Effect of climatic and agronomic factors on animal health, milk yield and quality parameters in Sfakiano dairy sheep production systems in Crete

Thesis submitted for the degree of Doctor of Philosophy

by

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Abstract

Due to the increasing demand of dairy products, many traditional, grazing-based Mediterranean sheep production systems have introduced intensified feeding and veterinary regimes. At the same time pastoralism was abandoned as a practice and many farmers invested high capital in settled facilities and increased their flock size in order to enhance productivity. However, compared with bovine milk production systems there is limited knowledge about the impact of these intensification practices on animal welfare/health and on the quality of dairy products. The aim of the study reported here was therefore to quantify the effects of a) production intensity/feeding regimes, and b) environmental background conditions during lactation, for ewes of different lambing period on health parameters and trade-offs between milk yield and quality parameters, in traditional Sfakiano sheep production systems in Crete. The findings of the reported study demonstrate that animal health, milk yield and quality in low-input dairy sheep production systems are affected by a series of agronomic and environmental factors. The different feeding practices between the semi-intensive and the extensive flocks on Crete had a significant effect on both milk productivity and FA profiles. In general, our results show that animals of the same genetic background, when reared under extensive management systems, produce less milk with a higher fat content and a more desirable milk FA profile but milk from extensive production systems was more variable throughout the seasons than milk from semi-intensive management systems. Moreover, clear correlations between management system and diseases and differences between the ewes of the different lambing periods were identified for animal health and milk quality parameters. Thus in order to optimize production and enhance product quality a detailed analysis of the characteristics of each production systems is required.

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Abbreviations used

ALA	α-linolenic acid (C18:3 *cis9.cis12.cis15*);
ANOVA	Analysis of variance;
BCS	Body Condition Score;
CFU	Colony Forming Units;
CLA	Conjugated linoleic acid;
CVD	Cardiovascular disease;
DMI	Dry Matter Intake
DPA	Docosapentaenoic acid (C22:5 n-3);
DPA	Docosahexaenoic acid (C22:6 n-3)
EL	Early lambing period;
EPA	Eicosapentaenoic acid (C20:5 n-3);
EPG	Eggs per gram (of faeces);
EX	Extensive production system;
FA	Fatty acid;
GIN	Gastrointestinal nematodes;
LA	Linoleic acid (C18:2 *cis9.cis12*);
LU	Lauric acid (C12:0);
LL	Late lambing period;
LME	Linear mixed-effects;
MA	Myristic acid (C14:0);
MUFA	Monounsaturated fatty acids;
NFS	Non-fat solid;
OA	Oleic acid (C18:1 *cis9*);
PA	Palmitic acid (C16:0)
PDO	Protected Designations of Origin;
PUFA	Polyunsaturated fatty acids;
RDA	Multivariate redundancy analysis;

SCC	Somatic Cell Count;
SI	Semi-intensive production system;
SFA	saturated fatty acids;
VA	Vaccenic acid (C18:1 *trans11*).

Chapter 1. Introduction

1.1 Sheep rearing in the Mediterranean basin

Small ruminants are reared all over the world but for the Mediterranean countries (Southern Europe, Northern Africa, Western Asia) the sector is of special importance. More than 50% of world's sheep milk and almost 20% of goat milk is produced in the area, with Turkey, Greece and Italy being the leader producers (Contreras *et al.*, 2007). This fact is not without reason since small ruminant are able to utilize the low-quality forage of the abundant marginal lands in these countries, where cow perform poorly, and crop cultivation is difficult (Zervas and Tsiplakou, 2011). Traditionally, sheep milk produced in the Mediterranean region has mainly been used to produce cheeses which have become regarded as high "sensory quality" artisan and often "Protected Designations of Origin" (PDO) cheeses such as Manchego (Spain), Roquefort (France), Pecorino Romano (Italy), Graviera Kritis (Greece), Feta (Greece), Halumi (Cyprus). The distinct sensory and nutritional quality characteristics of these products are the main drivers for demand for these cheese (Ozen *et al.*, 2014) and are known to be closely linked to the traditional breeds, the grazing-based extensive management systems and the local environmental conditions including the botanical composition of the semi-natural pasture/shrub vegetation used for grazing (Zervas and Tsiplakou, 2011). Demand for sheep milk and cheese products is expected to continue rising due to increasing demand in Northern Europe and North America and the growing consumer awareness about the nutritional value they hold (OECD, 2016).

As a result there has been a trend to intensify the traditional pastoral and extensive sheep production systems, with uncertain results regarding profitability and sustainability (Nahed *et al.*, 2006; Gelasakis *et al.*, 2012). There is also an increasing concern that changes in management may have a negative effect on milk quality and animal health/welfare status, as has been shown for many intensification measures introduced in bovine milk production (Lean *et al.*, 2008; Stafford and Gregory, 2008; Slots *et al.*, 2009; Stergiadis *et al.*, 2012). Specifically, there is concern that changes in production protocols may affect sensory and/or processing quality (e.g. protein and fat content, and cheese yield) (Bencini and Pulina, 1997) , microbial safety (e.g. total microbial and enteric pathogen loads) and/or nutritional quality parameters (e.g. concentrations of nutritionally desirable omega-3 fatty acids) (Martini *et al.*, 2010). Moreover, increased stocking densities and other intensification practices may have affected animal welfare by increasing the incidence of diseases such as mastitis (Albenzio *et al.*, 2002), lameness, gastrointestinal nematodes (GIN) and other infectious diseases (Casasús *et al.*, 2012). Finally, the environmental impact of such practices is a newly aroused concern.

Increase of input and stocking density may also increase "greenhouse gas" emissions (Llonch *et al.*, 2016) and negatively affect the landscape through overgrazing or absence of grazing (Kairis *et al.*, 2015), while climatic changes affects productivity and animal health in return (Kenyon *et al.*, 2009).

1.2 Sheep production systems

Worldwide two general systems of small ruminant farming can be considered - the pasture based and the indoor systems. However, between those two there is a wide variation reflecting the financial, cultural and climatic differences of each region, especially in the geographically diverse Mediterranean basin. This makes classification and analysis of the production systems extremely challenging. Nowadays there are 4 main management systems used; the traditional pastoral and extensive systems and the newly developed semi-intensive and intensive systems (de Rancourt *et al.*, 2006; Casasús *et al.*, 2012). In most European countries, including Greece, most of dairy sheep population is still reared in extensively managed flocks, but the contribution of semi-intensively and intensively managed flocks in overall milk and dairy production has likely increased (Sitzia and Ruiz, 2016). The actual contribution of each system in milk and dairy production is not easily estimated since there is no consistent method for farm classification between countries.

1.2.1 Pastoral systems

The pastoral systems are characterized by continues presence of the shepherd and constant movement of the flock (transhumance), where sheep must cover great distances to find the required resources. However, due to the high demands in labour and the low productivity (milk per ewe per year) this system is almost abandoned in Europe for milk production (Kilgour *et al.*, 2008).

1.2.2 Extensive systems

The main traditional system found nowadays is the extensive system. The common characteristic of all extensive systems is the continuous outdoor access to pastures, mainly unfenced, private or communal. Fences may exist to smaller fields and in some cases; these are sown either for animals to graze or for hay production. There are simple structures and the animals may be sheltered seasonally (e.g. during winter), during the night or when there are severe weather conditions (Molle *et al.*, 2004). Transhumant practices are still present with flocks switching pastures with season. For example in Southern Europe, in the areas near the coast, the flocks are moved to highlands from early summer until late autumn to be protected from the high temperatures in the lowlands (Zervas, 1998; de Rancourt *et al.*, 2006).

Feed supplementation is a common practice but limited to crucial phases of the productive cycle such as lambing and early lactation, with pastures having the major contribution to energy and nutrient intake on an annual basis. In most regions, ewes are milked twice a day either by hand or by mobile parlours and semi-automatic milking machines. The annual milk yield per ewe is around 100 litres and the lambs are slaughtered for meat at around 5-12 kg carcass weight, about two months after birth (de Rancourt *et al.*, 2006; Volanis *et al.*, 2007). In many countries the flocks are mixed with sheep and goats and flock size differs greatly from less than a hundred animals to a thousand heads. The ewes are kept in one flock and some rams are always kept with the ewes to "guide" the flock. Farmers may share rams during mating. Ewes enter breeding mainly at one year of age and replacement rate is moderate, typically 15%. The reared breeds are mostly local robust ones, suitable for the marginal lands the animal graze, adapted through "natural selection" (Sitzia and Ruiz, 2016).

1.2.3 Semi-intensive systems

By increasing the invested and annual capital many farmers switched from extensive production to a semi-intensive management system (Stefanakis *et al.*, 2007). The pastures in these farms are either improved, (cultivated with cereals) or natural and are located mainly on lowland and hilly areas. Most pastures are fenced, owned or rented, and rotational grazing is applied, however stocking density tends to be high (ewes per ha). The sheep will graze for some hours daily and will be sheltered for the rest of the day. There is a significant use of supplementary concentrate and conserved forage especially during critical time-periods (by "critical" we refer either to climate i.e. winter or to productive cycle phase i.e. mating, lambing, suckling, early lactation) (Molle *et al.*, 2004). There is also some evidence that semi-intensive sheep production systems compared with extensive production rely more on anthelminthics and antibiotics to maintain flock health, while not as much as intensive (Contreras *et al.*, 2007; Hoste and Torres-Acosta, 2011), but this has not been confirmed in many Mediterranean regions. Ewes are milked twice a day by machine, producing around to 200 litres per ewe annually. Lambs stay with the ewes for a few weeks and afterwards are weaned and either kept as replacement ewes or fattened and slaughtered for at 8-16 kg carcass meat (de Rancourt *et al.*, 2006). In some systems there is pre-weaning period when lambs are separated from the ewes during the day and the ewes are milked once a day (Stefanakis *et al.*, 2007).

