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Brief description of the Deliverable

Supporting innovation related to forage production utilization and feeding in organic and low-input agriculture based on participatory studies conducted in UK, Romania and Finland.

Target audience(s)

Researchers, farmers and extension services

Executive Summary

The SOLID project (Sustainable Organic Low-Input Dairying) carried out research to improve the sustainability of low-input/organic dairy systems in different ways. This deliverable is one of the three that are resulting from the WP1 of the SOLID project and presents and discusses a series of participatory on-farm studies that were conducted to test innovative approaches related to forage production, utilization and feeding in organic and low-input dairying systems. Specifically, the participatory studies focus on a) improvement of soil organic matter through grazing management and establishment of diverse swards b) on-farm strategies for the improvement of forage yield and protein content of the pasture c) utilisation of industrial by-products for dairy cow nutrition and d) the effects of dietary mineral supplementation on mineral concentrations in milk, with particular focus on iodine supplementation.

Improvement of soil organic matter through grazing and establishment of diverse swards

Amongst farmers, researchers and stakeholders there is renewed interest in the use of herbal leys and mixed swards and more information is needed on how to manage diverse swards and how to best exploit their potential for milk production on dairy farms. This is because mixed leys can have benefits for the pasture and animal productivity but also for the environment. Recent evidence shows that legume-based leys can maximise pasture productivity and other ecosystem services while functional diverse plant species can be optimised and fine-tuned to farm-specific needs. Diverse swards have increased above-ground biomass and provide greater stability of biomass production compared to monocultures while productivity increases over time.

In addition, "mob grazing" as a livestock management grazing strategy is considered to increase the organic matter in the soil which in turn results in increased forage productivity. Mob grazing is characterized by high stocking densities of livestock which are moved frequently from paddock to paddock with the aid of electric fences, trampling forage into the soil as they graze. The pasture land is then left ungrazed until it is fully recovered, allowing a whole host of plant species to establish in the sward. There is a growing notion that trampling of significant quantities of forage onto the soil surface provides a better environment for microorganisms and other soil life and increases the soil organic matter. The aim of the case study carried out in the UK was to determine a) the establishment and productivity of diverse swards compared with ryegrass-white clover on commercial dairy farms and b) to determine the effects of mob grazing on soil organic matter, and the performance of dairy cows in terms of energy utilisation and milk production. The study showed that soil improvement management through rotational high stocking grazing of bio-diverse pastures has a remarkable effect on the build-up of the soil organic matter; while microbial activity in the soil is moderate this can be improved by bio-treatment of slurry or farmyard manure. The results also show that pasture productivity of the diverse sward ley was slightly lower than that in the grass-clover ley. However, the total productivity remained relatively high suggesting that bio-diverse pastures serve as a viable alternative to conventional pastures (i.e. grass / clover pastures) as they can maintain animal productivity at high levels.

On-farm strategies for the improvement of the protein content of mixed lays for silage production

It is well known that high dry matter forage yield and high forage quality from the pasture is a key feature for the competitiveness of the organic and low-input sector as in these farming systems animals are normally pasture fed. In this respect farmers are always keen to research new approaches in order to increase both forage quantity and quality. On-farm experiments carried out in Finland aimed a) to assess the effects of topping of grass on the development of red clover in grass-clover mixtures and b) to test the effects of slurry application in the autumn on grass-clover pasture productivity. Two on-farm participatory experiments took place during 2013-2014. The exceptionally warm growing season in 2013 resulted in more vigorous and rapid early growth of red clover than usually. The difference in height between red clover and grasses was not sufficient in the early growth stage in May to top grass without damaging red clover. Thus, topping of grass (which happened later in the season) had a negative effect on clover content, total DM yield and protein content in the first harvest. This negative effect is attributed to the rather short period that intervened between the topping and the first harvest which was less than two weeks. The optimal timing for topping of grass to enhance red clover contribution cannot be determined by the results of this study and in this respect more research is needed. The results also show that estimation of DM by farm scale harvester is a reliable method as it determines the complete forage production of the harvested area compared to DM estimation by plot samples whose accuracy depends on the number of replicates obtained. The second on-farm trial showed that application of slurry (i.e. 20 tonnes of organic dairy cow digestate per ha including 62 kg soluble nitrogen) in the autumn has a positive significant effect both on soil nitrate and ammonium nitrogen and can increase crude protein content of grass and total forage crude protein yield allowing for better silage quality the following spring. The risk of nitrogen leaching seemed to be low, but it is unknown whether this is also the case for phosphorus. This study shows that on-farm participatory research is beneficial both for farmers and researchers and is proven to be an efficient approach to conduct research that addresses on-farm management problems and challenges.

Utilisation of industrial by-products for dairy cow nutrition

In Romania the two on farm experiments were conducted to assess the effects of camelina meal and dried grape marc on milk production and milk quality and to determine whether these by-products can serve as potential alternative feeds for dairy cows in low-input systems. These particular industrial by-products were selected because they are widely available in the market at competitive prices. Camelina meal is the extruded product remaining after cold extraction of the oil from the camelina (Camelina sativa) plant, which is a relative to Rapeseed (*Brassica napus*). Camelina meal has a nutritional value that is similar to other well-known protein meals, such as sunflower meal or rapeseed meal, but, there are a number of unknown aspects such as rumen degradability and influence of residual fats on milk quality that have to be studied. Grape marc is a by-product from the viticulture industry with a relatively low nutritional value, which consists of skin, pulp, seeds and stalks (Baumgärtel *et al*., 2007, Nair and Pullammanappallil, 2003); however, from a farmer's point of view its use could be beneficial in periods of feed shortages.

Two on-farm trials were conducted in order to assess the effects of these underutilised by-products on the performance of dairy cows. The results show that replacement of sunflower meal with camelina meal had no significant effect, neither on milk yield nor on milk protein or lactose. However, it decreased milk fat content by about 15%, which confirms previously reported findings in the literature. This outcome is important in systems where milk price at the farm gate is corrected in view of the milk fat content. The effects on milk fatty acids profile were positive, with an increase of PUFA (including the CLA). Replacement of one third of corn grains and barley grains with dried grape marc, did not affect milk yield and milk composition (milk fat, protein and lactose content) but it significantly increased milk PUFA, especially n-6 PUFA and, of these, linoleic acid. It is concluded that both camelina meal and dried grape marc can replace, at least in the short term, the more classical feedstuffs without noticeable adverse effects, except the decrease of milk fat in the case of camelina meal. It is important to underline that these results were obtained within a low input production system, on cows with a moderate production level. Whether these feedstuffs can be used extensively in the nutrition of the dairy cow will be determined by their price in the market.

Effects of dietary mineral supplementation on milk iodine concentrations.

Organic milk normally contains less iodine than conventional milk but the concentrations of iodine in organic milk are well within the optimal levels for human nutrition as it is evidenced by a number of European studies. Nevertheless, the relatively lower iodine concentrations in organic milk trigger discussions amongst stakeholders, farmers and researchers in view of the recent evidence that iodine intake has decreased due to a decrease in milk consumption in the UK. The aim of this study was to investigate the relationship between iodine concentrations in bulk milk samples with iodine concentrations in forage on organic dairy farms in view of farm practices. Ten organic dairy farms located in the sough-west of England participated in this farm comparative case-study and data on iodine and other mineral concentrations in milk, blood, urine and forage samples were collected during 2014 - 2015. The results show that the monthly milk iodine concentrations averaged over the farms remained within optimal levels, but in some farms milk iodine concentrations were systematically low throughout the monitoring period. The results show that urine iodine is indicative of dietary iodine intake; however, with regards to milk iodine, this was not the case: farms with low or average forage iodine concentrations had higher milk iodine compared to the farms with high forage iodine concentrations. This outcome reflects the fact that milk iodine concentrations are affected by the use of iodine-based teat disinfectants. Indeed, the data show that milk iodine concentrations were 2.3 times higher in the farms that use iodised post-dip teat disinfectants (mean average 195 ± 13 μg/L) compared with the farms that do not use iodised post-dip teat disinfectants (mean average 85 ± 8.9). In conclusion, this study showed that the use of iodised post-dip teat disinfectant is the most important influencing factor for the iodine concentration in milk and that where post-dip teat disinfectant is used the iodine concentrations in milk do not serve as a robust indicator in identifying shortfalls in iodine intake. Milk iodine concentrations fluctuated within farms across samplings but in some farms they were systematically low. This outcome deserves further attention in order to alleviate recent concerns that organic milk contains less iodine than conventional milk and to avoid that the health status of the animals might be negatively affected by low iodine intake. Where doubts about the iodine supply to animals exist, urine samples can be used to monitor the cow's iodine status.

Potential Stakeholder impact(s)

The studies presented herein bring forward innovative practices that can be used at a farm level in order to increase forage production and utilization and plan effective grazing and feeding strategies in organic and low-input dairy systems. The outcomes of these studies contribute towards the improvement of the self-reliance, and hence the competitiveness, of the organic and low input dairy farmers. They also contribute towards the developing of better tools to manage on-farm feed utilisation, feeding management and use of novel forage resources. The studies presented herein also address expectations of the consumers and society with regards to enhanced food security. This is essential for maintaining consumer confidence in organic and low input dairy products. In addition, they stimulate discussions on how to support innovation and how to further improve the engagement of farmers in order to produce high quality research that can influence the strategies.

Interactions with other WPs Deliverables / joint outputs			
WP no.	Relevant tasks	Partner(s) involved	Context of interaction
1	1.2	ABER, AU, DAPVET, BOKU, C-WEN, CSIC, INCDBNA, IOTA, LUKE, OMSCo, UNIVPM, EV-ILVO, ICEA, PROODOS, JLUOMO, WGOV, CABRA, THISE, AGR-SOL	Deliverable 1.1: Provides information on the identification of research projects through rapid sustainability assessments in organic and low-input dairy farms across Europe
3	3.1	CSIC, LUKE, INCDBNA, ORC	Deliverable 3.1: provides background information of the use of novel and under-utilised feed resources in organic and low-input dairy production
3	3.1	CSIC, LUKE, INCDBNA, ORC	Deliverable 3.3 provides information on novel and underutilized feed resources including by-products from processing of renewable raw materials
3	3.2	ORC	Deliverable 3.2 provides information on assessing an agroforestry system in terms of feed supply and multifunctionality

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Sustainable organic and low-input dairy production

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D 1 2. Innovative strategies related to forage production, utilization and feeding for dairy cow productivity

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1. Introduction

It has long been recognised that participatory research can integrate knowledge and action for the mutual benefit of all partners involved and offers ways of making scientific knowledge more applicable (Cornwall and Jewkes 1995). Over the last decade there has been an increase both in the number and the variety of participatory research projects within the agricultural sector in many parts of the world, for example in Africa, Asia, Australia and Central America (Pimbert, 2011). This reflects the notion that close collaboration and knowledge sharing between farmers and researchers appears to be a promising strategy to support innovation and find solutions within the agricultural sector, rather than technology transfer or dissemination alone (Padel *et al*., 2015). Participatory research is less widely recognised in Europe than the other continents mentioned above mainly because this type of research implies significant changes in dominant policies and practices both within research institutes and relevant EU commissions (Pimbert, 2011).

The participatory research and development approach adopted by the SOLID project was ideally suited to take full account of farmers' knowledge and creative potential in order to develop innovative solutions to a range of important challenges in the organic and low-input dairy sectors that relate to environmental impact and biodiversity, pasture management, breed choice and animal welfare and health. The research priorities were identified through sustainability assessments that were carried out on more than 100 (organic/low-input) dairy cow or dairy goat farms in nine countries across Europe. The results were discussed with farmers and stakeholders in national workshops where specific research topics were identified (see Leach et al. 2013 for further details). In total, eighteen on-farm participatory trials or case-studies were conducted in the UK, DK, FI, RO, ES, GR, AT and IT covering aspects related to feeding and forage, use of natural resources, environmental impact and animal management and health. This work was co-ordinated by the Organic Research Centre. This deliverable is one of the three that presents results from a selection of these studies (see also Vaarst et al., (2016) and Yanez et al., (2016).

The participatory studies presented and discussed in this report focus on challenges related to feed and forage production and utilisation. Particularly, they address issues related to a) the improvement of the protein content of forage for silage production, b) improved pasture utilization and soil management c) innovative animal feeding by the use of agro-industrial by-products and d) effects of dietary mineral supplementation on mineral concentrations in milk, with particular focus on iodine supplementation.

These aspects are crucial for pasture-based low-input dairy systems as they can impact on animal performance, animal health and welfare, and affect the overall sustainability of the system. In addition, feeding and nutrition of the dairy cow represents the highest cost in producing a litre of milk and therefore is one of the most important factors in efficient dairy production. Nutrition is also a key factor in the overall performance, health, and welfare of dairy cattle. In these respects, farmers, particularly within the organic and low-input sectors, must increasingly concern themselves with optimizing feed efficiency and nutrition. Given the high reliance of organic and low-input dairy cattle on forage resources and the various environments in which they are maintained, producers may inevitably have to adjust methods for forage production and adopt grazing strategies for better pasture utilisation while broadening the inclusion of alternative feed resources and diverse swards into their system (Zollitsch *et al.*, 2004).

The on-farm participatory studies presented herein were conducted in the UK, Finland and Romania under the European project "*Sustainable Organic and Low Input Dairying*" (acronym SOLID; www.solidairy.eu). In addition to the presentation and discussion of the outcomes of these studies, this report also aims to stimulate the discussion on how to support innovation related to forage production, utilization and feeding in organic and/or low-input agriculture and how to further improve the engagement of farmers in order to produce high quality research that can influence the strategies.

1.1. Advances for increased pasture productivity and nutritional value

There is an increasing number of farmers who are seeking ways to reduce their costs of production by using less fertiliser and by reducing the amount of purchased feed. To help to achieve this, some farmers, particularly from the organic or low-input sector, choose to grow diverse swards with high proportions of different legumes, grasses and herbs. Whilst there are many benefits from mixing leys, some farmers are not familiar with this practice and have reservations about their use compared to the typical grass-clover mixtures. This review addresses some of these concerns and provides information from relevant research projects that is regarded as important for farmers and advisors.

There are several EU projects such as "Multisward", "Eurolegumes" and "Legume futures" that provide documented information about increased pasture productivity from mixed species and diverse swards. In the UK a recently completed project (LEGLINK) aimed to determine the ease of establishment of mixed legume species and their efficiency of production over time. The project involved replicated field experiments in multiple locations and the trials tested the performance of 12 legume and 4 grass species that were sown in monocultures or as a mixture. In addition, the study mixture was compared with farmer-chosen ley mixtures. It was concluded that species-rich legume-based leys can maximise pasture productivity and other ecosystem services while functional diverse plant species can be optimised and fine-tuned to farm-specific needs. More specifically, the project revealed evidence that diverse swards have increased above-ground biomass and provide greater stability of biomass production compared to monocultures while productivity increases over time. In addition, they have greater resilience to adverse weather, climate and management conditions.

According to Döring *et al*. (2012), mixes with high agronomic productivity contain both lucerne and white clover while the overall performance improves with the inclusion of a third or fourth legume species. The three best multifunctional mixtures all contained black medic, lucerne and red clover. Some species such as meadow pea, winter vetch, large birdsfoot trefoil showed low performance on almost every occasion; however, these species can perform better under other climatic conditions as other European studies have shown (Clapham *et al.*, 1987; Döring *et al*., 2012). White clover consistently performed well in terms of yield and persistence and its creeping habit makes it the best legume adapted to grazing. Red clover is generally more productive than white clover, but, less persistent and less tolerant to high grazing pressure than white clover (Smetham, 1973; Cormack, 1996). Due to its long roots, red clover is regarded as drought tolerant (Knight *et al*., 2008). Lucerne also produces high quality feed, when dried or ensiled, although it is not commonly grazed. The

LEGLINK project showed that Lucerne is a high yielding species but not significantly higher than red or white clover. At some sites, Lucerne appeared to be relatively intolerant to being cut short, suggesting intolerance to grazing. Nevertheless, some farmers claim that they have managed their stock to graze it successfully. In terms of grass species, ryegrass is recommended for high yield while festulolium, which is a cross between a fescue and lolium (Perennial or Italian Ryegrass), provides a combination of high quality forage with good winter hardiness, persistence and stress tolerance.

Sweet clover seed performs best when inoculated with rhizobium bacteria. In the LEGLINK project it did not perform particularly well without inoculation. It is deep rooted, so would contribute to mining nutrients; factors making it less suitable for grazing are the risk of bloat, a bitter taste due to coumarin content, and the fact that it contains an anti-coagulant produced from the coumarin which can cause a bleeding disease. Sainfoin is attractive as a non-bloating legume, but does not survive well in competition from grasses.

