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### Legend session

The legend session – the aim of this session is to invite a senior member of the Society to present an address that covers “their” work. Aspects such as where they started/ended up, highlights, impacts of that work on industry, current and future philosophy.

We feel this new initiative will contribute to one of our Society objectives – promoting the value of science in animal production.

### Forage secondary compounds; past, present and future

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### ABSTRACT

Forages contain plant secondary compounds. These are primarily produced to protect the plant from attack by micro-organism, insects and herbivores. Research during the last 40 years has produced an insight into the biochemistry, metabolism and interaction of these compounds, specifically the unique amino acid S-methyl cysteine sulphoxide (SMCO) and condensed tannins (CT). Both occur within forage consumed by farmed livestock. SCMO induces anaemia in herbivores. Through plant breeding and effective management brassica crops can now be fed to livestock as a supplement without restricting growth rate. A wide range of plants contain CT. CT have been shown to have the capacity to bind to soluble proteins in the rumen enabling increased absorption of essential amino acids from the small intestine. This benefits body growth, milk production, wool growth and ovulation rate. CT have also been shown to have a direct effect on gastrointestinal parasites inhibiting their growth and fecundity, particularly in sheep. As a result of this detailed research we are now able to exploit the unique attributes of some plant secondary compounds and use them to our advantage to benefit animal production and the performance of our farmed livestock. Ways in which we able to do this are discussed.

**Keywords:** S-methyl cysteine sulphoxide; condensed tannin; sesquiterpene lactones; protein digestion; wool growth; reproduction; milk yield; parasites.

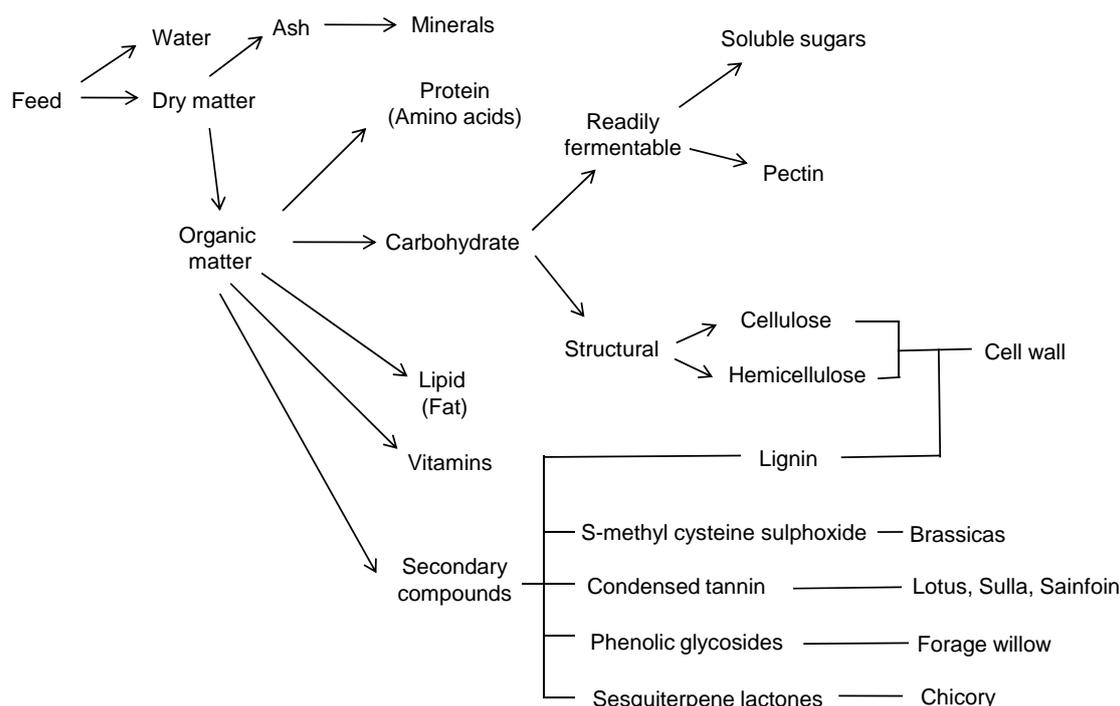
### INTRODUCTION

Work on secondary compounds in New Zealand (NZ) forages commenced in the 1970s. Where secondary compounds fit in the scheme of plant chemical composition is shown in Figure 1. Essentially they are components of the organic matter after the contents of readily fermentable carbohydrates, structural carbohydrate, protein, lipid and organic acids have been accounted for. The evolutionary role of many secondary compounds is to defend the plant against attack, either by micro-organisms or against being eaten by insects or herbivores. Some secondary compounds such as lignin occur in all plants but many others occur only in certain plants, as shown in Figure 1. Initial research identified some secondary compounds as causing animal health issues and losses in animal production; in the 1980s beneficial effects of manipulating secondary compounds became apparent.