Flock sizes vary from 100 to 500 animals and some of the flocks may have both sheep and goats. Ewes in different stage of the production cycle may be kept in different groups

(e.g. multiparous group and primiparous group). The rams are kept in a separate flock and are introduced to the ewes only prior mating. Artificial insemination may be applied as well as synchronization of oestrus. Local breeds adapted to the specific environments are preferred and are highly selected for production traits. However, introduction of high-performance breeds is not an uncommon practice. Ewes enter into breeding at one year of age and replacement rates are high at 20 to 25% (Sitzia and Ruiz, 2016) .

1.2.4 Intensive systems

Finally, there are some farmers that apply a purely intensive management system where the animals are kept indoors and there is a high investment in capital. There is no access to pasture and the animals are fed with roughage, silage and concentrate. The degree of supplementation varies but the concentrate to hay ratio tends to be high at 70/30 (Molle *et al.*, 2004). There is also an increased disease challenges and hence use of veterinary drugs (de la Concha-Bermejillo *et al.*, 1998; Scott and Menzies, 2011). Ewes are milked twice a day by automatic milking machines, producing from 200 litres to 300 litres per ewe annually (Epstein, 1985; Pollott and Gootwine, 2004). Lambs may stay with the ewes for a few weeks or are separated within the first week and reared using artificial milk supplements. Flock size varies, but the flocks tend to have less than 500 heads due to the high demands in capital. Ewes in different stage of the production cycle are kept in different groups. The rams are retained in low numbers in a separate flock. Artificial insemination is a common practice as well as synchronization of oestrus. Ewes enter breeding at one year of age and replacement rate is high, typically 25%. The breeds in the intensive systems are highly selected for milk yield (Sitzia and Ruiz, 2016).

1.2.5 Selection of management system for dairy sheep

Management of dairy sheep farms is a complex but necessary procedure in order to assure the viability of the farm. Management protocols have to take into account many variables and in many cases that leads to mistakes and inappropriate management by the farmers resulting in low productivity, poor health status, environmental deterioration and profit loos (Stefanakis *et al.*, 2007). An effective management system should always include suitable:

- Reproduction management protocols (breeding, pregnancy, lambing, weaning)
- Feeding and grazing strategies
- Milking management protocols
- Health management protocols

- Waste management and environmental sustainability

The main issues that must be considered before selecting and developing a management system and which are discussed in more detail later in the thesis, as indicated are:

- *Breed*. Productive performance (milk yield, prolificacy, lactation period), oestrus cycle, robustness are some of the important characteristics that differ between breeds and are essential in selecting a management system (***section 1.3*).**
- *Reproductive physiology* knowledge is essential in determining breeding management and nutritional requirements during the various phases of the reproductive cycle (Tzanidakis *et al.*, 2014)
- *Health care*. Preventive protocols are essential in helping ensure animal health and welfare, in minimizing productions loses and the use of antibiotics and veterinary drugs. Though some vaccination schemes and antibiotic administration protocols may be common between different management systems prevalence of diseases may differentiate (***section 1.4***).
- *Nutrition requirements*. Proper nutrition is important for ensuring high milk yield, milk quality and animal health and nutritional requirements differ, among else, between breeds, reproductive phase, lactation. Determining these requirements throughout the year is the first step before planning the grazing and feeding strategies. (Pulina and Bencini, 2004)
- *Pasture availability and landscape.* Sheep are effective grazers and can cover up to all their nutritional requirements from pasture. Thus, availability, accessibility and quality of pasture (arable or natural) will notably determine the needed grazing strategies as well as feeding requirements (Molle *et al.*, 2004)
- *Infrastructure quality*. As production intensifies the requirements for infrastructures and equipment increases. More space is needed for feed and hay storage, more indoor space per animal is required, milking parlours are vital and different weaning areas are recommended (Sevi *et al.*, 1999)
- *Flock size*. Though flock size may be influenced by many factors (breed, pasture facilities etc) intensification will affect the required annual capital per ewe upwards and change the needed labour, thus it should always be included in the design of management protocol (Zervas and Tsiplakou, 2011).
- *Marketing and Economics*. Since most of sheep milk is used for cheese production before selecting management systems the farmers should consider market and consumer

demands (Hadjigeorgiou *et al.*, 2002), since milk yield and quality may be affected (***section 1.5)*** and the required invested capital and production cost differs with intensification.

Though knowing and understanding the specifics of each variable is important it is similarly crucial to consider the interaction between them. Different breeds have different reproductive cycles which are linked to pasture availability and other climatic conditions. Moreover, the climatic conditions will determine also the demands for infrastructures in order to shelter animas form cold or heat indirectly affecting health status. Thus, when drawing recommendations for management system planning, it is important to refer to areas with similar climatic conditions and related breeding practices an example being the Mediterranean basin. Furthermore, every plan should start with breed selection

Management system	Lamb management	Ewe management (milking)	Adult male management	Nutrition	Human-animal relationship*	Genetic lines	Environmental conditions
Shepherding (pastoral and extensive)	Temporary separation on daily basis	Hand or machine milking (once or twice a day) Always kept in a small group Low replacement	As ewes, they remain with the group.	Pasture, depending on the environmental resources available Possibility of supplementation	Continuous, at animal level Absence of fear of stockperson High opportunity to recognize and treat welfare and health problems	Diverse, variable and with different degrees of adaptation to the environment	Usually carried out in marginal areas such as mountains or semi-arid open rangelands Low pasture quality
Semi-intensive	Separation could happen within a few days or weeks, until weaning for replacement and heavy lambs, or until slaughter	Machine milking (twice a day) Both natural and artificial insemination are practiced Kept in mixed groups with size in the hundreds High replacement Year-old animals enter into breeding	Kept in low numbers.	Improved or unimproved pasture and provision of feed.	Frequent, at animal level Daily unavoidable contact	Highly selected for milk yield and for local adaptation to the environment	Usually carried out in temperate and Mediterranean regions
Intensive	Separation within first days + artificial rearing, fattening	Automatic milking (twice a day) Artificial insemination may be practiced Kept in mixed groups with size in the hundreds High replacement Year-old animals enter into breeding	Kept in low numbers	No pasture Roughage and concentrates, provided by feeding	Continuous, at animal level Daily unavoidable contact High opportunity to recognise and treat welfare and health problems	Highly selected for milk yield	South-eastern Europe

* this refers to general management during the year, excluding lambing; (Casasús *et al.*, 2012)

Table 1.1. Main characteristics of a given milk-oriented management system

1.3 Sheep breeds used in the Mediterranean region

In most sheep rearing systems farmers choose breeds based on two main criteria; production performance and adaptability to local conditions (Boyazoglu and Morand-Fehr, 2001). There are breeds that are considered more suitable for milk production such as East Friesian, Lacaune, Sarda, Chios, British Milksheep, Awassi and Assaf and there are breeds that are considered more suited for meat (Dorset, Dorper, Cheviot, Hampshire, Suffolk) or wool production (Merino, Leicester, Karakul and Lincoln). At the same time adaptation to local breeding conditions is a significant consideration since background climatic conditions directly affect all ruminants (thermoregulation, biomass, pasture quality) (Feng-Hua *et al.*, 2014) and many breeding strategies have failed due to pure adaptability (Kosgey *et al.*, 2006). These are the same criteria that have since long been used by sheep farmers in their attempt to improve the local breeds through simple schemes such as selecting lambs from ewes with these traits e.g. high milk yield (Carta *et al.*, 2009). It is this practice of within breed selection that has led to the development of a large number of different breeds in the Mediterranean basin, where each breed reflects specifics of each area such as the local culture, the local environmental and geographical conditions (Ciani *et al.*, 2014). The same objectives were also used at sheep breeding programs introduced in the 1960s and the later developed cross-breeding programs which created new breeds for the farmers to choose from and at the same time screened out breeds that were deemed unsuitable for sustainable production (Carta *et al.*, 2009). By examining the different breeds utilized in the different management systems around the Mediterranean basin we can have a clearer estimation of the aforementioned practices.

1.3.1 Breeds in extensive and pastoral systems

In the extensive systems the farmers use mainly adapted local breeds distinguished for their robustness. There are more than 70 different breeds raised, where many of these are not clearly oriented to one production purpose. Such multipurpose breeds are found mostly in Turkey (Akkaraman and Morkaraman sheep) (Aytekin and Öztürk, 2012), North Africa (Barbary sheep) and Middle East (IFAD, 1999). But also in Europe many extensive farmers will milk their meat oriented ewes after weaning for a small period, such as the case of Merino in Spain (de Rancourt *et al.*, 2006). However, most extensively managed flocks in Europe raise exclusive milk breeds, such as the Manchega and Churra sheep in Spain (Morantes *et al.*, 2017), the Saloia in Portugal (Brooke and Ryder, 1979), the Corsican and the Manech breeds in France (France Génétique Elevage), the Sardinian in Italy (Sanna *et al.*, 2001), the Greek zackel breeds (e.g. Boutsiko) (Kondyli *et al.*, 2012) and the Sfakion sheep in

Greece (Volanis *et al.*, 2002). For these breeds milk is the main income, while lambs are also slaughtered at young age (5-12 carcass weight) adding to farm income (de Rancourt *et al.*, 2006).