A study by Lindstrom *et al*. (2013) has shown that legumes and herbs (i.e. red clover, white clover and chicory) compared with grasses, can provide considerably higher amounts of minerals per kg DM of grazed forage which is particularly important for the pasture-fed cow in organic or low-input dairy farms. The same study also showed that the micronutrient status of the soil, and variety within plant species has a minor effect on the mineral content of legumes and herbs, but the pH of the soil can affect mineral concentration in the herbage, particularly of manganese and molybdenum. In digestibility trials with sheep, Andueza *et al*. (2013) have found that swards rich in forbs have higher digestibility in the early season compared to swards rich in grasses, indicating nutritional advantages of mixed swards.

1.2. Use of industry by-products for dairy cow nutrition

The quantity and quality of available feed resources is a key determinant of total system output and overall profitability. Organic and low input dairy systems are unique in their high reliance on internal forage resources which will at least temporarily limit system productivity and inevitably may require production goals to be adjusted (Schiere *et al*., 1999; Zollitsch *et al*., 2004). This, together with increased volatility in feed prices, highlights the need for a broadening of feed resources and the utilization of novel feed components that are currently under-utilized. In addition, strategies that optimise the management of feed resources have the potential to reduce the risk inherent to organic and low input feed supply chains (e.g. seasonality of pasture/ forage production). The potential multi-functionality of organic and low input dairy production systems has to be addressed through assessing approaches that involve increasing the accessibility of feed resources to dairy farmers, and developing tools with the potential to optimise the management of internal resources.

A desk-top literature review that was carried out as part of the SOLID project (Deliverable 3.1) indicated the potential of novel feeds for inclusion in organic and low-input dairy production. There is a range of by-products and underutilized sources that can be used as animal feeds. However, there is also a necessity for additional research concerning the suitability of certain by-product as feeds for dairy cows. In this respect this report reviews the potential of two novel industry by-products that can be used as alternative feeding resources in organic and low-input dairying systems.

Camelina (*Camelina sativa*), a relative to canola, is a drought resistant seed that is adapted to cooler northern climates. It is an autumn or spring planted annual oil crop species with pale yellowish or brown coloured seeds which are quite small. Camelina germinates and emerges well before most cereal grains. Camelina meal is the extruded product remaining after cold extraction of the oil. It generally has high protein content and a variable proportion of residual oil, which adds to its energetic value. The nutritional value of camelina meal varies because of the variation in raw materials and processing technologies, and thus, its potential effects on animal performance may also vary. Camelina meal has a crude protein content that varies from 23 to 41% and neutral detergent fibre (NDF) content from 27% to 35% (Zubr, 2003; Hurtaud & Peyraud, 2007; Malgorzata *et al*., 2011; Moriel *et al*., 2011). Camelina meal is abundant in essential amino acids (Halmemies *et al*., 2011) whereas its residual oil has a high content of polyunsaturated fatty acids, having potential effects on milk quality. So far, only a few studies have been conducted on using camelina meal in the feeding of dairy cows. Moriel *et al*., 2011, reported an *in vitro* DM digestibility of 0.706, while Hurtaud & Peyraud (2007) reported shifts in acetate:propionate ratio in rumen fluid. There are only a few results on the use of camelina meal in cattle, two of which focus on the residual oil (Hurtaud and Peyraud, 2007, Halmemies *et al*., 2011). In both studies, milk yield was not influenced, whereas effects on milk fat were divergent: the first team reported a decrease whereas the second team found no effects. Divergent results were obtained also for DMI: no effects were found by Hamelmies *et al*. (2011) on dairy cows and Moriel *et al*., (2011) on heifers, whereas Hurtaud and Peyraud (2007) reported a decrease, following inclusion of camelina meal in diets. However, several authors referred to changes in FA profile of milk. The use of this crop is likely to increase in the future (Matthaus, 2004; Melcher, 2010) and its potential for organic production systems has already been identified (Henriksen and Prestløkken., 2008). However, there are limited studies that have tested the effects of camelina meal on animal performance and its suitability for incorporation in dairy cow feeding strategies.

The other underutilized feed source that is investigated in this report is the viticulture by-product grape marc. The viticulture industry produces huge quantities of this by-product (Nair and Pullammanappallil, 2003) and improper disposal of this by-product raises environmental concerns (Santos *et al*., 2014). The increasing concerns about the future availability of feed resources and stricter rules for environmental protection have stimulated discussion about the potential use of this by-product in animal feeding. It is estimated that grape marc contains 30% stalks, 23% seeds and 42% peels (Nerantzis and Tataridis, 2006). Although these figures provide a guide only, the proportions of the seeds, stalks and peels can influence its nutritional value. In addition, its high content of lignified cell walls and tannins is an important element from a nutritional point of view.

The crude protein content of grape marc varies between 12.5 and 17% while NDF content varies between 22 and 62%; the digestibility also varies, while its level is generally low (Sauvant., 2002; Pétriz-Celaya *et al*., 2010; Moghaddam *et al*., 2012). The nutritional value of grape marc varies in accordance with the different raw materials used such as red or white grapes (Zalikarenab *et al*., 2007) but also in accordance to the different processing methodologies used. Although grape marc as a by-product is widely available and its use in animal feeding is not uncommon in some European countries, the number of studies testing its effects on animal performance is surprisingly low. Greenwood *et al*. (2012) investigated the effects of grape marc on nitrogen partition and found that it contributes to high excretion of nitrogen in faeces. Pétriz-Celaya *et al*. (2010) reported higher DMI in lambs, presumably due to good palatability of grape marc. Santos *et al*. (2014), found no effects on DMI or milk production in dairy cows when grape marc was offered up to 10% of the diet (on DM basis), as silage. Nevertheless, the number of available studies is low and most of them do not cover adequately the effects of grape marc on animal performance nor on milk quality. The nutrition of the animal is a major factor influencing milk yield and milk quality. It is therefore important to assess the effect of these novels feeds on animal performance and milk quality in the context of organic and low-input dairying systems.

2. Diverse swards and mob grazing for dairy farm productivity

2.1. Introduction

Over the last fifty years or more, with the drive for high levels of production, the use of herbal leys and mixed swards became rare in European countries, surviving with a few enthusiasts, mainly in organic systems, or as part of specific habitat management schemes (Foster, 1988). Nowadays there is a renewed interest and farmers are seeking more information about how to manage diverse swards and how to best exploit their potential for milk production in dairy farms. The herb species commonly included in grazed leys are largely chosen for either their deep rooting nature and hence their ability to reach minerals from deep in the soil, or for palatability and possible medicinal properties. The most common are chicory, ribwort plantain or "ribgrass", sheep's parsley, burnett and achillea. The following notes are taken from Turner (1974): «*Burnett is highly palatable, deep rooting and has tonic properties. Plantain is also palatable, and contains a very high concentration of minerals. Achillea has high protein and tonic properties. Chicory can root up to ten feet deep, so is the ultimate collector of minerals from deep in the soil. It has remarkable powers of recovery after grazing in dry conditions. Sheep's parsley is included for its medicinal value, being high in minerals and in the compound "apiol" which is effective for treating kidney and bladder complaints*».

Farmers are interested in increasing soil organic matter (SOM) because it is well known that it serves as a reservoir of nutrients for crops, provides soil aggregation, increases nutrient exchange, retains moisture, reduces compaction, reduces surface crusting, and increases water infiltration into soil. The build-up of SOM can be influenced by the way in which the sward is managed (e.g. increasing the return of vegetation to the soil), and also by the plant species in the sward. In this respect, "mob grazing" is considered as a livestock management grazing strategy to increase the organic matter in the soil.

Mob grazing is characterized by high stocking densities of livestock which are moved frequently from paddock to paddock with the aid of electric fences, trampling forage into the soil as they graze. The pasture land is then left, ungrazed until it is fully recovered, allowing the whole host of plant species to establish in the sward. It is considered that leaving higher residues in the paddock can be a strategy for building up SOM, through the contribution of "liquid carbon" through plant roots. Plants with more above ground canopy are able to grow larger root systems than those that are grazed more severely and the long recovery time between grazing allows plants to establish a healthy root system. The roots grow deeper into the soil, bringing up nutrients and making the plant more drought-hardy. The long recovery time also leads to high volumes of above-ground forage, a mixture of leaf, seed and stem. In addition, it is claimed that high stocking density results in more than 50%

of the plant being trampled into the ground by the animals. Uneaten plant stems are trodden onto the soil surface and these stalks act both as mulch and as a food source for the soil microorganisms, building new soil in the process (Chapman 2011; Richmond 2011). It is also claimed that by turning animals out into a fully mature pasture, animal performance is improved as they can select the most nutritious parts of the plants and benefit from grazing the lush tops of the plants, seed-heads and upper leaves that are high in energy and protein.

It is regarded that "Mob Grazing" as a grazing system has its basis on the grazing patterns of some species of wild herbivores roaming unrestricted over large rangelands: animals spend a short time in a small area before moving on, leaving behind manure concentrated on a small area, and considerable plant residues, above and below ground, both of which contribute to SOM and to soil nutrients (Savory and Butterfield, 1999). Mob grazing tries to simulate the grazing behaviour of vast herds of wild herbivores found on the American plains, or in the African savannah. Some authors consider mob grazing to be similar to the holistic grazing approach (Savory, 2013), while McCosker (2000), in an extensive categorisation of grazing methods, uses the term "Cell Grazing" instead of "Mob Grazing" and places these two approaches in different categories. Allen *et al*., (2010) use the term "Mob Stocking" instead of "Mob Grazing" and refer to it as "a method of stocking at a high grazing pressure for a short time to remove forage rapidly as a management strategy". Amongst farmers another term is frequently used, that of "Tall Grass Grazing". This inconsistency in the terminology and the different perspectives on this grazing system create some confusion and in many cases make it difficult to compare and discuss its claimed benefits. Herein we will regard "Mob grazing" as a "short duration, high-density grazing followed by a long recovery period".

Picture 2.1. Suckler cows graze tall, mature pasture in Mississippi, USA (source, Tom Chapman, 2011).

Published work on such grazing systems to date has mainly been carried out in arid areas (Savory and Butterfield, 1999). According to Savory Institute (savoryinstitute.com) this management has been shown to provide environmental improvements on previously overgrazed areas in Africa, Australia and America, through the return of organic matter to the soil. Clatworthy, (1984) was the earliest publication which showed that "planned grazing under mob stocking principles" in Rhodesia doubled the number of animals which an arid area could carry, compared with a "government grazing system" (not defined), with no deterioration of the plant community. In another arid region, South Idaho, Weber and Gokhale (2011) demonstrated a statistically significant increase in soil moisture retention under "holistic planned grazing" (i.e. 3 day grazing at high stocking density) compared with both total rest of land and with a 30 day grazing with a lower stocking density.

The claimed benefits of mob grazing on SOM and animal performance have not been studied in scientifically robust experiments/studies and this gap in scientific knowledge is reflected in the literature. In the UK, there is a small but growing interest in this grazing method especially in the view that this method contributes to increasing SOM, but there is some uncertainty about the levels of production that may be achieved. Because mob grazing involves high stocking density for a short period of time with long recovery times between consequent grazing periods, there is some uncertainty as to how applicable is this grazing management approach under UK conditions.

This study aims to evaluate whether mob grazing on diverse swards can increase the organic matter of soil and provide information for those considering adopting this approach. Soil, forage and animal production data were collected from April to September in 2014 while preliminary assessments were also carried out in 2013.

Picture 2.2. Pasture with diverse swards (*Photo by K. Zaralis*).

2.2. Methodology and Data collection

The participating farm

A UK farmer has studied practical aspects of management relevant to the issue of increasing soil carbon and he introduced a mob grazing approach on diverse swards with the aim of increasing SOM. The farmer believes that longer intervals between grazing periods are likely to be best suited to swards that are more diverse than those based on ryegrass which are currently typical of UK dairy farms. According to the farmer, mob grazing holds the key to improving and maintaining soil fertility and forage productivity in his organic system.

The case study farm is a 220 ha mixed dairy /arable farm at approximately 260 m above sea level. It has a long history of arable use in many fields and was converted to organic production in 2005. The herd consists of Friesian-Shorthorn cross dairy cows that are spring calving, with a lactation period of 300 – 310 days. Full-time housing of the cows is limited to two months (i.e. December and January). Kale and fodder beet are grown for additional winter grazing.

The seed mixtures used

Diverse swards in the farm were established in 2007 and leys were reseeded as part of the rotation every five years with a diverse sward mixture that includes 10 different grass species, six legumes and five herbs as shown in Table 2.1. The best method for establishing the long-term diverse ley was found to be sowing under a spring cereal crop. The control sward (i.e. grass – clover) was established in equivalent conditions in terms of date, soil type, site location and establishment methods.

Table 2.1: Composition of the seed mixes used in the participating farm.

Species	Diverse Sward	Grass/Clover
Grasses	% by weight	% by weight
Cocksfoot *(Dactylis glomerata)*	7.8	
Creeping red fescue *(Festuca rubra)*	5.4	
Crested dogstail *(Cynosurus cristatus)*	1.6	
Italian ryegrass *(Lolium multiflorum)*	7.8	
Meadow fescue *(Phleum pratense)*	7.0	25
Perennial ryegrass *(Lolium perenne)*	7.5	25
Meadow grass *(Poa pratensis)*	1.6	
Tall fescue *(Festuca pratensis)*	3.9	
Timothy *(Festuca arundinacea)*	5.4	
Yellow oatgrass *(Trisetum flavescens)*	1.6	
Legumes		
Alsike clover *(Trifolium hybridum)*	1.6	
Sanfoin *(Onobrychis viciifolia)*	20.2	
Sweet clover *(Melilotus officinalis)*	5.4	
Birdsfoot trefoil *(Lotus corniculatus)*	2.3	
Red clover *(Trifolium pratense)*	2.3	25
White clover *(Trifolium repens)*	2.3	25
Herbs		
Burnet *(Sanguisorba minor)*	6.2	
Chicory *(Cichorium intybus)*	5.4	
Ribgrass *(Plantago lanceolata)*	0.8	
Sheeps Parsley *(Petroselenium Crispum)*	2.3	
Yarrow *(Achillea millefolium)*	1.6	

Pasture productivity, forage composition and feed intake estimation

Herbage yield and composition of the swards were assessed on a monthly basis in the same field (*Big Aero*) which was representative of the type and the age of the swards across the farm. The square-metre quadrat method was used to determine the productivity of the grazed pasture and thus allowed estimates of dry matter (DM) intake of the grazing cows. Briefly, a one-square-metre quadrat was placed randomly three times across the un-grazed plot (i.e. this was the next plot to be grazed by the animals within the next 12h). All the vegetation within the quadrat area was cut to approximately 5 cm height and the cut herbage from each quadrat was collected in separate bags; fresh weight was recorded. The same procedure was followed also in the residual forage in the grazed plot (i.e. this was the plot the animals had just grazed).

Dry matter content of the herbage both in the un-grazed and grazed plots were determined, allowing the calculation of the total DM productivity of the field while differences in DM productivity between un-grazed and grazed plots were used to estimate forage DM intake of the grazing cows. Sub-samples of the harvested forage were separated into four categories as follows: a) grass, b) clover c) other legumes and broadleaves and d) senescent material allowing for the determination of percentage of grass, clover, and broadleaved species on the grazing plots. Additional herbage samples were analysed by wet chemistry for metabolisable energy (ME) and Crude Protein (CP) content.

Picture 2.3. Assessing productivity of diverse lays with the quadrat method (i.e. 1, un-grazed plot; 2, grazed plot)

Monitoring of farm records and additional calculations

At the end of the monitoring period each year the farmer provided data and information regarding milk production and composition, grazing records (i.e. area and livestock numbers grazed daily) as well as supplementary feeding records regarding forage and concentrate supplementation, amounts and periods fed. These data in addition to chemical analysis data were used to estimate the ME intake of the cows over each season from the given field. Data from the sampled field were extrapolated to provide an estimate for the whole farm for each year.

Soil Samples

Historic data on the organic matter content of soil from three different fields are available from 2007, and 2012. At the end of the two years' monitoring, soil samples were taken again in spring 2015 from these fields to assess the change in soil organic matter (changes in SOM are likely to be slow, so maximising the time will increase the likelihood of detecting a change).

2.3. Results and Discussion

Pasture Productivity

The productivity of the diverse sward during the monitoring period (i.e. May to September 2014) averaged 10.5 tonnes per hectare. Herbage composition and monthly productivity of the diverse swards grazed by young stock (i.e. dairy bulls and heifers) from April to September are shown in Figure 2.1a. Preliminary data collection also showed a similar productivity for the same season in 2013.