### BRASSICA PLANTS

My research into secondary compounds commenced in the late 1970s at Invermay and was prompted by a survey of all NZ lamb grazing trials on *Brassica sp.* (Nicol & Barry, 1980). This showed that although the dry matter (DM) digestibility of swedes, turnips, kale and forage rape was high at 80-85%, growth of lambs grazing them was less than would be expected from this digestibility. It was in the range 100-150 g/day, with little difference between individual crops. A second finding was that low voluntary intakes always occurred during the initial five weeks following transfer of animals from pasture to brassica crops. Smith (1978) had just shown that all brassica plants contained the unique amino acid S-methyl cysteine sulphoxide (SMCO). They showed that SMCO was fermented by rumen micro organisms to dimethyl disulphide, which was absorbed from the rumen and then reacted with red blood cells. Kale contains high concentrations of SMCO and this mechanism was identified as the cause of kale anaemia in dairy cows.

**FIGURE 1:** Composition of animal feed.



Work in NZ (Barry *et al.*, 1982; 1984) showed supplementary SMCO to cause sub-clinical anaemia and depressed growth in lambs fed either fresh lucerne or kale, with the depression being much greater for lambs fed the kale diet and with all the growth depression being explained by reduced voluntary feed intake (VFI: Table 1). Subsequent grazing studies (Table 2) with kale containing normal and low SMCO concentrations, induced by growing it in low sulphur (S) plots, showed that use of low SMCO kale reduced blood dimethyl disulphide concentrations, eliminated the initial six weeks of low growth associated with the feeding of normal kale and substantially increased overall body growth rates. The SMCO work at Invermay focused

on kale, because it was the highest yielding of all the brassica forages. Methods of minimising the initial low growth, which lasted for about six weeks, were devised, including gradual adaption of animals, low S fertiliser treatment and choice of kale variety. These treatment options are still in use today.

### CONDENSED TANNINS

Condensed tannins (CT) first attracted attention in the 1970s when studies at Palmerston North showed CT-containing legumes to be non-bloating when grazed by cattle (Jones *et al.*, 1973). Subsequent studies with CT isolated from sainfoin showed that CT readily reacted with forage protein in the 5.5 to 7.5 pH range, the normal range for rumen pH, and reduced its solubility. The complex was however, dissociated at pH values below 3.5, the pH of the abomasum (Jones & Mangan, 1977). As bloat is caused by the accumulation of soluble protein in the rumen (Mangan, 1959), this provided a mechanism as to why CT-containing legumes were non-bloating. It is also indicated that the reversible binding of CT to forage protein might be able to be used as a means of protecting some soluble protein from rumen fermentation and so increasing the supply of

**TABLE 1:** Effect of S-methyl cysteine sulphoxide (SMCO) supplementation upon the voluntary digestible organic matter (DOM) intake and liveweight gain of young sheep fed fresh lucerne or forage kale, containing zero and 8 g/kg dry matter (DM) of SMCO respectively. Mean data for six weeks.

Measurement	Forage	SMCO added (g/kg DM)					Standard error of difference
		0	2	4	8	16	
DOM intake (g/kg W <sup>0.75</sup> /day)	Lucerne	65.2	68.15	69.3	65.6	57.9	2.5
	Kale	62.6	52.1	65.0	42.7	38.0	
Liveweight gain (g/day)	Lucerne	91	95	107	63	67	13
	Kale	64	4	54	-47	-42	

From Barry *et al.* (1982).

**TABLE 2:** Effects of grazing young sheep on kale of normal and low S-methyl cysteine sulphoxide (SMCO) concentration, induced by growing the crop in plots of normal and low soil sulphate-S concentration.