1.3.2 Breeds in semi-intensive systems

In the semi-intensive systems, the famers select breeds which are generally considered as good milkers. There are farmers that use the more intensively managed ewes (Lacaune, Awassi, Chios, Comisana and Sarda) and farmers that prefer traditionally extensively managed breeds such as Churra, Corsican, Sarda, Boutsiko and Sfakion (Casasús *et al.*, 2012). In many areas the local breeds were strongly selected for milk yield and quality traits under local environmental conditions (Casasús *et al.*, 2012) along with the transaction from extensive management to semi-intensive, or in some cases semi-extensive management (Sitzia and Ruiz, 2016). Nowadays, most of the sheep in the semi-intensive systems are mainly local breeds (de Rancourt *et al.*, 2006). Finally, there are cases where the local sheep were crossbred with either foreign ones or with native breeds of better productivity (Shrestha, 2011).

1.3.3 Breeds in intensive systems

For the intensive systems breed choice is more stable across countries, since the farmers prefer high yield breeds such as Lacaune (Duchemin *et al.*, 2012), East-Friesian, Awassi (Epstein, 1985) and Assaf (Pollott and Gootwine, 2004) . These breeds not only have high production traits, but also well documented management practices (feeding, medication, lambing etc.) thus are more easily incorporated in a highly intensive system (Pollott and Gootwine, 2004). Additionally, the farmers do not to have wait for several generation of within breed selection while in some cases the local genetic material is not appropriate for high yield milk production (Gabina and Ugarte, 2001).

In some regions the farmers have crossbred the local sheep with these high-yielders to improve the genetic material, such cases are the Churra breed in Spain (Gabina and Ugarte, 2001) and the Sarda in Sadinia (Boyazoglu *et al.*, 1979). Though the results in milk yield are generally good, problems have been addressed when foreign breeds were introduced in an area; (a) they require more careful management and higher inputs, (b) due to the higher input and the lack of grazing the environmental impact is considered to be negative, (c) they have a negative effect on the genetic material of the local well adapted breeds and (d) these breeds are not allowed to be used for the production of PDO cheese.

Rarely, the local farmer unions invested on improving the local breeds and now use them in intensively managed production system with some examples being the Frizarta (Shrestha, 2011) and Chios breed in Greece (Gelasakis *et al.*, 2012), the Sarda ewes in Sardinian (Congiu *et al.*, 2006) and the Comissana in Sicily (Albenzio *et al.*, 2002). In all cases the farmers intensively select under control conditions for milk yield and quality.

1.3.4 Novel aspects of dairy sheep breeding

Though the traditional quantitative and phenotypic approach in breed selection and breed genetic improvement has achieved acceptable results in productivity, recent research has highlighted the importance of other traits for farmers to consider before choosing a suitable breed or can be used in breeding programs. Firstly, robustness and resistance to diseases are considered as traits necessary in order to control diseases prevalence and moderate the use of drugs such as in the case of gastrointestinal parasites (Woolaston and Baker, 1996; Taylor, 2013) and mastitis (Contreras *et al.*, 2007). For example, within breed molecular marker assisted selection tools have been developed and are commercially used through service providers in New Zealand for foot-rot, scrapie, inverdale gene, carwell gene and texel muscling gene (Van der Werf, 2007).

Moreover, depending on the production orientation the farmers may also consider maternal instinct (meat production) or machine milking ability and udder morphology (milk production) as suitable traits that will further indirectly enhance productivity. Finally, while selecting for milk composition (mainly protein and fat content) is a challenging objective to incorporate in a breeding program due to the negative genetic correlation with milk yield, there are other quality traits such as milk fat fatty acid composition and bioactive peptides content that are deemed as more promising (Barillet, 2007).

1.4 Small ruminant health management

Health management in livestock production is an ethical obligation of the farmer and important measure to avoid production losses. An effective health management program should aim to lower mortality and morbidity rates and improve welfare which can be achieved by having prophylactic measures for disease prevention and control in sheep flocks.

These measure include having well-designed facilities, balanced feeding and grazing regimes, adequate and clean water in order to prevent potential problems (metabolic diseases, injuries etc) and facilitate the control of other hazards (e.g. viral infections) (EFSA NDA Panel, 2010). Cleanliness and disinfection protocols are also required in order to prevent and control the spread of infectious agents (Albenzio *et al.*, 2002). Afterwards a written health and

welfare programme for all animals should be prepared that will include as a minimum, vaccination policy and timing and control of external and internal parasites (DEFRA, 2013).

However, the incidence of diseases and their prevention depends on multiple factors, and in many cases the role of each one or their interdependence is not clear (Underwood *et al.*, 2015). Identifying the origin of diseases and other welfare problems alleviates the problem to some extent, by identifying measures that can affect more than one problem. Moreover, by identifying possible differences to the exposure risk in different management system will help in design of a more effective management system. For example, in the intensified productions (intensive and semi-intensive) the main health problems are expected to relate with facilities management, feeding and medication regimes, while in the extensive management systems the main health problems are suspected to originate from poor animal inspection - handling and high exposure to hazards (Casasús et al., 2012).

1.4.1 Health and welfare problems of dairy sheep in the Mediterranean basin

The health and welfare problems most dairy sheep farmers can be grouped in two main categories based on the origin. This approach focuses on the source of the problem making it easier to identify problems that can be mitigated or avoided through management practises and problems that have to be handled with management practices.

First there are problems originating from biotic factors. In this category are included:

- the infectious diseases caused by bacteria such as mastitis (*Staphylococcus spp*., *Streptococcus spp. E. Coli, Mycoplasma etc*), clostridium infections (Enterotoxaemia braxy), contagious agalactia (Mycoplasm), Leptospirosis (*Leptospira spp.*) paratuberculosis/Johne's disease (*Mycobacterium avium* subspecies *paratuberculosis*) and brucellosis (*Brucella ovis),* foot-rot (*Fusobacterium necrophorum* and *Bacteroides melaninogenicus),* chlamydiosis (*Chlamydophila abortus*) (Underwood *et al.*, 2015)
- the infectious diseases caused by viruses such as the contagious ecthyma (Parapoxvirus), bluetongue disease (Bluetongue virus), Maedi-Visna (ovine lentivirus), sheep pox (poxvirus**)** and Ovine rinderpest **(***small ruminants morbillivirus)* (Underwood et al., 2015)
- the endo-parasites infections from gastrointestinal nematodes such as *Telatosargia spp., Trichostrongylus spp.* and *Haemonchus spp.* and trematodes such as *Fasciola hepatica, Dicrocelium Dendriticum* as well as coccidiosis caused by *Eimeria species* and cryptosporidiosis. (Sykes, 2010)
- the ectoparasites such as ticks, mites, lice and flies that either cause discomfort or damage or transmit other diseases like babesiosis and bluetongue. (Sykes, 2010)

Second there are the problems related to abiotic factors. In this category are included:

- metabolic diseases such as hypocalcaemia, bloat and rumen acidosis as well as malnutrition that may be caused by incorrect feeding regimes (Landau *et al.*, 2005)
- problems caused by the facilities that due to incorrect design may not provide enough shelter, have poor ventilation and small available space or due to poor hygiene promote the spread of diseases (Caroprese, 2008).
- problems caused by background environmental conditions affecting directly (e.g. thermal stress) or indirectly (e.g. drought) the animals. (Casasús *et al.*, 2012)
- problems concerning animal welfare due to management practices such as castration, tail-docking, de-horning and ear tag placement (EFSA NDA Panel, 2010).

1.4.2 Differences in diseases prevalence and animal welfare, between different management systems

The most profound differences between the distinct management systems are found for various stress factors such as hunger, thirst, thermal stress, resting and chronic fear. In the intensified productions high stocking density during housing and poor ventilation may expose the sheep to thermal stress (Sevi *et al.*, 2002; Pennisi P *et al.*, 2010), movement restriction and resting problems (Caroprese *et al.*, 2009). On the other hand at the extensive systems the animals may have to cope with prolonged hunger and thirst (Elston *et al.*, 1999) or chronic fear caused by predators or poor human handling (Wemelsfelder and Farish, 2004) and with thermal stress (hot and cold) during extreme weather conditions (Sevi *et al.*, 2001).

In the case of infectious diseases, there are correlations with management systems an example being the high exposure of sheep to pathogens in the extensive systems that may lead to higher ecto- and endo-parasite infestations (Plant and Lewis, 2011) and incidences of reproductive disorders (Roger, 2012). Similarly, there are gastro-enteric disorders (e.g. rumen acidosis) that have been recorded in some areas to be more frequent in the extensively managed flocks compared with the intensively managed ones and are related to mistakes in grassland management and feed supplementation (Stefanakis, 2006). However, the effect is not always easily quantified. For instance, the main cause of reproductive disorders is in many cases poor nutrition (pregnancy toxaemia, hypocalcaemia) not accounted for litter size and this is true for both extensive and semi-intensive systems (Gootwine, 2016).