Herbage production reached a peak performance in June (i.e. 3.3 tonnes DM per ha) while the composition of the herbage fluctuated from month to month as shown in Figure 2.1a. Clover production accounted for about 46% while grass production accounted for 34% of the total herbage production. The productivity of other legumes and “broad leaves” represented 14% of the total production and senescent material was about 6% of the total production. Preliminary data collected in 2013 indicated a similar productivity of the diverse sward (data not shown).

Forage samples collected at farm visits were analysed for chemical composition. The diverse sward had an average of 15.7% DM, 9.7 MJ of ME and 21% of CP indicating a good quality forage. The average ME content was marginal as normal values for this type of forage are 11 to 13 MJ of ME per kg DM, but CP content was high and NDF within the expected levels.

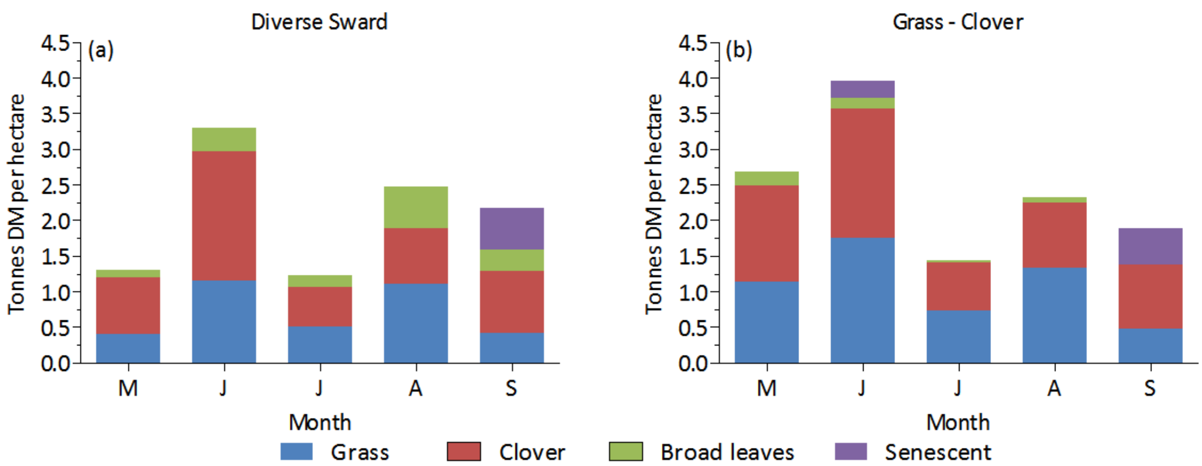

Figure 2.1. Monthly pasture productivity (tonnes of DM per hectare) and herbage composition of diverse swards (panel a) and grass-clover (panel b) grazed by dairy bulls and heifers.

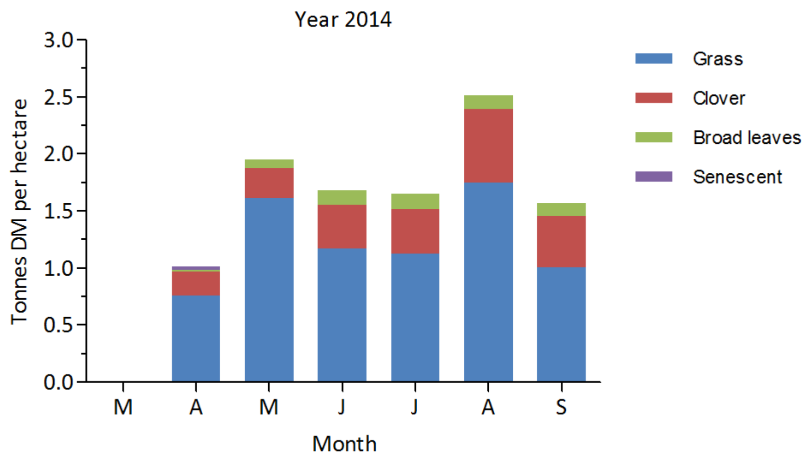

Figure 2.2. Monthly pasture productivity (tonnes of DM per hectare) and herbage composition of diverse swards grazed by dairy cows.

Herbage production of grass-clover ley during the monitoring period (i.e. May to September 2014) was better than those of diverse swards and averaged 12.3 tonnes per hectare. As expected, composition was dominated by grass and clover which accounted for about 44% and 46% respectively, while the productivity of other legumes and "broad leaves" represented just 4% of the total production (Figure 2.1b); as in the diverse swards, senescent material was about 6% of the total production. The grass-clover lay had an average of 17.2% DM, 10 MJ of ME and 21.5% of CP indicating a good quality forage.

The productivity of the sward grazed by dairy cows during the monitoring period (i.e. April to September 2014) averaged 10.3 tonnes per hectare. Herbage composition and monthly productivity of the diverse swards grazed by the cows from April to September are shown in Figure 2.2.

Herbage production increased from April to August while the composition of the herbage in summer months remained relatively constant as shown in Figure 2.2. Clover production accounted for about 24% while grass production accounted for 71% of the total herbage production. The productivity of other legumes and "broad leaves" represented 6% of the total production and senescent material remained below 3% of the total production. Preliminary data collected in 2013 indicated a similar productivity of the grazed sward but herbage composition fluctuated between months (data not shown).

Five forage samples collected at farm visits during April – September 2014 were analysed for chemical composition. The grazing diverse sward had an average of 19.1% DM, 10.8 MJ of ME, 21.3% of CP and 376 g of NDF indicating a good quality forage. The average ME content was marginal as normal values for this type of forage are 11 to 13 MJ of ME per kg DM, but CP content was high and NDF within the expected levels.

Gazing data, feed intake and cow productivity.

Grazing data from the 3rd of April to the 25th of September 2014 are shown in Table 2.2. On average 181 milking cows grazed a diverse sward field of total area of 12.5 ha for a total of 43 days in monthly rotation intervals. The duration of the grazing varied from 6 to 10 days based on herbage availability. The cows were moved on twice a day after each milking, grazing two adjacent plots of an average size of 0.9 ha delimited by electric fences. The average stocking density over the grazing period was about 200 dairy cows (of an assuming BW of 550 kg) per hectare. The resting period of the diverse sward between consecutive grazing periods averaged about 21 days with 16 and 25 days the shortest and the longest, respectively. These resting periods do not coincide with the principles of "mob grazing" where resting periods are of long duration (i.e. more than 50 days) but the stocking density was relatively high (Table 2.2). In year 2013 the farmer was applying a 40 to 50 days rotation management allowing the pastures to recover for longer but the total forage productivity was similar to 2014.

Daily ME requirements were calculated for an average LW of 550 kg and include ME requirements for maintenance (i.e. 65 MJ ME), reproduction (i.e. 26 MJ ME) and monthly milk yield based on monthly average milk fat and milk protein content (see Table 2.3). The estimated grazed intake per cow per day in each month as well as the calculated ME intake are shown in Table 2.4. Over the period the average daily grazed intake per cow was 17 ± 1.9 kg DM but it fluctuated from as little as 10.9 kg DM in July up to 23.8 kg DM in August. The average daily concentrate supplementation per cow was 2.9 ± 0.29 kg DM ranging from 4.3 kg DM in April to 2.2 kg DM in September.

Table 2.2: Summary of the grazing data during the monitoring period 3rd of April to the 25th of September 2014.

Grazing Period		Grazing duration (days)	Number of cows	ha grazed per day*	Total LW of grazing cows (t)**	Stocking Density (t LW per ha)
From	To					
03-Apr	09-Apr	6	150	2.08	82.5	79.2
05-May	12-May	6	180	2.08	99.0	95.0
02-Jun	11-Jun	8	189	1.56	104.0	133.1
05-Jul	23-Jun	10	189	1.25	104.0	166.3
09-Aug	19-Aug	6	189	2.08	104.0	99.8
12-Sep	25-Sep	7	189	1.79	104.0	116.4
On Average		7	181	1.81	99.6	115.0

*The total area was not grazed at once but it was divided into two plots
** Assuming a cow LW of 550 kg

Table 2.3: Average monthly milk production and composition during April to September 2014 and calculated ME requirements per cow.

Month	Milk Fat %	Milk Protein %	Milk Yield (kg)	MJ ME Req for Milk
April	3.47	3.15	24	113
May	3.40	3.22	26	122
June	3.53	3.21	25	119
July	3.49	3.19	22	104
August	3.69	3.27	20	97
September	3.98	3.43	17	87

Table 2.4: Estimated feed (kg DM) and energy intake (MJ) per cow per day during the grazing period from April to September 2014.

Month	Estimated grazed intake		Supplementary feed Intake*		Total ME Intake	Total ME requirements	Energy Balance
	DM intake (kg)	ME intake (MJ)	Kg DM	ME intake (MJ)			
03 – 09 April	14.0	152	4.3	56	208	204	4
05 – 12 May	22.6	244	3.0	39	283	213	70
02 – 11 June	13.9	150	3.0	39	189	210	-21
05 – 23 July	10.9	118	3.0	39	157	195	-37
09 – 19 August	23.8	257	2.2	28	285	188	96
12 – 25 September	14.8	160	2.2	28	188	178	11

*Natural Organic Green HDF 18 Nuts (BOCM PAUL LTD), 862g DM, 18% CP, 13 MJ ME.
** Assuming a cow LW of 550 kg

The estimated ME intake from the forage in addition to the ME Intake from the supplementary feed (i.e. Natural Organic Green HDF 18 Nuts (BOCM PAUL LTD), 862g DM, 18% CP, 13 MJ ME) covered the daily ME requirements of the cows in most months, but there was a nutritional shortfall in ME intake during the grazing periods in June and July as shown in Figure 2.3a. This is explained by the relatively low forage DM intake that is estimated for these periods (Table 2.4). The low DM intake is likely attributed to the low forage availability (see Figure 2.2) which in turn is attributed to the fact that the farmer applied a relatively short grazing rotation scheme and the pasture was not allowed to recover fully. Nevertheless milk yield did not seem to have been compromised (Table 2.3) by the relatively low intakes estimated for these months, which suggests that subsequent grazing in the next field in the rotation allowed for good DM intakes. It is well established that milk production has a linear positive relationship with DM intake as cows produce more milk at higher intakes (see Figure 2.3b). Over the monitoring period the estimated daily DM intake per cow averaged 19.6 kg DM while the daily milk yield averaged 22.3 kg. These intake and productivity data are consistent with each other and are in accordance with the predictions postulated by the literature and illustrated in Figure 2.3b.

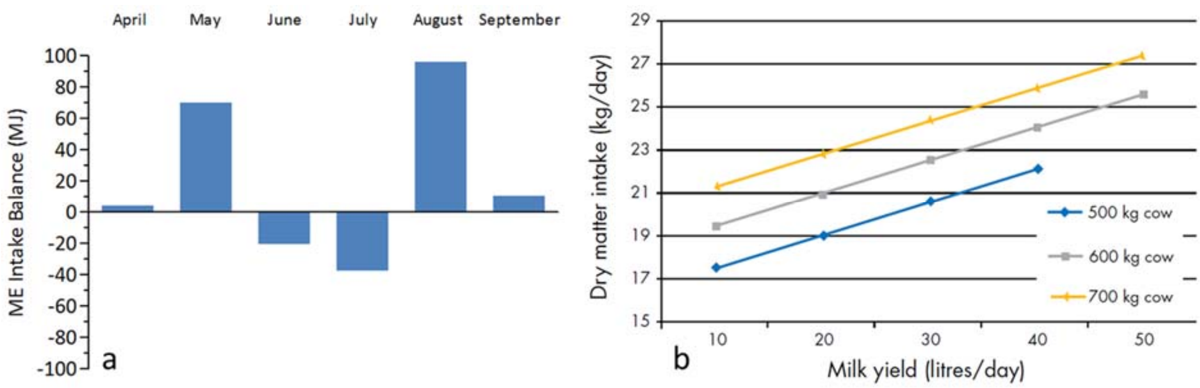

Figure 2.3. Monthly estimated ME intake of the cows from April to September 2014 (Panel a); relationship between dry matter intake and daily milk yield (Panel b; Source: DairyCo Feed into Milk, 2005)

Effects of mob grazing on soil organic matter.

It is well recognized that grassland soils with low organic matter content are characterised by poor fertility, are prone to compaction and flooding and are droughty and lacking soil microorganisms. As the organic matter rises and the soil becomes more fertile, the land grows more forage and the capacity of the land to carry higher stocking rates increases. It has been suggested that rotational grazing of bio-diverse pastures has the potential to build up carbon levels in the soil. The underlying hypothesis is that the stable environment under a diverse ley promotes the plant's ability to exude large amounts of sugars through its roots which in turn provides a food source for microbial activity resulting in increased soil carbon levels and building humus. The advocates of mob grazing suggest that this grazing system, by allowing plants to grow taller, results in the formation of large, complex and deep root systems and when they die off, they leave high amounts of organic matter in the soil. It is also advocated that trampling of significant quantities of forage onto the soil surface, provides a better environment for the microorganisms and other soil life and increases the soil organic matter.

In the case-study farm, the mob grazing approach on diverse swards was introduced with the aim of increasing soil organic matter. Despite the fact that monitoring of the performance of the diverse swards was conducted only in one field (i.e. Big Aero) soil samples were collected in 2015 from three different fields (i.e. Big Aero, Lanes Estate, Pinchins) and compared with earlier results from 2007 or 2012. The results of the soil analyses in these fields are shown in Table 3.4.

Table 3.4: Soil analysis results in three different fields (i.e. Big Aero, Lanes Estate, Pinchins)

Analysis Factor* Field	Big Aero		Lanes Estate		Pinchins	
Year	2007	2015	2012	2015	2012	2015
Standard Soil Analysis						
Soil PH	7.2	6.9	7.9	7.9	7.7	7.8
Phosphate (mg/l)	13	7	16	15	6	10
Potash (mg/l)	123	131	247	180	107	154
Magnesium (mg/l)	107	138	106	101	123	124
Physical Soil Structure (%)						
Sand (%)	7	15	21	17	15	20
Silt (%)	55	39	46	41	43	36
Clay (%)	38	46	33	42	42	44
Macro Nutrients						
Organic Matter %	**4.4**	**9.8**	**5.3**	**7.8**	**5.7**	**8**
Microbial Activity	13	25	33	22	27	23
Sulphate (mg/l)	35	56	37	29	49	58
Total Phosphorus	906	901	1025	1244	841	1069
Chemical						
CEC (meq/100)	41.2	36.0	28	30.5	32.9	30.7
Calcium (%)	84.7	78.9	88.1	88.2	87.7	86.1
Magnesium (%)	1.6	3.8	3.6	4	3.1	4.9
Ca:Mg ratio	53	21	24	22	28	18
Potassium (%)	0.8	1.2	1.8	1.9	1.1	1.6
Sodium (%)	0.2	0.4	0.5	0.3	0.4	0.7
Hydrogen (%)	0.0	0.0				
Others (%)	11.5	15.7	8.1	5.6	8.7	6.7

Trace elements (mg/l)						
Iron	33	64	40	57	49	65
Molybdenum	0.4	0.40	0.10	0.3	0.3	0.4
Copper	1.2	1.90	1.7	2.3	1.6	1.6
Selenium	0.68	0.68	0.39	0.38	0.53	0.46
Zinc	1.9	2.0	2.3	2.7	1.4	1.7
Manganese	11.5	22.8	11.8	14.1	16.6	24.6
Cobalt	0.3	0.3	0.2	0.2	0.3	0.4
Boron	1.10	1.60	1.5	1.4	1.3	1.3
Conductivity	2029	1913	2099	1977	2046	2056

*Samples were analysed by Kingshay Analysis Services, Kingshay, Bridge Farm, West Bradley, Glastonbury, Somerset BA6 8LU.

Soil organic matter increased by 122.7%, 47.2% and 40.4% in Big Aero, Lanes Estate and Pinchins fields, respectively. The relatively higher increase in soil organic matter in the Big Aero is attributed to the fact that samples collected in 2015 are compared with those collected in 2007 (i.e. 8 years earlier) and not in 2012, which is the case in the other fields (i.e. 3 years earlier). Yet, this is a marked improvement with more than double the levels of organic matter reserve. The results show that the build-up of the soil organic matter is remarkable and suggests that soil improvement can be achieved through high stocking rotational grazing of bio-diverse pastures.

2.4. Conclusions

This study shows that soil improvement management through rotational high stocking grazing of bio-diverse pastures has a remarkable effect on the build-up of the soil organic matter; while microbial activity in the soil is moderate this can be improved by bio-treatment of slurry or farmyard manure. An average 21-day grazing rotation was applied on the farm during 2014 which is regarded as rather a short period to allow plants to grow to the desired height that fulfils the expectations of mob grazing. The results also show that while pasture productivity of the diverse sward ley was slightly lower than that in the grass-clover ley, the total productivity remained relatively high suggesting that diverse pastures can serve as a viable alternative to conventional pastures (i.e. grass / clover pastures) as they can maintain animal productivity at high levels.

