6.

		Red clover (RC)						
		Trimming			Sec comp conc.			
		Ntrim	Trim	P-value[2]	High	Low	P-value[2]	SED[1]
RUN 1	**Height (cm)**							
	pre-grazing	12.7	10.5	0.016	12.3	10.9	0.1302	0.886
	removed[3]	1.3	0.9	0.3176	1.0	1.1	0.7911	0.390
	Plant mass (gDM/m²)							
	pre-grazing	321	322	0.9544	314	329	0.3733	16.9
	removed	35	33	0.8809	33	36	0.8584	15.3
	Plant area (cm²)							
	pre-grazing	1340	970	0.0122	1310	1010	0.0336	133.4
	removed	50	15	0.1284	50	20	0.2325	22.31
	Leafiness (%)							
	pre-grazing	71	68	0.3021	67	71	0.0793	2.19
	removed	9	6	0.2737	6	8	0.3604	2.25
	Habit							
	pre-grazing	2.7	2.7	1.0000	2.8	2.7	0.6465	0.180
	Density							
	pre-grazing	6.9	6.8	0.8153	6.6	7.1	0.1670	0.354
RUN 2	**Height (cm)**							
	pre-grazing	17.7	15.0	0.0044	16.7	16.0	0.4360	0.898
	removed	3.1	2.0	0.1140	2.5	2.6	0.7967	0.642
	Plant mass (gDM/m²)							
	pre-grazing	402	373	0.1179	377	397	0.2592	179.9
	removed	89	58	0.0655	65	82	0.2790	16.2
	Plant area (cm²)							
	pre-grazing	3090	1920	0.0001	2590	2420	0.5541	271.3
	removed	300	110	0.0018	230	170	0.2887	55.7
	Leafiness (%)							
	pre-grazing	71	68	0.1223	67	72	0.0083	1.71
	removed	17	13	0.1375	13	17	0.1375	2.87
	Habit							
	pre-grazing	2.7	2.9	0.4069	2.8	2.8	1.0000	0.198
	Density							
	pre-grazing	7.4	7.1	0.2472	7.0	7.5	0.0096	0.212

[1] SED – Standard error for differences of means when comparing means with the same level of treatment (trimming or sec. comp. conc.).

[2] P-value of the treatment (trimming or sec. comp. conc.) main effect.

[3] Removed = pre-grazing minus post-grazing assessment

4.3.2.2. Sward chemical composition

Extractable condensed tannin (ECT)

The averages of ECT concentration according to genotype, period and trimming effects are given in Table 4.25. There was no significant (P>0.05) interaction between genotype and period, and there was no significant difference in ECT concentration between runs. Cultivar Goldie had significantly lower concentration of ECT than accession PI273938 (Table 4.25). In proportional terms, accession PI273938 had on average 3.5 times more tannin than cultivar Goldie. Trimming did not affect significantly (P>0.05) the percentage of ECT (Table 4.25).

Table 4.25.Extractable condensed tannin (ECT) concentration (%DM) of birdsfoot trefoil genotype main effect and interactions with period and trimming effects (untrimmed: Ntrim; Trimmed: Trim) of Experiment 6.

		Goldie	*PI273938*	*SED*[1]	*P-value*[2]
Genotype		0.43	1.52	0.071	0.0001
Period	2	0.32	1.49	0.139	0.4677
	3	0.53	1.55		
Trimming	Ntrim	1.42	0.35	0.139	0.0734
	Trim	1.62	0.51		

[1]SED – Standard error for differences of means
[2] P-value of genotype main effect and interactions : period *genotype or trimming*genotype.
Number of observation contributing for each mean of the genotype main effect (n=32) and interactions (n=16)

Formononetin concentration

There was also a strong effect of genotype in relation to formononetin content. Cultivar Pawera had significantly higher concentration of formononetin than G-27. In proportional terms, overall Pawera had 2.3 times more formononetin than G-27. There was no effect of trimming and no interactions with different periods. The averages of formononetin content according to genotype, period and trimming effects are given in Table 4.26.

Table 4. 26. Formononetin concentration (%DM) of red clover genotype main effect and interactions with period and trimming effects (untrimmed: Ntrim; Trimmed: Trim) of Experiment 6.

		G-27	*Pawera*	*SED*[1]	*P-value*[2]
Genotype		0.26	0.61	0.017	0.0001
Period	2	0.23	0.60	0.024	0.1434
	3	0.29	0.62		
Trimming	Ntrim	0.26	0.59	0.024	0.3355
	Trim	0.26	0.63		

[1]SED – Standard error for differences of means
[2] P-value of genotype main effect and interactions : period *genotype or trimming*genotype.
Number of observation contributing for each mean of the genotype main effect (n=32) and interactions (n=16)

General chemical composition

The general chemical composition of each genotype is presented in Table 4.27. There were significant differences between genotypes in relation to the percentage of protein and neutral detergent fibre (NDF). The accession PI273938 had the lowest ($P<0.05$) proportion of protein and NDF compared to the other genotypes. However most of the component concentration differences were explained by differences in species main effect. Red clover had significantly ($P<0.05$) higher percentage of acid detergent fibre (ADF) and ash. Birdsfoot trefoil had significantly ($P<0.05$) higher concentration of lipid and in vitro dry matter digestibility (IVDMD). There was a significant effect of trimming for CHO (soluble sugars plus starch) content. Untrimmed plants had a significantly ($P<0.05$) higher concentration of CHO.

Table 4.27. Percentage of dry matter (DM), crude protein (CP), lipid, acid and neutral detergent fibre (ADF, NDF), carbohydrates (soluble sugars plus starch)(CHO), ash and *in vitro* dry matter digestibility (IVDMD) determined by Near Infrared Reflectance Spectroscopy (NIRS) of plants of birdsfoot trefoil and red clover of Experiment 6 (percentage of DM basis).

	Birdsfoot trefoil		*Red clover*		SED^1	SED^2	*P-value*^{3}
	Goldie	PI273938	G-27	Pawera			
Protein	22.0	17.5	23.1	23.0	0.736	0.521	0.0001
Lipid	3.3	3.2	2.7	2.6	0.104	0.073	0.2675
ADF	21.5	23.1	25.3	25.7	1.221	0.863	0.4092
NDF	28.2	21.8	27.4	29.2	2.095	1.482	0.0169
CHO	14.2	16.4	14.6	14.9	1.049	0.742	0.1303
Ash	8.4	8.5	10.2	10.1	0.359	0.254	0.9527
IVDMD	77.7	76.0	74.6	74.3	1.102	0.779	0.3265

[1]SED – standard error for differences of means when comparing means between genotypes.
[2]SED – standard error for differences of means when comparing means of species.
[3]P-value of genotypes within each species.
Number of observation contributing for each mean (n=6).

4.3.2.3. Number of bites per plant

The results of the number of bites taken from each plant are shown in Tables 4.28, 4.29, 4.30, and 4.31. Comparing all the treatments in the two different periods, there were no significant interactions between period and treatment [type of chemical added into the rumen, rumen chemical concentration (within each type of chemical), plant species, trimming and plant secondary compound concentration effects] (P>0.05) in relation to number of bites. However there was a significant interaction between trimming and secondary compound effects (Table 4.28) and between type of chemical inserted into the rumen and plant species (Table 4.29).

The interaction between trimming and plant secondary compound effects show that the highest number of bites was taken from untrimmed plants of cultivar Goldie (low ECT concentration) and untrimmed plants of both red clover cultivars (Table 4.28).

Table 4.28. Average of number of bites in birdsfoot trefoil (BT) and red clover (RC), in relation to secondary compound concentration (High and Low) and trimming (Ntrim = untrimmed plants Trim = trimmed plants) effect in Experiment 6.

	Birdsfoot trefoil		*Red clover*	
	High	Low	High	Low
Ntrim	2.5	10.4	8.2	8.0
Trim	2.1	3.1	4.9	4.7
SED[1] 1.112		P-value[2] 0.0001		

[1]SED – Standard error for differences of means
[2]P-value of the interaction: secondary compound concentration*trimming effect.

The interaction between type of material added into the rumen and plant species shows a greater number of bites in red clover when formononetin (red clover material) was added to the rumen, independent of the rumen chemical concentration (within each type of chemical) and plant secondary compound concentration (Table 4.29). There was no significant effect of rumen chemical concentration (within each type of chemical) on number of bites.

Table 4.29. Average of number of bites in birdsfoot trefoil (BT) and red clover (RC) plants in relation to rumen chemical modification (Tannin and Formononetin) effect in Experiment 6.

	BT	*RC*
Type of rumen chemical modification		
Tannin	4.4	4.1
Formononetin	4.7	8.8
SED[1] 0.786	P-value[2] 0.0001	

[1]SED – Standard error for differences of means
[2]P-value of the interaction specie*rumem chemical modification effect.

In order to clarify the effect of rumen concentrations (within each type of chemical modification: either tannin or formononetin) on number of bites, separate statistical analyses were performed for the two types of rumen chemical modifications (either tannin or formononetin) over both periods. There was no significant effect of rumen chemical concentration (within each type of rumen chemical) and no significant interaction with treatments (species, trimming and plant secondary compound effects).

Because there was a significant interaction between type of rumen chemical and plant species (Table 4.29), individual analyses considering only one type of rumen chemical with one specific species were carried out. The results of these analyses are presented in Table 4.30 and Table 4.31. All analyses show that there was no significant effect of rumen concentration or interaction with period effect on number of bites (P>0.05).

The number of bites taken from birdsfoot trefoil plants when the cows had either tannin or formononetin in the rumen were affected by a significant interaction between trimming and plant secondary compound concentration (Table 4.30.). Plants with low concentration of ECT and untrimmed had significantly higher number of bites, independent of the rumen chemical modification. The accession PI273938 (high concentration of ECT) also had the lowest number of bites in both rumen modification cases.

Table 4.30. Average of number of bites in birdsfoot trefoil (BT) in relation to plant secondary compound concentration (Plant Sec. Comp. Conc.: High and Low tannin concentration), plant trimming characteristic (Ntrim = untrimmed; Trim = trimmed plants), type of rumen chemical modification [Tannin and Formononetin (Form.)] and rumen concentration effect (within each type of rumen chemical modification) in Experiment 6.

		Plant Sec. Comp. Conc.				*SED*[1]	*P-value*[2]
		Low		High			
		NTrim	Trim	NTrim	Trim		
Type of rumen chemical							
	Tannin	9.4	3.1	2.9	2.3	1.241	0.0018
	Form.	11.5	3.2	2.2	1.9	1.629	0.0010
Rumen concentration effect							
Tannin	Low	8.3	3.0	3.2	2.6	1.755	0.5869
	High	10.4	3.2	2.6	2.1		
Form.	Low	12.6	2.1	0.4	1.8	2.303	0.0942
	High	10.3	4.2	4.0	2.0		

[1]SED – Standard error for differences of means when comparing means with the same level of type of rumen chemical modification.

[2]P-value of the interaction plant sec. comp. conc.*trimming, or plant sec. comp. conc.*trimming*rumen chemical concentration.

In relation to number of bites in red clover when modifying the rumen content either with tannin or formononetin, there was a significant trimming main effect in both situations (Table 4.31). In this case untrimmed plants had a significantly higher number of bites than trimmed ones. There was also a marginally significant interaction when modifying the rumen with tannin between period and trimming effects. There was a larger number of bites in the first (P1) than in the second (P2) period with larger number of bites for untrimmed plants (P1: 7.9 vs 3.7 bites/plant; P2: 3.0 vs 1.8 bites/plant, SED 1.076, P=0.0502).

Table 4.31. Average of number of bites in red clover in relation to type of rumen chemical modification [Tannin and Formononetin (Form.)] and plant trimming characteristic (Ntrim = untrimmed plants; Trim = trimmed plants) and, in relation to rumen concentration effect (within each type of rumen chemical modification), plant secondary compound concentration (Plant Sec. Comp. Conc.) and plant trimming characteristic, in Experiment 6.

		Plant Characteristics				*SED*[1]	*P-value*
Type of rumen chemical							
		NTrim		Trim			
	Tannin	5.4		2.7		0.761	0.0008
	Form.	10.7		6.9		1.304	0.0047
Rumen concentration effect							
		Plant Sec. Comp. Conc.					
		Low		High			
		NTrim	Trim	Ntrim	Trim		
Tannin	Low	3.8	2.5	4.7	1.7	1.5219	0.6048
	High	7.1	3.9	6.1	2.8		
Form.	Low	10.9	5.0	10.1	6.4	2.6078	0.6220
	High	10.0	7.3	11.7	8.7		

[1]SED – Standard error for differences of means when comparing means with the same level of type of rumen chemical modification.
[2]P-value of the trimming main effect or interaction plant sec. comp. conc.*trimming*rumen chemical concentration (within each type of rumen chemical modification).

For better understanding of the animals preferential grazing, correlation and covariance analyses were performed using number of bites per plant. These analyses are presented in section 4.3.2.5 and 4.3.2.6, respectively.

4.3.2.4. Rate of biting

An analysis was performed to verify the significant effects of interactions of period and rumen chemical modification, and to clarify differences between treatments (species, trimming and plant secondary compound effects) in rate of biting. There were no significant interactions between period and treatment (species, trimming and plant secondary compound effects). However there was a significant interaction between trimming and species effects, and a marginally significant difference between the four different genotypes (independent of trimming effect) (Table 4.32). Untrimmed plants of birdsfoot trefoil had significantly the lowest rate of biting, independent of ECT concentration effect. On the other hand, the low ECT concentration genotype (cultivar Goldie) had the lowest rate of biting, independent of trimming effect. There was no significant difference between RC plants in rate of biting.

Table 4.32. Average of number of bites per minute (rate of biting) in birdsfoot trefoil and red clover in relation to plant trimming characteristic (Ntrim = untrimmed plants; Trim = trimmed plants) and plant secondary compound concentration (Sec. Comp. Conc.: High and Low) in Experiment 6.

	Birdsfoot trefoil	*Red clover*	*SED*[2]
Trimming			
Ntrim	28.1	36.0	2.21
Trim	34.2	35.4	2.46
SED[1]	2.51	2.14	
P-value[3]	0.0285		
Sec. Comp. Conc.			
High	34.0	35.5	2.48
Low	28.3	35.8	2.13
SED[1]	2.50	2.11	
P-value[3]	0.0581		

[1]SED – Standard error for differences of means when comparing means with the same level of plant specie

[2]SED – Standard error for differences of means when comparing means with the same level of treatment (trimming or secondary compound concentration)

[3]P-value of the interaction treatment (trimming or secondary compound concentration)*specie effect.

4.3.2.5. Correlation Analyses

Correlation analyses were performed to investigate the relationships between general plant chemical characteristic, secondary compound concentration, number of bites and plant morphology. As in Experiment 5 (see section 4.3.1.5.), four correlation analyses were carried out: the correlations between number of bites and general plant chemical characteristics; secondary compounds (ECT and formononetin concentration) and either plant morphology or number of bites; and number of bites and plant morphology. The number of observations in each correlation was related to the number of samples bulked for the chemical analysis. In this case the average of the number of bites was used. The correlation between number of bites and plant morphology used all experimental plants. As in Experiment 5, correlation analyses together with covariance analyses were used to separate the effects of plant morphology and ECT concentration on number of bites. The full correlation coefficient matrices of each analysis is presented in Appendices 4.7, 4.8 and 4.9.

Because no significant interaction between rumen chemical with plant chemical characteristics was found for number of bites, the correlation analyses were performed within each period. In this way the effect of rumen manipulation was balanced within period and comparisons with Experiment 5 were possible.

<u>*Number of bites vs General plant chemical characteristics*</u>

The correlation coefficients and the probabilities of significance between general plant chemical characteristic and number of bites taken from birdsfoot trefoil and red clover plants are shown in Table 4.33. The number of bites in birdsfoot trefoil did not show significant correlation with any general plant chemical characteristic ($P>0.1$). However the number of bites in red clover plants had significant ($P<0.05$) negative correlation with acid detergent fibre and ash, and positive correlation with carbohydrates (soluble sugars plus starch) and *in vitro* dry matter digestibility.

Table 4.33. Pearson Correlation coefficients (*r*) from correlation analysis between number of bites per plant and percentage of protein, lipid, acid and neutral detergent fibre (ADF, NDF), carbohydrates (soluble sugars plus starch)(CHO), ash and *in vitro* dry matter digestibility (IVDMD) of plants of birdsfoot trefoil and red clover of Experiment 6 (percentage of DM basis).

	Protein	*Lipid*	*ADF*	*NDF*	*CHO*	*Ash*	*IVDMD*
Birdsfoot trefoil							
r	0.3970	0.2923	-0.3385	0.1251	0.1025	0.0252	0.3962
P-value	*0.1589*	*0.2719*	*0.1996*	*0.6444*	*0.7056*	*0.9262*	*0.1287*
Red clover							
r	-0.4169	-0.4231	-0.6093	-0.3043	0.5849	-0.5567	0.5982
P-value	*0.1082*	*0.1025*	*0.0122*	*0.2518*	*0.0173*	*0.0251*	*0.0144*

Number of observation contributing for each correlation (n=16)

<u>*ECT concentration vs Plant morphology and Number of bites in birdsfoot trefoil plants*</u>

The correlation analyses between ECT concentration and plant morphological characteristics and between ECT concentration and number of bites in Periods 1 and 2 are shown in Table 4.34. As in Experiment 5, there were significant negative correlations ($P<0.05$) between ECT concentrations and plant height, leafiness and number of bites in Period 1. However in Period 2 there was only a significant negative correlation between ECT concentration and leafiness. There was no significant ($P>0.05$) correlation between ECT concentration and number of bites in Period 2.

Table 4.34. Pearson Correlation coefficients (*r*) from correlation analysis between extractable condensed tannin concentration and plant area, height, volume, leafiness and number of bites per plant (N. Bites) of birdsfoot trefoil in Periods 1 and 2 of Experiment 6.

		Area	*Height*	*Volume*	*Leafiness*	*N.Bites*
Period 1	*r*	-0.0925	-0.4011	-0.2540	-0.6312	-0.3628
	P-value	0.6145	0.0229	0.1607	0.0001	0.0413
Period 2	*r*	0.0705	-0.1419	-0.0031	-0.6014	-0.2694
	P-value	0.7012	0.4384	0.9865	0.0003	0.1359

Number of observation contributing for each correlation (n=32)

Formononetin concentration vs Plant morphology and Number of bites in red clover plants

The correlation coefficient between formononetin concentration and either plant morphological characteristics or number of bites in Periods 1 and 2 are presented in Table 4.35. There was only a significant correlation between formononetin concentration and percentage of leaves in Period 2.

Table 4.35.Pearson Correlation coefficients (*r*) from correlation analysis between formononetin concentration and plant area, height, volume, leafiness and number of bites per plant (N. Bites) of red clover in Periods 1 and 2 of Experiment 6.

		Area	*Height*	*Volume*	*Leafiness*	*N.Bites*
Period 1	*r*	-0.0036	0.0596	0.0134	-0.0557	-0.0323
	P-value	*0.9846*	*0.7458*	*0.9421*	*0.7621*	*0.8608*
Period 2	*r*	0.0148	-0.0010	0.0190	-0.4746	-0.0733
	P-value	*0.9360*	*0.9955*	*0.9179*	*0.0061*	*0.6902*

Number of observation contributing for each correlation (n=32)

Number of Bites vs Plant Morphology

BIRDSFOOT TREFOIL

The correlation analysis between number of bites and morphological characteristics of birdsfoot trefoil plants in Periods 1 and 2 are given in Table 4.36. As in Experiment 5, there were significant ($P<0.05$) and positive correlations between number of bites and plant area, height, volume and leafiness in both periods.