For the intensive systems, because housing is continuous , there is an increased risk of respiratory diseases (Scott, 2011), if ventilation and microclimatic conditions are inappropriate and vaccination or antiparasitic treatments are not properly designed, leading to

the appearance of resistant pathogens being another concern (Casasús *et al.*, 2012). Lameness is another disease suspected to be more common among the intensively managed housed flocks mainly correlated to poor flooring , the lack of natural wear and tear (Raadsma and Egerton, 2013) and inappropriate nutrition (rumen acidosis through starch-rich diets and high dietary protein intake) (Morgante, 2004).

Regarding the semi-intensive systems, gastro-enteric disorders, either from poor feeding or infections (endoparasites), are problems considered more frequent than in other management systems. Regarding endoparasites, especially gastro-intestinal (GIN), the animals are likely to be exposed to pastures highly contaminated with endoparasites, due to high stocking densities and lack of rotational grazing (Torres-Acosta and Hoste, 2008). The improper use of anthelminthic has also led to the development of many resistance strains (Geurden *et al.*, 2014). In the extensive systems the main problems occurs with diets that are low in protein and are known to impair host resistance to GIN (Athanasiadou *et al.*, 2008; Torres-Acosta *et al.*, 2012). The feeding originated disorders include hypocalcaemia in parturient ewes (high yield - old ewes) (Oetzel, 1988) and pregnancy toxaemia, especially in cases of multiple-births (Brozos *et al.*, 2011). Rumen acidosis is another common problem caused mainly by increased consumption of concentrates, especially grains, but also grazing of fresh pasture or grain stubble after harvest (Commun *et al.*, 2009; Krehbiel, 2014). Similarly, grazing fresh pasture, or pastures rich in alfalfa and clover or elevated consumption of grains can also cause tympanism (bloat) (Colvin and Backus, 1988; Westendarp, 2006). The semi-intensively managed flocks are also more prone to lameness compared with the intensively managed flocks, because excess protein intake is more likely to occur with grazing causing a histamine release (immune response) in the rumen which results in heating up of the feet and, if skin integrity is not good, it can lead to lameness. Furthermore, high yield ewes are more susceptible to zinc deficiencies (Annicchiarico and Taibi, 2004) and also it is more difficult to identify and isolate contaminated animals (Raadsma and Egerton, 2013). However, the problem is also affected by breed choice (Emery *et al.*, 1984; Raadsma and Dhungyel, 2013).

There are also diseases such as mastitis (clinical or subclinical) considered an equally important problem in all dairy sheep systems. For the intensified production systems additionally to the general microclimatic condition during housing (Sevi *et al.*, 2003; Contreras *et al.*, 2007), the hygiene during milking routine and that of parlour between milking, mastitis is also affected by poor milking machine use (e.g. vacuum level, pulsation ratio, etc.) and maintenance (Olechnowicz, 2012). Thus it is suspected that intensification

may also increase mastitis incidences by exposing the ewes to higher risks (Ridler, 2008). However, it should be considered that during machine milking the animals are more likely to be efficiently inspected, compared with hand milking, allowing the farmers to easier isolate or treat animals (antibiotics, udder disinfection). Moreover, mastitis is irrespectively of management affected by drying-off practices (Contreras *et al.*, 2007) and there is also a correlation with genotype (Duchemin *et al.*, 2012) with high yield breeds considered to be more prone to mastitis than lower yield animals (Fragkou *et al.*, 2007) . Considering that many extensive systems now also use machine milking it is difficult to correlate the problem with a specific management system.

Another, aspect that may affect animal health regardless management is parity or age. Younger animals on initial exposure, are considered more susceptible to mastitis (Sevi *et al.*, 2000), to endoparasite infections (Hoste *et al.*, 2002) and other diseases but there is a lack of information on how this is affected by management choices.

1.5 Ovine milk quality

Contrary to bovine milk, ovine is not for liquid consumption, but is mainly further processed to dairy products. Thus, processing performance (cheese yield - kg of cheese per 100 kg of milk, clotting properties of milk etc.) and aromatic compound content (aroma, flavour) have been the main quality parameters traditionally evaluated for sheep milk (Bencini and Pulina, 1997). In the recent years, "microbiological quality" (bacterial load and pathogens) and "nutritional quality" (Fatty acid profile, vitamin content) are two new parameters vigorously examined. Regardless of the assessed quality parameter the main drivers that affect milk quality can be grouped to "genetic factors" such as breed or individuality, "physiological factors" such as age, parity, stage of lactation and prolificacy, "management factors" such as milking techniques, shearing, mating period and nutrition and "environmental factors" such as temperature and rainfall (Abrahamsen *et al.*, 2007). From those "management factors" are determined by the farmer and "environmental factors" can be manipulated by the farmers especially with the nowadays technological advances. On the other hand, "genetic factors" and "physiological factors" cannot be directly manipulated by the farmer and farming practices have to be adjusted accordingly.

1.5.1 Processing performance

Research on processing performance of milk has been carried out for more than five decades (Bencini *et al.*, 2010). Higher milk protein content and higher milk yield were common targets for the primarily research as also demanded by the dairy processing plants

(Abrahamsen *et al.*, 2007). Processing performance of milk is generally positively associated with the concentration of protein, fat and total solids in milk and negatively with microbial load (SCC) (Bencini *et al.*, 2010). Protein to fat ratio and casein to total protein ratio are two indicators that have been used to estimate processing quality of milk (Pappas *et al.*, 1994). Different ratios have been proposed depending on the dairy product, for example a protein fat ratio of 0.94 - 0.96 is considered suitable for full fat hard cheeses while casein to fat ratios have a proportional relation to cheese yield (Dovc, 2000). However, protein and especially casein content in milk is mainly determined by the genetic potential of each breed/animal (Ordás *et al.*, 1997) and it is not easily manipulated by environmental factors as the fat content (Morand-Fehr *et al.*, 2007). For example, high yield ewes compared with less productive breeds (Tsiplakou *et al.*, 2006), but also ewes of the same breed within the same flock that produce more milk when compared to the lower yield ewes (Soják *et al.*, 2013) tend to have lower fat and protein concentrations. As for the physiological factors - age, parity and number of lambs born are considered to affect milk composition but the results are often contrasting and farmers have little control over (Bencini *et al.*, 2010). Of the management factors milking system and milking interval may affect milk composition (McKusick *et al.*, 2002) and nutrition has a well-documented effect mainly on milk yield and milk fat concentration (Pulina *et al.*, 2006). When all factors are examined as part of a management system, it is supported that milk from pasture based systems have better coagulation properties and cheese yield than milk from intensively managed ewes (Nudda *et al.*, 2004).

As research progressed various genetic variants of milk protein that affect cheese renting properties were also identified, firstly in cow's milk (McLean *et al.*, 1984) and later for goats' (Martin *et al.*, 2002) and sheep's milk (Selvaggi *et al.*, 2014) giving new insights for improving milk processing quality. As a result genetic polymorphism of milk proteins was also examined as potential candidate to be included in breeding strategies (Barillet, 2007). It has to be noted that sheep and goat milk have compared with bovine milk different proportions of the four main casein fractions, along with greater between individuals variation (Selvaggi *et al.*, 2014). The most examined protein is as1-casein and eight genetic variants have been identified (A, B, C, D, E, F, H, and I) of which variant C showed the highest correlation with improved coagulation properties and variants D and H were correlated with lower cheese yield and lower milk protein expression respectively (Dovc, 2000). As for other caseins, Corral *et al.* (2010) associated in Merino sheep breed specific β- and κ-casein genotypes with milk composition and milk yield. However, the ability to apply these research findings into selection and breeding programs in order to improve processing performance is limited due to the infrastructure demands (Martin *et al.*, 2002).

Apart from milk composition and microbial quality the clotting properties of milk are also affected by cheese-making process. Nowadays, the dairy plants have methods to control clotting conditions by using different types of rennet and starting cultures (Albenzio *et al.*, 2010), by adjusting fat content, controlling clotting temperature or by adding calcium chloride (Bencini *et al.*, 2010). Thus, more parameters were included as quality characteristics that characterize milk processing performance. Since dairy products from sheep milk are considered delicacy products in many countries (Ozen *et al.*, 2014) cheese makers targeted to improve quality by enhancing the aroma and flavour of their products. The aromatic volatile compounds found in cheese, adding to their characteristic flavour, derive from several metabolic pathways such as lactose, lipid, protein, and citrate metabolisms. In this manufacturing process such as starter culture selection, local microbial flora (Albenzio *et al.*, 2010) and ripening process have an important affect (Calzada *et al.*, 2013). However, there are findings for bovine cheese (Buchin *et al.*, 1999; Carpino *et al.*, 2004) but also for ovine (Pulina *et al.*, 2006) that grazing of specific plants (e.g. Thyme) or grazing of natural local pastures are factors associated with the development of specific flavours. There are also off-flavours that may develop and are linked to feeding. Poorly conserved silage (silage taste), high quantities of concentrates with oat (unpleasant smells), citrus- and beet-pulp (fish flavour) are some feeds correlated with off flavours (Nudda *et al.*, 2004).