3. Enhancing on-farm forage yield and forage protein content

3.1. Introduction

Ruminant diets in northern Europe are strongly based on grass silage and cereals, and supplemental protein comes in the form of either domestic or imported rapeseed (*Brassica napus L.*) or imported soya bean. The use of legumes in ruminant systems in areas with a cold climate is mostly dependent on forage legumes and pea (*Pisumsativum L.*) or faba bean (*Viciafaba L.*), given the fact that the most important grain legume, soyabean (Glycinemax (L.) Merr.), does not thrive in such climates (Peltonen-Sainio and Niemi, 2012). In addition, in northern areas the late harvest time of legumes causes a risk both for the ripening of the crop and for the harvesting circumstances. In addition, the short growing season limits the choice of legumes to those that become available for harvest earliest.

In grass-clover mixtures, red clover is well suited for dairy cow feeding as its inclusion in the grass mixtures has several advantages, as reviewed before herein. However, in primary growth, the proportion of clover is lower compared to the regrowth (Rinne and Nykänen, 2000). Black *et al*., (2009) suggest that the first produced leaves of red clover are too small to intercept adequate light in the early stages, which results in a slow growth rate of red clover. Further, the spring is often rather cool, which may limit the growth of red clover compared to grasses. This can cause organic farmers concern about the low clover content in the first cut of red clover-grass and the protein content in the first-cut silage. This is particularly important as the first cut is the main forage batch for silage production in northern countries (i.e. Finland). In addition there is a need for high dry matter yield and high forage quality from the sward and therefore farmers are keen to implement new approaches in order to increase both forage quantity and quality.

The main interest of the farmers who participated in this project was to increase the protein content of the red clover-grass silage at farm level. Thus, two approaches were put forward:

a. The first approach (trial 1) involved topping of grass in order to allow more space and time for red clover to develop and thus increase the proportion of red clover in the herbage to be ensiled. It was expected that topping of grass will suppress grass growth in relation to clover in the grass-clover mixture.

b. The second approach (trial 2) aimed to increase forage yield and protein content of the grass-clover mix in spring by slurry application in the autumn of the previous year. Slurry is commonly used as a fertilizer, but it causes problems to clover stands when applied in spring, as plants are sensitive to physical damage and soil is prone to compaction (Stoddard *et al*., 2009). In addition, fields are often too wet for heavy machines to operate in spring. From a farmer's point of view slurry application in the autumn is better suited with time management compared to spring. It was also believed that low temperatures in the autumn would reduce ammonia emissions from slurry (Nykänen-Kurki *et al*., 1998).

3.2 Methodology and data collection

Two trials were conducted in eastern Finland (near Juva) for the purposes of this study. The first trial studied the effect of topping grass on improving the clover content and forage protein production. The second trial studied the effect of slurry application on protein content of a grass-clover field.

Trial 1: Effect of topping grass on clover production

The trial involved an early spring topping of grass to suppress grass growth in relation to red clover and was carried out on two different grass-clover fields on the same farm. Each field was divided into two parts: in one part of each field topping was carried out on May 31 by Lely Splendimo 550 P disc mowing machine (Figure 3.1) while the other part in each field was left untreated. The topping of the grass was expected to give more time for red clover to develop and thus the proportion of red clover in the first harvest for silage production was expected to be higher compared to that of the un-topped part of the field. Both fields were of moraine soils, which were quite warm with good water conditions. The soil type was coarser fine sand with moderate soil fertility (extraction with HAAc), and the pH (water) value and organic C content averaged 6.6 and 8.3 %, respectively. The clover species was *Trifolium pratense* L (red clover) and grass was *Phleum pratense* L (timothy) and *Festuca pratensis* L (meadow fescue)

In the previous year 20 tonnes per ha digestate of organic cattle slurry was directly injected into these fields after the first silage cut. The soluble nitrogen and the total nitrogen content of the slurry was 3.2 and 5.3 kg ton^{-1}, respectively. In slurry, 64 and 106 kg ha^{-2} soluble nitrogen and total nitrogen, respectively, were applied. In the spring of 2013 during which the trial took place no fertilization was applied.

Figure 3.1. Lely Splendimo 550 P disc mowing machine and John Deere 7250i harvester were used in the topping farm trial in Eastern Finland (Photo by P. Kurki).

The botanical composition, dry matter yield and height of clover and grasses, including timothy apex height, were measured at the time of topping. Crude protein content and forage digestibility were also determined. Samples were analysed in the commercial laboratory of Valio Ltd by NIR. The yield of the first harvest for silage production was measured by small plot hand sampling (0.25 m^2) with five replicates as well as by a farm scale harvester (John Deere 7250i, see Figure 3.1). Statistical analyses of the data were performed by SAS GLM procedure.

Trial 2: Effect of manure application on nitrogen content and forage protein

This trial was carried out on three different organic farms following identical procedures and involving farm scale machines for manure spreading and harvesting. In one of these farms two fields were used and therefore a total of four fields were used in the trial. The swards consisted of a mixture of organic red clover (*Trifolium pratense* L.) and grasses (*Phleum pratense* L., *Festuca pratensis* L.). In each farm, the trial fields were divided into two equally sized parts: in one part of the field organic slurry was applied while the other part was left untreated. Three fields were on moraine soils (fields 1, 2 and 3) with coarser fine sand and moderate soil fertility (extraction with ammonium acetate; HAAc). Water pH value and organic carbon content averaged 6.4 and 8 %, respectively. The fourth field (field 4) was of a half-bog soil with moderate soil fertility (extraction with HAAc), in which pH of water and organic C content averaged 6.1 and 50 %, respectively.

Figure 3.2. The slurry was injected directly into the sward by Joskin slurry tanker in the manure farm trial in Eastern Finland. (Photo by A. Laamanen).

Figure 3.3. Soil and small plot samplings in the farm trial in Eastern Finland (Photo by P. Kurki).

Fields 1, 2 and 3 were treated with 20 t per ha digestate of organic cattle slurry fermented by Juva's Bioson Ltd. The slurry was directly injected into the fields on 25-27 September 2013 by Joskin Solodisk 6880 slurry tanker with direct injection unit of 7 m carrying 32 solodisks with disk distance 22 cm (see Figure 3.2). The soluble nitrogen and the total nitrogen content of the slurry digestate was 3.1 and 5.3 kg per tonne, respectively. Thus, the organic slurry provided 62 and 106 kg per ha of soluble nitrogen and total nitrogen, respectively.

Field 4 (half-bog soil) was treated with 20 tonnes per ha of aerated organic cattle slurry (i.e. instead of digestate slurry) on soil surface on the 28th of August 2013 by a tanker of Koikkala's Machine and Iron, company. The soluble nitrogen and the total nitrogen content of the slurry was 2.5 and 4.0 kg ton^{-1}, respectively thus the slurry provided 50 and 80 kg per ha soluble nitrogen and total nitrogen, respectively.

The soil nitrogen content was determined at two depths, 0-25 cm and 25-50 cm (see Figure 3.3). Soil samples were collected and kept frozen pending analyses. Ammonium and nitrate nitrogen extracted by 2 M KCl were determined spectro-photometrically by an auto-analyser at the laboratory of Luke in Jokioinen, Finland.

Because of the extended rainy weather, all fields were harvested between the 11th to 20th of June in 2014. Fresh forage samples were collected at harvest for determination of botanical composition, dry matter yield, nitrogen content and digestibility. Samples were analysed in the commercial laboratory of Valio Ltd by NIR. Forage yield was measured by hand sampling of small plots (0.25 m^2) in five replicates (Figure 3.3) but also by a farm scale harvester (i.e. John Deere 7250i). Statistical analyses were performed by SAS GLM procedure.

Environmental conditions during the course of the trials

In eastern Finland, the growing season generally starts at about the end of April and finishes by October and this was the case also for year 2013. However, environmental temperature in the spring of 2013 was higher than the average temperature measured over the last 30 years (Table 3.1). The autumn and winter was clearly warmer than normally (Table 3.2). Instead of a typical five months' snow cover, it was exceptional short and thin (< 25 cm) appearing only in January - February. Similarly the soil frost stayed for as short a period as the snow cover.

Table 3.1. Mean temperature and monthly precipitation in 2013 in Eastern Finland.

	May		June	
	2013	1981-2010	2013	1981-2013
Mean temperature, °C	12.6	9.5	17.8	14.0
Monthly precipitation, mm	35	40	63	70

Table 3.2. Mean temperature and monthly precipitation sum over the period August 2013 - June 2014 in comparison with the average values of the period 1981-2010 in Eastern Finland.

	Average temperature °C		Precipitation mm	
	2013-2014	1981-2011	2013-2014	1981-2011
July	16.3	16.7	61	77
August	15.8	14.3	108	76

September	10.6	9.0	60	54
October	5.1	3.8	83	66
November	1.7	-1.7	84	56
December	-0.8	-5.9	56	52
January	-10.3	-8.1	20	49
February	-1.3	-8.2	26	35
March	0.8	-3.4	21	36
April	3.9	2.7	12	32
May	9.9	9.5	115	40
June	12.9	14.0	84	70
Mean / Sum	5.4	3.6	730	643

3.3 Results and Discussion

One of the challenges in conducting on-farm trials was to collect reliable quantitative data. In both trials reported here, the yield of the swards was estimated in two ways: a) by collecting forage samples manually, as typically is performed in on-station research experiments and b) by the farmer using a forage harvester equipped with scales (Figure 3.4). Both methods were satisfactory as indicated by the outcomes of the comparison of the treatments but DM yield data differed between the two methods. DM yield measured by large scale harvesting (method b) averaged 72 % of that from small plot sampling (method a) in all farm trials. However, the difference was constant between all comparisons that were made (Figure 3.5) indicating successful on-farm trial management and thus, the same conclusion derived from the two methods.

Figure 3.4. The area for John Deere 7250i farm scale harvesting was marked clearly. (Photo by P. Kurki).

Estimation of DM by the farm scale harvester is more reliable as this method determines the complete forage production of the harvested area while estimation by plot samples is affected by the number of replicates obtained. The difference between small plot and farm scale measurements has also been recognised earlier, and in accordance with these results a scaling factor of 0.7 has been used in extrapolating small plot results to farm scale.

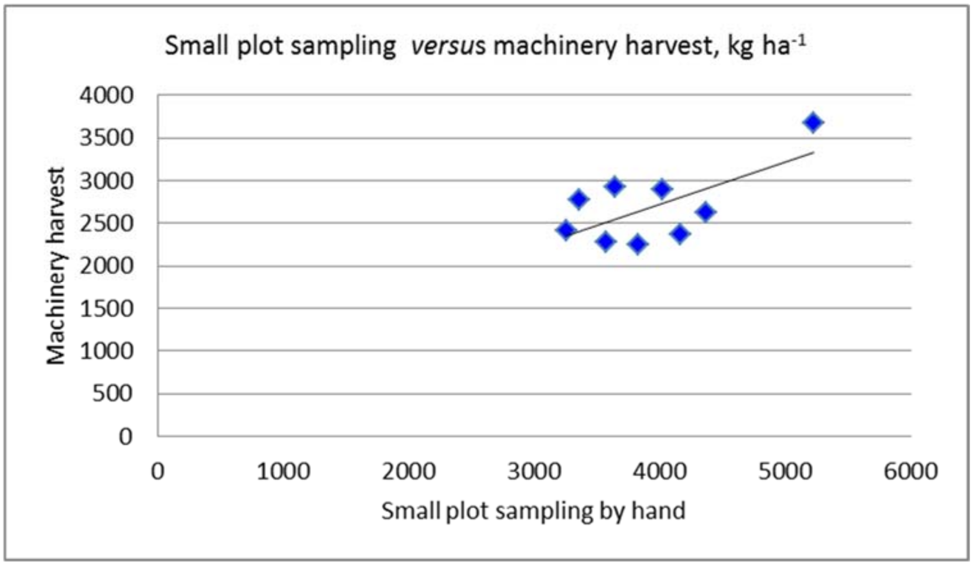

Figure 3.5. Effect of harvest method on estimation of dry matter production.

Trial 1: Effect of topping grass on clover production

The environmental temperature during the growing season in 2013 was higher than the average season temperature (Figure 3.6) resulting in more vigorous and rapid early growth of red clover than usual.

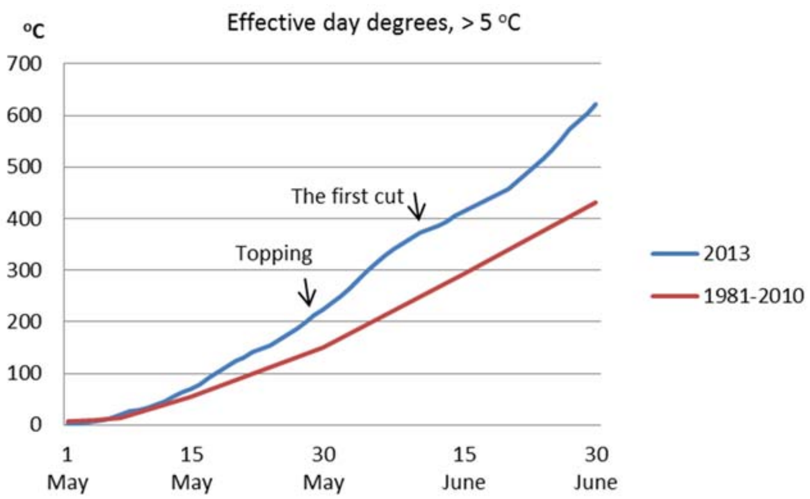

Figure 3.6. Effective day degrees (>5 °C) in spring in Eastern Finland.

Grasses started to grow in the beginning of May when daily mean temperature was above 0 °C. It is well recognized that growth of grass is correlated positively with effective day degrees above 0 °C. Calculated effective day degrees above 0 °C averaged 390 and 620 °C at topping and at harvest, respectively. Although red clover growth started later than that of grasses, the high temperature was more beneficial to red clover growth than that of grasses. However, growth of red clover is correlated better with effective day degrees above 5 °C than with day degrees above 0 °C. Calculated effective day degrees above 5 °C averaged 240 and 400 °C at topping and at harvest, respectively (Figure 3.6).

The annual variation in weather conditions is a challenge in all agricultural research and this is evident from the topping trial reported here. The results might have been different in a cooler spring, and repetition over several years would give more definitive answers to most questions related to forage production.

The difference in height between red clover, timothy and meadow fescue was not great enough in the early growth stage during May 2013 so as to allow topping of the grass to remove the timothy apex without damaging red clover with the mowing machine. Therefore, it was decided to perform the topping at a later stage when the height of the grass averaged about 44 cm (Figure 3.7). In May the apex height of timothy averaged 8 and 20 cm and the height of red clover averaged 26 and 30 cm, in the two study fields, respectively. Red clover content averaged about 21 % in both fields at topping.

Figure 3.7. Topping at the height of 30 cm (Photo by P. Kurki).

The topping of grass had a significant negative effect (*P*=0.025) on the overall DM forage production; the average DM per ha was of 3868 kg and 4798 kg per ha for the topped and up-topped fields, respectively (Table 3.3). Contrary to our expectation the nitrogen yield from grass as well as the total nitrogen yield was lower in the topped fields (53.4 and 68.8 kg per ha, respectively) compared to the equivalent nitrogen yields in the un-topped fields (63.3 and 88 kg per ha; for P-values refer to Table 3.3). On average, one cut from the un-topped grass-clover fields yielded 4800 kg per ha dry matter, 3270 kg per ha digestible organic matter, 560 kg per ha crude protein and 380 kg per ha metabolizable protein. That cut gave an average of 52,300 MJ per ha metabolizable energy.

The negative effect of topping on both DM and nitrogen yields is attributed to the rather short period that intervened between the topping and the first harvest which was less than two weeks. This short period did not allow the red clover to adequately develop and increase its mass, protein content or to have a positive effect on nitrogen yield. Forage quality data did not differ between fields of the same treatments (Table 3.3). As expected, grass height was decreased due to topping. However, topping of the grass caused damage to the red clover in one of the fields, in which the height of the red clover was above 30 cm.

With regards to the identification of the best possible period to perform grass topping, Virkajärvi (2003) reports that timothy tiller production differs from that of other forage grass species. In accordance with Virkajärvi (2003) both generative tillers and elongated vegetative tillers were recognized in our samples. Timothy tillers were generative at about 90% at harvest while the rest were elongated vegetative ones. Those tillers have an erect growth habit, which indicates a good ability to intercept light and produce a high dry matter yield.