Table 4.36.Pearson Correlation coefficients (*r*) from correlation analysis between number of bites per plant and area, height, volume and leafiness of birdsfoot trefoil plants in Periods 1 and 2 of Experiment 6.

		Area	*Height*	*Volume*	*Leafiness*
Period 1	*r*	0.6060	0.4310	0.6374	0.6222
	P-value	*0.0001*	*0.0001*	*0.0001*	*0.0001*
Period 2	*r*	0.6674	0.5110	0.6968	0.7397
	P-value	*0.0001*	*0.0001*	*0.0001*	*0.0001*

Number of observation contributing for each correlation (n=96)

RED CLOVER

The correlation analysis between number of bites per plant and morphological characteristics of red clover plants in Periods 1 and 2 is shown in Table 4.37. In Period 1 the number of bites was significantly correlated to plant leafiness but not to plant area, height and volume. In Period 2 there were significant correlations between number of bites and all four plant morphological characteristics.

Table 4.37.Pearson Correlation coefficients (*r*) from correlation analysis between number of bites per plant and area, height, volume and leafiness of red clover plants in Periods 1 and 2 of Experiment 6.

		Area	*Height*	*Volume*	*Leafiness*
Period 1	*r*	0.1260	0.0608	0.1193	0.2476
	P-value	*0.2214*	*0.5563*	*0.2469*	*0.0150*
Period 2	*r*	0.7779	0.6989	0.7889	0.4090
	P-value	*0.0001*	*0.0001*	*0.0001*	*0.0001*

Number of observation contributing for each correlation (n=96)

The correlation matrices showed that correlation coefficient results from the smaller number of observations used in the analyses involving ECT and Formononetin concentration was not substantially different from the analyses carried out with individual plant data (more number of observations). Small differences observed in correlation coefficient did not alter the significance of the relationship.

4.3.2.6. Use of covariates

The analyses of covariance were performed to distinguish the plant morphological characteristic effect from the extractable condensed tannin (ECT) concentration effect on number of bites in birdsfoot trefoil. As in Experiment 5, in the first analysis, individual morphological characteristics (leafiness or volume) data of all experimental plants (number of observations (n) = 96) were used as covariate and genotype effect was a class variable (described as high and low concentration of ECT). The effect of covariates or class variable added to the analysis of variance was assessed in terms of the R-square changes. The covariates were added to a basic model formed by the effect of day variation, individual cow variation, rumen chemical added to the rumen, rumen chemical concentration, sequence of plants, block of plants within each sequence and trimming. Leafiness and plant volume were used as covariates. In the second analysis, the number of samples was restricted to the untrimmed plants (n=48) to verify the effects of the natural variation. In this case the trimming effect was not included in the basic model. In the third, ECT concentration, and plant leafiness and volume were all used as covariates. The degrees of freedom of this analysis were limited by the number of samples used for the chemical determination of ECT concentration (n=32).

Genotype effect (class variable) vs Plant morphological characteristics (covariate)

TRIMMED AND UNTRIMMED PLANTS

The R-square changes when adding genotype effect (class variable) and either the percentage of leaves or plant volume in the first and second periods are given in Figure 4.8 and 4.9. The basic model explained about 43% in Period 1, and 30% in Period 2 of the total variation. The improvement in R-square after fitting covariates and genotype effect varied from 27 to 42% (adding both plant volume and genotype effects) in Periods 1 and 2, respectively. Leafiness explained more variation than volume in both periods (Period 1: 33 vs 26 % and Period 2: 39 vs 33 %). However both leafiness and volume effects explained important amounts of the variation in number of bites, more than the genotype effect, in both periods.

PERIOD 1

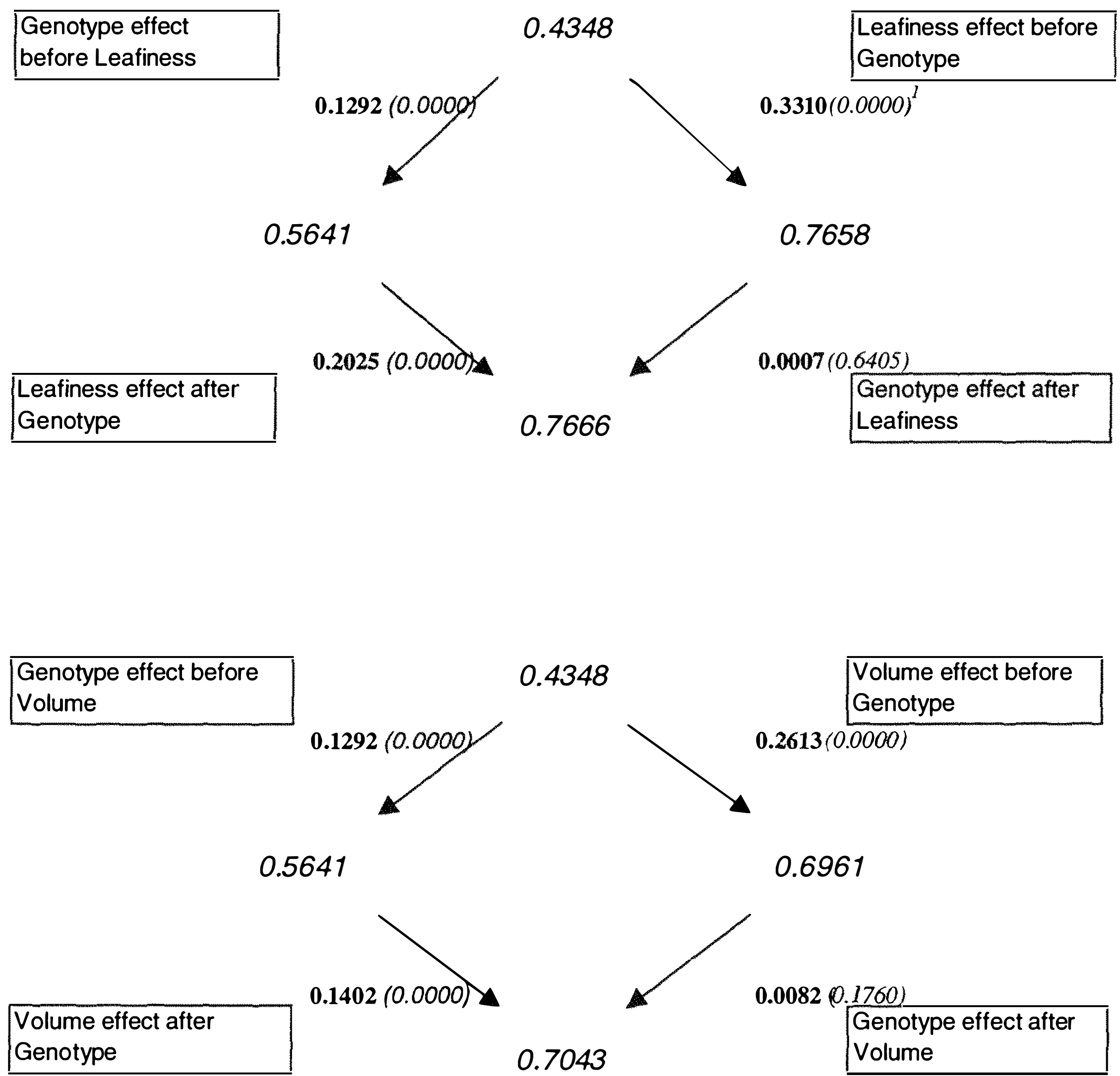

Figure 4.8. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or genotype effect had been added. Considering all experimental plants of birdsfoot trefoil in Period 1 of Experiment 6.

[1] P-value for the differences of R-squares.

PERIOD 2

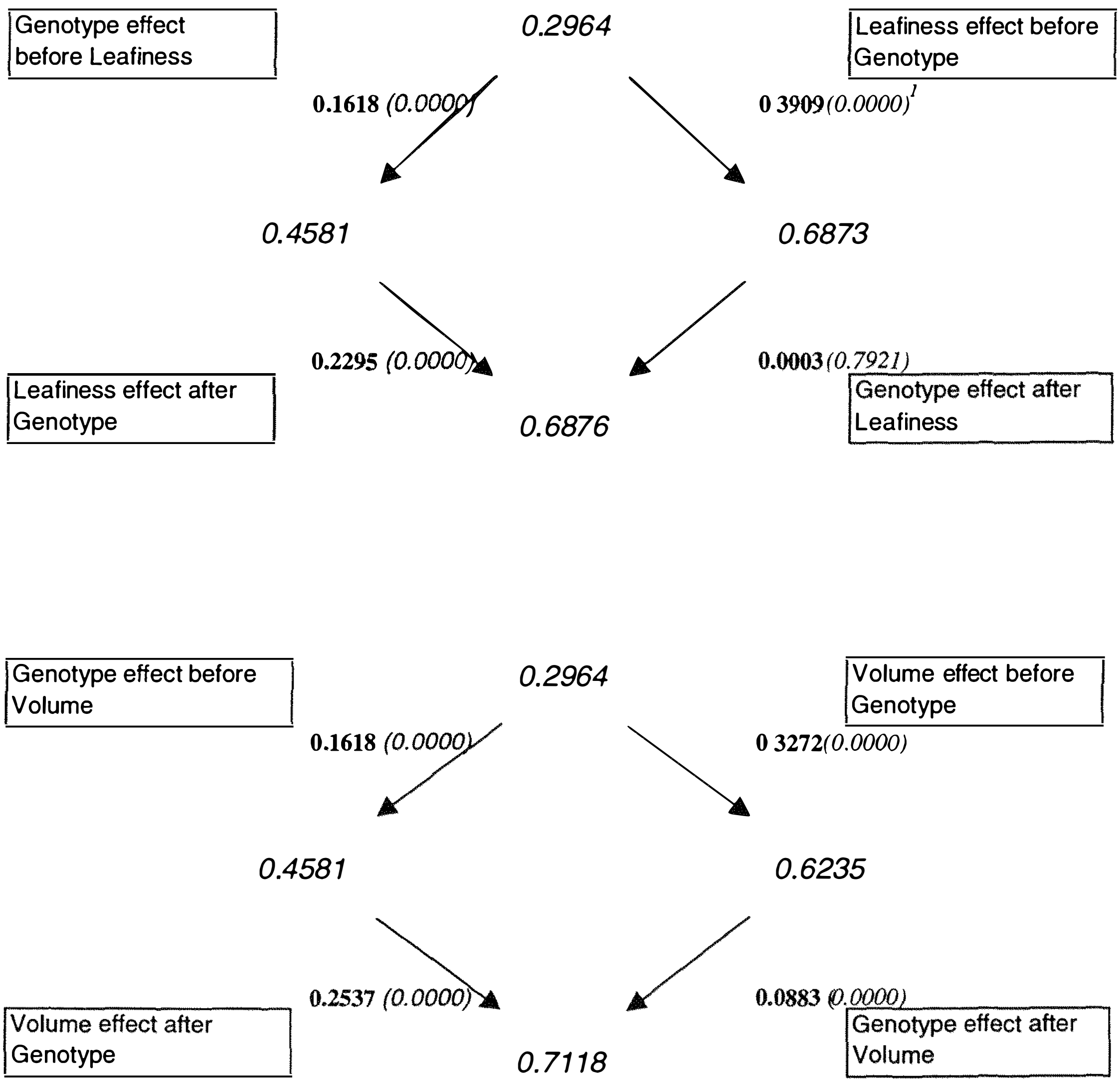

Figure 4.9. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or genotype effect had been added. Considering all experimental plants of birdsfoot trefoil in Period 2 of Experiment 6.

[1]P-value for the differences of R-squares.

In both periods there was a highly significant effect of genotype, plant leafiness and volume when initially added to the model. However in Period 1 leafiness and volume were important for explaining the variation between genotypes in relation to number of bites. After adding the covariates leafiness or volume, the genotype effect became non significant (P>0.05). In Period 2 , although volume had a substantial effect on changes of R-square, it did not seem to be important for explaining the variation between genotypes. After adding volume, the genotype effect was still highly significant. In this period leafiness was more effective than volume for explaining the differences between genotypes in relation to number of bites. After adding the covariate leafiness, the genotype effect became non significant (P>0.05). Plant morphological characteristics (leafiness and volume) had greater effect on R-square changes than genotype effect in both periods.

UNTRIMMED PLANTS

The R-square changes when adding genotype effect and either the percentage of leaves or volume in Periods 1 and 2, considering only untrimmed plants, are presented in Figure 4.10 and 4.11. The basic R-square (without the addition of genotype effect or any covariate) in Period 1 was greater than in Period 2. The R-squares of the basic models in both periods were smaller than in the first analyses. The improvement in R-square after fitting covariates and genotype effect varied from 40 to 65% (adding both plant volume and genotype effects in Periods 1 and 2, respectively). As in the previous analysis, the variation in R-square was also greater in Period 2 than in Period 1. In both periods, the changes in R-square promoted by the covariates and genotype effect were greater than the basic R-square. However morphological characteristics had greater effect on changes in R-square than the genotype effect in both periods.

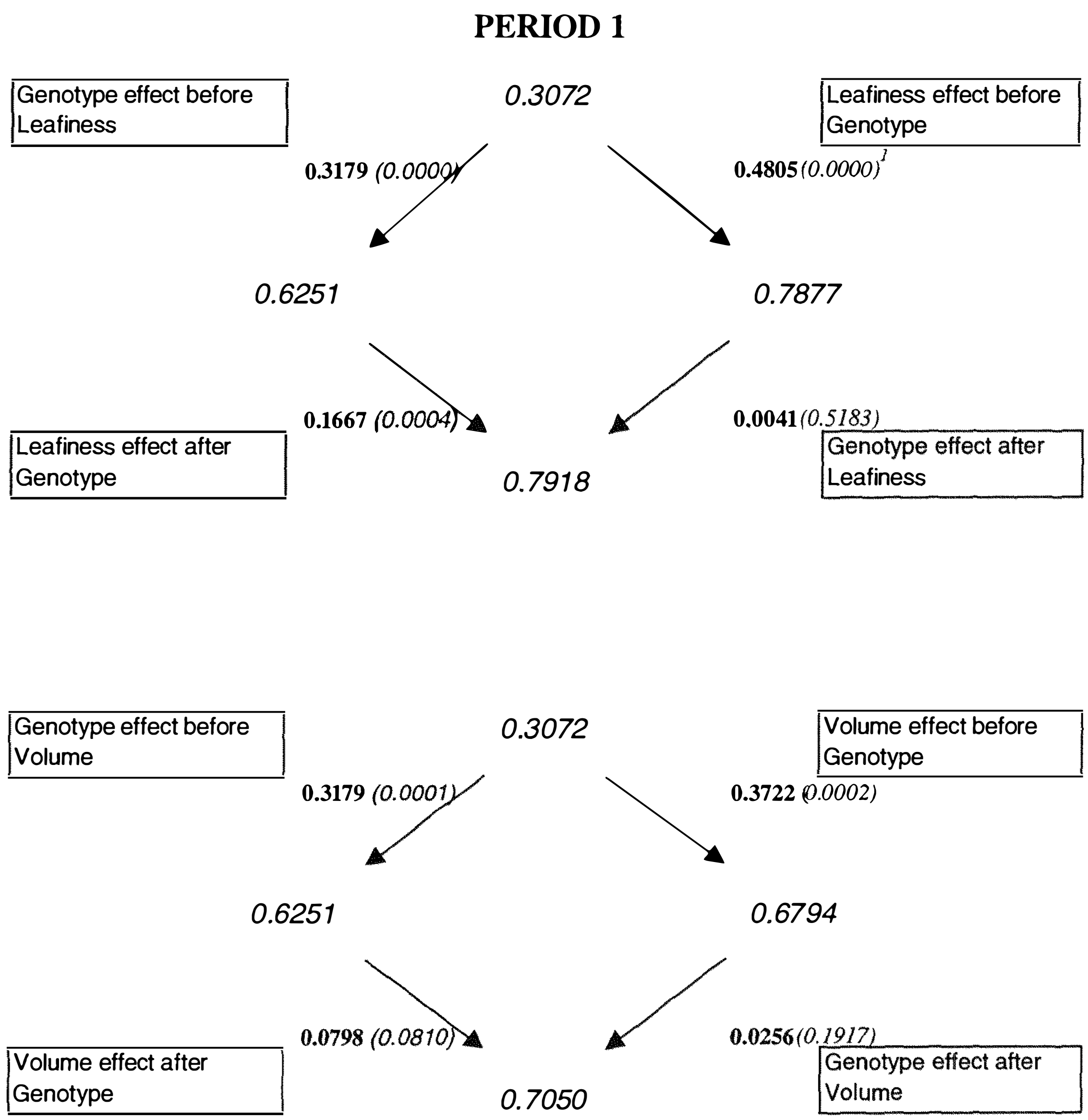

Figure 4.10. Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology (leafiness or volume) or genotype effect had been added. Considering only untrimmed birdsfoot trefoil plants in Period 1 of Experiment 6.

[1]P-value for the differences of R-squares.

PERIOD 2

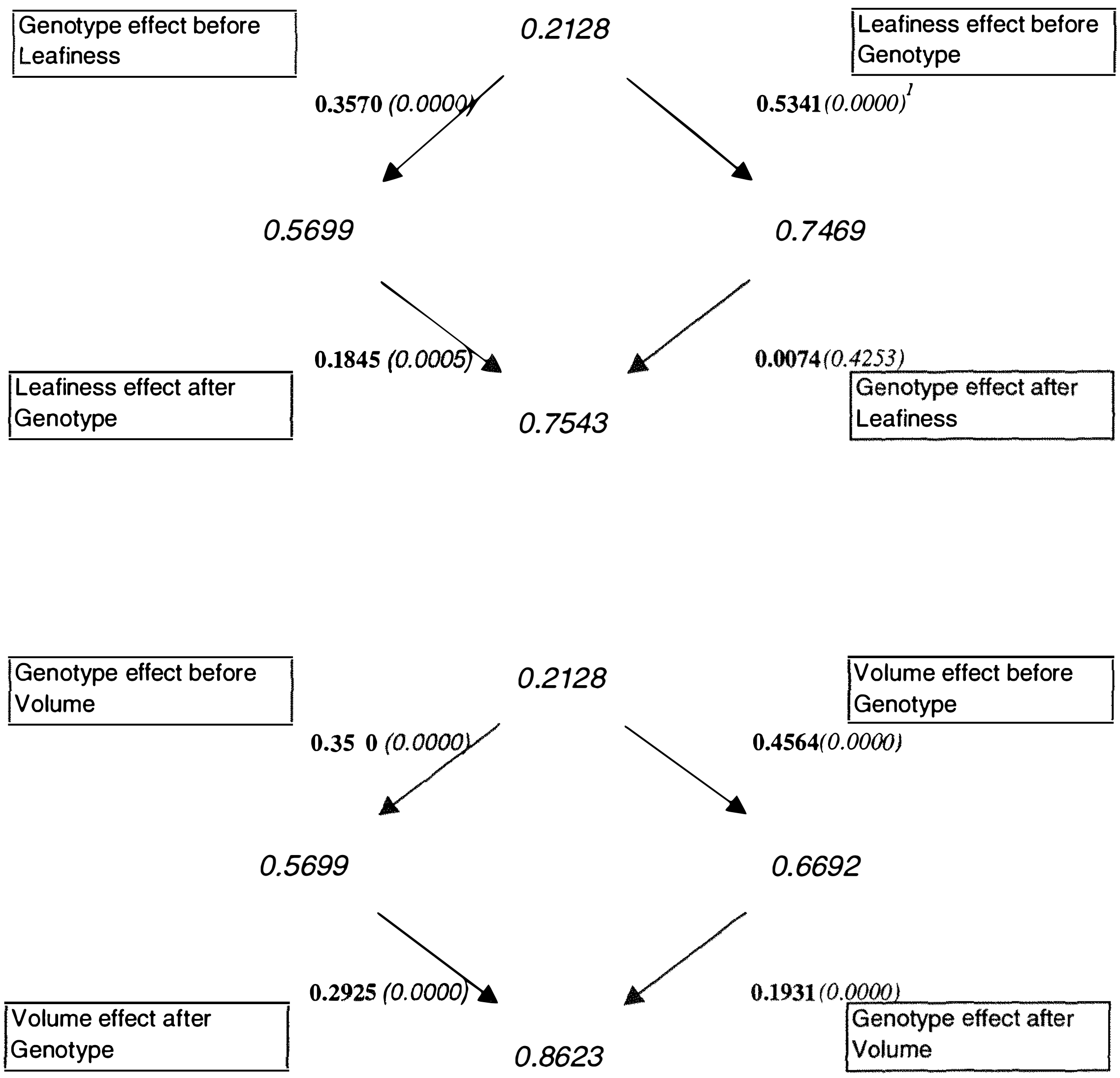

Figure 4.11 Effect of genotype (class variable) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology (leafiness or volume) or genotype effect had been added. Considering only untrimmed birdsfoot trefoil plants in Period 2 of Experiment 6.