Finally, the concentration of compounds with antioxidant activity is a recent parameter associated with milk processing quality by dairy industry and research. A main focus in studies has been the correlation between antioxidant concentration in milk and fat stability during ripening and product self-life (Alenisan *et al.*, 2017). Enhancing, alpha-tocopherol and carotenoids concentration in milk through grazing has been a proposed method for increasing stability in bovine milk (Havemose *et al.*, 2004) and other dairy products (de Renobales *et al.*, 2012). However, grazing also enhances the concentration of milk unsaturated FA in milk making it more vulnerable to lipid oxidation making this approach quite challenging. The direct addition of antioxidants such as tocopherols and selenium during cheese making is similar approach with promising results (Batool *et al.*, 2018).

1.5.2 Microbiological quality

Hygienic aspects of "Milk microbial quality" is a newly emerging concept of the last 20 years (Boyazoglu and Morand-Fehr, 2001). Previously there were specific pathogens considered as hazards for human health mainly *Mycobacterium bovis* (cause of bovine tuberculosis and a form of human tuberculosis), *Brucella abortus* (brucellosis) and *Coxiella burnetii* (Q fever) (Fox *et al.*, 2017). Many strategies have been applied to control the

prevalence of these diseases and nowadays these are considered to be largely eliminated from the flocks in many countries though remerging cases and endemic problems still remain (Kenny, 2013). The presence of such pathogens is also one of the main reasons for the obligatory pasteurization of milk intended for cheese or dairy production.

However, many more human pathogens are now correlated with milk and dairy consumption (Table 1.2) and the European Union has established specific criteria for the presence of many of these in milk and dairy products (European Comission, 2005). Though most risk assessments are based on evidence from dairy cows the risks is considered to be similar for dairy sheep and goats (Donnelly, 1990). On-farm management practices recommended to control these pathogens are similar for all systems including hygienic husbandry, management of animal wastes and effluents from dairy farms, herd health management, milking hygiene and mastitis control (European Food Safety Authority, 2009). However, since disease prevalence, waste production and stocking density differ between different management systems it is safe to assume that the respective risk will also change.

Furthermore, high microbial loads and presence of psychrotrophs microbes, Coliforms, Clostridia and fungi in milk have been linked to many defects in cheeses (Ledenbach and Marshall, 2009). Good hygiene practice during milking and control of mastitis are measures that can alleviate these problems regardless of production intensity and other factors (Oliver et al., 2005). Moreover, there are objective indicators of milk microbiological quality reflecting both animal health and sanitary conditions. Specifically, Somatic Cell Count (SCC) and Microbial Colony Count (MCC) are two indicators for which the European Union has established specific criteria and monitoring practices in raw milk (Regulation (EC) No 853/2004), with the SCC being accounted only for bovine milk at the moment.

Pathogen	Main source of infection	Main means of on-farm control	Main means of control in processing and food handling
Bacillus cereus	Via milk	No effective control measures presently available	Good manufacturing and hygiene practices. Holding cooked foods at either >60 °C or <4 °C
Brucella abortus	Contact infection (handling infected animals/materials). Also via raw milk	Herd health management \ (vaccination, serological screening)	Milk pasteurization* Hygiene precautions for at-risk workers
Cronobacter spp.	Associated with powdered infant formula		Good manufacturing and hygiene controls in the production environment and during rehydration/reconstitution of the product. Control storage temperature and time of reconstituted product
Shigatoxin producing *Escherichia coli* (STEC) also known as verotoxin-producing *E. coli* (VTEC)	Mainly via raw milk	Hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. * Good manufacturing and hygiene practices
Campylobacter Jejuni	Mainly via raw milk	Hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. * Good manufacturing and hygiene practices
Listeria monocytogenes	Mainly via raw milk and soft cheeses. Also contact infection from handling infected animals/materials	Hygienic husbandry, herd health management	Milk pasteurization. * Good manufacturing and hygiene practices. Prevention of postprocessing contamination
Mycobacterium bovis	Mainly via raw milk	Hygienic husbandry, herd health management, tuberculin testing and slaughter of positive reactors	Milk pasteurization*
Salmonella spp.	Mainly via raw milk	Hygienic husbandry and management of animal wastes and effluents from dairy farms	Milk pasteurization. * Good manufacturing and hygiene practices
Staphylococcus aureus	Mainly via raw milk	Milking hygiene, mastitis control	Milk pasteurization. * Good manufacturing and hygiene practices. Process and storage control
Streptococcus zooepidermicus S. agalactiae	Mainly via raw milk	Milking hygiene	Milk pasteurization*
Yersinia enterocolitica	Mainly via raw milk	Impractical (wide range of animal hosts)	Milk pasteurization*
Coxiella burnetii	Via aerosol and milk. Also, possibly tick bites Mainly via raw milk	Tick control, herd health management	Milk pasteurization. * Hygiene precautions for at-risk workers

* Thermal pasteurization or processes determined to be equivalent to thermal processing.
Source: adapted from FAO, 2013.

Table 1.2. Main pathogenic micro-organisms associated with milk and dairy products

1.5.3 Nutritional quality

"Nutritional quality" is also a relatively new concept with respects to milk and dairy. The high saturated fat and cholesterol content of dairy products has been a concern, because of linkages to increased risk of certain chronic diseases in humans, including overweight, hypertension, and cardiovascular disease (CVD) (Schaefer, 2002) and generally has been considered as a unnecessary source of calories (Dietary Guidelines Advisory Committee, 2015). In particular high intakes of the saturated fatty acids (SFA), lauric (LU, C12:0), myristic (MA, C14:0) and palmitic (PA, C16:0) acid have been linked to an increases in total and LDL cholesterol and some haemostatic/ thrombotic factors that promote thrombosis (Lichtenstein *et al.*, 2006). However, the view that milk consumption, especially milk fat, has a negative impact on health has been challenged, since more recent scientific data indicate that milk or dairy product consumption may have a protective effect against certain diseases (Elwood *et al.*, 2008). Additionally, it should be always taken into account that milk and dairy products remain one of the main sources of "nutritionally desired" animal protein, calcium, potassium, magnesium, iodine, and vitamin A in human diets all over the world and are also good sources of carotenoids and tocopherols, significant provitamins and natural antioxidants with several biological functions (Kenny, 2013).

Beneficial health impacts have been attributed to a range of monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA) found in milk, including several shown to have a protective effect against CVD. Most importantly, milk contains omega-3PUFA including eicosapentaenoic acid (EPA), docosapentanoicacid (DPA) and docosahexaenoic acid (DHA). These long-chain PUFAs are linked to healthy development of the brain and eyes during childhood, lower rates of childhood adiposity (Scharf *et al.*, 2013), improved neurological and immune function (Robinson *et al.*, 2010), protection against CVD (Wang *et al.*, 2006), and improved insulin sensitivity (Franz *et al.*, 2004). Milk from ruminant animals also contains conjugated linoleic acid (CLA),which has potential positive health effects, including anticarcinogenic, antiatherogenic, antiobesity effect or modulation of immune system, although most evidence is from animal studies (Rainer and Heiss, 2004; Bhattacharya *et al.*, 2006). It should also be noted that from the at least 28 different isomers rumenic acid (C18:2 *cis9.trans11*) is the one mostly studied, either for possible health effects or for the concentration in ruminant products, since it is the predominant of the isomers in the dairy products, accounting for 85-90% of total CLA (Ferlay *et al.*, 2017). Furthermore the most predominant FA in sheep milk (Zervas and Tsiplakou, 2011) and thereof dairy products (Mele *et al.*, 2011; Balthazar *et al.*, 2016) is oleic acid making them an important source for the

nutritionally desired *cis*-monounsaturated fatty acids (Astrup *et al.*, 2016). Apart from these fatty acids, there several other bioactive proteins and peptides, that originate from them, and are formed during microbial fermentation, cheese ripening or digestion and are studied for potential health benefits (Table 1.3).

However, the overall impact of these compounds into human health is not easily estimated since the concentration in dairy products is small and easily varies from product to product (EFSA NDA Panel, 2010). Nonetheless enhancing the concentration of nutritionally desirable compounds and improving sheep milk FA profile should remain an important aim. Dairy products are already consumed by a large portion of the population in many European countries (Sanchez-Villegas *et al.*, 2003) and are increasing in demand in North America (Bentley and Kantor, 2018) thus can be consider a significant source of nutrients. By improving the nutritional profile of sheep milk populations that already have a preference towards sheep dairy products can be benefited, as long as consumption is within recommendations (Dietary Guidelines Advisory Committee, 2015).

The level of these beneficial Fatty Acids has been closely linked to animal nutrition. Higher concentrate use is found to have a negative on milk fatty acid profile (Marques and Belo, 2001; Min *et al.*, 2005) while milk from ruminants with high fresh forage intakes has a higher content of PUFA and omega-3 fatty acids and/or lower myristic and lauric acid content (Dewhurst *et al.*, 2006; Chilliard *et al.*, 2007; Tsiplakou and Zervas, 2008; Tsiplakou *et al.*, 2010) compared with ruminants fed diets high in concentrate. Additionally, CLA concentrations in milk from dairy cows were shown to be highly responsive to the percentage of daily Dry Matter Intake (DMI) that is from high-quality forages in contrast to grain supplements (Dewhurst *et al.*, 2006; Butler *et al.*, 2008; Butler *et al.*, 2011; Stergiadis *et al.*, 2012; Stergiadis *et al.*, 2015.). The botanical composition of grazing swards may also affect FA profile of milk with studies reporting higher PUFA in sheep milk when ewes graze pastures rich in legumes compared with pure grass swards (Cabiddu *et al.*, 2005; Nudda *et al.*, 2014). As for the genetic (breed, animal etc), physiologic (age, parity, stage of lactation etc) or environmental factors there is not clear evidence quantifying the effect they have on sheep milk FA profile mainly due to the interaction with other factors such as nutrition (Tsiplakou *et al.*, 2006; de La Fuente *et al.*, 2009; Sinanoglou *et al.*, 2015).