Measurement	Length of feeding period (weeks)	Type of kale		Standard error of mean
		Normal SMCO	Low SMCO	
SMCO (g/kg DM)	1-6	5.2	3.1	0.3
	7-12	6.8	3.8	0.4
Liveweight gain (g/day)	1-6	116	176	11
	7-12	180	183	10
Empty body gain (g/day)	1-12	113	170	7

From Barry *et al.* (1984).

**TABLE 3:** Effect of abomasal casein infusion on protein deposition (g/d) in weaned lambs fed fresh spring ryegrass/white clover pasture *ad libitum*. SE = Standard error of mean.

Tissue	Control lambs		Protein-infused lambs		Significance
	Mean	SE	Mean	SE	
Wool	3.9	0.8	6.6	0.8	*
Carcass	4.9	0.5	8.6	0.5	***
Wool-free body	8.7	0.8	14.3	0.7	***
Total deposition	12.6	0.9	21.0	0.9	***

From Barry (1981).

“bypass protein” in grazing livestock. These studies begun 35 years ago marked the start of research into CT and the nutrition of ruminants grazing fresh forages, which still continues today. It is convenient to categorize progress made in this area into that made in each decade.

This section reviews the effect of low and medium CT concentration in temperate forages only; high CT concentrations which are detrimental are not considered. Effects of CT in tropical forages are summarized by Barry *et al.* (2001).

### Indoor nutrition and laboratory studies

Studies conducted in the 1970s (MacRae & Ulyatt, 1974) identified the large loss of nitrogen (N) across the rumen in sheep fed fresh forages, with about one-third being absorbed from the rumen as ammonia. Despite the large protein intakes of NZ grazing animals, this raised the possibility that protein absorbed could be limiting productivity in some instances. An abomasal protein infusion experiment with lambs fed fresh pasture *ad libitum* showed this was the case (Table 3), with the infusion markedly increasing protein deposition without affecting VFI. This provided the impetus to examine the reversible protein binding properties of CT as a means of increasing the absorption of essential amino acids (EAA) in sheep fed fresh forages. Most of this research occurred in the 1980s and early 1990s

Initially the legumes *Lotus corniculatus*, *Lotus pedunculatus*, sulla (*Hedysarum coronarium*) and sainfoin (*Onobrychis viciifolia*) were identified as CT-containing (25-80 g/kg DM), with all other legume species being used as forages believed not to contain CT. Developments in methodology to include measurement of CT bound to protein and fibre subsequently showed that small concentrations of CT (1-4 g/kg DM) were present in the leaves of most of the other temperate legumes, herbs and grasses (Terrill *et al.* 1992). In all cases CT extracted and purified from *Lotus pedunculatus* was used as the standard. This was confirmed using <sup>13</sup>C nuclear magnetic resonance and other specific CT methodology for perennial ryegrass and red clover, with chicory, lucerne and plantain also probably containing CT (Jackson *et al.*, 1996).

Two milestone studies by Waghorn *et al.*, (1987; 1994) determined the effects of CT upon the flow and absorption of EAA in sheep fed fresh *Lotus corniculatus* and fresh *Lotus pedunculatus* (Table 4). Effects attributable to CT were established through infusing polyethylene glycol (PEG; MW 3,350) into the rumen of sheep fed each forage. Condensed tannin binds to PEG in preference to protein, so comparing control (CT-acting) sheep with PEG sheep allows the effect of CT to be calculated. Action of CT in both legumes reduced rumen ammonia concentration and increased EAA flowing into the abomasums. Effects in the small intestine differed between legumes. Condensed tannins present in *Lotus corniculatus* markedly increased EAA absorbed but there was little increase with CT in *Lotus pedunculatus*. This gave the first indication that CT structure is important in determining effects in ruminant nutrition as well as CT concentration. Further studies (Bermingham *et al.*, 2001) showed that CT in sulla (64 g/kg DM) increased EAA absorbed by about 20% but no increase was obtained with CT from sainfoin (38 g/kg DM).

### Field studies

During the 1990s and early 2000s a range of field experiments were conducted in NZ, with and without PEG supplementation, to establish the effects of forage CT upon animal production. Most of these used *Lotus corniculatus*, because its CT produced the biggest increase in EAA absorption as discussed earlier. Effects upon wool production

**TABLE 4:** Effect of condensed tannins (CT) on the intake and absorption of essential amino acids from the small intestine of sheep fed on fresh *Lotus corniculatus* containing 22 g CT/kg dry matter (DM) and *L. pedunculatus* containing 55 g CT/kg DM, with or without an intra luminal infusion of polyethylene glycol to inactivate the CT.