[1]P-value for the differences of R-squares.

There were significant effects of genotype, leafiness and volume in both periods. However genotype effect was not independent of leafiness in either period, and of volume in Period 1. In this case, after adding leafiness or volume, the genotype effect became non significant. The genotype effect was independent of volume in Period 2. In both periods, plant leafiness and volume had greater effects than genotype in R-square change, but leafiness was more important than volume to explain the R-square changes.

ECT concentration (covariate) vs Plant morphological characteristics (covariate)

The R-squares and the differences of R-squares when plant leafiness or volume and ECT concentration were added to the model as covariate in the analysis of variance, considering either Period 1 or Period 2, are given in Figures 4.12 and 4.13. The basic model (without the covariate effects) explained a higher percentage of the variation in Period 1 (56%) than in Period 2 (27%). The improvement in R-square after fitting covariates was about 28 and 43% in Periods 1 and 2, respectively. Leafiness, volume and ECT concentration explained important variation in number of bites. However leafiness and volume had greater effect on R-square change than ECT concentration. Although ECT concentration showed similar effect in both periods, leafiness and volume effects explained higher variation of the R-square in Period 2 than in Period 1.

In both periods, addition of ECT concentration, leafiness or plant volume to a basic model significantly increased R-square. However the ECT concentration was not independent of the plant leafiness because after adding leafiness to the model the effect of ECT concentration became non significant ($P>0.05$). However after adding volume, the ECT concentration effect was still marginally significant.

PERIOD 1

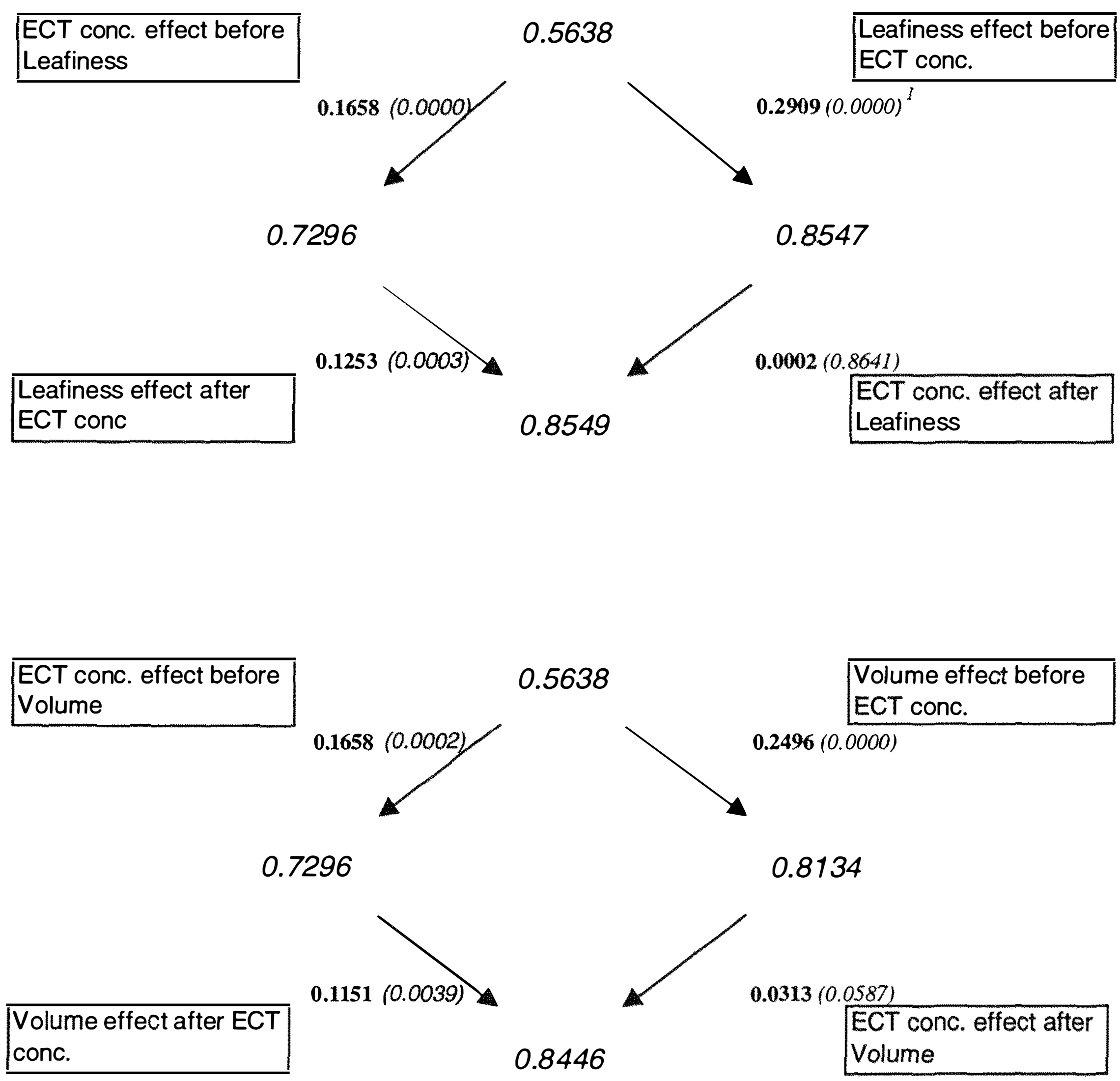

Figure 4.12. Effect of extractable condensed tannin concentration (covariate) (ECT conc) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or ECT conc. had been added. Considering plants of birdsfoot trefoil in Period 1 of Experiment 6.

[1]P-value for the differences of R-squares.

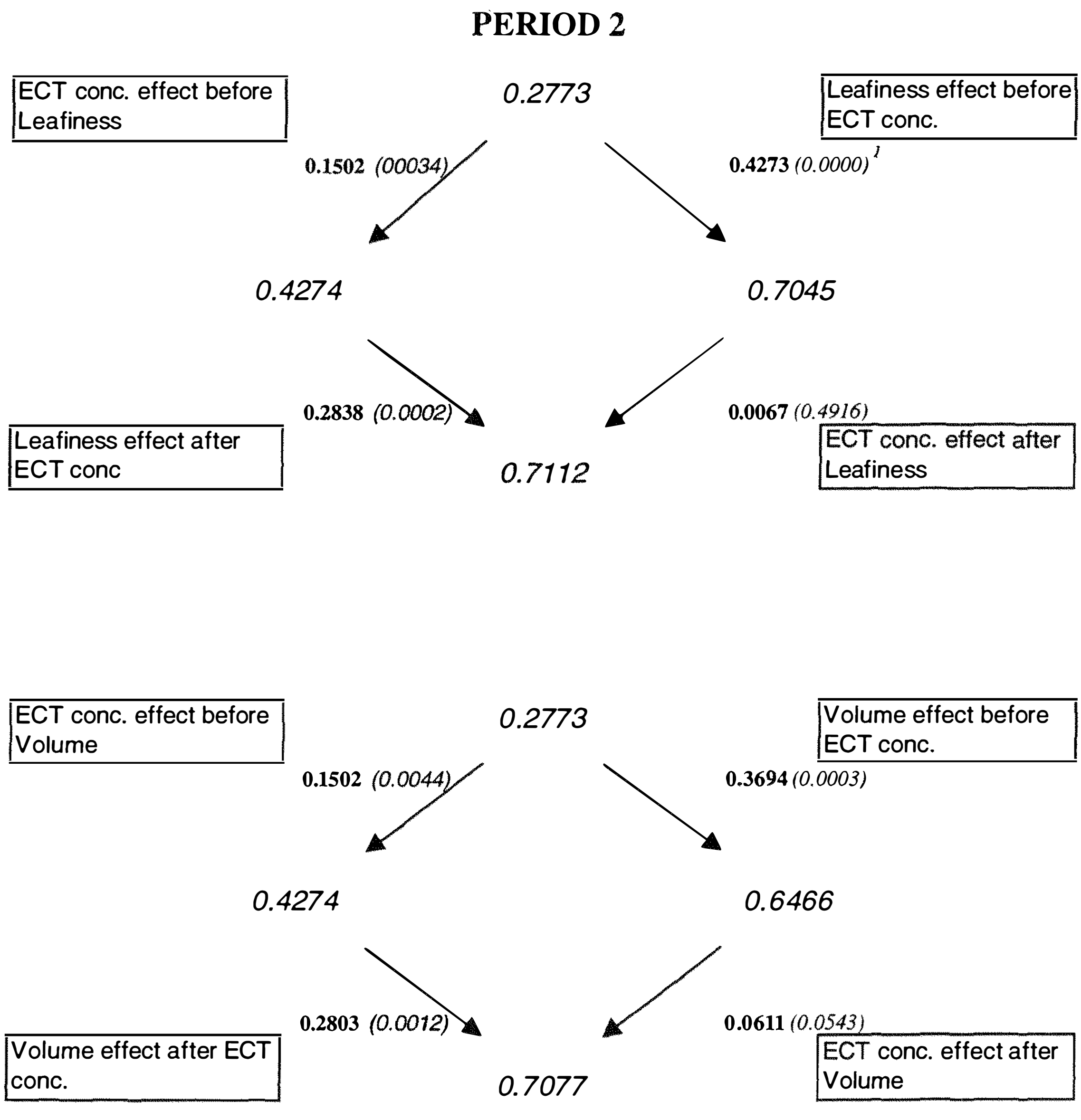

Figure 4.13.Effect of extractable condensed tannin concentration (covariate) (ECT conc.) and plant morphology (covariate) (Leafiness or Volume) on R-squares (from the analysis of variance of number of bites), when added to the model before (independent) or after plant morphology or ECT conc. had been added. Considering plants of birdsfoot trefoil in Period 2 of Experiment 6.

[1]P-value for the differences of R-squares.

This analysis was similar to the analysis where individual plant data and genotype effects (rather than ECT concentration) were used. In both periods, the effect of leafiness was greater than the effect of volume. In both analyses plant morphology (leafiness and volume) had greater effects on the R-square changes than ECT concentration (or genotype effect). The difference between the effects of plant morphology and ECT concentration (or genotype effect) was greater in Period 2 than in Period 1.

4.4. DISCUSSION

4.4.1. Evaluation of experimental procedures

The grazing preference of cows was assessed using a procedure based on techniques developed by Laca et al. (1993) and Griffiths et al. (1996) to determine grazing behaviour on patches of ryegrass pasture, and on techniques developed by Real-Ferreiro (1997) to assess pre and post grazing characteristics of spaced plants of red clover. These experiments were designed to determine animal preference, rather than selection, by maximising opportunity and offering free choice (see Chapter 3, section 3.4.2 for definition of preference and selection). The technique of assessing behaviour as an animal grazes along a sequence of spaced plants differs from the conventional technique where groups of animals demonstrate their preference by selecting from several plants or swards in a field (Simon, 1974; Marten and Jordan, 1974; Hedges et al. 1978; MacGraw et al., 1989; van Santen, 1992; Shewmaker et al., 1997). With this technique a close assessment of animal preference was possible. It allowed the precise measurement of the number of bites, grazing time and rate of biting for each plant. In addition it provided information about behaviour at a specific locus in relation to conditions at the preceding and the succeeding loci in a sequence, and the animals were not influenced by previous grazing of each plant, deposition of feces and treading. The other advantage of this technique is that the assessment period is short. However it requires the participation of at least two people for monitoring behaviour and it is very time consuming in relation to animal training and pre and post grazing assessment. The other constraint is that the animals need to be accustomed to handling and close grazing control.

Using this technique it was possible to explain a substantial proportion of the variation in the number of bites per plant. From 62 to 92 % of the total variability in number of bites was explained when analysing only one plant species and including covariates in the model (see covariance analyses, section 4.4.4). The combined analysis of number of bites per plant including all periods within each experiment explained approximately 40% of the total variability. Both Experiments 5 (E5) and 6 (E6) also explained similar variability (similar R-square). The accuracy reached with this technique, explaining up to 92% of the variation, is better than that achieved by Shewmaker et al. (1997) using visual preference score (44% of variation) when assessing cattle preference for eight tall fescue cultivars planted in swards. However, comparison of this technique with other techniques is difficult because of other sources of variation among the different experiments (such as differences in animal species; plant species, maturity, morphology, biochemistry) and because of the scarcity of information published. The small variability between E5 and E6 is in agreement with what was found by van Santen (1992), assessing cattle preference of twenty-five tall fescue cultivars and populations, where preference rating agreed very closely within and between years.

Preferential behaviour was assessed mainly by the comparison of number of bites the animals took per plant. Although the variability of animal response was explained equally well by either number of bites or grazing time, number of bites was more precisely measured than grazing time. Grazing time per plant was used in the analyses of rate of biting.

Plant morphology had an important effect on preference. The measurement of plant morphology was a useful measurement for better understanding of choice. The assessment of plant characteristics, other than herbage mass, before and after grazing followed a modified technique described by Real-Ferreiro (1997). This technique provided individual assessment of the plants in terms of what was offered to the animal, and the reduction of each plant variable by grazing. The use of visual assessment of plant leafiness, habit and density provided non-destructive and useful information with minimum use of time and resource. Real-Ferreiro (1997) when assessing the amount removed from several cultivars of red clover by sheep concluded that visual assessment of post-grazing leafiness was the best estimation of forage removal.

The assessment of herbage mass was one of the main difficulties in this research. It was only possible to have an indirect assessment for each plant. Prediction equations developed for the pasture probe from plants in the spare sequences showed R-squares of 0.37 for birdsfoot trefoil (BT) and 0.20 for red clover (RC) (see Appendix 4.2). Although the R-squares were relatively low for both species, the herbage mass measurements agreed relatively well with visual comparisons between plants. Big and dense plants usually had greater herbage mass than small and sparse plants. This observation was confirmed by correlation analyses that showed that in most periods of both experiments, herbage mass had a significant correlation (R-square generally better than 0.5) with plant area, height, volume and leafiness (Appendices 4.4, 4.5, 4.6, 4.8, 4.9). The only exceptions happened with RC plants in Periods 1 and 2 of E5. However, there were significant correlations in Period 1 between herbage mass and leafiness and in Period 2 between herbage mass and height. In these cases the lowest correlation found in both periods was between herbage mass and area. This low correlation is probably related to the fact that the probe device used is mainly sensitive to pasture height and density.

Plant trimming was used as a way to assess, and separate into morphological and chemical components, the effects of plant genotype on preferential behaviour. In both experiments, trimming made the alternative genotypes within each species morphologically uniform. However trimming usually reduced the plant size and leafiness, which in part also altered the animal preference and needed to be considered in the analyses. Trimming did not affect the ECT concentration of BT plants in either experiment (Tables 4.8, 4.25) or the formononetin concentration in E6 (Table 4.26). However, in E5 trimmed plants of cultivar Pawera had higher concentration of formononetin than untrimmed plants (Tables 4.9), though this difference did not affect the substantial contrast between cultivars G-27 and Pawera. The higher formononetin concentration of trimmed plants of Pawera may be explained by the fact that the old leaves were trimmed and there was a proportionate increase of young leaves. Rossiter and Beck (1967) and Keogh (1995) demonstrated that concentration of formononetin in individual leaves decreased from emergence to senescence. In addition Anwar (1994) observed that Pawera have greater variation in formononetin concentration between young and old leaves than did G-27.

Previous experience was necessary for the animals to get used to the plant species and sequences. Although Lascano et al. (1988) affirm that in cafeteria trials where the objective is to rank forage species in terms of palatability it is not necessary to subject animals to short-term previous experience on the individual species under evaluation, it was important in E5 that the cows had previous experience of grazing BT. During the training period, one week before running the experiment, the animals were not willing to graze BT. This result contrasts with E6, using different cows, where the animals had only the normal training with the same plant genotypes one week before starting the trial, but they did not reject BT. The probable reason is that the cows used in E6 were used to a greater variety of foods and had experienced BT before.

The use of sequences of spaced plants with one animal per sequence did not allow assessment of between-animal variation in preference. Because different animals grazed different sequences, the animal effect was confounded with sequence variation. However, the variation of plant sequence and cow were considered in the statistical model before treatment effects. In this way variation of cows or plant sequence were not included in the treatment effect.

The analysis of extractable condensed tannin (ECT) was done mainly on samples of BT plants, and formononetin analysis on samples of RC plants. This was because concentrations of ECT in samples of RC, and of formononetin in samples of BT were negligible. Other studies have also shown that the amount of ECT in RC is very small (Jackson et al., 1996). The analysis of ECT and formononetin concentration followed the same procedures described in Chapter 3 (see section 3.4.1).

The addition of two different materials (*Lotus* species and red clover cultivars) with different ECT and formononetin concentrations into the rumen of cows in E6 demonstrated the effects of manipulation of rumen content on cattle diet selection. Although other studies (e.g. Cooper et al., 1995; Carter and Grovum, 1990) using penned animals have been carried out to determine the effect of rumen manipulation on diet selection, this study is believed to be one of the first attempts to use rumen manipulation in grazing trials. The cows had a significantly higher number of bites in RC when RC (independent of the cultivar) was inserted into the rumen (see discussion section 4.4.4). However, comparing the concentration effect within each material inserted in the rumen (*Lotus* species or red clover cultivars), there was no significant effect of the different concentrations on cattle preference (Tables 4.30, 4.31). This result was apparently a reflection of the low concentrations and small contrast between high and low concentrations of either tannin or formononetin added to the rumen (Appendix 4.10). The low concentrations and small contrasts were related in part to the addition of pellets of alfalfa to facilitate the mincing process (see section. 4.2.6.2) and in part to the amount of weeds present in some of the materials used.

The contrasts between different plant species and genotypes provided the opportunity for investigation of the effect of plant morphological and biochemical characteristics on cattle preference. The range of variation found in each experiment is summarised in Table 4.38. Although ECT concentration and plant area had the greatest range of variation, the correlation analysis showed that the sward morphological characteristics were significantly correlated among themselves (Appendices 4.4, 4.5, 4.6, 4.8, 4.9). The correlations of plant morphological and biochemical characteristics and their importance on cattle preference are discussed in section 4.4.4.

Table 4.38. Range of values (minimum and maximum) of individual plant morphological and biochemical characteristics observed in Experiments 5 and 6. These values were extracted from averages presented in Tables 4.5, 4.6, 4.7, 4.19, 4.20, 4.21, 4.22, 4.23 and 4.24.