Compound	Health effect	Recommended intake[a]	per 100 ml of sheep milk fat [b]
Saturated FA (SFA)[1]		7 – 22 g / day	60 – 64 g
lauric, myristic, palmitic acid	Raises total LDLa and HDLb cholesterol and increases some haemostatic/thrombotic factors that promote thrombosis		
Monounsaturated FA (MUFA)[1]		18 – 56 g / day	30 – 32 g
palmitoleic, oleic, acid	Decreases total and LDL cholesterol when substituted for saturated fat and decreases total cholesterol compared with dietary carbohydrate		
Polyunsaturated FA (PUFA)[1]			
n-6 fatty acids	Decreases total and LDL cholesterol	7 – 22 g / day	5 – 7 g
linoleic acid arachidonic acid	Precursor for eicosanoids (prostaglandins, thromboxanes, leukotrienes)		
Conjugated Linoleic Acid (CLA)	Has anti-cancer properties, decreases body fat in growing animals		0.4 – 2.8 g
n-3 fatty acids	Decreases cardiovascular risk through multiple mechanisms including platelet function,	1.3 – 2.7 g / day	1.0-1.5 g
a-linolenic acid	inflammation endothelial cell function, arterial compliance and arrhythmia		
Eicosapentaenoic acid (EPA), Docosapenteonoic acid (DPA), Docosahexaenoic acid (DHA)	Decreases risk of sudden death through multiple mechanisms including platelet function, endothelial cell function, arterial compliance and arrhythmia and has beneficial effects on nervous system, development and health	Total of 500 mg / day	
Bioactive proteins and peptides			
Immunoglobulins[2], lactoferrin[3]	Has anti-microbial activity, modulate of inflammatory response	n/a	
Growth factors (EGF, TGF-b)[4]	Prevents development of inflammatory bowel disease	n/a	
PP and VPP peptides[4] (fermented milk)	Antihypertensive activity	n/a	
Casoxins: A, B, C and D[4,5]	Regulates appetite and reduces feeding	n/a	
Caseinophosphopeptides[4,5]	Enhances calcium absorption	n/a	
Milk basic protein (whey)[4,6]	Promotes bone formation and inhibit bone resorption	n/a	

[1](Kris-Etherton *et al.*, 2007); [2] (Mehra *et al.*, 2006); [3](Iwasa *et al.*, 2002); [4](Rutherfurd-Markwick, 2012); [5] (Korhonen and Pihlanto, 2006); [6](Madureira *et al.*, 2007)

[a] Recommendations based on a 2,000 calorie diet

[b] (Zervas and Tsiplakou, 2011; Markiewicz-Kęszycka M. *et al.*, 2013)

Table 1.3. Health effects of milk's bioactive compound

1.6 Aims of the Study

Given that sheep rearing in certain areas utilizes natural resources (pasture, etc.) which cannot be exploited by other species, producing food of high biological value, sheep farming was, is and will be present to challenging geographic areas. Since animal health, productivity and product quality, therefore sustainability of the systems, is affected by various factors, a holistic study on the field of their interaction and interdependence is necessary.

Thus, the main objectives of the PhD study were to:

a) Quantify the effect of feeding regimes on milk composition (including on FA profiles) in two contrasting management systems (extensive and semi-intensive), at flock and animal level for ewes of two different lambing periods.
b) Quantify the effects of changing environmental conditions throughout the production season on milk composition, at farm and animal levels.
c) Assess the combined impact of agronomic factors affected by intensification and of background environmental conditions on animal health and welfare and on veterinary medicine use.
d) Identify associations between nutritional (amount and type of concentrate, amount and type of forage, grazing time-area) and environmental drivers (climatic conditions: average temperature, average rainfall) on milk yield and composition.
e) Identify associations between nutritional (amount and type of concentrate, amount and type of forage, grazing time-area) and environmental drivers (average temperature, average humidity, average rainfall) on animal health.

All effects should be unbiased by breed choice and reflect the intensification of a traditional dairy sheep system. Thus, the studies were conducted on the island of Crete, with a long history on dairy sheep husbandry, with farms rearing the major local Sfakion breed.

Chapter 2. Materials and Methods

2.1 Study area

As discussed, there are many areas in the Mediterranean basin with significant sheep production. However, having a genetically homogenous population of sheep in the same area, reared under different conditions is difficult since many breeds are adapted to specific production systems. Thus the island of Crete, Greece was an ideal study area since a large population of sheep is reared, estimated to be 1.58 million Sheep that counts for approximately 18.6% of the national sheep population of Greece (ELSTAT, 2014b), though the percentage is lower at 9% when non-producing animals (rams, non-milked ewes, replacement lambs) are excluded, while milk production is around 9.5% of Greek total (ELSTAT, 2014a).

Furthermore, the main breed reared is the traditional 'Sfakiano', representing over 80% of the island's one million milked ewes (Stefanakis, 2016; Heraklion, Crete, personal communication), allowing us to focus on the impact on animal health, milk yield and quality from changes in animal nutrition and management and avoid potential complications due to genetic influences.

Crete provided also another advantage. The island has a typical Mediterranean climate like many coastal areas in the basin, characterized by a mild winter, rainfalls occurring in autumn and spring, and a very dry summer. Being at the southern part of Europe climatic change has already started affecting Crete (Koutroulis *et al.*, 2013) providing a suitable environment to assess the effect of environmental factors on animal health, milk yield and quality, since the severe environmental changes, such as climatic alterations (warming and precipitation decrease), desertification and pasture degradation are demonstrated to significantly affect milk production (Giorgi and Lionello, 2008; Kenyon *et al.*, 2009)

The study area was further narrowed to the provinces of Rethymno and Chania where the highest sheep population density on the island is found (Figure 2-1).

2.2 Study breed - Sfakion sheep

The breed takes its name from relevant region where it was first raised, the region Sfakia of Chania prefecture on Crete. From there it was spread to the rest of Crete, with farmers selecting the breed for its special characteristics. The "Sfakiano" sheep is well adapted to the harsh, semi-arid, mountainous conditions making it suitable for traditional extensive systems although is also used in more intensive production systems. The skin colour

and the productive traits are like Italian breeds which were probably brought to the island during its occupation by the Venetians during Medieval, as happened to other regions of Greece. The Sfakiano sheep is probably a crossbreed between those Italian breeds and the Zackel category breeds introduced to Greece from South-East Europe. It is classified as a Thin-Long-tailed breed. The genetic relationship with other major Greek sheep breeds is shown in Figure 2.2 (Rogdakis *et al.*, 1995)

Figure 2-1. Sheep distribution in Crete in 2005 (1:75000 resolution; data source: FAO GeoNetwork), with yellow dots are pinpointed the areas where the farms in this study were located

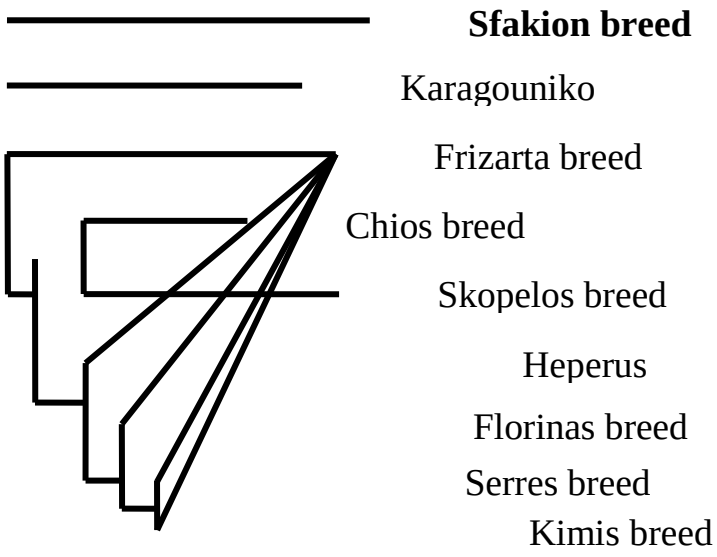

Figure 2-2. Dendrogram resulting from the application Neighbhor-joining method on genetic distances D2 (Rogdakis et al., 1995)

Ewes have a medium-sized head with a roman nose (Figure 2-3). The ears are middle sized, horizontally placed on the head and covered by short fleece. Other naked parts of the

sheep covered with small wool are the head, the neck, the chest, the belly and the legs. The dominant colour on these parts is white, while black dots may be present, especially around the eyes and at the legs. Head characteristics are more intense for rams and the wool covering the naked parts of the body is slightly thicker compared with females. The wool is characterized as carpet wool type; hairs 3 to 5 cm thick and of spiral or wave shape (Kominakis *et al.*, 2001). Mature ewes average around 44kg - 64kg live weight and are 64cm – 74cm tall for ewes-rams, respectively.