At the harvest both the grass and clover appeared to be at the appropriate stage of development to give good forage quality: 60 % of generative tillers of the Timothy grass were heading and the rest were still developing and the red clover was at the growth stage of green flower-bud. Taken together this suggests that the growth stage of the organic red clover-grass mixture was optimal for forage quality as can be seen in the forage quality results (Table 3.3). These results were supported by results on organic crop rotation based on red clover leys (Nykänen, 2008).

Table 3.3. Effect of topping two weeks before harvesting on red clover-grass yield and quality.

	n	Topped	Not topped	SEM	P-value
Dry matter content, g per kg	20	188	182	4.6	0.397
Red clover content, g per kg DM	20	119	167	2.95	0.266
Dry matter yield, kg per ha					
Grasses	20	3377	3957	215.0	0.074
Red clover	20	478	837	166.5	0.146
Total dry matter including weeds	20	3868	4798	268.4	0.025
Nitrogen yield, kg per ha					
From Grasses	20	53.4	63.3	2.84	0.026
From Red clover	20	14.1	24.5	4.87	0.149
In total dry matter including weeds	20	69.8	88.0	5.20	0.024
Feed analysis from mixed samples including weeds, g/kg DM (ARTTURI®)					
Ash	20	79	78	1.2	0.689
Crude protein	20	114	116	5.2	0.840
Water soluble carbohydrates	20	116	125	3.5	0.078
Neutral detergent fibre (NDF)	20	560	542	12.3	0.318
Indigestible NDF	20	84.8	83.4	2.67	0.715
D-value*	20	675	681	3.4	0.249
Metabolizable energy, MJ/kg DM	20	10.8	10.9	0.06	0.285
Metabolizable protein**	20	77.8	78.5	0.85	0.568
Protein balance in the rumen**	20	-3.1	-2.6	4.19	0.934

SEM = Standard error of the mean.
*Digestible organic matter in dry matter. **Calculated according to Luke (2015).

Trial 2: Effect of manure application on nitrogen content and forage protein

In three organic farms (with moraine soils) slurry digestate was injected into the soil at a depth of 3 to 4 cm in late September (Figure 3.8); prior to the application, forage DM averaged 1200 kg per ha. Three weeks later most of the slurry was filtered into the soil. In the fourth farm (i.e. with half-bog soil) slurry was broadcast into the ley surface in late August; forage DM prior to the application averaged 2200 kg per ha. Due to the warm autumn, ley growth was still occurring in October 2013, three weeks after slurry application (Figure 3.9). Although autumn temperatures were warm enough to risk the proper cold-hardening of plants for winter, no damage was caused and grass-clover leys established well in the following spring. Slurry application significantly increased the crude protein content of grass ($P<0.001$) as indicated by forage analysis obtained at first harvest for silage in the following year (Table 3.4). Thus, the overall crude protein yield of the field was affected positively ($P=0.021$). In addition, slurry application significantly increased the total nitrogen yield per ha ($P=0.021$) indicating that more soluble nitrogen was available in the soil; however increase in soil nitrogen concentration did not affect the proportion of grass or red clover in the leys (Table 3.4).

Figure 3.8. The ley after slurry digestate injection of 20 t per ha and three weeks later (Photo by P. Kurki).

Figure 3.9. Red clover- grass in October 2013 and the same ley in the beginning of the following June (Photo by P. Kurki).

Prior to the slurry application in the autumn of 2013, soil nitrate nitrogen, ammonium nitrogen and soluble nitrogen (sum of nitrate and ammonium nitrogen) content averaged 0.7, 7.4 and 8.1 mg per l soil (top layer of 0-25 cm cut), respectively (Table 3.5). In 2014, following harvest, the summer soil nitrogen figures were again at the same level as in autumn 2013. These values are typical for Finnish mineral soils with red clover grass production before fertilization and after harvest (Nykänen-Kurki *et al.*, 1998).

Concentration of soil nitrogen differed between fields ($P<0.001$) due to soil type but within individual fields no difference was found between treatment plots before the start of the trial. Slurry application significantly increased both soil nitrate ($P<0.001$) and ammonium nitrogen ($P=0.002$) as indicated by samples collected five weeks following the application (Table 3.5). The effect on soil soluble nitrogen (*P*=0.044) was similar as indicated in samples collected the following spring. The difference of soluble N in soil measured in October and in May indicates that natural mineralization (decomposition) of organic matter also occurred. The soil nitrogen results show that slurry application in the autumn increased grass nitrogen content of the following spring.

Prior to the slurry application, nitrate nitrogen, ammonium nitrogen and soluble nitrogen were determined as 2, 18 and 20 kg per ha in a soil layer of 0-25 cm (depth of ploughing); this layer contains 25 million litre of soil per ha. Over 90 % of soil nitrogen was ammonium nitrogen. Soluble nitrogen was also measured at the deeper soil layer prior to the start of the trial and averaged 25 kg per ha in a soil layer of 0-50 cm. Following slurry application at 20 tonnes per ha at a depth of 4 cm (Bioson Ltd), soil soluble nitrogen at the ploughing layer increased approximately by 15 kg per ha within five weeks.

Table 3.4. Effect of autumn slurry application on clover grass production in the following spring.

	N	With slurry	No slurry	SEM	P-value
Dry matter yield, kg per ha					
Red clover	40	725	698	89.0	0.833
Grasses	40	2373	2180	136.6	0.326
Red clover + grasses	40	3098	2879	107.1	0.157
Total (includes unsown species)	40	3433	3244	108.2	0.226
Crude protein concentration, g per kg DM					
Red clover	40	225	218	3.5	0.195
Grasses	40	125	116	2.1	<0.001
Crude protein yield, kg per ha					
From grass and clover	40	455	397	16.7	0.021
Nitrogen yield, kg per ha					
Red clover	40	25.8	23.3	2.96	0.562
Grasses	40	47.0	40.3	2.73	0.091
Red clover + grasses	40	72.7	63.6	2.67	0.021
Proportion of red clover	40	0.215	0.217	0.0278	0.968
Proportion of unsown species	40	0.095	0.106	0.0121	0.514

In the current study, nitrogen yield from grass only and grass-clover mix at the first harvest for silage production was 47 and 73 kg per ha, respectively. Biological nitrogen fixation and nutrient cycling underground in the rooting system was not estimated. Our results show that there was no risk of nitrogen leaching during winter, although slurry application took place in autumn when the growing season is at the end and plants do not make excessive use of the soil nutrients and therefore, the soil itself was catching most of nutrients. These results are in accordance with preliminary results by Virkajärvi and Räty (2014) who also did not observe differences in nitrogen loss between summer and autumn application of slurry in a lysimeter field trial that included two surface runoff collector ditches. Although in the current study runoff of the phosphorus was not investigated, Virkajärvi and Räty (2014) reported that this can be an issue with autumn applications of slurry.

In 2014, the Finish Government reviewed the restriction of discharges from agriculture and horticulture including organic manure and organic fertilizer products, with effect from the 1st of April 2015 (Government Decree 1250/2014). According to this legislation from the 15th of September to 31st of October each year, only direct injection of slurry is allowed. No application is allowed from the 1st of November until the 31st of March. After the 1st of September application of a maximum 35 kg per ha of soluble nitrogen of organic origin is allowed.

Table 3.5. Soil nitrate nitrogen, ammonium nitrogen and soluble nitrogen concentration.

	n	With slurry	No slurry	SEM	P-value
Soil depth of 0-25 cm					
Nitrate N, mg l^{-1} soil					
September before slurry	30	0.7	0.8	0.11	0.526
October five weeks after slurry	40	3.2	0.8	0.35	<0.001
May after winter	40	2.3	2.1	0.33	0.745
June after silage harvest	30	0.8	0.9	0.88	0.698
Ammonium N, mg l^{-1} soil					
September before slurry	30	7.4	7.4	0.35	0.988
October five weeks after slurry	40	9.8	6.2	0.76	0.002
May after winter	40	11.3	10.7	0.42	0.373
June after silage harvest	30	8.7	8.8	0.31	0.872
Soluble N, mg l^{-1} soil					
September before slurry	30	8.1	8.2	0.41	0.879
October five weeks after slurry	40	13.0	6.9	0.90	0.001
May after winter	40	13.5	12.8	0.62	0.044
June after silage harvest	30	9.6	9.7	0.34	0.806
Soil depth of 25-50 cm					
Nitrate N, mg l^{-1} soil					
September before slurry	30	0.2	0.2	0.06	0.735
May after winter	38	3.7	2.2	0.64	0.087
Ammonium N, mg l^{-1} soil					
September before slurry	30	2.0	1.9	0.11	0.976
May after winter	38	7.3	5.3	1.06	0.171
Soluble N, mg l^{-1} soil					
September before slurry	30	2.1	2.2	0.13	0.897
May after winter	38	11.0	7.5	1.65	0.124

3.4. Conclusions

The optimal timing for topping of grass to enhance red clover contribution could not be determined by the results of Trial 1. However, it is apparent that a period of two weeks between topping and the first harvest is rather too short to allow for the clover to develop adequately and increase its mass and protein content.

The results of the second trial show that slurry application in autumn can increase crude protein content of grass and total forage crude protein yield allowing for better silage quality. Application of slurry in the autumn can positively affect both soil nitrate and ammonium nitrogen but not the concentration of soil nitrogen which is driven more by the soil type. The risk of nitrogen leaching seemed to be low, but it is unknown whether this is the case also for phosphorus. In this respect more research is needed.

The results from both trials also show that estimation of DM by the farm scale harvester is a reliable method as it determines the complete forage production of the harvested area compared to DM estimation by plot samples whose accuracy depends on the number of replicates obtained. This study shows that on-farm participatory research is beneficial both for farmers and researchers and is proven to be an efficient approach to conduct research that addresses on-farm management problems and challenges.

4. Effects of inclusion of industry by products on the productivity of dairy cows

4.1. Introduction

Sustainability assessments that were carried out on dairy farms in Romania revealed that, with few exceptions, industrial by-products are insufficiently known and used by the farmers. A comprehensive literature review showed that two industrial by-products, camelina meal and dried grape marc have the potential to be fed to dairy cattle, given their nutritional value, availability for purchasing and willingness of the farmers to offer them to their animals. However, the optimum proportion of these feedstuffs to be added into the ration as well as their effects on animal productivity and health was unknown.

Camelina meal has a practical interest as the production of this crop is likely to increase as it is largely used for biofuel production. The chemical composition of camelina meal suggests a nutritional potential that is similar to other well-known protein meals, such as sunflower meal or rapeseed meal, but there are a number of unknown aspects such as rumen degradability and influence of residual fats on milk quality that have to be studied. Camelina is currently a non-improved species, with low yields, yet it is a resilient, low-input cultivar with low nutrient requirements. On the other hand, dry grape marc as an industry by-product is widely available and its potential as alternative feedstuff in case of feed shortages has a practical interest in low-input dairy farms. The nutritional value of dry grape marc is not particularly high; however from a farmer's point of view the challenge is to test whether it can be used for short-term replacement of conventional feeds in periods of feed shortages, without noticeable adverse effects on milk production.

The two on farm experiments were conducted to assess the effects of camelina meal (on-farm trial 1) and dried grape marc (on-farm trial 2) on the productive performance of dairy cows (milk production and milk quality) and to determine whether these by-products can serve as potential alternative feeds for ruminants. The availability of both by-products is likely to increase in the future due to the increased biofuel production and because there is a need for the viticulture industry to dispose of "waste" products (i.e. grape marc) in an environmentally friendly manner.

4.2. Methodology and data collection

The general experimental procedure was similar for both feeding trials: statistically comparable groups of animals received diets including camelina meal (trial 1) or grape marc (trial 2) and their performance was compared to control groups that received a basal diet that is normally offered under practical conditions. The experimental diets were formulated to be of the same nutritional value as the control diets. Feed intake data and milk production were recorded and feed and milk samples were collected periodically and analysed for nutritional value and quality, respectively.

The experiments took place from July to October in 2013 (trial 1) and from March to June 2014 (trial 2). Both on-farm feeding trials took place at Agro Solomonescu farm (SME partner from Romania), in Miron Costin village, Botosani county, North-East of Romania and lasted for 12 weeks.

Figure 4.1. On-farm Feeding trial on by-products (Photo by S. Toma).

Figure 4.2. Milk samplings in the farm feeding trial on by-products (photo by S. Toma)

Trial 1: Feeding trial on camelina meal

The on-farm trial was organized in a mono-factorial experimental design and aimed to assess the potential of replacing sunflower meal with camelina meal in dairy cows' diet and investigate its effects on milk yield and quality. The farm in which the trial took place is a commercial farm not designed for sophisticated experiments thus, it was not possible to measure nor individual intakes, neither individual cow milk yield on a daily basis.

Fifty Holstein Friesian cows of low production level (i.e. milk yield averaged about 14 litres of milk per cow per day), were used in the trial and kept in free stalls. In order not to interfere greatly with the normal on-farm management the cows were randomly allocated into two different diet groups in terms of concentrate supplementation. The control diet was constituted of barley, oat, pea and sunflower meal (24.2% in the concentrate mixture). The experimental diet contained the same ingredients with the difference that sunflower meal was entirely replaced by camelina meal (Table 4.1). The diets had similar nutritional value in terms of protein and energy and were formulated in agreement with the farmer, who opted to maintain his usual feeding strategies, while agreeing to try the use of camelina meal. All animals received the same forages in the diet.

Animals were adapted to the diets for two weeks prior to recording milk production daily for 12 weeks on a treatment group basis. Cow individual milk production was recorded periodically. In addition to production data, milk, blood and feed samples were taken and transported to INCDBNA for biochemistry analyses. Milk samples were analysed for protein, fat, milk lactose and milk fatty acids while additional milk samples were retained for determination of milk protein fractions. Blood samples were analysed for plasma immunoglobulins and total antioxidant capacity in order to assess the effects on immune status (these results are not discussed herein and have been reported in the SOLID deliverable 3.3 as part of WP3).

Table 4.1. Experimental diets in the camelina meal trial (kg DM / d)

	Control Diet	Camelina Diet
Forages		
Corn green biomass	7.95	7.95
Alfalfa hay	2.10	2.10
Concentrates		
Barley grains	1.30	1.30
Oat grains	1.74	1.74
Pea	0.86	0.86
Sunflower meal	1.34	
Camelina meal		1.36

Trial 2: Feeding trial on grape marc

The objective of this on-farm trial was to assess the potential of temporarily replacing cereals with dried grape marc in dairy cows' diets. In this trial 30 Holstein Friesian and Fleckvieh cows of a similar production capacity as in trial 1 were used and kept in free stalls. The cows were randomly allocated into two statistically comparable groups. In a similar design as in trial 1, both groups of cows received the same basal forage diet that consisted of corn silage (*ad libitum*) and alfalfa hay (limited amount of 3 kg per cow per day). Cows in the control group received a diet that was constituted of corn, barley and sunflower meal; cows in the experimental group received a diet with similar ingredients as the cows in the control group with the difference that 1/3 of the quantity of the corn and 1/3 of that of barley were replaced by dried grape marc (Table 4.2). The replacement of corn and barley by 1/3 was chosen in order to maintain similar nutritional values in the diets.

Table 4.2. Experimental diets in the grape marc trial (kg DM / d)

	Control Diet	Grape marc Diet
Forages		
Corn Silage	10.23	9.65
Alfalfa hay	2.51	2.51
Concentrates		
Corn grains	0.83	0.56
Barley grains	1.73	1.16
Sunflower meal	1.78	1.78

Dried grape marc	0	2.70

The experimental conditions were identical to those of camelina feeding trial. However, following a researchers' advice, the farmer upgraded their milking parlour in February 2014 and individual cow milk production was recorded daily. This trial followed an identical protocol in terms of data collection and data analysis as in trial 1.

4.3 Results and Discussion

Trial 1: Feeding trial on camelina meal

The results show that replacement of sunflower meal with camelina meal had no significant effect on overall DM intake for the herd. However, this outcome should be interpreted with caution given the fact no individual DM intake data were obtained from the experiment. Replacement of sunflower meal with camelina meal resulted in a slight decrease in milk yield of about 5%, but the difference between the treatments was not statistically significant (Table 4.3). Nevertheless, the overall performance of the cows in terms of milk yield is regarded as low in view of the cows' potential and the diets' nutritional value, indicating an insufficient feed conversion efficiency.

Table 4.3. The effect on milk yield and composition - Camelina trial

	Treatment		*SEM*	*P value*
	Control	**Camelina**		
Milk yield, l/d	13.09	12.40	0.512	0.507
% fat	4.070	3.551	0.128	0.038
% protein	3.523	3.385	0.087	0.447
% lactose	4.679	4.543	0.061	0.283

Cows fed on the diet that contained camelina meal had significantly lower milk fat content (P=0.038) compared to their counterparts fed on the basal diet; milk protein and milk lactose did not differ between the treatments (Table 4.3). Earlier studies with fistulated animals also reported that inclusion of camelina meal in diets can decrease milk fat content in dairy cows (Hurtaud and Peyraud, 2007). This effect should be considered by farmers as part of the feeding strategy as in some countries (e.g. Romania) milk fat is the only variable for correcting milk price at farms' gates.