Measurement	<i>L. corniculatus</i>		<i>L. pedunculatus</i>	
	CT-acting group	CT-non-acting group	CT-acting group	CT-non-acting group
Rumen ammonia (mgN/L)	367	504	175	460
CT intake (g/day)	98.9	98.9	103.2	116.8
Essential amino acids				
Abomasal flow (g/day)	84.7	55.5	121.1	105.6
Proportion intake	0.86	0.56	1.17	0.90
Apparent absorption from small intestine (g/day)	58.8	36.2	81.4	83.5
Proportion abomasal flow	0.67	0.67	0.66	0.79
Proportion intake	0.59	0.37	0.79	0.72

From Waghorn *et al.* (1987; 1994).

were investigated first, because clean wool is 100% protein. Min *et al.* (2003) summarized the 11 studies in this area and concluded that CT in *Lotus corniculatus* in the range 22-38 g/kg DM increased wool production in grazing sheep by 10-15%.

These grazing studies at Massey University then went on to look at effects upon ovulation rate (OR) and lambing % in ewes. Averaged over three studies, grazing ewes for six to eight weeks on *Lotus corniculatus* just before and during mating increased OR by 22%, with approximately half of the response due to the action of CT (Table 5; Min *et al.*, 2003). Subsequent studies, summarised by Ramirez & Barry (2005) indicated that grazing on *Lotus corniculatus* during early pregnancy may also reduce embryonic losses and post natal lamb losses. Further work is needed in this area.

Wang *et al.*, (1996) showed that grazing lactating ewes on *Lotus corniculatus* increased milk production by approximately 20%, relative to ewes grazing Lucerne (*Medicago sativa*) that contained only traces of CT. Studies with dairy cows at DairyNZ (Table 6) have confirmed this with cows

fed lotus having a higher DM intake, a greater milk solids production (33%) and producing 17% less methane/kg DM consumed relative to cows fed ryegrass pasture. The use of PEG showed that approximately half of these responses to lotus feeding can be explained through the action of CT.

Niezen *et al.*, (1995; 1998) first reported the effect of grazing CT-containing legumes upon internal parasites in sheep (Table 7). They found that the growth of parasite-free animals was similar on CT-containing and

non-CT-containing legumes, but that growth of parasitized animals was greater on CT-containing legumes. This was especially so for lambs grazing sulla, where worm burdens at slaughter were less than for lambs grazing lucerne. It has been proposed that effects such as these can be explained through direct action of forage CT in inhibiting parasites and their larvae and by indirect effects through some, but not all, CT by increasing the absorption of EAA, which then stimulate the immune system (Mupeyo *et al.*, 2011).

**Systems studies**

Twelve month whole system studies have been conducted from 2000 to 2007 at Massey University’s Riverside farm in the Wairarapa, on the integration of CT-containing *Lotus corniculatus* and forage willow into grazing systems. Under these conditions *Lotus corniculatus* delivered similar production responses to those reported earlier in this paper and in addition were associated with greatly reduced dag scores (Ramirez & Barry 2005). However, its greatly reduced persistency under grazing was identified as a problem, with stands

**TABLE 5:** The effect of grazing ewes on *Lotus corniculatus* or perennial ryegrass/white clover (pasture) and of supplementation with polyethylene glycol (PEG) on ovulation rate.

Reference	Number of ewes	Mean live weight (kg)	Ovulation rate			Response to lotus feeding (%)
			Pasture ± PEG	<i>L. corniculatus</i>		
				+ PEG	- PEG	
Min <i>et al.</i> (1999) (Second cycle)	200	54	1.33 <sup>a</sup>	1.56 <sup>b</sup>	1.76 <sup>c</sup>	32.3
Luque <i>et al.</i> (2000) (Second cycle)	240	60	1.45 <sup>a</sup>	1.66 <sup>b</sup>	1.64 <sup>b</sup>	13.1
Min <i>et al.</i> (2001) (Third cycle)	225	51	1.48 <sup>a</sup>	1.66 <sup>b</sup>	1.80 <sup>c</sup>	21.6
Mean	222	55	1.42	1.62	1.73	22.3

Different superscripts within a row indicate values that are significantly different (P <0.5).