	Experiment 5		*Experiment 6*	
Plant Characteristics	Minimum	Maximum	Minimum	Maximum
Height (cm)	10.2	18.9	9.7	30.4
Area (cm^2)	970	5540	980	6880
Leafiness (%)	37	75	31	75
Herbage mass (gDM/m^2)	325	494	228	419
ECT (%)	0.47	3.28	0.35	1.62
Formononetin (%)	0.29	0.73	0.26	0.63

Although the cows and experimental site differed between E5 and E6, the behavioural responses in these two experiments were similar. The similar approach for each experiment, the limited effect of rumen manipulation, and the similar animal response justify a combined discussion for the two experiments. Differences between experiments will be explained where appropriate.

4.4.2. Plant morphological characteristics

There were important variations in plant morphology in both experiments (Tables 4.5, 4.6, 4.7, 4.19 - 4.24). Red clover (RC) had greater density and percentage of leaves than birdsfoot trefoil (BT) in all runs and periods. Within species, BT genotypes showed greater morphological contrast than RC genotypes. Untrimmed plants of Goldie were on average the largest plants and accession PI273938 were the smallest. Trimmed plants usually were smaller and had less leaves and density than untrimmed. In fact, the animals were faced with a complex decision involving variations not only in plant biochemistry but also in plant morphology.

The analysis of the reduction by grazing in magnitude of each plant variable showed that in part the animals responded to the plant morphology (Tables 4.5, 4.19, 4.20). The animals tended to remove more from the larger and leafier plants, showing a preference for plants with a greater proportion of leaves (as in Theron and Booysen, 1966; O'Reagain and Mentis, 1989, O'Reagain, 1993), and greater height and density (as in Illius et al., 1992; Demment et al., 1993). This result confirms what was found by Clark (reported in Illius and Gordon, 1990) where cattle were very sensitive to sward height and they could discriminate well between alternative swards and select across a broad range of height contrasts the taller one. It also indicates, as demonstrated by Clark (in Illius and Gordon, 1990) and Illius et al. (1992), that the preference was affected by higher levels of plant height and herbage mass than values reported in other studies as limiting intake (Holmes, 1987; Hodgson, 1990; Gibb et al., 1997). More detailed analyses including analysis of variance of number of bites, correlation and covariance analyses were carried out for a better understanding of the effect of plant morphology on animal preference (see section 4.4.4).

4.4.3. Plant chemical composition

There were significant contrasts in ECT concentration between genotypes of BT in both experiments (Tables 4.8, 4.25, summarised in Table 4.39). As expected, the ECT concentration was significantly higher in accession PI273938 than in the cultivar Goldie. The accession PI273938 had, on average, 4.2 times (in E5) and 3.5 times (in E6) higher ECT concentration than the cultivar Goldie (Table 4.39). The concentration of ECT in E6 either in January or April was similar to the concentration found in April in E5. However the concentration found in March (E5) was notably higher. The increase in concentration of tannin during summer months and a decline from summer to autumn was also reported in several studies for different species (Clark et al., 1939; Stit and Clark 1941; Donnelly, 1959; Cope et al. 1971; Windham et al. 1988; Iason et al., 1995). The smaller difference in ECT concentration found in E6 between January and April can therefore be explained by the fact that the comparison was done in the beginning of summer and in autumn. The higher percentage of ECT in March (E5) showed that probably the ECT concentration increased from January to March and decreased from March to April. The lowest concentration in autumn was also found in E1, E2 and E3 (Tables 3.4, 3.13, 3.25, summarised in Table 4.39). However, in these three previous experiments the cultivar Goldie had higher concentration in November (spring) than in February and April-May.

Table 4.39. Extractable condensed tannin (ECT) concentration (%) in birdsfoot trefoil (cultivar Goldie and accession PI273938) between November and April-May of Experiments 1, 2 and 3 (only leaves) and Experiments 5 and 6 (intact stems: leaf and stems).

Genotype	*Experiment*[†]	*November*	*January*	*February*	*March*	*April-May*
Goldie	1, 2 and 3	1.69		0.58		0.54
	5				0.72	0.47
	6		0.32			0.54
PI273938	5				3.28	1.81
	6		1.49			1.55

[†] The Experiments 1,2 and 3 were carried out in 1995/1996, and the Experiments 5 and 6 in 1997.

Comparing the ECT concentration of cultivar Goldie in the five experiments it can be observed in Table 4.39 that there was a decrease in concentration from spring to summer, an increase during the summer months and a decline from summer to autumn. However the variation observed between experiments and within each experiment can also be affected by several factors discussed below.

Previous studies showed variation of ECT concentration for BT between 0.25 to 3.58 % of DM (Douglas et al., 1993, 1995; Jackson et al., 1996; John and Lancashine, 1981; Li et al., 1996; Lowther et al., 1987; Terrill et al., 1992; Wang et al., 1995; Waghorn et al. 1987ab). However analyses of cultivar Goldie reported by Douglas et al. (1993, 1995) showed greater concentrations of ECT (1.18 to 2.52 % DM) than those found in E5 and E6 (0.32 to 0.72 % DM). This difference is probably explained by seasonal variation (Roberts et al., 1993), site variation (Douglas et al., 1993), soil fertility (Barry, 1989), tannin analysis procedures (Furstenburg and van Hoven, 1994; McNabb, *pers. comm.*) or differences in plant morphological characteristics (Iason et al., 1995; Douglas et al., 1993). The fact that the plants in E5 and E6 were sown in a glasshouse and were planted out in the field probably influenced the percentage of ECT. Studies with *Lotus pedunculatus* and *Lotus corniculatus* (John and Lancashire, 1981; Barry and Forss, 1983; Barry, 1985; Lowther et al, 1987) showed that growing conditions have an important effect on condensed tannin concentration. In addition, the DMACA-HCL procedure used in these studies is more specific for condensed tannin than butanol-HCL (McNabb, *pers. comm.*) used by Douglas et al. (1993, 1995), so values of ECT measured using butanol-HCL protocol tend to be higher than with DMACA-HCL. Douglas et al. (1993, 1995) also used condensed tannin from *Lotus pedunculatus* as a standard curve, while in the analyses for these studies condensed tannin extracted from *Lotus corniculatus* was used (Appendix 3.1). The difference in absorbance curves between differing species was reported by Furstenburg and van Hoven (1994), who explain that condensed tannin composition is diverse and species specific. Given the differences in the standard curves, there is always a tendency to predict a higher concentration when using *Lotus pedunculatus* as the standard compared to *Lotus corniculatus* (W.C. McNabb, *pers. comm.*).

The concentration of formononetin was very similar in both experiments (Table 4.9 and 4.26, summarised in Table 4.40). As expected, there was a substantially higher formononetin concentration in cultivar Pawera than in cultivar G-27. The concentrations found in both cultivars were similar to levels reported in other studies (Kelly et al., 1979; Anwar, 1994). In both experiments Pawera had on average 2.3 times more formononetin than G-27, but the concentration of formononetin in cultivar Pawera seemed more variable than the concentration in G-27. Anwar (1994) explained that the decline in formononetin concentration of leaves with age was greater in Pawera than in G-27. However, there was no significant variation between different periods. This result agrees with the results found in E1, E2 and E3 with cultivar Colenso (Tables 3.5, 3.14, 3.26, summarised in Table 4.40). The variation of formononetin concentration in cultivars Pawera and G-27 seemed more related to the age of each plant part (Rossiter and Beck, 1967; McMurray et al., 1986; Keogh, 1995) than to seasonal variation. The plant parts probably had a similar age in different periods and experiments.

Table 4.40. Formononetin concentration (%) in leaves of red clover cultivar Colenso and intact stems (leaf, petiole and stems) of cultivar G-27 and Pawera between November and April-May of Experiments 1, 2 and 3 and Experiments 5 and 6.

Cultivars	*Experiment*[†]	*November*	*January*	*February*	*March*	*April-May*
Colenso	1, 2 and 3	0.61		0.50		0.70
G-27	5		0.28		0.30	0.30
	6		0.29			0.23
Pawera	5		0.67		0.72	0.64
	6		0.60			0.62

[†] The Experiments 1,2 and 3 were carried out in 1995/1996, and the Experiments 5 and 6 in 1997.

The four genotypes showed a relatively high nutritional value in terms of general chemical composition in both experiments (Tables 4.10, 4.27). All four genotypes had digestibility above 74 %. The data showed lower ADF and NDF than standard values published by NRC (1989, 1996), but similar concentrations of the other components. The high nutritive values for BT and RC were also found in E1, E2 and E3 (Tables 3.6, 3.15, 3.27). Correlation analyses were carried out to investigate the influence of the plant nutritive value on animal preference (Tables 4.14, 4.33). Although there was a significant correlation between the number of bites and lipid concentration in BT (E5, Table 4.14), and between the number of bites and ADF, carbohydrates and ash in RC (E6, Table 4.33), the four genotypes in both experiments did not differ significantly in concentrations of lipid, ADF, carbohydrate and ash. It can be concluded, therefore, that nutritive value did not have an important effect on preference in relation to the four different cultivars.

4.4.4. Grazing behaviour

The rate of biting showed little variation between different plant genotypes in E5, but was affected by plant morphology in E6. In both experiments there were also no significant interactions with the Period effect. In E5 the difference in plant characteristics, mainly density, was not large enough to affect rate of biting (Tables 4.12, 4.13). This result suggests that the time spent grazing each plant was proportional to the number of bites taken (Griffiths et al., 1996). However in E6 the rate of biting demonstrated that the animals spent more time to obtain the same number of bites from untrimmed plants of BT, and from BT cultivar Goldie, than from the other plants (Table 4.32). This result apparently reflected the plant morphology. Untrimmed plants of cultivar Goldie were large, but with lower density than RC plants. As several studies (Chacon and Stobbs, 1976; Hodgson and Jamieson, 1981; Milne et. Al., 1982; Philips and Leaver, 1985; Penning et al. 1991; Mitchell et al., 1993) have also shown, there was a negative relationship between rate of biting and sward height, or rate of biting and herbage mass. In this case the animals had lower rate of biting in tall sparse swards than on short dense ones of equal mass.

Comparing the number of bites taken per plant in BT and in RC within E5, there was a significant preference for RC plants in Period 2, but not in Period 3 (Table 4.11). The preference for RC in the second period might be influenced by the higher concentration of ECT in BT in this period. In E6 the preferential behaviour between BT and RC was modified by an interaction between trimming and secondary compound concentration effects (Table 4.28) and by the rumen content manipulation (Table 4.29). The fact that the animals took more bites from untrimmed plants of RC and untrimmed plants of cultivar Goldie reflected the preference the animals had for bigger, denser plants and with greater proportion of leaves (O'Reagain and Mentis, 1989; Illius et al. 1992; O'Reagain, 1993; Demment et al., 1993). The fact that in both experiments the animals always left a great portion of herbage mass behind and never completely grazed a plant (Tables 4.5, 4.6, 4.7, 4.19 - 4.24) probably demonstrated that the amount of herbage mass did not limit the number of bites. On the other hand, the fact that the animals took significantly fewer bites from accession PI273938, independent of the trimming effect, could also indicate that the animals were affected by the concentration of ECT. Individual analyses of variance including only one species at a time, followed by correlation and covariance analyses were carried out to clarify these results. In E6 the animals were also influenced by the type of material inserted into the rumen. The animals took more bites from RC plants than BT when RC material was inserted in the rumen. The effects of rumen manipulation on animals diet selection will be discussed later.

The variation in number of bites between RC genotypes in both experiments demonstrated that morphological effects were more important than formononetin concentration in determining preferential behaviour (Tables 4.11, 4.31). In all analyses of variance, trimming had a substantially more important effect than the difference between genotypes. Cows were apparently not directly influenced by the formononetin concentration in RC. This result disagrees with results reported by Francis (1973). This author, working with variants of subterranean clover cultivar Geraldton with different isoflavone composition (formononetin concentration varied from 0.15 to 1.05 % DM), postulated that unhydrolyzed glycosides in subterranean clover could contribute to unpalatability. However Harborne (1993) argued that isoflavones are not sufficiently repellent in taste to deter feeding.

In both experiments there was substantially more contrast between genotypes in number of bites per plant in BT than in RC. This seemed to be related either to the contrasts in morphological characteristics of the two genotypes of BT or to the contrasts in ECT concentrations. In E5 and E6 the number of bites taken from BT was affected by both trimming and genotype effects (Tables 4.11, 4.30). The fact that trimmed plants had lower number of bites is probably associated with the smaller size and lower percentage of leaf found in trimmed plants than in untrimmed plants. As in other studies, the animals seemed to show an immediate reaction against small and stemmy plants or patches in a sward (Arnold, 1960, Arnold, 1981; L'Huiller et al., 1984; L'Hiuller, 1986; O'Reagain and Mentis, 1989; Illius et al. 1992; O'Reagain, 1993; Edwards, 1994). However, in E5 Period 2 ECT concentration (represented by differences in genotypes), independent of trimming effect, had an immediate effect on selection, and had a substantially greater effect on diet selection than did formononetin. This result probably reflects the higher concentration of ECT in this period than in Period 3 and in both periods of E6. However, further analyses are important to clarify the effects of plant morphology and plant biochemistry and the effect of their relationship with preference.

Correlation analysis between number of bites and either ECT or formononetin concentration was one of the first steps to clarify the effect of plant morphology and biochemistry on grazing preference. In both experiments there was low correlation between ECT concentration and number of bites in April, but there were significant correlations in March (E5) and January (E6) (Tables 4.15, 4.34). However, number of bites were also highly correlated with plant morphological characteristics (area, height, volume (area x height) and leafiness of BT plants) (Tables 4.17, 4.36). This result reflects the difference in plant morphology between BT genotypes. The significant morphological contrasts between genotypes with high and low concentration of ECT can also be detected by the significant correlation between ECT concentration and plant morphology. Covariance analysis then was necessary to determine the relative magnitude of the ECT concentration and plant morphology effects on selective behaviour and how they were interrelated.

In contrast, formononetin did not have an important effect on preferential behaviour. There was no significant correlation between number of bites and formononetin concentration in RC plants in either experiment (Tables 4.16 and 4.35). Although there were no significant correlations between formononetin concentration and RC morphological characteristics (except in Period 2 of E6), there were, in both experiments, significant correlations between number of bites and plant morphology (Tables 4.18 and 4.37). The low correlation between formononetin concentration and plant morphology reflects the fact that the RC genotypes (with high and low concentration of formononetin) were morphologically similar. However, most of the variation in number of bites probably could be explained by the variation in RC morphology. Thus, it is concluded that morphological characteristics were much more important than formononetin content in influencing preference between cultivars of RC.

Covariance analyses were carried out to clarify the effects of ECT concentration and plant morphology on preference in BT which were observed in the analyses of variance and correlation analyses. The effect of BT genotype and ECT concentration on animal preference was tested in the covariance analysis against two of the most important plant morphological characteristics (leafiness and volume) that affected preference in BT. Similar analyses were not necessary for RC since formononetin clearly had a very minor influence on preference. Although the covariance analyses did not separate completely the effect of plant morphological characteristics from the effects of ECT concentrations in BT, the use of three different analyses of covariance helped in understanding of this relationship. The addition of plant morphology and genotype effect to a basic model had greater effect (greater R-square improvement) in the analysis where only untrimmed plants were considered than in the analysis where all experimental plants were used (Figures 4.2 - 4.5, 4.8 - 4.11). This result reflects the greater morphology differences between untrimmed than trimmed plants. Therefore, plant morphology had an important effect on selective behaviour in both experiments and all periods. The use of means of the subset of plants, according to the sets of plants bulked for ECT chemical determination, reduced the R-square improvement with the addition of the covariates to the basic model (Figures 4.6, 4.7, 4.12, 4.13). This reduction can be explained by the greater variability in number of bites explained by the basic model. Although the average number of bites within each sequence reduced the variation within and between sequences of plants, ECT concentration as a covariate had a similar effect to that of the genotype effect in the analysis where all experimental plants were used. The effect of the covariate ECT concentration increased as the contrast between genotypes increased.

Although the two experiments had similar design, they need to be discussed separately because of the difference in causes of variation in each experiment. While the basic model of E5 was formed by the variation of sequences of plants, blocks (within sequences) and trimming, in E6 it was formed by the variation of day, cow, chemical inserted in the rumen, rumen chemical concentration, sequence of plants, blocks (within sequences) and trimming. The significance of the effect of the covariates on R-square changes in E5 and E6 are summarised in Tables 4.41, 4.42, respectively.

Table 4.41. Summary of the R-square improvement when the covariates plant leafiness, volume or ECT concentration were added before and after fitting the alternative covariate to the model in analyses 1, 2 and 3 for Periods 2 and 3 of Experiment 5.

Analyses[†]	*Period*	*Leafiness*		*Volume*		*ECT*[††]		
		Before ECT	After ECT	Before ECT	After ECT	Before leaf.	After leaf.	After vol.
1	2	+++[1]	++	++	+	++	+	+
	3	++	+	++	++	+	+	+
2	2	+++	+	+++	+	+++	+	+
	3	+	+	+++	++++	+	+	++
3	2	++	+	++	+	++	+	+
	3	+	++	+	+	+	+	+

[†]Analyses 1 - full number of observation were used (n=48); Analyses 2 - only untrimmed plants were used (n=24); Analyses 3 - averages according to the bulking for ECT concentration analyses were used (n=16).

[††] ECT concentration in analyses 1 and 2 corresponded to the genotype class variable

[1]R-square changes: + - from 0 to 10%; ++ - from 11 to 20%; +++ - from 21 to 30%; ++++ - > 30%

Table 4.42. Summary of the R-square improvement when the covariates plant leafiness, volume or ECT concentration were added before and after fitting the alternative covariate to the model in analyses 1, 2 and 3 for Periods 1 and 2 of Experiment 6.

Analyses[†]	*Period*	*Leafiness*		*Volume*		*ECT*[††]		
		Before	After ECT	Before	After ECT	Before leaf.	After leaf.	After vol.
1	1	++++[1]	++	+++	++	++	+	+
	2	++++	+++	++++	+++	++	+	+
2	1	++++	++	++++	+	++++	+	+
	2	++++	++	++++	+++	++++	+	++
3	1	+++	++	+++	++	++	+	+
	2	++++	+++	++++	+++	++	+	+

[†]Analyses 1 - full number of observation were used (n=96); Analyses 2 - only untrimmed plants were used (n=48); Analyses 3 - averages according to the bulking for ECT concentration analyses were used (n=32).

[††] ECT concentration in analyses 1 and 2 corresponded to the genotype class variable

[1]R-square improvement: + - from 0 to 10%; ++ - from 11 to 20%; +++ - from 21 to 30%; ++++ - > 30%

In E5, the first covariance analysis, where the full number of observations were used and the genotype effect was tested, leafiness was shown to be more effective (greater improvement in R-square) in explaining variation in number of bites between genotypes than volume (Table 4.41, Figures 4.2, 4.3). In the second analysis (only untrimmed plants - plant natural variation) Period 2, both leafiness and volume had important and similar effects on the variation in number of bites, but they did not have a significant effect after genotype had been added to the model (Figures 4.4, 4.5). This result demonstrates that the variations between genotypes were unlikely to be completely explained by either leafiness or volume. In the third analysis (ECT as covariate) Period 2, ECT concentration and leafiness both had important effects on the improvement of R-square (Table 4.41). However, both variables reduced the effect of the other, so that after adding one covariate to the model the other covariate was not significant (Figure 4.6). In this analysis, Period 3, variation between R-squares was small and ECT concentration and morphological characteristics were not important in explaining the number of bites (Figure 4.7). Most of the variation was probably explained by the variation between sequences of plants, which had the greatest sums of square in the analysis of variance in this period.

In E6, the first and second analyses showed that in the first period either leafiness or volume were important to explain the variation between genotypes in number of bites (Table 4.42, Figures 4.8, 4.9, 4.10, 4.11). However in the second period leafiness was more effective than volume in explaining the difference between genotypes. Volume in this case was important to explain the variation in number of bites but was not related to the genotype effect. The third covariance analysis showed that most of the variation in number of bites between BT genotypes apparently was related more to plant morphological characteristics than to ECT concentration (Table 4.42, Figures 4.12, 4.13). ECT concentration did not have a significant effect when added after plant morphological characteristics. However, when adding either leafiness or volume after ECT concentration, they still showed a significant effect on R-square improvement. Although volume also had an important effect on variation in number of bites, it did not seem to be related to ECT concentration.