Figure 2-3. Sfakion breed. Photos by Voutzourakis Nikos and Tzanidakis Nikos

Most flocks on Crete lamb mature ewes in October/November (early lambing period) and the 20–25% ewes lamb, later in January/February (late lambing period). Suckling lasts for 30 to 60 days before male (and surplus female) lambs are slaughtered for meat. After weaning, ewes are milked twice a day until early- to mid-summer, either by hand (still widely practiced in extensively managed flocks) or by machines (typical for semi-intensive systems) (Figure 2-4). The Sfakion sheep ewes produces 1.2-1.5 lambs per ewe per birth plus around 109.8± 40.8 l of milk for a lactation period of 156.5± 29.3 days annum (Volanis and Tzerakis, 1997; Volanis *et al.*, 2002). Is some areas the annual harvested milk is recorded to reach almost 200 kg ewe^{-1} (Stefanakis *et al.*, 2007).

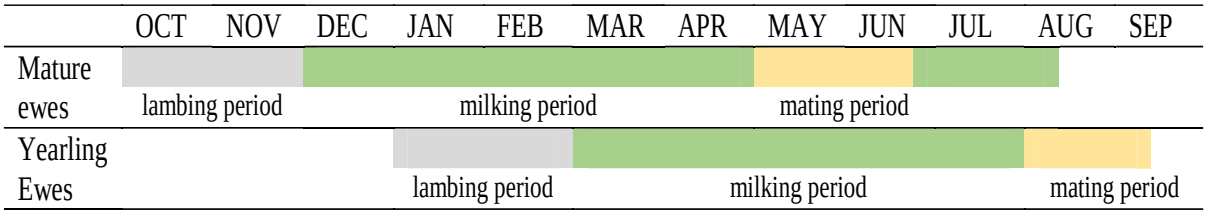

Figure 2-4. Production cycle of the Sfakiano dairy ewes, raised on Crete.

2.3 Experimental design

Aiming to assess the impact of intensification on milk yield and composition and animal health and welfare, 20 commercial sheep flocks were included in the study, ten represented the extensive (**EX**) and ten represented the semi-intensive production (**SI**) systems (***section 2.4***). The 20 flocks were monitored over two consecutive lactation periods (from December 2009 until December 2011) and were visited on monthly intervals (*Figure 2-5*). At each visit, information on flock productivity, nutrition, health problems and on farming practices were collected (***section 2.6***) aiming to clarify and quantify the differences between the two management systems. Moreover, records of the environmental conditions were retrieved (***section 2.7***) to examine for associations between farming practices and background environmental conditions along with their combined effect on, animal health, welfare and veterinary medicine use. In order to investigate for potential effects on milk quantity and quality, milk production was measured and recorded while milk samples were also collected for laboratory analysis (***section 2.5)***. The continues monitoring of the flocks for two years allowed us to examine within and between season variations on the recorded practices and the observed milk productivity and milk quality traits.

Considering the between animal variations recorded in the literature review for milk production, milk quality and animal health, 20 lactating ewes were selected within each flock in order to further investigate on animal level the effect that management system has on milk productivity and quality as well as on important production reducing diseases such as mastitis and gastrointestinal nematodes. Moreover, since there are two different lambing periods that exploit ewes of different age and for which milking overlaps, not including animals from both periods would result in overlooking a significant factor. Thus, ten of the ewes were from the late lambing period (**LL**) and were primiparous (ewe lambs) and 10 of them were from the early lambing period (**EL**) and were multiparous (second or third lactation specifically) (***section* 2.4**). Finally, the animals selected the 2nd year of the study had to be different from the ones selected the 1st since replacement ewes are the ones lambing late in winter. From the selected ewes at each sampling date individual faecal samples were collected; body condition

score was evaluated and FAMACHA anaemia record was assessed. During lactation individual milk samples were collected at the evening milking and for each ewe milk yield was measured. Half udder milk samples were also aseptically collected to be examined for mastitis related pathogens (***section 2.5***).

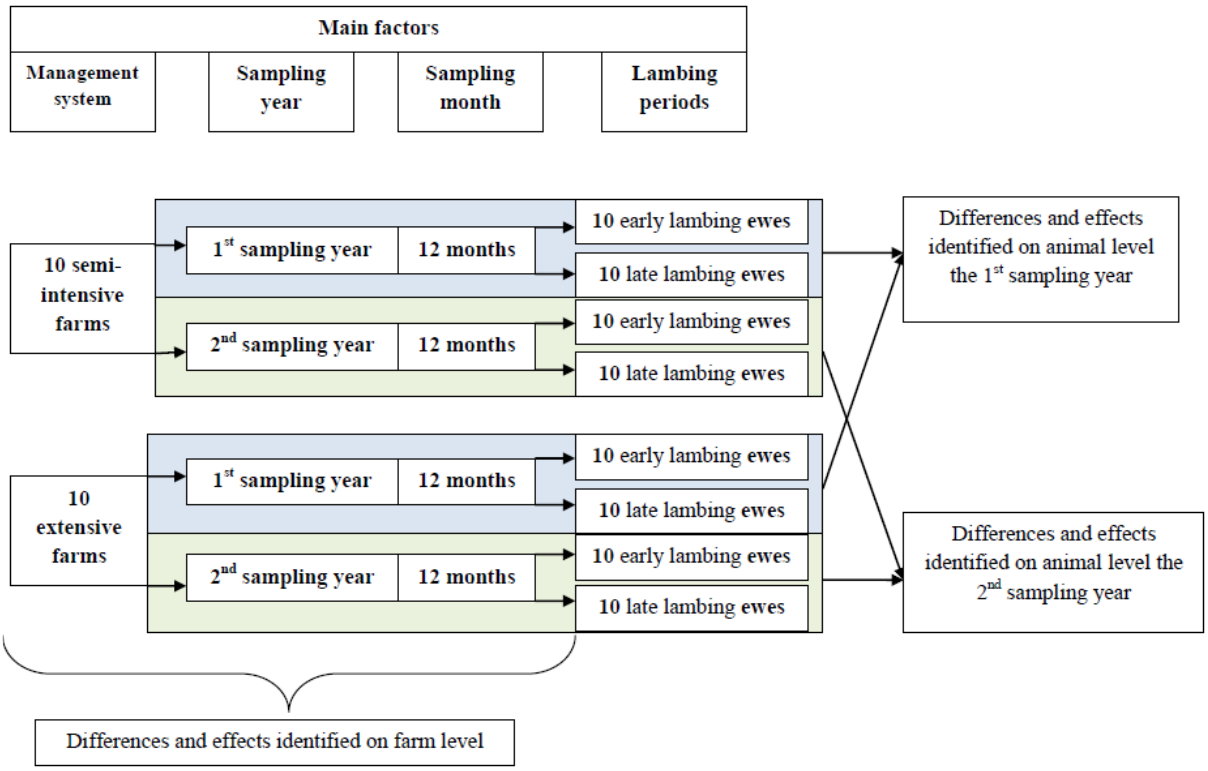

Figure 2-5. Experimental design of study

2.4 Farm and animal selection

Three meeting with local agricultural union members, were held, in Rehtymno, Anogia and Sfakia, in order to identify and select the farms that will participate in the experiments. During the meetings the aims of the study were presented and explained to the farmers present and their insight was discussed. Moreover, information on farm management and breed selection were collected for every farmer. The farms included in this survey were selected based on the following criteria: a) the genotype being pure-bred "Sfakiano" (with pedigree information available), and b) management practices applied being either "extensive" or "semi-intensive".

Pedigree information was based on the holding registers that farmers must keep as well as the farm's record that the veterinarian in charge held. Additionally, the morphological characteristics of the animals in the flock were examined later and crosschecked with those described for the breed.

Systems were classified as extensive when flocks had low stocking densities (0.5 ha/ewe), spent at least 300 days each year grazing marginal land with semi-natural vegetation, and used less than 200 kg supplementary concentrates per ewe per year and had low invested capital in facilities (≤2 m^2 per animal) (Volanis *et al.*, 2007.). In contrast, semi-intensive systems were characterised by higher stocking densities (0.25 ha/ewe) with 200-300 days grazing on improved grassland per year and feeding more than 250kg supplementary concentrate per ewe per year and had high invested capital in facilities (≥2.5 m^2 per animal) and earlier lambing periods than the extensive farms (Stefanakis *et al.*, 2007).

2.5 Sampling procedure

During lactation sampling took place in the evening prior to milking while during dry period sampling took place in the morning. Animals were identified by numbered collars on their necks and by their eartag (Figure 2-6).

For each ewe, milk yield was assessed with the use of a volumetric canister (summary of morning carried by the farmer and evening carried by sampler). Firstly, the udder was disinfected with a 70% ethanol solution and the ewe was aseptically hand milked in the canister. After the first three drops of milk were ejected half udder milk samples aseptically collected in a 10 ml vacuum tube (Vacutainer™, BD Vacutainer Systems, UK) to be examined for mastitis related pathogens. After milking each ewe, two additional milk samples were then collected from the canister in two 50 ml plastic vials (Milk vials, P.Spyropoulos & CIA Lp. Greece). The 1st would be used for measuring the chemical composition (fat, protein, lactose, solids non-fat) and somatic cell counts and the 2nd contained a tablet of Sodium azide (Sodium azide tablets, Merck, Germany) and would be used for total bacterial counts

Moreover, each lactation period two sampling dates were selected, and an additional vial was collected for milk FA profile analysis. In order to select the two periods, we examined a) when ewes from both lambing periods are milked in both management systems, b) when feeding includes both grazing and concentrate and c) in which season there is the lowest possible for extreme weather conditions (snow, drought, storm etc). The first sampling took place in the beginning of March, in order to have data from both lambing groups and a stable ration based on supplementary concentrate and grazing. The second was in the beginning of May before the mating season after which, ewe's reproductive stage may change, milk yield of early lambed ewes is expected to drop sharply, and environmental temperature is expected to increase.