However, the results show that the inclusion of camelina meal in the diet had a positive effect on the milk's fatty acids composition with a more pronounced effect for the polyunsaturated fatty acids. For example α-linolenic acid increased 1.24 times ($P<0.01$) while the concentration of FA n-3 increased 1.76 times ($P<0.001$). Content of some FA has increased even more: 4.8 times the eicosatrienoic acid, 2 times the CLA, etc. Overall, the PUFA increased by 41.81% compared to the control ($P<0.001$).

Trial 2: Feeding trial on grape marc

The inclusion of dried grape marc at a level of 15% of the diet (on DM basis) did not negatively affect intake. However, it has to be noted that the cows were in a low input system, with a moderate production level (at about 14 l/d on average). Milk production was not influenced by inclusion of dried grape marc in the diet (13.90 l/d in control group vs. 14.07 l/d in the grape marc group), an outcome that suggests the potential of grape marc to replace part of the conventional dietary ingredients, despite its low nutritional value (Table 4.4).

Milk fat, protein and lactose concentrations were recorded but no differences were observed between the two treatments. The polyunsaturated fatty acids content was increased by the inclusion of grape marc in the diet (*P*=0.045), particularly the linoleic acid (*P*=0.017). A slight increase was also observed for α-linolenic acid (*P*=0.091), although the total n-3 PUFA was not significantly affected. The saturated fatty acids slightly decreased, based on the decrease of palmitic acid whereas stearic acid, of the same category, has increased. The latter is not considered to be a negative effect, as the stearic acids are regarded as having a rather neutral activity in terms of affecting consumers' health, compared to other saturated fatty acids.

Table 4.4. The effect on milk yield and composition – grape marc trial

	Treatment		*SEM*	*P value*
	Control	**Grape marc**		
Milk yield, l/d	13.90	14.07	0.563	0.483
% fat	3.946	3.792	0.175	0.687
% protein	3.559	3.447	0.048	0.306
% lactose	5.017	4.999	0.038	0.976

4.4. Conclusions

Replacement of sunflower meal with camelina meal had no significant effect, neither on milk yield nor on milk protein or lactose. On the other hand, it decreased milk fat content by about 15%, which confirms previously reported findings in the literature. The effects on milk fatty acids profile were positive, with an increase of PUFA (including the CLA). Replacement of one third of corn and barley grains with dried grape marc, while maintaining the nutritional supply of the diets did not statistically influence milk yield and milk composition (milk fat, protein and lactose content). However, it significantly increased milk PUFA, especially n-6 PUFA and, of these, the linoleic acid (P=0.017).

It is concluded that both camelina meal and dried grape marc can replace, at least in the short term, the more classical feedstuffs without noticeable adverse effects, except the decrease of milk fat in the case of camelina meal. Overall, positive effects on the quality of milk fat were observed for both by-products. It is important to underline that these results were obtained within a low input production system, on cows having a moderate production level. Whether these feedstuffs can be used extensively in the nutrition of the dairy cow will be likely to be determined by their price on the market.

5. Effects of dietary iodine supplementation on iodine concentrations of milk in dairy cows

5.1. Introduction

The levels of micro and macro elements in milk depend largely upon the content of these elements in soil (which affects levels in pasture) and animal feed, which varies considerably among and within countries. In general, the mineral content of milk is not constant through the lactation period of a cow and can be influenced by both genetic and environmental factors. Variation in the reported concentrations of many minerals in milk can also be due to analytical errors and contamination from milk collection and processing equipment and procedures (Cashman, 2006, Flachowsky *et al*., 2014). Representative values for the average mineral content of milk in the UK are presented in Table 5.1.

Table 5.1*: Optimal mineral concentration of bulk milk samples (µg/L)

Mineral	Guide Values
Manganese	20 – 25
Zinc	3000 – 4000
Copper	50 – 60
Molybdenum	40 – 50
Iodine	**60 – 100**
Selenium	15 – 20

**According to Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, Norwich, NR12 8QN, UK*

Iodine is an essential trace element for animals and humans because it is necessary for the synthesis of the thyroid hormones triiodothyronine (T3) and thyroxine (T4) which have multiple functions in energy metabolism, growth and brain development. The daily iodine requirement for dairy cows is estimated to be about 0.33 mg/kg DM or about 0.6 mg dietary iodine/100 kg of body weight (NRC, 2001). Pregnancy does not increase the requirement for iodine for thyroxine production to any significant degree (Miller *et al*., 1988). Late gestation cows incorporate about 1.5 mg iodine/day into thyroid hormone while during lactation thyroid hormone production is increased, especially in high producing cows and iodine incorporation into thyroid hormones may reach 4 to 4.5 mg iodine/day (NRC, 2001). The percentage of the dietary iodine that is incorporated into the thyroid gland is inversely related to the iodine content of the diet. In diets with adequate iodine content, about 20 percent of the dietary iodine is incorporated into the thyroid gland; when intake of dietary iodine is marginal the thyroid gland will incorporate about 30 percent of the dietary iodine and up to 65 percent of the dietary iodine in iodine deficient diets (Miller *et al*., 1988). Dietary iodine that is not taken up by the thyroid gland is excreted in urine and milk, making the iodine content of milk a possible indicator of iodine status (Berg *et al*., 1988).

It is well documented that high concentrations of dietary iodine in the diet proportionally increase iodine concentrations in milk but also in urine and faeces. Franke *et al*. (2009) tested the effect of six dietary iodine supplementation levels (between 0.5 and 5 mg/kg DM) and found that iodine content in the milk increases in a dose-dependent manner. This is in accordance with earlier studies that show that there is a linear increase in the iodine concentration of milk with increasing iodine intake of the cows (see Figure 5.1). The types of iodine used as nutritional additives do not seem to affect the iodine content of the milk differently (Franke *et al.*, 2009; Flachowsky *et al.*, 2014) but simultaneous use of different iodine sources must be avoided because of the possible oxidation reactions that can occur under the acidic conditions of the rumen (EFSA, 2013).

Feeds containing goitrogens or glucosinolates, when fed to cows negatively affect the iodine concentrations of the milk (Flachowsky *et al.*, 2014). Goitrogens are substances that suppress the function of the thyroid gland by interfering with iodine uptake, which can, as a result, cause an enlargement of the thyroid (i.e. a goiter). Plants that contain goitrogenic substances are those of the cruciferous family, including rape, canola and kale, as well as raw soybean, beet pulp, millet, linseed, cyanogenic strains of white clover, and sweet potato (Borucki Castro *et al.*, 2011). Glucosinolates are secondary plant metabolites which occur in almost all plants of the order *Brassicales* such as rape, mustard and cabbage. Glucosinolates inhibit iodine accumulation from the blood to the thyroid and the mammary gland (Franke, 2009).

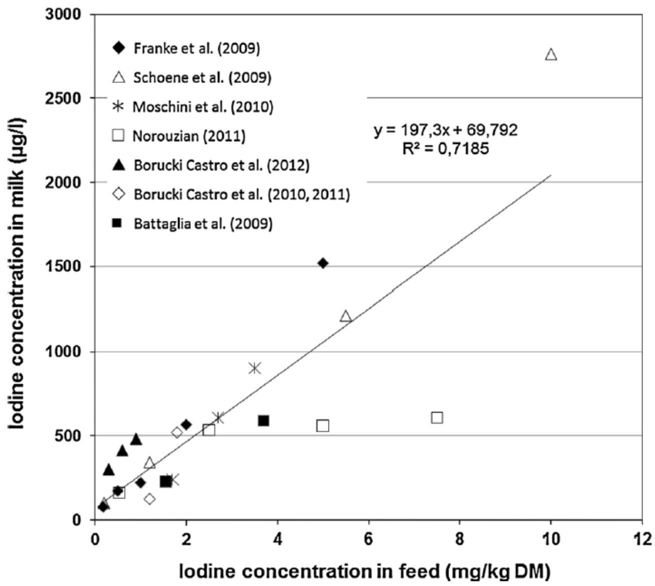

Figure 5.1: Influence of iodine concentration in the feed of dairy cows (mg/kg DM) on the iodine concentration of milk (µg /L) by various authors [Source: Flachowsky *et al.*, (2014)]

The current study provides information about dietary factors and farm management practices that influence the iodine content of milk in organic dairy herds based on data collected from case study farms during 2014/2015. More specifically the study investigates the relationship between iodine concentrations in bulk milk samples with iodine concentrations in forage on organic dairy farms in view of farm practices.

5.2. Methodology and data collection

Twelve case-study farms were selected and farmers agreed to a monitoring protocol that allowed data collection on iodine and other mineral concentrations in milk, blood, urine and forage samples. The study lasted from June 2014 to January 2015.

Selection of the farms

Determination of trace elements in milk samples from more than 800 organic dairy herds throughout the UK have been carried out on behalf of the Organic Milk Suppliers Cooperative (OMSCo) during September 2013 – January 2014. This initial data set was analysed and results were used as a basis to identify participating farms for the current project. Based on these data, the farms were categorised as L (low), O (optimal) or H (high) in milk iodine when milk iodine concentrations were below 60 µg/L, between 60 to 120 µg/L or above 120 µg/L, respectively. To facilitate farm visits and regular contact with the farmers, those farms that were located more than 200 miles from the ORC (Elm Farm, Newbury, RG20 0HR, Berkshire) were excluded from the selection. From the remaining farms, 4 farms from each one of the L, O or H groups were selected randomly for participation in the study. All the selected farmers (i.e. 12 in total) agreed to participate. However, two farms out of the twelve, one from the O and one from the L group, voluntarily withdrew from the study shortly after the start of the monitoring and no data were collected from these farms.

Milk, forage and feed samples for iodine and mineral determination

Bulk milk samples were collected via OMSCo's routine farm milk collection from the participating farms from May 2014 to January 2015. These samples were analysed for iodine and other minerals every 35 – 45 days. In addition, the farmers were asked and agreed to provide a representative sample of the grazed forage or diet (TRM, silage) once every month for iodine and mineral analysis (Freepost sample bags, and input sheets were provided). Laboratory analyses on the milk, forage and feed samples were carried out by Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK.

Blood and urine samples for iodine and mineral analysis

For each farm, blood and urine samples from 10 milking cows were obtained under normal vet visits in three occurrences over the study period in 2014. Table 5.2 summarises the sampling schedule and the type of laboratory analysis carried out in the samples obtained. The first sampling took place during August/September, the second during October/November and the third during December/January. In each occurrence the cows with the highest milk yield (measured over the previous week) were selected for sampling. This was because milk Iodine was analysed in bulk samples: it was anticipated that the cows with the highest milk yield will have the greater contribution to the total milk yield, hence the selection of the cows with the highest milk yield for further testing.

On all occasions, blood samples were analysed for glutathione peroxidase (GSHPx), which is a selenium dependent enzyme. Selenium is required for the conversion of the thyroid hormone triiodothyronine (T3) to thyroxine (T4), hence the measurement of selenium status. In the first sampling occasion only (i.e. August/September), supplementary blood samples from 4 of these cows were analysed for full trace element profile. Urine samples obtained from the same 10 cows were analysed for Iodine concentrations. The results are reported in µg/L and are standardised to a creatinine concentration of 5000 µmol/L to account for the different dilution of urine samples collected. All samples were dispatched from the farms within 48 hours of collection. These laboratory analyses were carried out by the School of Veterinary Medicine and Science, University of Nottingham, Loughborough.

Table 5.2: Summary of the sampling schedule

Type of laboratory analysis	Sampling period		
	August/September	October/November	December/January
Urine Iodine[1]	Yes	Yes	Yes
Glutathione Peroxidase (GSHPx)[2]	Yes	Yes	Yes
Full Trace Element[3]	Yes	-	-

[1]Urine samples were collected from 10 cows by free catch
[2]Blood samples were collected into lithium heparin (LH) vacutainers from the same 10 cows on the same day in each farm
[2]Blood samples were collected into plain (z) or clot activator tubes from 4 of the 10 cows sampled for urine iodine and GSHPx

Farmer's Questionnaire

To obtain an overview of the management and practices of the case-study farms, the farmers were asked to fill in a questionnaire. This was developed specifically for the purposes of the study and aimed to collect information primarily in relation to the provision of iodised rock salt and the use or not of iodine-based pre- or post- dip teat disinfectants. According to the literature, these practices are related to or can affect the iodine concentration in the milk (Borucki Castro *et al*., 2012). The questionnaire also collected information on farm topography, livestock, crop production, health, fertility and housing (these data are beyond the scope of the study and are not presented).

5.3 Results and Discussion

Iodine and mineral concentrations in milk

Mineral analysis of milk samples from dairy herds is widely recognised to be a useful indicator of the status of trace elements, particularly selenium, iodine and molybdenum.

The iodine and other mineral concentrations of bulk milk samples obtained from May to December 2014 are shown in Tables 5.3 and 5.4, respectively. Calculated mean iodine concentrations over the sampling period (i.e. May to December 2014) varied considerably across the farms; in four farms (i.e. 3, 5, 7, 8) the mean iodine concentrations were below the optimal levels (<60 µg/L; see Figure 5.2, panel a), in two farms (i.e. 1 and 10) they were within optimal levels (60 to 120 µg/L) and in four farms mean iodine concentrations were above optimal levels (>120 µg/L; see Figure 5.2, panel a). The highest iodine concentration of 1025 µg/L was observed in a sample collected from Farm 6 (average milk iodine of 576 ± 104.1 µg/L) in September 2014. The lowest iodine concentrations were observed in Farms 8 and 5 with 7 µg/L and 10 µg/L, and mean iodine concentrations of 26 ± 15.3 µg/L and 23 ± 5.3 µg/L, for each farm respectively. Farm 6 had significantly higher milk iodine concentrations compared with Farms 3 ($P \leq 0.05$), 5 ($P \leq 0.001$), 7 ($P \leq 0.01$) and 8 ($P \leq 0.001$); milk iodine concentrations in Farm 4 were significantly higher than Farm 8 ($P \leq 0.05$).

Monthly averaged milk iodine concentrations over the 10 study farms indicate that iodine concentrations in organic milk dropped from early spring to late summer and increased again from autumn towards winter (Figure 5.2, Panel b). Providing that the farmers do not change farm practices in terms of the use of iodine-based teat disinfectants (this effect will be discussed later), this outcome is in accordance with the literature which suggests that winter milk normally contains higher iodine than summer milk, likely because animals spend more time indoors during winter and have access to diets with higher mineral content (Flachowsky *et al*., 2014). The monthly milk iodine concentrations averaged over the 10 study farms remained within optimal levels (60 to 120 µg/L) or above (>120 µg/L). The lowest average was observed in August with 64 ± 27.7 µg/L and the highest in December with 225 ± 42.6 µg/L (Figure 5.2, Panel b). Calculated average milk iodine concentrations in all farms from May to August and from September to December 2014 were 112 ± 28.6 µg/L and 166 ± 40.7 µg/L, respectively and the overall average milk iodine concentrations was 135 ± 24.2 µg/L (data not shown). These data suggest that despite milk iodine concentrations being systematically low in some study farms (i.e. Farms 5, 7 and 8), the overall milk iodine concentration in organic milk remains within or above optimal levels defined in the literature for cows that are fed with about 0.33 mg iodine/kg of dietary DM (Berg *et al*., 1988; Holland *et al*., 1995; Franke, 2009; Haug *et al*., 2012; Borucki Castro *et al*., 2012; EFSA, 2013; Flachowsky *et al*., 2014).

Table 5.3: Iodine concentrations of bulk milk samples obtained from May to December 2014 in the study farms and average farm iodine concentrations over the same period. Dara are reported as µg/L.

Farm	*n*	Month[1]							Mean ± SE
		M	J	J	A	S	N	D	
1	6		103	13	40	44	89	134	71 ± 18.6
2	6	174		40	42	62	312	279	152 ± 50.0
3	6	18	28		32	11	142	83	52 ± 20.7
4	6	407	121	55		79	144	401	201 ± 65.4
5	6	23	27	10	10		25	45	23 ± 5.3
6	6	645	601	481	289	1025		412	576 ± 104.1
7	6	29	38	56	13	19	63		36 ± 8.2
8	7	7	17	7	5	13	17	117	26 ± 15.3
9	5	143		90	77	73		276	132 ± 38.2
10	6		71	35	72	95	71	277	104 ± 35.6
Farms Sampled		8	8	9	9	9	8	8	

[1]Samples were collected from May 2014 to December 2015 every 35 – 45 days; No sample collection occurred in October; Monthly values are raw data as reported from the lab based on which mean mineral content and SE were calculated for each farm; Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK

Selenium concentrations in bulk milk samples remained within optimal levels if not slightly higher on all study farms (Table 5.4). Given the fact that the concentration of selenium in milk is dependent on selenium intake (Walker *et al*., 2010) the data show no nutritional shortfalls in selenium in the case-study farms. It should be mentioned that increased concentrations of selenium in milk may have positive effects on calf and human health (NRC, 2001). Molybdenum concentrations in milk samples

(Table 5.4) averaged from optimal to high which reflects the high Molybdenum concentrations in the forage (see section below). The current status of molybdenum does not indicate a practical concern, however when dietary molybdenum levels reach as little as 5mg/kg DM they can inhibit absorption of copper (NRC, 2001).