Conclusions from the covariance analysis can be summarised as follows. In E5 Period 2, plant morphology and ECT concentration both had important effects on number of bites. The modification of one characteristic might change the effect of the other in cattle diet selection. In E5 Period 3 and E6, plant morphology had the major effect on variation in number of bites. In this case, most of the apparent effect of ECT concentration on number of bites could be accounted for by correlated differences in plant morphology.

The correlation and covariance analyses showed similar results. Both analyses demonstrated that the greater part of the variation in number of bites between genotypes was explained by leafiness. They also agreed in showing the greatest importance of the ECT concentration effect on number of bites in Period 2 of E5, which was associated with the highest ECT concentration found over the two experiments. Condensed tannins have been thought to decrease forage intake by inhibition of digestion (Fenny, 1969; Roades and Cates, 1976; Swain, 1979; Barry, 1989). However, this result indicates that they also have an immediate effect on preference. Provenza and Malechek (1984) also observed rejection by goats of shrubs containing high concentrations of tannin and high levels of energy. They concluded that goat nutrition was affected more by the adverse effects that tannins had on palatability than by the negative effects they had on digestibility.

A concentration of 3.2% of ECT was apparently high enough to reduce preference. Studies, involving different animal and plant species and different management conditions have shown different responses to tannin concentration. Donelly and Anthony (1969), for example, reported that the condensed tannin level required for rejection by grazing animals was as low as 0.2% of DM. Barry (1989), summarising several studies reported that concentrations of condensed tannin at or above 0.63 % depress food intake by herbivores. More recently, Waghorn et al. (1990) suggested values exceeding 5.5% depress voluntary intake. However, there is no evidence in the literature suggesting levels of condensed tannin that promote an immediate effect on cattle preference.

According to previous studies the immediate effect of condensed tannin observed in these experiments could be explained either by the taste of tannin (Arnold et al. 1980; Van Soest, 1994) or by an association between the taste and aversive post-ingestive consequences (Provenza and Malechek, 1984; Provenza et al., 1990; Provenza, 1995).

There is no evidence in the literature that animals can smell tannin. Provenza et al. (1990) explain that animals learn to avoid plants with high concentration of condensed tannin (CT) because of the internal malaise promoted by CT and not because of its flavour. According to Provenza (1995) this post-ingestive feedback is a rapid process, being less than one hour in goats. However Van Soest (1994) argued that tannin has astringent properties caused by the precipitation of salivary mucoprotein, which has an important effect on taste. According to what was reported by Provenza (1995) the difference of one hour between rumen manipulation and the preferential behaviour observation in this study was probably enough for the animal to show aversive consequences of tannin. However the low concentration of ECT and small contrast between the materials added to the rumen in E6 (see section 4.4.1 and Appendix 4.10), precluded a conclusive explanation of the influence of rumen manipulation on preferential behaviour. In addition, the animal training experience with similar plant sequences, one week before running each trial probably did not provide each cow with enough herbage to cause post-ingestive feedback. Futher research is still needed to explain how the immediate effects of condensed tannin content influence animal preferential behaviour.

In the context of rumen manipulation, as discussed in section 4.4.1, the low response to different concentrations of each material (either *Lotus* species or red clover cultivars) inserted into the rumen is probably the reflection of the limited secondary compound concentrations and contrasts found in each material (see Appendix 4.10). However, higher preference for RC when red clover was added to the rumen (Table 4.29) might show that cattle diet selection could be influenced positively by the rumen content composition. The positive post-ingestive feedback in cattle diet selection observed in this research was also reported by Pfister et al. (1997), where highly nutritious plants caused positive animal response toward a greater intake of the same plant. The influence of rumen content on animal diet selection has been demonstrated by recent studies with sheep. Carter and Grovum (1990b) and Hou (1991) in Cooper et al. (1995) have shown that sheep can make rapid changes in their diet selection as a result of manipulation of rumen environment. Cropper (1987) in Cooper et al. (1995) and Parsons et al. (1994a) suggested that ruminants appear to select, from two feeds that differ in nutrient density or digestibility, a diet that enables their rumens to remain in a fit and adaptive state. However, future studies need to be carried out to confirm and give a better understanding of these effects.

4.5. CONCLUSIONS

1) The technique using plant sequences with substantial genotype contrasts in morphology and secondary compound concentration was effective in determining the preference of cattle.

2) Plant morphology played a very important role in diet selection. The animals seemed to prefer large, dense and leafy plants to small, sparse and stemmy plants, and showed an immediate discrimination between plants on the basis of morphology.

3) Differences in formononetin concentration between red clover plants were influenced more by plant morphological characteristic than by seasonal effects. Plant morphology was substantially more important than formononetin concentration in affecting diet selection by cattle.

4) The concentration of 3.2% of ECT in birdsfoot trefoil was apparently high enough to cause an immediate negative effect on preference. Concentrations below 1.8 % apparently did not have an immediate influence on cattle preference. However this effect was also confounded with plant morphology characteristics (mainly leafiness) between genotypes. On the other hand, the contrast of animal preference between periods was associated with variation in ECT concentration. This suggests a basis for seasonal variations in preference for birdsfoot trefoil.

5) The composition of rumen content affected diet selection by cattle. The preference for red clover when red clover was inserted in the rumen suggests that there is a

positive post-ingestive feedback that affects cattle preference. Although no effects of tannin and formononetin concentration were found when inserted in the rumen, this conclusion needs to be treated with caution since only low concentrations and contrasts of these secondary compounds were used in these studies. Further research is required to clarify this result.

CHAPTER 5

GENERAL DISCUSSION

The main objective of Experiments 5 and 6 (E5 and E6) was to provide an explanation for the selective behaviour observed in Experiments 1, 2 and 3 (E1, E2 and E3) in relation to how physical and biochemical plant characteristics influenced animal preference and to what extent this influence explained seasonal variations in preference. This general discussion will therefore focus on these issues. However, it is important to distinguish animal response between experiments. In E1, E2 and E3 the animals expressed their selection ("preference modified by opportunity", Hodgson, 1979). In E5 and E6, although preferential behaviour was also modified by variations in plant morphology or structure, similar opportunity for access to a range of spaced plants was provided and the animals could express their preference with little constraint. This discussion will deal first with the general pattern of selective behaviour observed in E1, E2 and E3, and then on the deviation from the general behaviour in response to specific sward characteristics and possible explanations for this behaviour.

The overall partial preference demonstrated between the birdsfoot trefoil/white clover (BW) and red clover (RC) swards was close to 50:50 (based on grazing time) in E1 and E3 and 40:60 in E2 (section 3.4.4). There was thus relative stability of partial preference across experiments, matching well with the non-significant difference in preference between birdsfoot trefoil (BT) and RC demonstrated in an overall analysis of E5 (5.9 vs 5.4 ± 0.637 bites/plant of BT and RC respectively, P = 0.1066) and to the value close to 50:50 reported by Torres-Rodiguez (1997). However, the partial preference was shown to be sensitive to manipulations of sward height, herbage mass and plant morphology. This pattern of general stability but responsiveness to specific manipulation is also apparent in the series of studies on combinations of perennial ryegrass and white clover swards by Newman et al. (1992); Parsons et al. (1994a) and Cosgrove et al., (1996).

Observations showed that animals did not graze randomly but modified behaviour to meet their preference for a mixed diet. This was demonstrated on Day 1 of E1, when animals allocated a disproportionately large time to graze in the minor swards (Table 3.8, Figure 3.3), even though they could have satisfied appetite by grazing only in one sward. This result is in agreement with Parsons et al. (1994a) who found that sheep did not graze at random when different proportions in ground area of white clover in relation to perennial ryegrass were offered. In addition, although E5 and E6 showed that the preference between BT and RC was largely influenced by plant height and herbage mass, in E1 Day 1, when herbage mass and height of both swards were relatively high and both sward were in similar maturity stage, the animals allocated proportionally more time (in relation to herbage mass offered) grazing on the sward with lower mass (Table 3.9, Figure 3.8). This behaviour also demonstrated that, with high levels of herbage mass and height, animals preference for a mixed diet was stronger than the desire to graze swards which were taller or with greater herbage mass.

The objective of obtaining a mixed diet was also modified by sward physical characteristics. E5 and E6 clarified these effects. In E1, E2 and E3, the animals demonstrated selection for swards with greater herbage mass and height (except in E1 Day 1 when the swards were in similar maturity stage, and sward height and herbage mass was high - see above) and proportion of leaves. This selective behaviour was illustrated by the selection of swards with greater herbage mass in Day 3 of E1 (Table 3.9, Figure 3.8), and Day 1 and 3 of E3 (see section 3.3.3.1 - *Grazing time per kg of DM offered)*, by the selection of swards with greater height in Day 1 of E2 (Tables 3.11, 3.19) and E3 (Tables 3.23, 3.30) and by the selection of swards with greater leaf/stem ratio in E2, mainly in Day 1 (Tables 3.12, 3.19). In E5 and E6, when given a greater opportunity to demonstrate their preference, the animals also showed preference for plants with greater height, volume, density and higher proportion of leaves (Tables 4.5, 4.19, 4.20). These results therefore confirmed that the objective of obtaining a mixed diet in E1, E2 and E3 was strongly influenced by the sward physical characteristics.

The preference observed in E5 and E6 for taller plants, with larger area and a greater proportion of leaves can explain the sward maturity effect on selection in E2. The selection for RC rather than BW sward in E2 was probably related to the greater herbage mass, height and higher proportion of leaves of RC than of BT. The high correlation in E5 and E6 between leafiness and number of bites, mainly for BT plants (Tables 4.17, 4.36), the substantial effect of leafiness when included as a covariate in the analysis of variance of number of bites taken from BT plants (covariance analyses Figures, 4.2 - 4.13) and the pattern of behaviour across experiments in the discussion of Chapter 3 (Table 3.36) showed that plant leafiness (or leaf/stem ratio) had a strong effect on selection. Therefore, considering the fact that variation in selective behaviour between seasons was closely related to plant maturity (see discussion in Chapter 3), it can be concluded that in swards formed mainly by BT or RC the variation in selection across seasons is mainly related to variation of leaf/stem ratio contrasts between swards, though this effect may be modified by sward structure (mainly height and herbage mass). This complex of physical effects on diet selection agrees with the conclusion of Real-Ferreiro (1997), where preference of RC cultivars could not be determined by simple morphological characters alone.

In addition to sward physical characteristics, biochemical characteristics could also have affected selection (Hodgson et al., 1994; Provenza, 1995; Launchbaugh, 1996). In E1, E2 and E3 it was not clear how secondary compounds affected selective behaviour, how they were related to the sward physical characteristics or whether they made any contribution to the variation in selective behaviour observed in different periods of the year. E5 and E6 clarified the effect of the formononetin concentration in RC and ECT concentration in BT on animal preference.

In E5 and E6 the animals were substantially more directly influenced by the morphological characteristics of RC than by the concentration of formononetin, agreeing with the conclusions of Harborne (1993). In both sets of experiments there was a relatively small variation, within each RC genotype among different experiments and periods, in formononetin concentration, smaller than that observed by Anwar (1994), suggesting that formononetin concentration would not have influenced the seasonal variation in selection observed in E1, E2 and E3. In addition to the small direct effect of formononetin concentration observed in E5 and E6, it also did not have an important post-ingestive feedback effect in E6: considering only the case where RC was inserted into the rumen, there was no significant effect of formononetin concentration on preference for RC cultivars. Therefore, it can be concluded that the concentration of formononetin apparently did not contribute to the variation in selective behaviour observed in E1, E2 and E3.

E5 and E6 also provided an understanding of the effect of ECT concentration on preference and its relation with plant morphological characteristics. The correlation and covariance analyses carried out in E5 and E6 showed that animals responded immediately by selecting against the BT genotype containing high ECT concentration. However, the negative effect of ECT concentration on preference was confounded with variation in plant morphology. The main confounding effect observed was between plant leafiness and ECT concentration: plant leafiness had a positive effect on preference, ECT concentration had a negative effect. These experiments also showed that the effect of ECT concentration on animal preference was observed only in concentrations of approximately 3.2% (Period 2 of E5). Concentrations below approximately 1.8% (Period 3 of E5) did not have an immediate effect on preference. In addition to these results, the modification of rumen content in E6 also demonstrated that low levels of ECT concentration in the rumen did not have an important effect on preference for BT genotypes (Table 4.30). Although several studies and reports (Provenza and Malechek, 1984; Provenza et al., 1990; Provenza et al., 1994; Provenza, 1995) argue that condensed tannin causes a negative post-ingestive feedback on preference, in E6 preference was not affected by the low rumen loading with ECT. It can be concluded that the selective behaviour in E1, E2 and E3, where there was a relatively low range of variation of ECT in BT (0.54 to 1.69 %) (Table 3.36), was unlikely to have been affected by ECT concentration. However, further studies on the effect of higher ECT concentrations in the rumen on the post-ingestive feedback of condensed tannin need to be carried out for a better understanding of the cattle selective response to condensed tannin.

Whittaker and Feeny (1971), Roades and Cates (1976) and Lindroth (1989) suggested that condensed tannin acts as a protection against herbivory. The higher concentration of ECT in leaves than in stems (Table 4.8, 4.25), demonstrated also by the significant correlations between ECT concentration and leafiness (Tables 4.15, 4.34), and the fact that relatively high concentrations of ECT reduce preference for BT, suggest a hypothesis that the plant produces ECT concentration in the most preferred plant part to inhibit grazing. Thus, it can be inferred that relatively high ECT concentration in leaves of BT may increase the persistence of birdsfoot trefoil in a mixed sward by reducing the preference for this species, if persistence of BT is adversely affected by selective grazing. However, the fact that the animals were attracted by leaves of BT and rejected stems in E1, E2 and E3 indicated that the low ECT concentration was not effective in protecting the plants against grazing.

The importance of leaf/stem ratio and secondary compound concentration in affecting the selective and preferential behaviour in these studies shows scope for manipulation of preferential behaviour. In erect legumes like BT and RC, stems make an important contribution to the total herbage mass, but also affect negatively preferential behaviour. Therefore, it can be inferred that sward managements or plant breeding programmes that increase the leaf/stem ratio of either BT or RC will result in an improvement in animal preference. However the increase in proportion of leaves in BT needs to be associated with low ECT concentration. Leaves of BT have much higher concentration of ECT than stems (Tables 4.8, 4.25), indicating a potentially negative effect on preference.

The selective behaviour observed in E1, E2 and E3, where the animals grazed to obtain a mixed diet and were also influenced by sward physical characteristics, may be explained by several hypotheses proposed in the literature. The hypothesis that selection is related to the secondary compound concentration present in the sward and that the animals prefer a mixed diet to minimise the risk of toxicity (Freeland and Janzen, 1974; Laycock et al., 1988) was not relevant in this experiment because the two most important secondary compounds in the swards did not have any apparent effect on diet selection. However it is important to recognise that there is a potential effect on preference of ECT when in high concentrations in BT and that the current studies were limited only to the effects of the major secondary compounds (ECT and formononetin) of BT and RC.

The other possible hypothesis is that animals graze to obtain a balanced diet (Newman et al., 1992). The overall stability of partial preference across E1, E2 and E3, discussed in the beginning of this chapter, associated with the fact that the animals grazed the minor sward component when both swards had relatively high mass and height and were in a similar stage of maturity, suggests that the animals may have targeted a balanced diet reflecting partial preference for BW and RC close to 50:50. However, at relatively low herbage mass and sward surface height, the animals modified their behaviour and selected the taller and greater herbage mass sward, grazing in proportion to the herbage mass offered (E1 -Table 3.9, Figure 3.7; E3 - section 3.3.3.1 - *Grazing time per kg of DM offered)*. This behaviour may indicate that the animals adjusted their selection towards swards that provided potentially greater intake rate. This result also suggests that there is a trade off between preference and intake. Parsons et al. (1994b) and Newman et al. (1995) models explain that changes in diet selection behaviour, to include more of the less preferred species in the diet, may allow animals to maintain intake as the total herbage mass available declines. This interpretation also agrees with studies showing that a choice between alternative forages or patches strongly favours those with greater potential intake rate (Laca et al., 1993; Kenney and Black, 1984; and Black and Kenney, 1984).

The hypothesis that the animal sampled in both swards to constantly reinforce awareness of the sward conditions (Illius and Gordon, 1990; Illius et al., 1987) could also help to explain selective behaviour in these studies. Edwards (1994), working in carefully controlled conditions demonstrated that when food "patches" (bowls containing varying types of pellets) remained in the same location, sheep learned the location quickly and did not need to continue a sampling strategy. However, he also pointed out that changeable sward conditions could make reliance on memory alone of limited effectiveness. In fact, the current studies suggest that sampling was a consistent feature that helped animals to adjust their selection according to changing sward conditions.

GENERAL CONCLUSIONS

The overall conclusions in relation to the most significant findings from these studies on the effects of morphological and biochemical characteristics of birdsfoot trefoil and red clover on cattle preference are summarised below.

1) The methodologies used in the two sets of experiments were effective in determining and explaining grazing behaviour in studies involving animal response to physical and biochemical sward characteristics.

2) There was overall a relative stability in preference between birdsfoot trefoil and red clover across experiments with an average partial preference of approximately 50:50. However, this preference was shown to be flexible once it was modified by sward height, herbage mass, plant morphology and ECT concentration.

3) Birdsfoot trefoil/white clover and red clover sward structures had important effects on selective behaviour. The animals were attracted by the tallest swards with the greatest herbage mass. However, on tall and high mass swards (in these studies, approaching 4000 kg/ha) this selection was modified by the preference for a mixed diet.

4) Plant maturity had an important effect on preferential grazing behaviour, mediated through effects on sward structure and leaf/stem ratio.

5) Formononetin concentration between 0.26 and 0.73 % on a DM basis did not affect preference for red clover. Differences in physical characteristics of the two genotypes of red clover had greater effects on preference than differences in formononetin concentration.

6) Preference of cattle was affected by ECT concentration in birdsfoot trefoil. However this effect was confounded with associated contrasts in plant morphology, mainly leafiness, and was only apparent in relatively high concentrations of ECT (in these studies 3.2% on a DM basis, but not below 1.8% on a DM basis).

7) The absence of discrimination between red clover and a birdsfoot trefoil genotype with low ECT concentration contrasts with the lower preference for a birdsfoot trefoil genotype with high ECT concentration. It may be inferred that animals would not show preference between red clover and birdsfoot trefoil cultivars with low ECT concentration if the physical characteristics were similar. Therefore, it can be concluded that birdsfoot trefoil and red clover were suitable species for determining the effects of sward physical characteristics on selective behaviour.

8) Leaf/stem ratio was one of the most important plant morphological characteristics that affected selection between swards based on birdsfoot trefoil and red clover species. The fact that animals usually preferred leaves and rejected stems caused variations in selection between swards differing in maturity. The preference for leaf also influenced the effect of ECT concentration on preference.

9) Seasonal variability in selection between swards formed by strips of birdsfoot trefoil and red clover species could be explained by the effect of plant maturity where the animals demonstrated preference mainly for swards with high leaf/stem ratio, provided the ECT concentration in BT was not high enough to adversely affect this preference.

10) In addition to the fact that the animals were attracted by swards with greater leaf/stem ratio, three hypotheses provide possible explanations for the selective behaviour observed in E1, E2 and E3: (i) animals tried to obtain a balanced diet; (ii) animals selected swards that provided the potentially higher rate of intake; (iii) animals sampled to constantly reinforce awareness of the sward conditions.