**TABLE 6:** Cow live weight, dry matter intake, milk yield, milk composition, milksolids yield, and methane production of groups of eight Friesian dairy cows fed either perennial pasture containing 0 g CT/kg dry matter (DM), or birdsfoot trefoil (*Lotus corniculatus*) containing 26 g CT/kg DM and drenched twice daily (3.6 L/day) with either 50% polyethylene glycol (PEG) or water. The means and standard error of the difference (SED) for individual treatments are given (n=8). MS = Milksolids.

Measurement	Ryegrass	Ryegrass + PEG	Lotus	Lotus + PEG	SED
Live weight (kg/cow)	538	540	536	537	4
Intake (kg DM/cow/day)	14.9	14.9	17.4	17.1	0.5
Milk yield (kg/cow/day)	18.5	19.0	24.4	22.1	0.7
Fat (%)	4.5	4.7	4.6	4.6	0.2
Protein (%)	3.59	3.56	3.63	3.61	0.05
Milksolids (kg/cow/day)	1.49	1.55	2.01	1.81	0.05
Methane per unit intake (g CH <sub>4</sub> /kg DM)	24.2	24.7	19.9	22.9	0.8
Methane per unit production (g CH <sub>4</sub> /kg MS)	250	244	171	216	11
Methane energy (% of gross energy intake)	7.5	7.7	6.0	6.9	0.3

From Woodward *et al.* (2004).

only lasting three to four years under favourable dry conditions.

Forage willow was shown to contain approximately 30 g CT/kg DM, 20-35 g/kg DM phenolic glycosides and 10-15 g/kg DM flavenoid monomers. It was very successful as a supplement in drought conditions, when fed to ewes just before and during mating resulting in the number of lambs weaned/100 ewes mated being increased by 13-29% (McWilliam *et al.*, 2005). Weaned ram lambs grazing willow fodder blocks showed reduced burdens of *Teladorsagia*, *Trichostrongylus* and *Nematodirus* parasites relative to ram lambs grazing pasture (Diaz-Lira *et al.*, 2008). Subsequent studies (Mupeyo *et al.*, 2011) showed that willow feeding controls internal parasites through killing some, but not all, established parasites, reducing female worm fecundity and reducing egg hatching and development into L1 larvae. This research did not identify the cause of these reductions but from information in the literature the authors considered it likely that secondary compounds were involved.

## CHICORY

New Zealand produced the world's first forage chicory (Rumball, 1986) and nutritional work with it commenced in the 1990s. I have been involved in

**TABLE 7:** A summary of the results from three trials reporting the effect of grazing forages that contain condensed tannin (CT) on the growth and parasite status of anthelmintic drenched (parasite free) and non-drenched (parasitized) lambs.

Experiment/Measurement	Forage type		
	Lucerne	Sulla	<i>Lotus pedunculatus</i>
Experiment 1 (28 days duration)			
Total condensed tannins (g/kg DM)	1	120	-
Liveweight gain (g/day)			
Anthelmintic drenched	263	316	-
Non-drenched	28	231	-
Faecal egg counts (eggs/g), non-drenched	2,220	1,320	-
Experiment 2 (42 days duration)			
Total condensed tannins (g/kg DM)	2	99	-
Liveweight gain (g/day)			
Anthelmintic drenched	184	200	-
Non-drenched	-39	129	-
Total worm burden, non-drenched	19,268	8,016	-
Experiment 3 (42 days duration)			
Liveweight gain (g/day)			
Anthelmintic drenched	243	226	232
Non-drenched	121	175	160
Total worm burden (non-drenched)	18,084	13,090	23,665

From Niezen *et al.* (1995; 1998).

nutritional work with farmed deer and also summarised the feeding value of chicory for all animal species (Barry, 1998). My involvement with chicory was to find a forage that promoted high growth rates in young stags so that they attained 50-60 kg target slaughter weight by one year of age. Like white clover, chicory contains a high ratio of readily fermentable to structural carbohydrate (approximately 1.2:1.0), which allowed it to be broken down rapidly in the rumen and to have a high digestibility and voluntary feed intake.