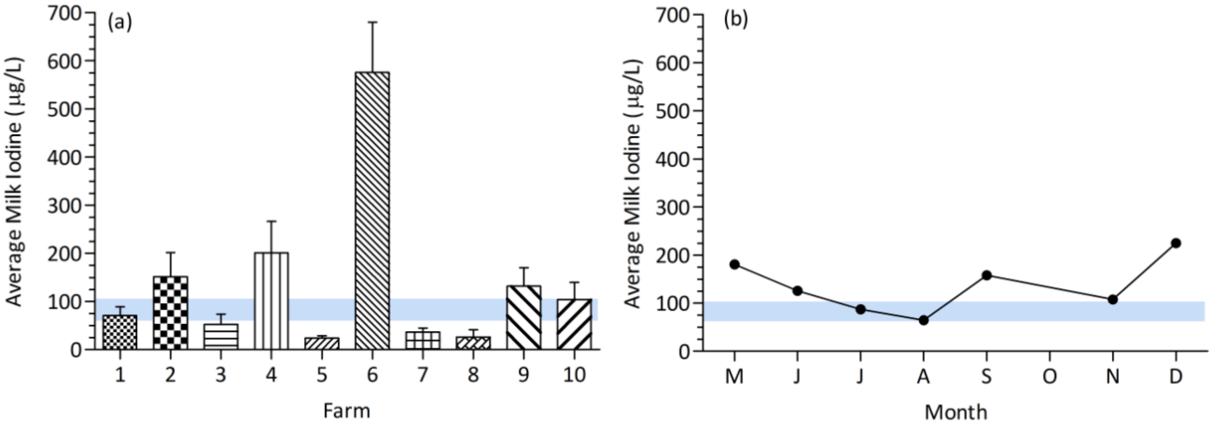

Figure 5.2: Panel (a), Milk iodine concentrations in each farm averaged over the sampling period (i.e. May to December 2014); Panel (b), fluctuation of the average milk iodine concentrations collected from 10 organic farms from May to December 2014.

Table 5.4: Average mineral concentration in bulk milk samples in 10 organic dairy farms

Farm	*n*	Micro-element (µg/L)[1]					
		Manganese (Mn)	Copper (Cu)	Zinc (Zn)	Iodine (I)	Molybdenum (Mo)	Selenium (Se)
1	*6*	21 ± 1.5	35 ± 1.5	3531 ± 82	71 ± 18.6	54 ± 5.7	24 ± 3.3
2	*6*	19 ± 1.0	38 ± 4.8	3295 ± 27	152 ± 50.0	56 ± 4.8	22 ± 1.4
3	*6*	23 ± 3.8	35 ± 2.7	3776 ± 78	52 ± 20.7	54 ± 1.1	16 ± 1.8
4	*6*	28 ± 5.8	35 ± 4.5	3340 ± 111	201 ± 65.4	43 ± 2.4	17 ± 1.1
5	*6*	27 ± 1.2	45 ± 1.2	3365 ± 53	23 ± 5.3	47 ± 1.2	23 ± 1.8
6	*6*	25 ± 1.5	44 ± 1.9	3998 ± 80	576 ± 104.1	48 ± 1.4	15 ± 0.3
7	*6*	23 ± 1.0	38 ± 5.1	3211 ± 126	36 ± 8.2	47 ± 4.8	17 ± 3.4
8	*7*	64 ± 4.0	47 ± 3.0	4139 ± 48	26 ± 15.3	47 ± 0.8	18 ± 0.9
9	*5*	20 ± 1.7	54 ± 16.3	3327 ± 292	132 ± 38.2	66 ± 10.1	20 ± 1.2
10	*6*	16 ± 1.1	35 ± 3.1	3939 ± 85	104 ± 35.6	49 ± 3.2	15 ± 2.2
Optimal levels		20 - 25	50 – 60	3000 - 4000	60 - 100	40 - 50	15 - 20

[1]Six milk bulk samples were collected from May 2014 to January 2015, with exception of farms 8 and 9 from which 7 and 5 samples were collected, respectively. Results are expressed as mean mineral content ± SE; Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK

Urine iodine and mineral concentrations in blood samples in view of milk iodine

Average urine iodine concentrations in each farm and for each sampling occasion are shown in Figure 5.3 and average blood plasma mineral concentration in Table 5.5.

As expected there was variation in urine iodine concentrations between farms but urine iodine also fluctuated considerably within farms across samplings. Determination of iodine in urine is a reliable parameter for the assessment of the iodine supply and low levels reflect nutritional shortfalls of iodine intake. Urine iodine concentrations were above optimal levels (i.e >100 µg/L) in most of the farms but in Farms 7 and 8 urine iodine was marginal or below optimal levels in the first two samplings (Figure 5.3, panels (a) and (b); for Farm 6 there were below optimal levels in the last sampling (Figure 5.3, panel (c), which may suggest some dietary losses of iodine intake. However, the average urine iodine concentrations over the sampling period (i.e. May to December 2014) were above optimal levels on all farms (Figure 5.4, panel (b).

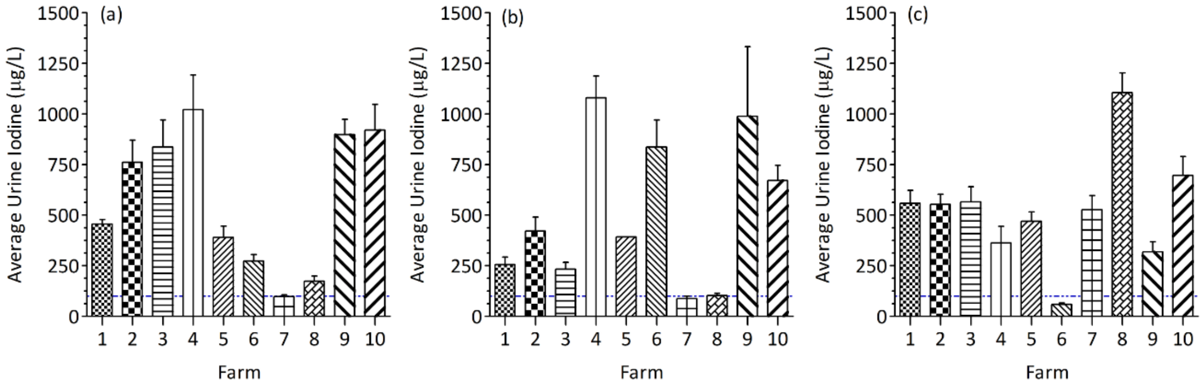

Figure 5.3: Average urine iodine concentrations in each farm during August/September (Panel a), October/November (Panel b) and December/January Panel (c). The results reported are standardised to a creatinine concentration of 5000 µmol/l.

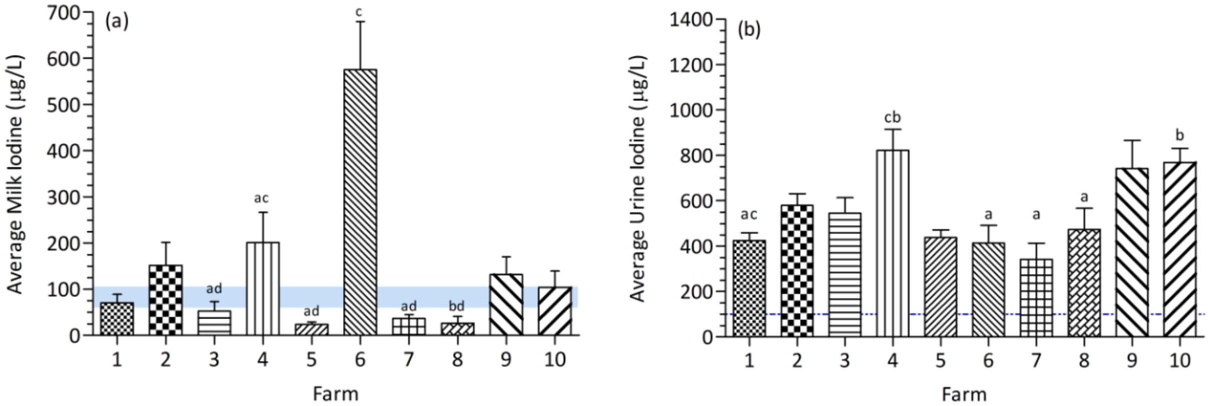

Figure 5.4: Milk iodine (panel a) and urine iodine (panel b) concentrations in each farm averaged over the sampling period (i.e. May to December 2014). Urine iodine is standardised to a creatinine concentration of 5000 µmol/l. (Means with different letters differ significantly by Kruskal-Wallis H test)

Because urine iodine was determined in individual cow samples on three occasions and milk iodine concentrations were determined in bulk milk samples every 35 – 45 days, correlation analysis between the two variables is not possible. Farm comparisons in urine iodine concentrations showed that cows in farm 10 had significantly higher urine iodine compared to those in farms 1 ($P \leq 0.05$), 7 ($P \leq 0.01$), 8 ($P \leq 0.01$) and 6 ($P \leq 0.001$); urine iodine concentrations in farm 4 were significantly higher compared to farm 6 ($P \leq 0.01$), 7 ($P \leq 0.05$) and 8 ($P \leq 0.01$). These results show that the differences between farms in milk iodine (as described in Section 5.1) do not follow the same pattern as the farm differences in urine iodine (Figure 5.4). Unlike urine iodine, milk iodine concentrations are affected -in addition to iodine intake- by farm practices and in particular by the use of iodised-based teat disinfectants (discussed later). In view of that the present results suggest that urine iodine concentrations are not indicative of milk iodine concentrations.

Blood plasma mineral analysis did not show mineral deficiency in any of the farms studied as average plasma concentrations of minerals were within optimal levels (Table 5.5). It should be noted that plasma selenium concentrations of 0.5 to 1 µmol/L is required to maintain target GSHPx concentrations and these were marginal in Farms 10 and 7.

Table 5.5: Average blood plasma mineral concentration

Farm	Micro-element (µg/L)[1]						
	Selenium (µmol/l)	Zinc (µmol/l)	CP Activity (mg/dl)	Copper (µmol/l)	CP:Pl Cu	SOD (U/g Hb)	GSHPx (U/ml PCV)
1	1.1 ± 0.08	11.1 ± 0.7	29.6 ± 1.1	13.1 ± 0.7	2.3 ± 0.1	2533 ± 53	118 ± 3.8
2	1.2 ± 0.05	12.3 ± 0.4	19.8 ± 2.3	10.1 ± 0.4	2 ± 0.2	2373 ± 104	109 ± 3.5
3	0.8 ± 0.02	16.5 ± 1.3	30.3 ± 4.1	12.1 ± 0.7	2.5 ± 0.3	2416 ± 80	141 ± 3.7
4	1 ± 0.07	11.8 ± 0.4	19.8 ± 1.3	11 ± 0.5	1.8 ± 0.1	2764 ± 103	121 ± 2.9
5	1.3 ± 0.03	12.6 ± 1.5	35.8 ± 1.2	11.5 ± 0.4	3.2 ± 0.2	2606 ± 107	120 ± 3.6
6	1 ± 0.07	13.6 ± 0.3	26.6 ± 1.5	12.2 ± 1.0	2.3 ± 0.3	2754 ± 609	102 ± 3.9
7	0.6 ± 0.03	13.7 ± 0.6	23.4 ± 1.1	10.2 ± 0.6	2.3 ± 0.1	2425 ± 57	51 ± 3.3
8	0.7 ± 0.09	11.7 ± 1	29.7 ± 1.1	12.8 ± 0.5	2.3	2342 ± 182	70 ± 2.6
9	1.1 ± 0.03	16.3 ± 1.1	29 ± 4.1	14 ± 0.2	2.1 ± 0.3	2340 ± 99	117 ± 2.8
10	0.5 ± 0.07	11.9 ± 0.4	30.3 ± 3.7	11.3 ± 0.8	2.8 ± 0.6	2524 ± 137	65 ± 3.4
Optimal levels	Norm>0.2	12.3–18.5	Norm>15	9.4-19	Norm>1.7	Norm >2000	Norm > 40

[1]Blood samples were collected from 4 cows in each farm during August/September. GSHPx data are based on 30 samples per farm. Results are expressed as mean mineral content ± SE; Laboratory analyses were carried out by the School of Veterinary Medicine and Science, University of Nottingham, Loughborough UK

Iodine and mineral concentrations in forage samples

Data on forage (or diet) mineral concentrations are missing from three farms as samples were not collected. Over the study period only two farms submitted four samples and three farms submitted one sample for mineral analysis. Table 5.6 shows the average mineral concentration of the forage/diet fed to the animals over the study period in each farm for those farms where it was possible to calculate.

Calcium in forage is an indicator of the soil pH conditions. In all studied farms the average forage calcium concentrations were above optimal levels to high (>0.8%; Table 5.6). Although calcium tends to rise as the plant matures due to its association with the fibre fraction, alkaline soil, over liming or naturally calcaerous soil remain the major influence. Calcium is important for optimal trace element uptake but at high concentrations can reduce trace mineral absorption (especially zinc) in animals (NRC, 2001). The forage analysis results (Table 5.6) show that across the study farms, copper, zinc, cobalt, iodine and selenium were relatively low but molybdenum levels were above optimal levels.

Iodine as an element is essential for animals, but plants have no requirement for iodine. On three farms (i.e. Farms 2, 5 and 6) the average iodine concentrations in forage were below optimal levels (< 0.5 mg/kg) while in another three farms (i.e. Farms 4, 7 and 9) average iodine concentrations were marginal or optimal (0.5 to 0.8 mg/kg). On one farm average forage iodine concentrations were relatively high (1.5 mg/kg). Across farms the average iodine concentration in the forage samples was 0.63 ± 0.2 mg/kg DM. The relatively low average iodine concentrations of the forage samples in the case-study farms can be seen as reflecting the fact that British soils are low in iodine. The transfer ratio of iodine from soil to plant is low and, with the exception of coastal zones, it is suggested that most of the land surface is actually low in iodine (Johnson, 2003). It is important to note that, when animals are given a choice, they will select for forages that are high in protein, calcium, and phosphorus. However, a study that evaluated clipped pasture samples and steer selected forage showed that this is not the case for trace elements (Corah, 1995). This outcome can partially explain nutritional shortfalls in terms of trace elements in grazed cattle.

A comparison both in milk iodine and urine iodine concentrations was performed between farms with low, average and high iodine concentrations in forage. Results show that urine iodine concentrations were significantly higher in the farms with average or high forage iodine compared with the farms with low forage iodine ($P \leq 0.001$), adding to the existing body of evidence that iodine excreted in urine is indicative of dietary iodine intake (Figure 5.5, panel b). With regards to the milk iodine concentrations this was not the case as farms with low or average forage iodine concentrations had higher milk iodine compared to those with high forage iodine values (Figure 5.5, panel a). This outcome is not surprising in view of the data presented in Section 5.2 and reflects the notion that milk iodine concentrations are affected by farm practices such as the use of iodine-based teat disinfectants which is discussed in the next section.