11) Sward managements or plant breeding programmes that increase the leaf/stem ratio of either birdsfoot trefoil or red clover will result in an improvement in animal preference. However this improvement in proportion of leaves in birdsfoot trefoil needs to be associated with low ECT concentration.

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APPENDICES

APPENDIX 3. 1. DMACA-HCL Protocol -using plate reader

Li *et al.* (1996) developed a protocol using HCl-acidified 4-dimethylaminnocinnamaldehyde (DMACA) for screening condensed tannins (proanthocyanidins). They developed a reliable and sensitive method to detect condensed tannin at concentrations lower than 0.4 mg g^{-1} dry matter (DM). The DMCA-HCL protocol is recommended for the detection of condensed tannins in plants with low concentrations.

Considering the methodology described by Li *et al.* (1996) and Terrill *et al.* (1992), a DMACA-HCL protocol was developed to utilize a plate reader with Softpro software. This protocol was used to determine the concentration of extractable condensed tannins in *Lotus corniculatus* L.

FREE OR ACETONE EXTRACTABLE CONDENSED TANNIN

Solutions:

1. **67 mM glycine HCl pH 3.0**
   Dissolve 5.0 g of glycine in about 900 ml of milliQ H_2O. Adjust the pH to 3.0 with conc HCl and adjust the final volume to 1 litre. Filter through a 0.2 μm filter.

2. **Aqueous acetone (20mM glycine-HCl pH 3.0; acetone (30:70 v/v); ascorbic acid (1g l^{-1}))**
   Per litre, combine 700 ml of acetone, 300 ml of 67 mM glycine HCl pH 3.0 and 1 g of ascorbic acid. Adjust pH to 3.0 with conc HCl. Store in a brown bottle away from direct sunlight.

Method - Extraction of CT

1. Weigh 500 mg of freeze dried and very finely ground sample (0.5 mm sieve) into a 50 ml Oakridge centrifuge tube. Do a dry matter (DM) on each sample as well so that the CT concentration can be corrected for DM.

2. Add 10 ml of aqueous acetone solution and homogenize on ice with the Utraturex for about 1 min.

3. Centrifuge at 15,000 rpm (about 22,000x*g*) for 10 min.

4. Transfer the supernatant to a 50 ml bluetop tube. Keep on ice.

5. Add 10 ml of aqueous acetone solution to the residue remaining in the Oakridge tube, and re-homogenize. Centrifuge again and add the supernatant to the bluetop tube from step (4).

6. Add 20 ml of methylene chloride. Vortex vigorously.

7. Leave the tube standing until the phases are completely separated.

8. Transfer the upper aqueous phase to a 50 ml tube, leaving only the lower solvent phase as residual.

9. Rewash the residue remaining in the tube with milliQ H_2O. Leave the phases to separate completely. The washing with milliQ H_2O is important mainly in the first wash to recover any condensed tannin trapped by lipid in the solvent phase.

10. Rewash with the combined aqueous phases with methylene chloride. Continue washing the aqueous phases with methylene chloride until all the chlorophyll and lipids are removed.

11. Once the lower solvent phase is clear (transparent), transfer the aqueous phase to a 250 ml round-bottom flask and remove excess acetone and methylene chloride at 40^0C under reduced pressure.

12. Decant into a 50 ml transport storage tube and make up to 50 g with milliQ H_2O. The sample can be stored at -20^0C at this stage.

COLOURMETRIC DETECTION OF CONDENSED TANNINS - DMACA-HCL PROTOCOL

The colourmetric detection of condensed tannins is performed using a standard curve with a range of condensed tannin concentrations.

Solutions

1. **6M HCl**

Very slowly add 262 ml of concentrated HCl to 200 ml of milliQ H_2O. Make up to 500 ml with milliQ H_2O.

2. **Methanol:6M HCl (1:1 v/v)**

Very slowly add 100 ml of cold 6M HCl to 100 ml of methanol.

3. **2% DMACA-HCl (w/v)**

Dissolve 0.2 g of 4-dimethylaminocinnamaldehyde (DMACA) in 10 g of methanol:6M HCl (1:1 v/v). Make fresh each time that the reagent is required and store in a dark bottle when in use.

Method

Thawing of samples

It is possible to loose sample in the thawing process. Therefore it is essential that all tubes are well closed and no tubes have cracked during freezing.

Standard Curve (applicable for *Lotus corniculatus* L.)

1. Weigh 10 mg of Sephadex LH-20 extract (*Lotus corniculatus* condensed tannin extracted according to Jackson et al., 1996) into a 5 ml bluetop tube. Make up to 5 g with milliQ H_2O. This stock CT solution and is 2 mg ml^{-1}.

2. Using the stock CT solution to make the following standards in 2 ml microcentrifuge tubes.

3.

0 µg ml^{-1}	0 µl of the CT stock and 2000 µl of milliQ H_2O
12.5 µg ml^{-1}	12.5 µl of the CT stock and 1987.5 µl of milliQ H_2O
25 µg ml^{-1}	25 µl of the CT stock and 1975 µl of milliQ H_2O
37.5 µg ml^{-1}	37.5 µl of the CT stock and 1962.5 µl of milliQ H_2O
50 µg ml^{-1}	50 µl of the CT stock and 1950 µl of milliQ H_2O
75 µg ml^{-1}	75 µl of the CT stock and 1925 µl of milliQ H_2O
100µg ml^{-1}	100 µl of the CT stock and 1900 µl of milliQ H_2O
125 µg ml^{-1}	125 µl of the CT stock and 1875 µl of milliQ H_2O
150 µg ml^{-1}	150 µl of the CT stock and 1850 µl of milliQ H_2O
175µg ml^{-1}	175 µl of the CT stock and 1825 µl of milliQ H_2O
200 µg ml^{-1}	200 µl of the CT stock and 1800 µl of milliQ H_2O
225 µg ml^{-1}	225 µl of the CT stock and 1775 µl of milliQ H_2O
250 µg ml^{-1}	250 µl of the CT stock and 1750 µl of milliQ H_2O
275 µg ml^{-1}	275 µl of the CT stock and 1725 µl of milliQ H_2O
300µg ml^{-1}	300 µl of the CT stock and 1700 µl of milliQ H_2O

Plate Reader

1. Add 100 µl of each standard to a separate well in the culture or microtitre plate. Use the first 15 wells in the plate for the standard curve (A1-B3).

2. Add 100 µl of each sample to a separate well in the culture plate. Analyze each sample in duplicate.

3. Add 100 µl of milliQ H_2O to each well containing standards and samples.

4. Add 100 µl of methanol to each well containing standards and samples.

5. Add 50 µl of fresh DMACA-HCl reagent to each well containing standards and samples.

6. With the addition of the DMACA-HCL reagent, ensure that the contents in each well are thoroughly mixed with a multi-channel pipette.

7. Allow the assay to develop at room temperature for 20 minutes.

8. Read the absobance at 643 nm.

Computer and plate reader

Turn on the computer and the plate reader at least 15 minutes before using.

Adjust the wavelength to 643nm and enter the concentration of each standard and indicate the location of the standards and unknowns in the culture plate into the softpro software.

A standard with a concentration of 25 µg ml^{-1} will have 2.5 µg in the well.

$25\ \mu g\ ml^{-1} = 25\ ng\ \mu l^{-1}$

using 100 µl

$25 ng\ \mu l^{-1} \times 100\ \mu l = 2500\ ng = 2.5\ \mu g$ in the well

APPENDIX 3. 2. Rainfall and soil temperature – Experiments 1, 2, 3 (Chapter 3)

Table 3.1. Daily rainfall and average soil temperature (10 cm depth) during the experiment 1 at Flock House - from 30 October/95 to 27 November/95.

Date	*Average soil temp. (^{0}C)*	*Rainfall (mm)*
30-Oct	11.2	0.0
31-Oct	11.5	1.5
01-Nov	11.7	28.1
02-Nov	10.8	16.9
03-Nov	10.4	0.0
04-Nov	11.1	4.6
05-Nov	11.0	1.8
06-Nov	10.9	1.8
07-Nov	10.6	0.0
08-Nov	11.3	0.0
09-Nov	11.9	6.7
10-Nov	12.6	15.4
11-Nov	12.3	0.3
12-Nov	11.7	0.8
13-Nov	11.7	0.0
14-Nov	12.0	0.0
15-Nov	11.6	0.0
16-Nov	11.5	0.0
17-Nov	12.0	0.0
18-Nov	11.9	0.0
19-Nov	11.7	0.0
20-Nov	11.3	8.2
21-Nov	10.5	0.8
22-Nov	10.4	0.0
23-Nov	10.5	0.0
24-Nov	11.1	3.6
25-Nov	12.3	11.8
26-Nov	11.9	5.4
27-Nov	10.4	9.7

APPENDIX 3.2. Rainfall and soil temperature – Experiments 1, 2, 3 (Chapter 3)

Table 3.2. Daily rainfall and average soil temperature (10 cm depth) during the experiment 2 at Flock House - from 5 February/96 to 1 March/96.

	Average soil temp. (^0c)	*Rainfall (mm)*
5-Feb	14.10	0.60
6-Feb	14.10	0.00
7-Feb	14.40	16.10
8-Feb	14.50	0.00
9-Feb	14.00	0.00
10-Feb	14.00	0.00
11-Feb	13.50	0.00
12-Feb	14.80	0.00
13-Feb	15.30	1.00
14-Feb	15.10	0.00
15-Feb	14.60	0.00
16-Feb	13.90	0.00
17-Feb	13.80	0.00
18-Feb	14.30	4.90
19-Feb	15.30	4.40
20-Feb	14.50	18.90
21-Feb	13.20	1.50
22-Feb	11.80	18.70
23-Feb	11.90	3.60
24-Feb	10.80	0.00
25-Feb	11.10	0.00
26-Feb	11.80	0.00
27-Feb	12.10	0.00
28-Feb	12.80	0.00
29-Feb	13.10	0.00
1-Mar	13.10	0.00

APPENDIX 3.2. Rainfall and soil temperature – Experiments 1, 2, 3 (Chapter 3)

Table 3.3. Daily rainfall and average soil temperature (10 cm depth) during the experiment 3 at Flock House - from 15 April/96 to 10 May/96.

	Average soil temp. (^0c)	*Rainfall (mm)*
15-Apr	11.80	3.80
16-Apr	11.70	2.60
17-Apr	11.00	0.00
18-Apr	9.90	0.00
19-Apr	10.20	8.70
20-Apr	11.00	9.50
21-Apr	11.40	19.40
22-Apr	10.10	0.00
23-Apr	9.30	0.00
24-Apr	9.20	0.00
25-Apr	8.30	0.00
26-Apr	7.70	0.00
27-Apr	8.60	0.00
28-Apr	9.40	0.80
29-Apr	9.30	0.30
30-Apr	9.60	6.40
1-May	8.90	3.60
2-May	6.90	0.00
3-May	6.90	0.00
4-May	6.40	0.00
5-May	6.70	0.00
6-May	6.80	0.00
7-May	7.60	0.00
8-May	8.10	2.20
9-May	7.80	2.10
10-May	6.10	0.00

APPENDIX 3. 3. Experiment 1 (Chapter 3)

Table 3. 4. Herbage mass (kg DM/ha), sward height (cm) and bulk density (mg DM/cm^3) before and after grazing, and estimation of the herbage mass removed (kg DM/ha) of birdsfoot trefoil and white clover (BW) and red clover (RC) swards according to treatment (area ratios) in Experiment 1.

	Treatments A		B		C		D		*SED*[1]	*P-value*[2]
	BW 20%	RC 80%	BW 33%	RC 67%	BW 67%	RC 33%	BW 80%	RC 20%		
Herbage mass (Kg DM/ha)										
Pre-graz	3930	4500	4020	4580	3930	4670	3880	4530	328	0.9802
Post graz	1930	3060	2450	3360	2560	3650	2580	3280	291	0.7261
DM rem.	2000	1450	1560	1210	1370	1020	1310	1250	412	0.8599
Sward height (cm)										
Pre-graz.	19.8	26.1	19.0	29.1	19.3	29.2	18.4	26.3	2.68	0.7204
after 1 day	11.5	24.5	11.5	23.3	12.6	21.2	13.6	19.3	1.71	0.0597
Post graz.	6.8	13.8	6.8	13.4	8.0	14.2	8.3	11.7	0.70	0.0098
Bulk density (mg DM/cm^3)										
Pre-graz	2.00	1.77	2.12	1.57	2.04	1.60	2.10	1.74	0.222	0.7732
Post graz	2.73	2.16	3.70	2.48	3.18	2.63	3.08	2.78	0.433	0.5002

[1] SED– Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

Table 3.5. The effect of treatments (area ratios) on rate of biting (bites/minute) in the first, second and third days (Day 1, 2 and 3) (total 55 hours) of grazing assessment in Experiment 1.

	Treatments A		B		C		D		*SED*[1]	*P-value*[2]
	BW 20%	RC 80%	BW 33%	RC 67%	BW 67%	RC 33%	BW 80%	RC 20%		
Rate of biting (bites/min)										
Day 1	49.5	45.4	52.7	45.0	48.2	44.1	47.6	45.8	1.45	0.0702
Day 2	51.2	43.8	53.8	46.9	53.5	45.9	52.1	48.0	2.70	0.7468
Day 3	55.1	49.0	54.7	46.8	55.3	47.3	52.2	47.1	5.60	0.9794

[1] SED– Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

APPENDIX 3. 3. Experiment 1 (Chapter 3)

Table 3.6. Botanical characteristics of birdsfoot trefoil and white clover (BW), and red clover (RC) swards before and after grazing, according to the treatments (area ratios) (DM basis): (a) percentage of components in live fraction, (b) percentage of live matter in total DM of each sward and (c) ratio of the total live matter of birdsfoot trefoil and white clover (B:W) in the BW sward, Experiment 1.

	Treatment A		*Treatment B*		*Treatment C*		*Treatment D*			
	BW 20%	RC 80%	BW 33%	RC 67%	BW 67%	RC 33%	BW 80%	RC 20%	SED[1]	P-value[2]
Pre-grazing										
(a) Leaf	41.2	39.2	44.0	37.6	41.6	40.4	49.2	47.2	5.78	0.9135
Petiole	13.6	17.8	25.4	14.7	26.0	16.8	13.1	13.8	5.71	0.3208
Stem	15.9	34.1	17.9	41.9	12.9	33.9	15.4	27.2	5.17	0.4100
Flower	0.7	0.7	0.8	1.2	1.3	0.8	0.4	1.1	0.61	0.6000
Grass	10.6	1.1	2.7	0.0	8.9	1.9	11.2	4.4	5.73	0.8880
Other species	16.7	5.7	7.8	2.9	11.2	8.0	12.6	8.4	5.97	0.7900
(b) Total live matter	97.3	91.7	97.0	92.9	96.1	91.4	92.4	89.8	1.85	0.7185
(c) B:W ratio	0.82		0.79		0.45		0.66		0.436	0.8220
Post-grazing										
(a) Leaf	29.3	20.0	25.6	16.6	28.1	17.7	28.2	14.8	5.12	0.9237
Petiole	19.9	16.9	28.9	14.8	37.7	13.8	30.0	19.4	3.05	0.0128
Stem	23.2	50.4	42.3	51.3	20.4	52.3	24.9	38.4	6.74	0.0707
Flower	0.4	0.4	0.5	0.5	1.4	2.1	0.5	0.3	1.06	0.9101
Grass	12.5	0.3	0.4	0.1	5.3	5.3	2.9	16.4	7.63	0.1522
Other species	12.9	10.3	0.4	16.3	8.6	9.4	14.3	11.6	5.81	0.0940
(b) Total live matter	86.4	85.9	93.0	81.3	94.1	83.3	91.7	86.1	3.48	0.1065
(c) B:W ratio	0.47		0.47		0.25		0.41		0.221	

[1] SED – Standard error for differences of means when comparing means with the same level of treatments.
[2] P-value of the interaction: treatment*sward type effect.

APPENDIX 3.4. Experiment 2 (Chapter 3)

Table 3.7. Formononetin concentration (%) of leaf, petiole, stem and flower of birdsfoot trefoil (BT) and white clover (WC) in birdsfoot trefoil and white clover sward (BW), and red clover (RC) in red clover sward (RC), according to the treatments (sward maturity: Imm = immature; Mat = mature) (DM basis) in Experiment 2.

	BW sward				*RC sward*			
	BT		*WC*		*RC*		*SED*[1]	*P-value*[2]
	Imm	Mat	Imm	Mat	Imm	Mat		
Leaf	0.11	0.13	0.14	0.20	0.54	0.46	0.745	0.9000
Petiole	-[3]	-	0.18	0.24	0.50	.35	0.064	0.7460
Stem	0.11	0.10	-	-	0.41	0.25	0.051	0.0938
Flower	0.07	0.06	0.15	0.11	0.11	0.10	0.021(BT)[3] 0.018(WC)[4] 0.015(RC)[5]	0.3840

[1] SED — Standard error for differences of means when comparing within each species
[2] P-value of the interaction: species*maturity effect
[3] - no sample
Number of observations contributing to the mean of each specie (n=4), but n=3(immature) and n=2(mature) for birdsfoot trefoil, n=3 for white clover and n=4 for red clover in flower assessment.

Table 3.8. The effect of treatments (maturity: Imm = immature, Mat = mature) on grazing time (minutes) in the first, second and third days of grazing (Days 1, 2 and 3), and average DM intake per animal per day (kg dm/hd/day) during 55 hours of grazing in Experiment 2.

	Treatments									
	A		B		C		D		*SED*[1]	*P-value*[2]
	BW Imm	RC Imm	BW Imm	RC Mat	BW Mat	RC Imm	BW Mat	RC Mat		
Grazing time (min)										
Day 1	28.7	42.9	39.5	41.5	29.4	49.0	29.7	46.1	9.93	0.6236
Day 2	34.5	49.0	31.2	46.7	34.4	59.2	34.3	54.4	8.81	0.8312
Day 3	38.7	53.1	22.5	59	41.2	51.3	26.7	58.9	10.23	0.2260
Intake (Kg DM/hd/day)	4.29	2.33	5.20	5.69	5.28	3.30	4.66	4.47	1.437	0.5293

[1] SED– Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

APPENDIX 3. 4. Experiment 4 (Chapter 3)

Table 3. 9. Herbage mass (kg DM/ha), sward height (cm) and bulk density (mg DM/cm^3) pre-grazing (Pre-graz.), and sward height post grazing (post graz.) of birdsfoot trefoil and white clover (BW) and red clover (RC) according to treatment (sward position - side: close to the fence; central: in the middle of the plot; altern.= alternative sward) in Experiment 4.

	Treatments								SED[1]	P-value[2]
	A		B		C		D			
	BW side	RC altern.	BW central	RC altern.	BW altern.	RC side	BW altern.	RC central		
Herbage mass (Kg DM/ha)										
Pre-graz	2024	2280	2150	2270	2110	2190	1910	2070	307	0.9829
Sward height (cm)										
Pre-graz.	7.0	8.8	7.1	7.0	7.0	7.5	7.0	6.8	0.743	0.2655
Post graz	4.6	5.0	4.1	4.7	4.1	4.0	4.4	4.1	0.5454	0.6565
Bulk density (mg DM/cm^3)										
Pre-graz	2.82	2.64	3.05	3.36	3.09	2.96	2.76	2.97	0.4324	0.8170

[1] SED– Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

Table 3.10. The effect of treatments (strip position - side: close to the fence; central: in the middle of the plot; altern.= alternative sward) on grazing time (minutes in three hours of observation) in Experiment 4.