Consequently animal growth is high on chicory, especially during autumn, making it a good feed for weaned lambs or deer calves. Relative feeding value during autumn for perennial ryegrass, chicory and white clover was 100, 161 and 191 respectively for the growth of weaned lambs and was 100, 141 and 126 respectively for the growth of weaned deer grazed on perennial ryegrass, chicory and red clover (Barry, 1998). Experiments during autumn have consistently shown reduced parasite problems with young lambs/deer grazing chicory than grasses or lucerne and a reduced need for anthelmintic drench (Scales *et al.*, 1995; Hoskin *et al.*, 1999). Laboratory experiments have confirmed anti-parasite properties of chicory (Table 8) and shown sesquiterpene lactones and low concentrations of CT to be two of the factors involved (Schreurs *et al.*, 2002; Molan *et al.*, 2003). There could be others. Management systems for chicory have been defined and promoted to the farming industry. These include sowing with a companion legume and not in grass mixtures, lax rotational grazing from spring to autumn and not grazing chicory stands during winter when they are dormant, as this causes plant death and reduced persistence. Currently the anti-parasite properties of chicory are receiving attention in many countries.

## FUTURE DEVELOPMENTS

From the foregoing it is clear that forages containing secondary compounds have the potential to improve a whole range of aspects of the nutrition and production of grazing livestock, including animal health aspects such as the control of bloat and internal parasites, environmental aspects associated with reduced methane production and urinary N excretion as well as increased animal productivity from wool, milk and reproduction.

Since the 1980s there have been advances in brassica plant breeding; notably the development of hybrid leafy turnips. These can be repeat grazed from eight weeks after sowing and are not associated with brassica anaemia. Liveweight gain at 200 g/day is still less than would be expected from their metabolisable energy concentration.

Waghorn (2008) emphasised the critical importance of CT structure in understanding the action of CT in ruminant nutrition. It may well be that a slightly different structure is needed to give optimum effects on parasite control compared by what is needed to increase EAA absorbed. It is important that we continue to derive information on CT structure and how it influences reversible reactivity with forage proteins in future studies.

**TABLE 8:** Effect of source of faeces, source of fluid and type of fluid upon the viability of deer L1 Lungworm larvae measured by the larval migration inhibition assay for deer fed either chicory or pasture.

Source of faeces to extract larvae	Source of rumen fluid	Percentage of larvae not passing through sieves	
		Rumen fluid	Abomasal fluid
Chicory	Chicory	49.1	39.9
Chicory	Pasture	43.8	39.1
Pasture	Chicory	40.4	29.6
Pasture	Pasture	27.7	21.2
Standard error of mean		2.0	2.0

With the existing CT-containing legumes, and also willow fodder blocks, their poor persistency under NZ grazing conditions means that widespread uptake by farmers is unlikely. What is really needed is to increase the CT concentration in the grasses and legumes that are commonly grazed in NZ from approximately 1-2 g/kg DM into the range, 5-10 g/kg DM to prevent bloat (Li *et al.*, 1996), 20-40 g/kg DM for *Lotus corniculatus*-type CT to increase EAA absorbed and a 30-40 g/kg DM minimum to control internal parasites (Hoste *et al.*, 2006). Barry and Reid (1985) first proposed the transfer of DNA coding for CT production in leaves from plant species such as lotus into white clover and lucerne, to fulfil this objective. After many attempts to do this using transgenic techniques, success has finally been achieved by inserting a CT transcription factor (masterswitch) isolated from hares foot clover (*Trifolium arvense*) into the genome of white clover and lucerne, resulting in the expression of significant CT concentration in the leaves of both species (J. Caradus, Personal communication). The trace levels of CT present in normal white clover and lucerne are expressed in the trichomes (leaf hairs) only, but the DNA responsible for CT production is probably present in all plant tissues, but is not expressed. The masterswitch transferred from hares foot clover turns on the expression of CT-production in leaf tissue. These developments are in the early stages. Progress is awaited with interest. If successful they would have great application for improving the health, welfare and productivity of grazing ruminants in NZ, whilst also benefiting the environment.

The future uptake of chicory in NZ grazing systems will continue to depend on selling a management system for grazing chicory as well as selling the seed. In the case of chicory its higher metabolisable energy concentration does translate into higher live weight gain with improved animal health and comparatively strong agronomic qualities providing additional benefits.

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