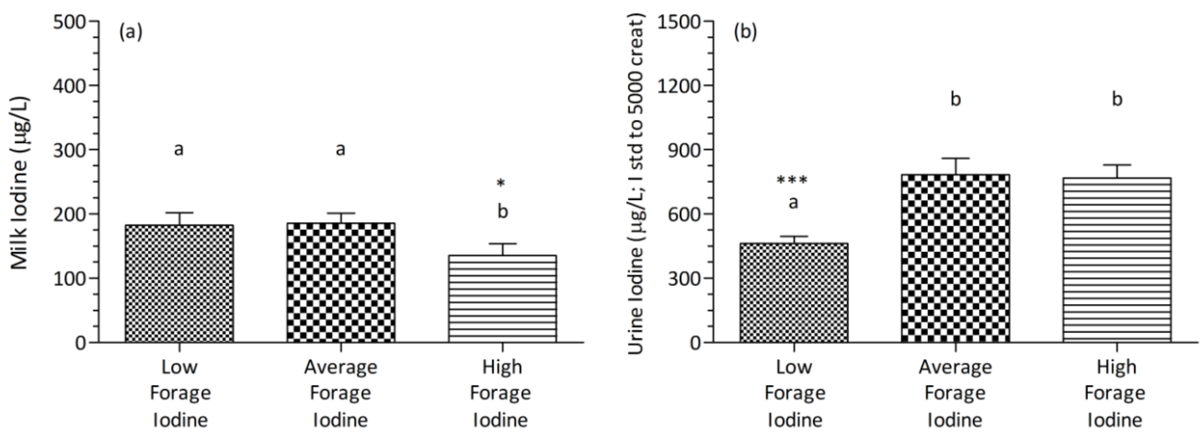

Figure 5.5: Effect of iodine content of forage on bulk milk iodine (panel a) and urine iodine (panel b) concentrations (means with different letters differ significantly by t-test, * P ≤ 0.05; *** P ≤ 0.001)

Half of the farms supplement the animals with iodised rock salt (IRS). Differences in milk iodine and urine iodine concentrations between farms that offer IRS or not were also tested. Analysis of these data showed that there were no differences in urine iodine concentrations between the farms that offer IRS and those that do not (530 ± 35.6 µg/L vs. 612 ± 39 µg/L, respectively; *P*=0.06) but milk iodine concentrations were higher in the farms that supplement the animals with IRS (179 ± 16 µg/L *vs.* 121 ± 8.8 µg/L, respectively; *P*<0.001). However, this comparison does not account for the use or not of iodised teat disinfectants in the farms compared and this result is rather confounded by the effect of iodised teat disinfectants on concentrations of iodine in milk.

Table 5.6: Average mineral concentration of forage/diet in each participating farm.

Mineral[1] *Macro-elements (% DM Basis)*		Optimal levels	Farm									
			1	2	3	4	5	6	7	8	9	10
Calcium	Ca	0.5 - 0.7	-	0.9	-	0.8	1.1	0.9	0.9	-	1.0	1.5
Phosphorus	P	0.3 - 0.4	-	0.1	-	0.3	0.4	0.3	0.3	-	0.4	0.4
Magnesium	Mg	0.15 - 0.25	-	0.1	-	0.2	0.2	0.2	0.2	-	0.3	0.3
Potassium	K	1.5 - 2.5	-	2.3	-	3.0	3.2	2.3	2.4	-	1.8	2.4
Sodium	Na	0.2 - 0.3	-	0.1	-	0.2	0.1	0.1	0.2	-	0.1	0.4
Chloride	Cl	0.6 - 1.4	-	0.7	-	1.4	0.9	0.7	0.8	-	0.6	1.0
Sulphur	S	0.15 - 0.25	-	0.2	-	0.3	0.2	0.2	0.2	-	0.2	0.2
Micro-elements (mg/kg DM)												
Manganese	Mn	75 - 125	-	32	-	72	102	92	80	-	258	100
Copper	Cu	08 - 12	-	6.8	-	13.3	8.9	8.3	8.3	-	21.0	61.2
Zinc	Zn	40 - 80	-	31	-	39	26	33	34	-	73	194
Cobalt	Co	0.2 - 0.3	-	0.1	-	0.2	0.1	0.1	0.1	-	0.1	0.9
Iodine	I	0.5 - 1.5	-	0.3	-	0.8	0.4	0.2	0.5	-	0.7	1.5
Selenium	Se	0.1 - 0.2	-	0.0	-	0.1	0.1	0.1	0.0	-	0.3	1.0
Iron	Fe	100 - 200	-	59	-	561	252	296	377	-	197	551
Molybdenum	Mo	0.35 - 1.25	-	1.5	-	1.4	2.5	1.6	2.0	-	1.3	1.5
Forage samples submitted for analysis			0	1	0	4	3	2	4	0	1	1
Farm Iodine in milk[2]			O	O	O	H	L	H	L	L	H	H

[1]Laboratory analyses were carried out by the Thomson & Joseph Ltd, Albion Laboratory Services, Hoveton, NR12 8QN, UK except for farm Number 2 for which samples were analysed by Sciantec Analytical Services, Stockbridge Technology Centre, Selby YO8 3SD
[2]L=Low, O=Optimal and H=High with milk iodine concentrations below 60 µg/L, between 60 to 120 µg/L or above 120 µg/L, respectively

Effect of iodised teat disinfectants

Six out of the 10 case-study farms use iodised post-dip teat disinfectants, while the remaining 4 farms do not follow that practice. Comparison between the two groups of farms indicated that milk iodine concentrations were 2.3 times higher (Figure 5.6, panel a; *P*<0.0001) on the farms that use iodised post-dip teat disinfectants (mean average 195 ± 13 µg/L) compared with the farms that do not use this practise (mean average 85 ± 8.9). Similarly, urine iodine concentrations were significantly higher on the farms that use iodised post-dip teat disinfectants compared with those that do not (618 ± 34 µg/L *vs.* 481 ± 37 µg/L; Figure 5.6, panel b, *P* ≤ 0.01).

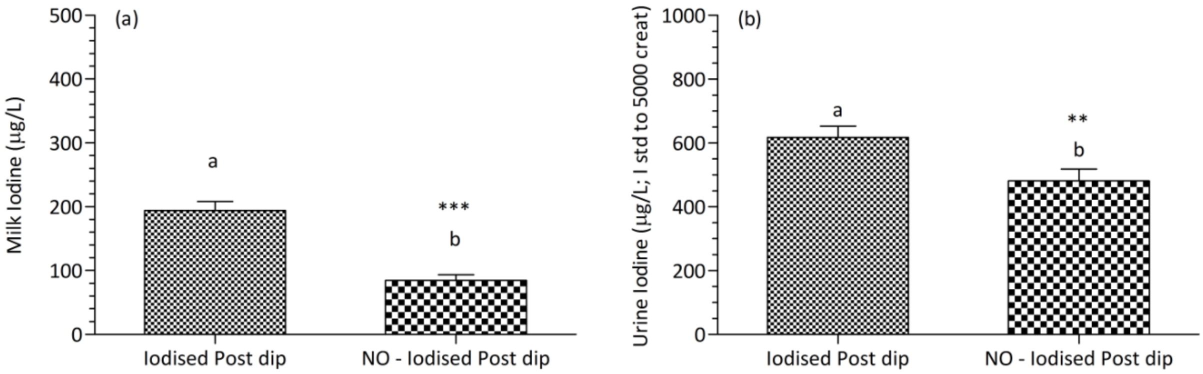

Figure 5.6: Effect of iodised post-dip teat disinfectants on bulk milk iodine (panel a) and urine iodine (panel b) concentrations (means with different letters differ significantly by t-test, ** P ≤ 0.01; *** P ≤ 0.0001)

The results show that the use of iodised post-dip teat disinfectants has a major effect on milk iodine concentrations, which is in accordance with the current literature. According to the review of Flachowsky *et al*. (2014) there are different views about the mode by which the iodine enters into the milk; some authors suggest that iodine residues in milk originate mainly from the contamination of the teat surface (Rasmussen *et al*., 1991) but earlier studies proposed that iodine enters into the milk by the milk synthesis process due to absorption through the skin (Conrad and Hemken, 1978). The content of iodine in the disinfectants is a more important factor than the timing of the application (i.e. pre- or post- dip) in the increase of iodine concentration in milk (Flachowsky *et al*., 2014).

It has been discussed earlier that absorption of dietary iodine is a key factor affecting urine and milk iodine concentrations. The finding that urine iodine concentrations were also significantly higher in the farms that use iodised post-dip teat disinfectants is of particular importance as it can reflect the fact that iodine can be also absorbed in the lungs or via the skin (Conrad and Hemken, 1978; Flachowsky *et al*., 2014).

5.4. Conclusions

This study shows that the monthly milk iodine concentrations averaged over the farms remained within optimal levels, but, in some farms milk iodine concentrations were systematically low through the monitoring period. Urine iodine concentrations were significantly higher in the farms with high (i.e. 1.5 mg/kg) or optimal (i.e. 0.5 to 0.8 mg/kg) forage iodine concentrations compared to the farms with low forage iodine (i.e < 0.5 mg/kg). This outcome reflects the well-established evidence that urine iodine is indicative of dietary iodine intake. With regards to milk iodine, this was not the case: farms with low or average forage iodine concentrations had higher milk iodine compared to the farms with high forage iodine concentrations.

Although this outcome is surprising, it reflects the fact that milk iodine concentrations are affected by the use of iodine-based teat disinfectants. Indeed, six out of the 10 case-study farms use iodised post-dip teat disinfectants, while the remaining 4 farms do not. Comparison between the two groups of farms indicated that milk iodine concentrations were 2.3 times higher in the farms that use iodised post-dip teat disinfectants (mean average 195 ± 13 µg/L) compared with the farms that do not use iodised post-dip teat disinfectants (mean average 85 ± 8.9). This outcome indicates that iodised post-dip teat disinfectants have a major positive effect on milk iodine concentrations and can wipe-out any effect that dietary iodine intake might have on milk iodine concentrations.

In conclusion, this study shows that the use of iodised post-dip teat disinfectant is the most important influencing factor for the iodine concentration in milk and that where post-dip teat disinfectant is used the iodine concentrations in milk do not serve as a robust indicator in identifying shortfalls in iodine intake.

However, forage iodine concentration is an important factor in maintaining milk iodine concentrations at optimal levels, in addition to its importance in maintaining animal health and performance at optimum levels. Milk iodine concentrations fluctuated within farms across samplings but in some farms they were systematically low. This outcome deserves further attention in order to alleviate recent concerns that organic milk contains less iodine than conventional milk and to avoid the health status of the animals being negatively affected by low iodine intake. Where doubts about the iodine supply to animals through their feed exists, urine samples can be used to monitor the cow's iodine status.

6. General discussion and conclusions

This report describes a series of participatory studies that were carried out in three European countries (Romania, Finland and UK) as part of WP1 in the SOLID project. This report consolidates the outcomes of these studies with the aim to provide an insight to the end user (i.e. farmer, stakeholder, researcher or student) on these innovative strategies studied with regards to forage production, utilization and feeding for dairy cow productivity.

The results of the study on diverse swards and mob grazing for dairy farm productivity show that although pasture productivity of the diverse sward ley was slightly lower than that in the grass-clover ley, the total productivity remained relatively high. This suggests that bio-diverse pastures are sufficiently productive to serve as a viable alternative to conventional pastures (i.e. grass / clover pastures) as they can maintain animal productivity at high levels. However, these results are based on data collected from one farm only and therefore this outcome should be interpreted with care. Future research in this area should focus on gathering more information and evaluating the economics of diverse swards versus standard mixtures. The suitability of mixtures, species or varieties to different soil types but also the impact of diverse leys on soil fertility (nutrients, carbon, structure, water) is of particular importance. The feed value of diverse leys as forage for the dairy cow merits further investigation in terms of milk production, livestock performance and animal health.

The farmer who participated in this study claimed that the grazing system he applies in his farm falls within the principles of “mob grazing”. The grazing rotations in year 2014 were of about 40 to 45 days. The average 21-day rotation he applied on his farm during 2015 is regarded as rather short to allow plants to grow to a desired height that fulfils the expectations of mob grazing. However, it should be acknowledged that stocking density always remained high. The overall soil analysis data suggest that management through rotational high stocking grazing of bio-diverse pastures appears to have a beneficial impact on soil organic matter. Microbial activity in the soil does not seem to have been improved considerably over the years but it can be accelerated by bio-treatment of slurry or farmyard manure in short-term. In all fields tested soil trace element status is generally low which may indicate a potential need for zinc and copper supplementation in the ration of the herd.

The studies conducted in Finland aimed at enhancing forage productivity and the protein self-sufficiency of organic dairy farms by studying two cultivation practices for forage production. This is particular relevant for northern European countries as the growing season in cold climates is short but intensive and the cold winters are a challenge to overwintering forage legumes. This limits the choice of available forage legumes for cultivation to species that are winter-hardy and can withstand low temperatures. Overwintering of forage legumes is affected by occasionally occurring warm, wet autumns, mid-winter freeze-thaw cycles, freezing, frost heave, flooding, ice encasement and low temperature fungal attack. Overwintering of red clover is better in mixed swards with grasses, but the benefits may be lost during the growing season, when grasses compete for the resources (Riesinger, 2010).

Red clover is well suited for dairy cow feeding in northern European countries and its inclusion in the grass mixtures at about 30% to 50% for silage production has several advantages. The most compatible companion grass for red clover is timothy (*Phleum pratense L.*), but tall fescue (*Festuca arundinacea Schreb.*) also seems to be a feasible alternative. Grass-clover mixtures are cut twice a year producing up to 10,000 kg of DM per year. The proportion of clover is typically lower in the primary growth than in the regrowth, and declines as the ley gets older. Red clover compared to grasses has a lower digestibility and cell wall concentration, but higher crude protein (CP) and indigestible neutral detergent fibre (iNDF) concentrations enhancing the feed intake potential by ruminants (Kuoppala, 2010). However, red clover is vulnerable to clover rot (Sclerotinia trifoliorum Erikss.) and root rots (Fusarium spp.) and a grass-clover mixture is slow to establish causing problems with weeds as there is a lack of efficient herbicides for clover mixtures (Finckh *et al*. 2015).

In dairy production, topping of the grass to enhance clover production was a novel idea while slurry application is commonly used to enhance forage productivity. The studies descried herein show that topping of grass two weeks before forage harvesting did not increase the proportion of clover in the forage. Although the optimal cutting time cannot be identified by this study this outcome indicates that in practice farmers should allow more time for the red clover to develop adequately in order to increase its mass, protein content and have a positive effect on nitrogen yield. The study shows that slurry application in autumn can increase crude protein content of grass and total crude protein yield in the first harvest for silage production (i.e. in the spring of the coming year). Concentration of soil nitrogen is influenced merely by the soil type as no difference in N concentrations was found before and after of the slurry application in the trial. However, slurry application did affect both soil nitrate and ammonium nitrogen five weeks after the application.

The participatory research studies presented herein also addressed feeding opportunities related to the use of industrial by-products focusing on the utilization of camelina meal and grape marc. It is acknowledged that the feeding on-farm trials conducted as part of this research project had some limitations in terms of estimating individual animal feed intake; however, in view of the limited studies conducted in the area, they do provide a first estimate of the effects of these industry by-products on milk yield and milk quality. The results show that these feedstuffs have a potential to be used in dairy cow nutrition. The total replacement of sunflower meal with Camelina meal did not affect milk yield and milk composition, but it did significantly decrease the fat content of milk by about 15%. This result coincides with earlier results and raises concerns in systems where milk price at the farm gate is corrected in view of the milk fat content. Even so, the use of Camelina meal remains a viable alternative in view of its relatively low cost on the market and it can be a viable alternative in cases of scarce nutritional resources in low-input systems. In addition, the effects on milk fatty acids profile were positive, with an increase of PUFA (including the CLA). Future studies need to identify the mechanism responsible for the decrease in fat content and eliminate obstacles in fully valorising its potential for ruminant nutrition.

The partial replacement of corn grains and barley grains with dried grape marc, while maintaining the experimental diets at the same nutritional value did not affect milk yield and primary composition of milk in terms of fat, protein and lactose content. The grape marc diet did significantly increase n-6 PUFA, especially linoleic acid with a tendency to also increase α-linolenic acid. These outcomes support the notion that grape marc can be used for a short term (weeks, months) replacement of cereals without noticeable adverse effects on cows' performances. On the contrary, it has positive effects on the health value of milk, by increasing the milk content in PUFA. In general, the trials have shown that both feeds have some potential to be used as replacements for energy and protein components of the diet of dairy cows, particularly in low-input systems. It is important to note that these trials were conducted on low-input farms in which animals have a moderate production level (about 14 litres of milk per cow per day) and therefore the results cannot be extrapolated for the intensive, high producing conventional dairy systems.

With regards to the effects of dietary mineral supplementation on milk iodine concentrations, the comparative case studies revealed evidence that use of iodised post-dip teat disinfectant is the most important factor influencing the iodine concentration in milk. In this respect, the iodine concentration in milk does not serve as a robust indicator in identifying shortfalls in iodine intake or dietary iodine deficiencies especially on farms that use iodised post-dip teat disinfectants. Forage iodine concentration is an important factor in maintaining milk iodine concentrations at optimal levels, in addition to its importance in maintaining animal health and performance at optimum levels. Therefore, dietary iodine supplementation is recommended to the farms in which iodine concentrations in forage are below 0.5 mg/kg. Over the course of the study average iodine concentrations in organic milk remained within or above optimal levels defined in the current literature (i.e. >120 µg/L). Milk iodine concentrations fluctuated within farms across samplings but in some farms they were systematically low. This outcome deserves further attention in order to alleviate recent concerns that organic milk contains less iodine than conventional milk.

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