	Treatments								SED[1]	P-value[2]
	A		B		C		D			
	BW Imm	RC Imm	BW Imm	RC Mat	BW Mat	RC Imm	BW Mat	RC Mat		
Grazing time (min)										
Day 1	46.3	95.0	44.5	103.1	92.2	46.9	87.6	43.3	5.948	0.0000

[1] SED-- Standard error for differences of means when comparing swards within each treatment.
[2] P-value of the interaction between treatment and sward type.

APPENDIX 3. 5. Grazing time – morning observation (Chapter 3)

Table 3.11.Treatment (20, 33, 67 and 80 % of the total area offered) effects on the proportion of grazing time (in relation to the total grazing time spent in plot) devoted to birdsfoot trefoil plus white clover swards (BW) in Experiment 1.

	Proportion of area of BW				*Mean*
	20%	*33%*	*67%*	*80%*	
Day 1 – evening	0.35	0.46	0.60	0.69	
Day 2 – morning	**0.20**	**0.33**	**0.67**	**0.80**	**0.50**
Day 2 – evening	0.15	0.26	0.70	0.80	
Day 3 - evening	0.09	0.20	0.66	0.79	
Means Day 1 and Day 2 evening	**0.25**	**0.36**	**0.65**	**0.75**	**0.50**

Table 3. 12.Treatment effects on the proportion of grazing time (in relation to the total grazing time spent in the plot) devoted to birdsfoot trefoil and white clover swards (BW) in Experiment 2 Treatment A= BW and RC immature; Treatment B=BW immature and RC mature; Treatment C= BW mature and RC immature; Treatment D= BW and RC mature).

	Treatments				*Means*
	A	B	C	D	
Day 1 –evening	0.39	0.51	0.39	0.41	
Day 2 – morning	**0.46**	**0.44**	**0.42**	**0.36**	**0.42**
Day 2 – evening	0.42	0.39	0.38	0.38	
Day 3 - evening	0.42	0.27	0.46	0.34	
Means Day 1 and Day 2 evening	**0.41**	**0.45**	**0.38**	**0.40**	**0.41**

Table 3.13.Treatment effects on the proportion of grazing time devoted to birdsfoot trefoil and white clover swards (BW) in Experiment 3 Treatment A= BW and RC short; Treatment B=BW short and RC tall; Treatment C= BW tall and RC short; Treatment D= BW and RC tall)

	Treatments				*Means*
	A	B	C	D	
Day 1 –evening	0.46	0.29	0.63	0.46	
Day 2 – morning	**0.54**	**0.37**	**0.52**	**0.49**	**0.48**
Day 2 – evening	0.52	0.41	0.59	0.54	
Day 3 - evening	0.44	0.34	0.51	0.50	
Means Day 1 and Day 2 evening	**0.49**	**0.35**	**0.61**	**0.50**	**0.45**

APPENDIX 3. 6. Grazing time – morning observation (Chapter 3)

ANALYSES OF VARIANCES (ANOVA)

ANOVA of total grazing time of morning observation (GTMORN1) of Day 2, Experiment 1

DATA = GTMORN1

Error: PERIOD:GROUP

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
TREATM	3	160.6480	53.54934	0.6148842	0.6224212
Residuals	9	783.7964	87.08849		

Error: Within

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
SPECIE	1	444.443	444.443	8.91360	0.008738614
TREATM:SPECIE	3	4202.219	1400.740	28.09278	0.000001299
Residuals	16	797.779	49.861		

ANOVA of total grazing time of morning observation (GTMORN2) of Day 2, Experiment 2

DATA = GTMORN2

Error: PERIOD:GROUP

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
TREATM	3	270.781	90.2604	0.5596006	0.6548896
Residuals	9	1451.649	161.2944		

Error: Within

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
SPECIE	1	2363.906	2363.906	9.954745	0.0061291
TREATM:SPECIE	3	480.747	160.249	0.674831	0.579962
Residuals	16	3799.444	237.465		

APPENDIX 3.6. Grazing time – morning observation (Chapter 3)

ANOVA of total grazing time of morning observation (GTMORN3) of Day 2, Experiment 3

DATA = GTMORN3

Error: PERIOD:GROUP

	Df	Sum of Sq	Mean Sq	F Value	Pr(F)
TREATM	3	546.8519	182.2840	1.750791	0.2262231
Residuals	9	937.0370	104.1152		

Error: Within

	Df	Sum of Sq	Mean *Sq*	*F* Value	Pr(F)
SPECIE	1	71.111	71.1111	0.329472	0.5739513
TREATM:SPECIE	3	797.778	265.9259	1.232089	0.3306272
Residuals	16	3453.333	215.8333		

APPENDIX 4. 1. Rainfall and temperature – Experiments 5 and 6 (Chapter 4)

Table 4.1. Monthly rainfall and average soil temperature (10 cm depth) from July/1996 to June/1997 compared with 60 years average values at the site.

	Total monthly rainfall	*Total monthly rainfall (av. 60-years)*	*Av. daily 10cm soil temp. (^0C)*	*Av. daily 10cm soil temp. (^0C) (av. 60-years)*
Jul-96	104.8	89.0	8.0	6.7
Aug-96	82.3	89.0	8.1	7.6
Sep-96	102.8	75.0	11.5	9.9
Oct-96	95.6	88.0	13.7	12.5
Nov-96	100.5	78.0	14.4	15.1
Dec-96	91.1	94.0	16.7	17.3
Jan-97	68.0	79.0	17.6	18.5
Feb-97	58.0	67.0	17.7	18.1
Mar-97	68.1	69.0	15.8	16.3
Apr-97	144.7	81.0	12.5	13.2
May-97	24.3	89.0	11.6	10.1
Jun-97	60.4	97.0	8.1	7.7

APPENDIX 4. 2. Equations to estimate herbage mass per plants of birdsfoot trefoil and red clover, using probe GrassMaster reading (X)

BIRDSFOOT TREFOIL

Herbage mass = 1.3317 X - 9.6061 (R-square = 0.3653)

RED CLOVER

Herbage mass = 0.7902 X + 913.82 (R-square = 0.1958)

APPENDIX 4.3. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 5 - plant nutritional characteristics

BIRDSFOOT TREFOIL PLANTS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 8

	PROTEIN	LIPID	ADF	NDF	CHO	ASH	INVITRO	BITES
PROTEIN	1.00000 0.0	0.83385 0.0101	-0.80969 0.0149	0.70706 0.0498	-0.46862 0.2415	-0.31500 0.4473	0.81069 0.0146	0.62870 0.0950
LIPID	0.83385 0.0101	1.00000 0.0	-0.77012 0.0254	0.43637 0.2797	-0.23585 0.5739	0.08864 0.8347	0.77082 0.0252	0.79053 0.0195
ADF	-0.80969 0.0149	-0.77012 0.0254	1.00000 0.0	-0.19217 0.6484	-0.11968 0.7777	0.33045 0.4240	-0.99992 0.0001	-0.30720 0.4592
NDF	0.70706 0.0498	0.43637 0.2797	-0.19217 0.6484	1.00000 0.0	-0.90728 0.0019	-0.33505 0.4172	0.19188 0.6490	0.58885 0.1246
CHO	-0.46862 0.2415	-0.23585 0.5739	-0.11968 0.7777	-0.90728 0.0019	1.00000 0.0	0.08362 0.8439	0.12054 0.7762	-0.52746 0.1791
ASH	-0.31500 0.4473	0.08864 0.8347	0.33045 0.4240	-0.33505 0.4172	0.08362 0.8439	1.00000 0.0	-0.32645 0.4300	0.38395 0.3477
INVITRO	0.81069 0.0146	0.77082 0.0252	-0.99992 0.0001	0.19188 0.6490	0.12054 0.7762	-0.32645 0.4300	1.00000 0.0	0.31154 0.4526
BITES	0.62870 0.0950	0.79053 0.0195	-0.30720 0.4592	0.58885 0.1246	-0.52746 0.1791	0.38395 0.3477	0.31154 0.4526	1.00000 0.0

RED CLOVER PLANTS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 12

	PROTEIN	LIPID	ADF	NDF	CHO	ASH	INVITRO	BITES
PROTEIN	1.00000 0.0	0.91763 0.0001	-0.03276 0.9195	0.11415 0.7239	-0.77440 0.0031	0.74333 0.0056	0.01473 0.9638	-0.46503 0.1277
LIPID	0.91763 0.0001	1.00000 0.0	-0.03108 0.9236	0.17897 0.5779	-0.66781 0.0176	0.67739 0.0155	0.01856 0.9544	-0.17374 0.5892
ADF	-0.03276 0.9195	-0.03108 0.9236	1.00000 0.0	0.53408 0.0737	-0.51880 0.0839	0.48006 0.1142	-0.99925 0.0001	-0.19320 0.5474
NDF	0.11415 0.7239	0.17897 0.5779	0.53408 0.0737	1.00000 0.0	-0.44129 0.1510	0.20651 0.5196	-0.55574 0.0606	0.33273 0.2906
CHO	-0.77440 0.0031	-0.66781 0.0176	-0.51880 0.0839	-0.44129 0.1510	1.00000 0.0	-0.90577 0.0001	0.53698 0.0718	0.55228 0.0626
ASH	0.74333 0.0056	0.67739 0.0155	0.48006 0.1142	0.20651 0.5196	-0.90577 0.0001	1.00000 0.0	-0.49066 0.1053	-0.54047 0.0696
INVITRO	0.01473 0.9638	0.01856 0.9544	-0.99925 0.0001	-0.55574 0.0606	0.53698 0.0718	-0.49066 0.1053	1.00000 0.0	0.19550 0.5426
BITES	-0.46503 0.1277	-0.17374 0.5892	-0.19320 0.5474	0.33273 0.2906	0.55228 0.0626	-0.54047 0.0696	0.19550 0.5426	1.00000 0.0

APPENDIX 4. 4. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 5 Period 1 – characteristics of red clover plants.

RED CLOVER MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.21268 0.1467	0.84572 0.0001	-0.32021 0.0265	-0.01337 0.9281
HEIGHT	0.21268 0.1467	1.00000 0.0	0.70129 0.0001	0.17703 0.2287	0.22725 0.1203
VOLUME	0.84572 0.0001	0.70129 0.0001	1.00000 0.0	-0.13693 0.3534	0.11435 0.4390
LEAF	-0.32021 0.0265	0.17703 0.2287	-0.13693 0.3534	1.00000 0.0	0.45162 0.0013
MASS	-0.01337 0.9281	0.22725 0.1203	0.11435 0.4390	0.45162 0.0013	1.00000 0.0

RED CLOVER FORMONONETIN CONCENTRATION, PLANT MORPHOLOGY AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 16

	FORM	AREA	HEIGHT	VOLUME	LEAF	BITES
FORM	1.00000 0.0	-0.15965 0.5548	-0.10634 0.6951	-0.17185 0.5245	-0.18536 0.4919	-0.10244 0.7058
AREA	-0.15965 0.5548	1.00000 0.0	0.26850 0.3147	0.88169 0.0001	-0.42843 0.0978	0.70743 0.0022
HEIGHT	-0.10634 0.6951	0.26850 0.3147	1.00000 0.0	0.69124 0.0030	0.25200 0.3464	0.61915 0.0105
VOLUME	-0.17185 0.5245	0.88169 0.0001	0.69124 0.0030	1.00000 0.0	-0.19796 0.4624	0.83397 0.0001
LEAF	-0.18536 0.4919	-0.42843 0.0978	0.25200 0.3464	-0.19796 0.4624	1.00000 0.0	0.04955 0.8554
BITES	-0.10244 0.7058	0.70743 0.0022	0.61915 0.0105	0.83397 0.0001	0.04955 0.8554	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.21268 0.1467	0.84572 0.0001	-0.32021 0.0265	0.55954 0.0001
HEIGHT	0.21268 0.1467	1.00000 0.0	0.70129 0.0001	0.17703 0.2287	0.44735 0.0014
VOLUME	0.84572 0.0001	0.70129 0.0001	1.00000 0.0	-0.13693 0.3534	0.65253 0.0001
LEAF	-0.32021 0.0265	0.17703 0.2287	-0.13693 0.3534	1.00000 0.0	0.11256 0.4462
BITES	0.55954 0.0001	0.44735 0.0014	0.65253 0.0001	0.11256 0.4462	1.00000 0.0

APPENDIX 4. 5. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 5 Period 2 – characteristics of birdsfoot trefoil and red clover plants.

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.39755 0.0051	0.93175 0.0001	0.67672 0.0001	0.41193 0.0036
HEIGHT	0.39755 0.0051	1.00000 0.0	0.70358 0.0001	0.50352 0.0003	0.60727 0.0001
VOLUME	0.93175 0.0001	0.70358 0.0001	1.00000 0.0	0.72332 0.0001	0.55932 0.0001
LEAF	0.67672 0.0001	0.50352 0.0003	0.72332 0.0001	1.00000 0.0	0.71943 0.0001
MASS	0.41193 0.0036	0.60727 0.0001	0.55932 0.0001	0.71943 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.45853 0.0010	0.94412 0.0001	-0.13048 0.3767	0.05364 0.7173
HEIGHT	0.45853 0.0010	1.00000 0.0	0.72581 0.0001	0.00415 0.9776	0.58554 0.0001
VOLUME	0.94412 0.0001	0.72581 0.0001	1.00000 0.0	-0.09946 0.5012	0.25869 0.0758
LEAF	-0.13048 0.3767	0.00415 0.9776	-0.09946 0.5012	1.00000 0.0	0.09735 0.5104
MASS	0.05364 0.7173	0.58554 0.0001	0.25869 0.0758	0.09735 0.5104	1.00000 0.0

BIRDSFOOT TREFOIL ECT CONCENTRATION, MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 16

	TANNIN	AREA	HEIGHT	VOLUME	LEAF	BITES
TANNIN	1.00000 0.0	-0.09732 0.7199	-0.60540 0.0130	-0.26827 0.3151	-0.67276 0.0043	-0.50307 0.0470
AREA	-0.09732 0.7199	1.00000 0.0	0.49337 0.0521	0.95691 0.0001	0.72687 0.0014	0.24270 0.3651
HEIGHT	-0.60540 0.0130	0.49337 0.0521	1.00000 0.0	0.72113 0.0016	0.81835 0.0001	0.53044 0.0345
VOLUME	-0.26827 0.3151	0.95691 0.0001	0.72113 0.0016	1.00000 0.0	0.83587 0.0001	0.36427 0.1654
LEAF	-0.67276 0.0043	0.72687 0.0014	0.81835 0.0001	0.83587 0.0001	1.00000 0.0	0.59289 0.0155
BITES	-0.50307 0.0470	0.24270 0.3651	0.53044 0.0345	0.36427 0.1654	0.59289 0.0155	1.00000 0.0

RED CLOVER FORMONONETIN CONCENTRATION MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 16

	FORM	AREA	HEIGHT	VOLUME	LEAF	BITES
FORM	1.00000 0.0	0.14113 0.6021	-0.04916 0.8565	0.09622 0.7230	0.09261 0.7330	0.17249 0.5229
AREA	0.14113 0.6021	1.00000 0.0	0.51480 0.0413	0.96082 0.0001	0.17522 0.5163	0.80100 0.0002
HEIGHT	-0.04916 0.8565	0.51480 0.0413	1.00000 0.0	0.73224 0.0013	0.03475 0.8983	0.14306 0.5971
VOLUME	0.09622 0.7230	0.96082 0.0001	0.73224 0.0013	1.00000 0.0	0.15043 0.5782	0.68256 0.0036
LEAF	0.09261 0.7330	0.17522 0.5163	0.03475 0.8983	0.15043 0.5782	1.00000 0.0	0.26445 0.3223
BITES	0.17249 0.5229	0.80100 0.0002	0.14306 0.5971	0.68256 0.0036	0.26445 0.3223	1.00000 0.0

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.39755 0.0051	0.93175 0.0001	0.67672 0.0001	0.28467 0.0499
HEIGHT	0.39755 0.0051	1.00000 0.0	0.70358 0.0001	0.50352 0.0003	0.43985 0.0018
VOLUME	0.93175 0.0001	0.70358 0.0001	1.00000 0.0	0.72332 0.0001	0.39452 0.0055
LEAF	0.67672 0.0001	0.50352 0.0003	0.72332 0.0001	1.00000 0.0	0.57495 0.0001
BITES	0.28467 0.0499	0.43985 0.0018	0.39452 0.0055	0.57495 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.45853 0.0010	0.94412 0.0001	-0.13048 0.3767	0.78368 0.0001
HEIGHT	0.45853 0.0010	1.00000 0.0	0.72581 0.0001	0.00415 0.9776	0.47889 0.0006
VOLUME	0.94412 0.0001	0.72581 0.0001	1.00000 0.0	-0.09946 0.5012	0.78423 0.0001
LEAF	-0.13048 0.3767	0.00415 0.9776	-0.09946 0.5012	1.00000 0.0	0.04855 0.7431
BITES	0.78368 0.0001	0.47889 0.0006	0.78423 0.0001	0.04855 0.7431	1.00000 0.0

APPENDIX 4. 6. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 5 Period 3 – characteristics of birdsfoot trefoil and red clover plants.

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.66337 0.0001	0.95504 0.0001	0.49994 0.0003	0.43495 0.0020
HEIGHT	0.66337 0.0001	1.00000 0.0	0.85539 0.0001	0.50360 0.0003	0.62644 0.0001
VOLUME	0.95504 0.0001	0.85539 0.0001	1.00000 0.0	0.54559 0.0001	0.54928 0.0001
LEAF	0.49994 0.0003	0.50360 0.0003	0.54559 0.0001	1.00000 0.0	0.62960 0.0001
MASS	0.43495 0.0020	0.62644 0.0001	0.54928 0.0001	0.62960 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.50765 0.0002	0.95924 0.0001	0.24888 0.0880	0.56170 0.0001
HEIGHT	0.50765 0.0002	1.00000 0.0	0.73042 0.0001	0.33649 0.0194	0.71314 0.0001
VOLUME	0.95924 0.0001	0.73042 0.0001	1.00000 0.0	0.30766 0.0334	0.67918 0.0001
LEAF	0.24888 0.0880	0.33649 0.0194	0.30766 0.0334	1.00000 0.0	0.57524 0.0001
MASS	0.56170 0.0001	0.71314 0.0001	0.67918 0.0001	0.57524 0.0001	1.00000 0.0

BIRDSFOOT TREFOIL ECT CONCENTRATION, MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 15

	TANNIN	AREA	HEIGHT	VOLUME	LEAF	BITES
TANNIN	1.00000 0.0	0.03988 0.8878	0.14485 0.6065	0.09258 0.7428	-0.48602 0.0662	-0.01384 0.9609
AREA	0.03988 0.8878	1.00000 0.0	0.86008 0.0001	0.98304 0.0001	0.59211 0.0200	0.51227 0.0509
HEIGHT	0.14485 0.6065	0.86008 0.0001	1.00000 0.0	0.93598 0.0001	0.63244 0.0114	0.60250 0.0175
VOLUME	0.09258 0.7428	0.98304 0.0001	0.93598 0.0001	1.00000 0.0	0.61934 0.0138	0.55326 0.0324
LEAF	-0.48602 0.0662	0.59211 0.0200	0.63244 0.0114	0.61934 0.0138	1.00000 0.0	0.55464 0.0319
BITES	-0.01384 0.9609	0.51227 0.0509	0.60250 0.0175	0.55326 0.0324	0.55464 0.0319	1.00000 0.0

RED CLOVER FORMONONETIN CONCENTRATION, MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 16

	FORM	AREA	HEIGHT	VOLUME	LEAF	BITES
FORM	1.00000 0.0	0.01195 0.9650	-0.17947 0.5060	-0.05222 0.8477	-0.10481 0.6993	0.25581 0.3389
AREA	0.01195 0.9650	1.00000 0.0	0.64001 0.0076	0.96512 0.0001	0.60020 0.0140	0.70836 0.0021
HEIGHT	-0.17947 0.5060	0.64001 0.0076	1.00000 0.0	0.81886 0.0001	0.52794 0.0356	0.65241 0.0062
VOLUME	-0.05222 0.8477	0.96512 0.0001	0.81886 0.0001	1.00000 0.0	0.62827 0.0092	0.75148 0.0008
LEAF	-0.10481 0.6993	0.60020 0.0140	0.52794 0.0356	0.62827 0.0092	1.00000 0.0	0.50826 0.0444
BITES	0.25581 0.3389	0.70836 0.0021	0.65241 0.0062	0.75148 0.0008	0.50826 0.0444	1.00000 0.0

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.66337 0.0001	0.95504 0.0001	0.49994 0.0003	0.43815 0.0018
HEIGHT	0.66337 0.0001	1.00000 0.0	0.85539 0.0001	0.50360 0.0003	0.50888 0.0002
VOLUME	0.95504 0.0001	0.85539 0.0001	1.00000 0.0	0.54559 0.0001	0.50491 0.0003
LEAF	0.49994 0.0003	0.50360 0.0003	0.54559 0.0001	1.00000 0.0	0.43414 0.0020
BITES	0.43815 0.0018	0.50888 0.0002	0.50491 0.0003	0.43414 0.0020	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 48

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.50765 0.0002	0.95924 0.0001	0.24888 0.0880	0.56760 0.0001
HEIGHT	0.50765 0.0002	1.00000 0.0	0.73042 0.0001	0.33649 0.0194	0.57313 0.0001
VOLUME	0.95924 0.0001	0.73042 0.0001	1.00000 0.0	0.30766 0.0334	0.63793 0.0001
LEAF	0.24888 0.0880	0.33649 0.0194	0.30766 0.0334	1.00000 0.0	0.34966 0.0148
BITES	0.56760 0.0001	0.57313 0.0001	0.63793 0.0001	0.34966 0.0148	1.00000 0.0

APPENDIX 4. 7. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 6 - plant nutritional characteristics

BIRDSFOOT TREFOIL PLANTS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 16

	PROTEIN	LIPID	ADF	NDF	CHO	ASH	INVITRO	BITES
PROTEIN	1.00000	0.82647	-0.75202	-0.18241	-0.04070	0.56706	0.74957	0.36957
	0.0	0.0001	0.0008	0.4989	0.8810	0.0220	0.0008	0.1589
LIPID	0.82647	1.00000	-0.85616	-0.62122	0.39394	0.71549	0.86038	0.29233
	0.0001	0.0	0.0001	0.0102	0.1311	0.0018	0.0001	0.2719
ADF	-0.75202	-0.85616	1.00000	0.57506	-0.51325	-0.49017	-0.97650	-0.33854
	0.0008	0.0001	0.0	0.0198	0.0420	0.0539	0.0001	0.1996
NDF	-0.18241	-0.62122	0.57506	1.00000	-0.74256	-0.66864	-0.58705	0.12507
	0.4989	0.0102	0.0198	0.0	0.0010	0.0046	0.0168	0.6444
CHO	-0.04070	0.39394	-0.51325	-0.74256	1.00000	0.26097	0.56136	0.10249
	0.8810	0.1311	0.0420	0.0010	0.0	0.3289	0.0237	0.7056
ASH	0.56706	0.71549	-0.49017	-0.66864	0.26097	1.00000	0.58122	0.02521
	0.0220	0.0018	0.0539	0.0046	0.3289	0.0	0.0182	0.9262
INVITRO	0.74957	0.86038	-0.97650	-0.58705	0.56136	0.58122	1.00000	0.39623
	0.0008	0.0001	0.0001	0.0168	0.0237	0.0182	0.0	0.1287
BITES	0.36957	0.29233	-0.33854	0.12507	0.10249	0.02521	0.39623	1.00000
	0.1589	0.2719	0.1996	0.6444	0.7056	0.9262	0.1287	0.0

RED CLOVER PLANTS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 16

	PROTEIN	LIPID	ADF	NDF	CHO	ASH	INVITRO	BITES
PROTEIN	1.00000	0.88656	0.58518	0.63669	-0.85542	0.86413	-0.57851	-0.41688
	0.0	0.0001	0.0173	0.0080	0.0001	0.0001	0.0189	0.1082
LIPID	0.88656	1.00000	0.56582	0.39133	-0.86009	0.90217	-0.56364	-0.42314
	0.0001	0.0	0.0223	0.1339	0.0001	0.0001	0.0230	0.1025
ADF	0.58518	0.56582	1.00000	0.68076	-0.85678	0.80302	-0.99942	-0.60927
	0.0173	0.0223	0.0	0.0037	0.0001	0.0002	0.0001	0.0122
NDF	0.63669	0.39133	0.68076	1.00000	-0.62268	0.53448	-0.67367	-0.30432
	0.0080	0.1339	0.0037	0.0	0.0100	0.0329	0.0042	0.2518
CHO	-0.85542	-0.86009	-0.85678	-0.62268	1.00000	-0.97046	0.85243	0.58488
	0.0001	0.0001	0.0001	0.0100	0.0	0.0001	0.0001	0.0173
ASH	0.86413	0.90217	0.80302	0.53448	-0.97046	1.00000	-0.80038	-0.55674
	0.0001	0.0001	0.0002	0.0329	0.0001	0.0	0.0002	0.0251
INVITRO	-0.57851	-0.56364	-0.99942	-0.67367	0.85243	-0.80038	1.00000	0.59817
	0.0189	0.0230	0.0001	0.0042	0.0001	0.0002	0.0	0.0144
BITES	-0.41688	-0.42314	-0.60927	-0.30432	0.58488	-0.55674	0.59817	1.00000
	0.1082	0.1025	0.0122	0.2518	0.0173	0.0251	0.0144	0.0

APPENDIX 4. 8. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 6 Period 1 – characteristics of birdsfoot trefoil and red clover plants.

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.38059 0.0001	0.88601 0.0001	0.39506 0.0001	0.29482 0.0035
HEIGHT	0.38059 0.0001	1.00000 0.0	0.76597 0.0001	0.38425 0.0001	0.68364 0.0001
VOLUME	0.88601 0.0001	0.76597 0.0001	1.00000 0.0	0.46730 0.0001	0.54773 0.0001
LEAF	0.39506 0.0001	0.38425 0.0001	0.46730 0.0001	1.00000 0.0	0.71891 0.0001
MASS	0.29482 0.0035	0.68364 0.0001	0.54773 0.0001	0.71891 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.48451 0.0001	0.94801 0.0001	0.04390 0.6710	0.39031 0.0001
HEIGHT	0.48451 0.0001	1.00000 0.0	0.73771 0.0001	-0.05826 0.5729	0.56313 0.0001
VOLUME	0.94801 0.0001	0.73771 0.0001	1.00000 0.0	0.01269 0.9024	0.50608 0.0001
LEAF	0.04390 0.6710	-0.05826 0.5729	0.01269 0.9024	1.00000 0.0	0.36849 0.0002
MASS	0.39031 0.0001	0.56313 0.0001	0.50608 0.0001	0.36849 0.0002	1.00000 0.0

BIRDSFOOT TREFOIL ECT CONCENTRATION, MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 32

	TANNIN	AREA	HEIGHT	VOLUME	LEAF	BITES
TANNIN	1.00000 0.0	-0.09251 0.6145	-0.40110 0.0229	-0.25397 0.1607	-0.63116 0.0001	-0.36284 0.0413
AREA	-0.09251 0.6145	1.00000 0.0	0.63146 0.0001	0.92830 0.0001	0.34409 0.0538	0.69709 0.0001
HEIGHT	-0.40110 0.0229	0.63146 0.0001	1.00000 0.0	0.86923 0.0001	0.59623 0.0003	0.60167 0.0003
VOLUME	-0.25397 0.1607	0.92830 0.0001	0.86923 0.0001	1.00000 0.0	0.50190 0.0034	0.73580 0.0001
LEAF	-0.63116 0.0001	0.34409 0.0538	0.59623 0.0003	0.50190 0.0034	1.00000 0.0	0.68781 0.0001
BITES	-0.36284 0.0413	0.69709 0.0001	0.60167 0.0003	0.73580 0.0001	0.68781 0.0001	1.00000 0.0

RED CLOVER FORMONONETIN CONCENTRATION, MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 32

	FORM	AREA	HEIGHT	VOLUME	LEAF	BITES
FORM	1.00000 0.0	-0.00356 0.9846	0.05963 0.7458	0.01337 0.9421	-0.05570 0.7621	0.03227 0.8608
AREA	-0.00356 0.9846	1.00000 0.0	0.60621 0.0002	0.95593 0.0001	-0.08904 0.6279	-0.24196 0.1821
HEIGHT	0.05963 0.7458	0.60621 0.0002	1.00000 0.0	0.80188 0.0001	-0.12831 0.4840	-0.24736 0.1723
VOLUME	0.01337 0.9421	0.95593 0.0001	0.80188 0.0001	1.00000 0.0	-0.13525 0.4605	-0.28522 0.1136
LEAF	-0.05570 0.7621	-0.08904 0.6279	-0.12831 0.4840	-0.13525 0.4605	1.00000 0.0	0.33105 0.0642
BITES	0.03227 0.8608	-0.24196 0.1821	-0.24736 0.1723	-0.28522 0.1136	0.33105 0.0642	1.00000 0.0

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.38059 0.0001	0.88601 0.0001	0.39506 0.0001	0.60601 0.0001
HEIGHT	0.38059 0.0001	1.00000 0.0	0.76597 0.0001	0.38425 0.0001	0.43104 0.0001
VOLUME	0.88601 0.0001	0.76597 0.0001	1.00000 0.0	0.46730 0.0001	0.63741 0.0001
LEAF	0.39506 0.0001	0.38425 0.0001	0.46730 0.0001	1.00000 0.0	0.62218 0.0001
BITES	0.60601 0.0001	0.43104 0.0001	0.63741 0.0001	0.62218 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.48451 0.0001	0.94801 0.0001	0.04390 0.6710	0.12597 0.2214
HEIGHT	0.48451 0.0001	1.00000 0.0	0.73771 0.0001	-0.05826 0.5729	0.06079 0.5563
VOLUME	0.94801 0.0001	0.73771 0.0001	1.00000 0.0	0.01269 0.9024	0.11933 0.2469
LEAF	0.04390 0.6710	-0.05826 0.5729	0.01269 0.9024	1.00000 0.0	0.24763 0.0150
BITES	0.12597 0.2214	0.06079 0.5563	0.11933 0.2469	0.24763 0.0150	1.00000 0.0

APPENDIX 4.9. Correlation matrices with Pearson Correlation Coefficients and P-values of Experiment 6 Period 2 – characteristics of birdsfoot trefoil and red clover plants.

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.50705 0.0001	0.96024 0.0001	0.53047 0.0001	0.40525 0.0001
HEIGHT	0.50705 0.0001	1.00000 0.0	0.72752 0.0001	0.36859 0.0002	0.58281 0.0001
VOLUME	0.96024 0.0001	0.72752 0.0001	1.00000 0.0	0.54164 0.0001	0.51136 0.0001
LEAF	0.53047 0.0001	0.36859 0.0002	0.54164 0.0001	1.00000 0.0	0.75514 0.0001
MASS	0.40525 0.0001	0.58281 0.0001	0.51136 0.0001	0.75514 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	MASS
AREA	1.00000 0.0	0.79143 0.0001	0.97611 0.0001	0.26509 0.0090	0.56918 0.0001
HEIGHT	0.79143 0.0001	1.00000 0.0	0.90534 0.0001	0.30731 0.0023	0.67775 0.0001
VOLUME	0.97611 0.0001	0.90534 0.0001	1.00000 0.0	0.29342 0.0037	0.63638 0.0001
LEAF	0.26509 0.0090	0.30731 0.0023	0.29342 0.0037	1.00000 0.0	0.49951 0.0001
MASS	0.56918 0.0001	0.67775 0.0001	0.63638 0.0001	0.49951 0.0001	1.00000 0.0

BIRDSFOOT TREFOIL ECT CONCENTRATION, MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 32

	LNTANNIN	LNAREA	LNHEIGHT	LNVOLUME	LNLEAF	LNBITES
LNTANNIN	1.00000 0.0	0.07054 0.7012	-0.14193 0.4384	-0.00312 0.9865	-0.60142 0.0003	-0.26943 0.1359
LNAREA	0.07054 0.7012	1.00000 0.0	0.58124 0.0005	0.97079 0.0001	0.32934 0.0657	0.63113 0.0001
LNHEIGHT	-0.14193 0.4384	0.58124 0.0005	1.00000 0.0	0.74500 0.0001	0.47503 0.0060	0.59118 0.0004
LNVOLUME	-0.00312 0.9865	0.97079 0.0001	0.74500 0.0001	1.00000 0.0	0.39643 0.0247	0.68226 0.0001
LNLEAF	-0.60142 0.0003	0.32934 0.0657	0.47503 0.0060	0.39643 0.0247	1.00000 0.0	0.69065 0.0001
LNBITES	-0.26943 0.1359	0.63113 0.0001	0.59118 0.0004	0.68226 0.0001	0.69065 0.0001	1.00000 0.0

RED CLOVER FORMONONETIN CONCENTRATION MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 32

	FORM	AREA	HEIGHT	VOLUME	LEAF	BITES
FORM	1.00000 0.0	0.01478 0.9360	-0.00105 0.9955	0.01898 0.9179	-0.47464 0.0061	-0.07327 0.6902
AREA	0.01478 0.9360	1.00000 0.0	0.86200 0.0001	0.97773 0.0001	0.15808 0.3875	0.84889 0.0001
HEIGHT	-0.00105 0.9955	0.86200 0.0001	1.00000 0.0	0.94392 0.0001	0.09680 0.5982	0.76122 0.0001
VOLUME	0.01898 0.9179	0.97773 0.0001	0.94392 0.0001	1.00000 0.0	0.12955 0.4798	0.85153 0.0001
LEAF	-0.47464 0.0061	0.15808 0.3875	0.09680 0.5982	0.12955 0.4798	1.00000 0.0	0.27593 0.1263
BITES	-0.07327 0.6902	0.84889 0.0001	0.76122 0.0001	0.85153 0.0001	0.27593 0.1263	1.00000 0.0

BIRDSFOOT TREFOIL MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	LNAREA	LNHEIGHT	LNVOLUME	LNLEAF	LNBITES
LNAREA	1.00000 0.0	0.50705 0.0001	0.96024 0.0001	0.53047 0.0001	0.66744 0.0001
LNHEIGHT	0.50705 0.0001	1.00000 0.0	0.72752 0.0001	0.36859 0.0002	0.51102 0.0001
LNVOLUME	0.96024 0.0001	0.72752 0.0001	1.00000 0.0	0.54164 0.0001	0.69680 0.0001
LNLEAF	0.53047 0.0001	0.36859 0.0002	0.54164 0.0001	1.00000 0.0	0.73967 0.0001
LNBITES	0.66744 0.0001	0.51102 0.0001	0.69680 0.0001	0.73967 0.0001	1.00000 0.0

RED CLOVER MORPHOLOGICAL CHARACTERISTICS AND NUMBER OF BITES

Pearson Correlation Coefficients / Prob > |R| under Ho: Rho=0 / N = 96

	AREA	HEIGHT	VOLUME	LEAF	BITES
AREA	1.00000 0.0	0.79143 0.0001	0.97611 0.0001	0.26509 0.0090	0.77790 0.0001
HEIGHT	0.79143 0.0001	1.00000 0.0	0.90534 0.0001	0.30731 0.0023	0.69892 0.0001
VOLUME	0.97611 0.0001	0.90534 0.0001	1.00000 0.0	0.29342 0.0037	0.78892 0.0001
LEAF	0.26509 0.0090	0.30731 0.0023	0.29342 0.0037	1.00000 0.0	0.40899 0.0001
BITES	0.77790 0.0001	0.69892 0.0001	0.78892 0.0001	0.40899 0.0001	1.00000 0.0

APPENDIX 4. 10. Rumen manipulation – Experiment 6

Table 4. 2. Botanical composition (% in DM of total material) and tannin and formononetin concentrations (%) of the minced material added to cow's rumen in the Period 1.

Period 1	Leaf	stem	Flower	Dead matter	Weeds	DM	Tannin	Formononetin
Day 1								
Red clover								
Astred	61	39	0	0	0	28	0.04	0.39
Pawera	53	46	0	0	1	59	0.04	0.59
Lotus spp.								
Goldie	19	18	4	8	51	32	0.07	0.15
Maku	29	59	9	2	1	29	0.59	0.2
Day 2								
Red clover								
Astred	61	39	0	0	0	28	0.05	0.39
Pawera	53	46	0	0	1	59	0.05	0.40
Lotus sp.								
Goldie	42	46	5	3	4	30	0.23	0.2
Maku	31	47	12	1	9	29	0.35	0.2

Table 4.3. Botanical composition (% in DM of total material) and tannin and formononetin concentrations (%) of the minced material added to cow's rumen in Period 2.

Period 2	Leaf	Stem	Flower	Dead matter	Weeds	DM	Tannin	Formononetin
Day 1								
Red clover								
Astred	71	6	0	16	7	29	0.04	0.45
Pawera	79	1	0	10	10	32	0.04	0.50
Lotus sp.								
Goldie	56	28	0	2	14	28	0.19	0.16
Maku	47	21	0	3	29	28	0.16	0.21
Day 2								
Red clover								
Astred	77	8	2	11	2	19	0.04	0.43
Pawera	86	3	0	3	8	16	0.06	0.50
Lotus sp.								
Goldie	60	33	0	1	6	29	0.22	0.18
Maku	52	21	0	2	25	26	0.15	0.23

APPENDIX 4.10. Rumen manipulation – Experiment 6

Table 4. 4. Kilograms (fresh weight) of rumen content taken out and of minced material put into cow's rumen with correspondent amount of lucerne pellets (kg) and secondary compound [extractable condensed tannin (ECT) or formononetin] (g) added to the minced material in Period 1.

	Rumen content modification (kg)			
Period 1	*Take out*	*Put into*	*Lucerne pellets*	*Secondary Compound*
Day1	*(Kg)*	*(Kg)*	*(Kg)*	*(g)*
Lotus sp.				
				ECT
Goldie	21.2	14.5	2.5	3
Maku	27.3	15.2	3.0	26
Day2				
Lotus sp.				
Goldie	24.8	17.0	2.8	12
Maku	20.0	15.0	2.8	15
Day1				
Red clover				*Formononetin*
Astred	28.0	15.1	3.0	16
Pawera	28.0	16.0	3.5	56
Day 2				
Red clover				
Astred	15.5	14.4	3.0	16
Pawera	17.7	15.5	3.0	62

APPENDIX 4.10. Rumen manipulation – Experiment 6

Table 4. 5. Kilograms (fresh weight) of rumen content taken out and of minced material put into cow's rumen with correspondent amount (kg) of lucerne pellets and secondary compounds [extractable condensed tannin (ECT) or formononetin] (g) added to the minced material in Period 2.

	Rumen content modification (kg)			
Period 2	*Take out*	*Put into*	*Lucerne pellets*	*Secondary Compound*
Day1	*(Kg)*	*(Kg)*	*(Kg)*	*(g)*
Lotus sp.				
				ECT
Goldie	11.7	15.4	2.5	7
Maku	23.3	16.0	3.0	9
Day2				
Lotus sp.				
Goldie	10.4	17.2	2.7	9
Maku	6.5	14.0	2.5	8
Day1				
Red clover				*Formononetin*
Astred	14.9	6.7	1.2	9
Pawera	15.8	11.6	2.2	19
Day 2				
Red clover				
Astred	24.3	6.7	1.1	5
Pawera	9.9	13.9	2.2	11