Samples for chemical and microbiological examination were refrigerated for 12 to 24 hours until analysis while samples for FA profile analysis were freeze-dried and kept frozen (-18^{o}C) until analysis. During sample collection and transfer samples were kept cool with the use of portable electric cool boxes (Crivit^{TM}, Germany) and icepacks.

Figure 2-6. Various photos of sampling and recording procedures. Photos by Voutzourakis Nikos and Tzanidakis Nikos

At each visit, body condition score of the ewe was evaluated (MAFF, 1996) and FAMACHA anaemia record was assessed using the FAMACHA© system card (Mohammed *et al.*, 2016). Finally, rectal faecal samples were collected in plastic gloves (Figure 2-6). Faecal samples were immediately stored in a portable electric cool box (Crivit^{TM}, Germany) with icepacks and was later vacuum packed and refrigerated until analysis, maximum 10 days after sampling. All samples and measurements were recorded in "monthly measurement sheet" (Figure 2-7) on site. After sampling from individual animals was finished milk sample

was collected from the bulk tank (evening and previous morning milking) in two 50 ml plastic vials (Milk vials, P.Spyropoulos & CIA Lp. Greece) one containing again a tablet of Sodium azide. The total number of samples collected, and the analysis carried out are presented in Table 2.1 while the obtained records are presented in Table 2.2.

Samples collected	number of samples	Variables measured for each sample
Bulk tank milk samples	**236**	milk fat, protein, lactose and solids non-fat and contents, milk fat fatty acid profile, SCC, CFU
Individual milk samples	**4,853**	milk fat, protein, lactose and solids non-fat content, SCC, CFU
of which	**1.592**	milk fat fatty acid profile
Half udder milk samples	**9,576 collected (5.432 examined)**	SCC, CFU and if SCC>500,000 microbiological examination (pathogen detection and enumeration)
Individual faecal samples	**6,495**	Egg per Gram (EPG) for Gastrointestinal nematodes, Nematodirus, Moniezia, Trichuris, Hemonchus

Table 2.1. Samples collected, and analysis carried out for each category

2.6 Records obtained

Each year prior to the 1st sampling a detailed questionnaire the "Farm main questionnaire" regarding (Figure B.4-9, appendix B) the characteristics of the farm was completed. The questionnaire had 5 sections. The 1st section regarded general and contact information of the farmer. The 2nd section recorded detailed information on agronomical characteristics of each farm. These included flock size, available facilities and machinery, grazing land characteristics, grazing management and feeding regimes. The 3rd section regarded flock health management (vaccinations, preventive treatments etc). The 4th section included health issues and prevalence of diseases in the flock and in the 5th more detailed information on udder health management and treatment of mastitis were recorded. In the next three sections information on flock management during mating, lambing and milking were recorded. These included feeding regimes for each period, conception rates, lamb mortality, lamb management etc.

During each monthly farm visit a short "Farm surveillance questionnaire" was completed (Figure 2-8), containing information about animal health status (prevalence of mastitis and other diseases), nutrition (grazing hours, use of conserved forage, use of concentrate feed), interventions to the flock (vaccination, matting etc.) and productivity (last 7 days daily milk yield, monthly milk yield, lambs born per ewe).

For all farms the Days in Milk (DIM) indicator was recorded representing the days since milking started. All grazing areas were classified on a floristic code between 1 and 5, as a proxy for altitude and plant communities with sites higher up the hills having higher values

(Vogiatzakis *et al.*, 2008). In the classification, one (1) represents the coastal zone where plants are affected by sea saltiness and humidity, the altitude is between 0 and 100 meters and some common species are the sea lily P*ancratium maritimum,* and the tree *Tamarix cretica* and the Cretan palm tree *Phoenix theophrastii*. Two (2) represents the plains, where altitude is between 100-400 meters and many areas are cultivated. Local flora consists mainly of shrubs and aromatic plants and herbs such as *Quercus coccifera, Nerium oleander, Vitex agnus-castus, Chamomilla recutica, Mentha spicata, Myrtus communis, Erica, Alcea pallida, Papaver rhoeas, and Ebenus cretica.* Three (3) represents the semi-mountainous areas with altitudes between 400 - 800m. There, at the slopes of the mountain's shrubs and phrygana such as *Quercus coccifera, Thymus capitatus, Arbutus unedo,* and *Phlomis cretica* dominate the flora along with wild flowers such as *Cyclamen creticum, Iris cretica, Tulipa orphanidea,* and *Muscari commosun* and occasionally there are clusters of carob trees (*Ceratonia siliqua*) or oak trees *(Quercus).* Four (4) represents the mountainous areas with altitudes between 800 - 1000 m. Trees such as oak (*Quercus coccifera*) and maple (*Acer sempervirens*) are the main flora along with herbaceous and bushes such as *Erysimum creticum, Tulipa cretica, Achillea cretica, Viola cretica* and *Crocus oreocreticus*. Finnaly, five (5) represents the subalpine and alpine zone with altitudes above 1,000 mwhere a multitude of herbaceous or bulbous plants and small shrubs grow. Common species found in the areas are *Crocus sieberi, Chionodoxa nana, Anchusa caespitosa, Arabis alpine, Astragalus angustifolius, Corydalis rutifolia, Prunus prostrate, Viola fragrans* and in higher altitudes (above 2,200 meters) there are psychotropic plants such as *Veronica thymifolia, Draba cretic, Cynoglossum sphacioticum,* and *Dianthus sphacioticus.*

Recorded variable	number of records	Period records obtain / frequency
Recorded variables of monitored animals		
Milk yield	4,904	monthly during lactation period
Body conditions Score	6,603	monthly all year around
FAMACHA score	6,603	monthly all year around
Main recorded variables for farm		
Information contained in the Farm main questionnaire	40	each year before weaning
Information contained in Farm surveillance questionnaire	480	monthly all year around
Environmental conditions (Temperature, relative humidity)	~350,000	Continues monitoring all year around
Environmental conditions (rainfall)	3,650	Daily values all year around

Table 2.2. Records obtained during the study

FARM:			Date:							°C
Ewe	SIDE	TAG	Famacha	BCS	Fecal score	MILK	milk/ yield	PROB LEMS	no.	pH
1	R								1	
	L								2	
2	R								3	
	L								4	
3	R								5	
	L								6	
4	R								7	
	L								8	
5	R								9	
	L								10	
6	R								11	
	L								12	
7	R								13	
	L								14	
8	R								15	
	L								16	
9	R								17	
	L								18	
10	R								19	
	L	71							20	

										°C
Ewe	SIDE	TAG	Famacha	BCS	Fecal score	MILK	milk/ yield	PROB LEMS	no.	pH
11	R								21	
	L								22	
12	R								23	
	L								24	
13	R								25	
	L								26	
14	R								27	
	L								28	
15	R								29	
	L								30	
16	R								31	
	L								32	
17	R								33	
	L								34	
18	R								35	
	L								36	
19	R								37	
	L								38	
20	R								39	
	L								40	
21	BULK SAMPLE								41	
22	MAST								42	
23	MAST								43	

Figure 2-7. Monthly measurement sheet

MONTHLY FARM QUSETIONNAIRE LowInputbreed	
DATE:	FARM:
WEATHER CONDITIONS: oC RH	
DAILY MILK PRODUCTION:	MORNING EVENING GOAT
MONHLTY MILK PRODUCTION	
NUMBER OF ANIMALS THAT ARE MILKED	SHEEP GOAT
NUMBER OF ANIMALS THAT ARE NOT MILKED	
CURRENT FEEDING: (what and how much?)	CONCETRATE:
HAY / PRESERVED FORAGE: (bought/ harvested)	
GRAZING improved areas/ plant : wild areas / plant :	
FLOCK MOVEMENTS (other regions): WHEN:	WHERE
LAST MONTH INTERVENTIONS - TREATMENTS TO THE FLOCK	
MATING. ENTRY DATE: MATING DATE: NUMBER OF RAMS:	
LAMBING. DATES: NUM. EWES: NUM LAMBS: live / dead	
VACCINATION (which/dose):	
ANTIPARASITIC TREATMENT (which/dose):	
CHANGES TO THE FACILITIES	
OTHER	
LAST MONTHS PROBLEMS	
MASTITIS Where there any mastitis?	0. NO 1. YES
How many & which sheep (! Experiment animals?)	
Treatment?	0. Nothing 1.Antibiotics im 2.Intrammamary 3. Intram & im Antibiotics
FOOT ROT	0. NO 1. YES
How many animals:	
Specify the problem	0. Lameness 1. Wound 2. Interdigital dermatitis 3. other problem:
DEATHS: nom	CAUSE
OTHER PROBLEMS (respiratory, abortions, fever, other)	
	Signature :

Figure 2-8. Farm surveillance questionnaire

2.7 Environmental conditions

At each farm, a temperature and humidity recorder (T&D Recorders®, RTR-53) was placed in the yard outside the main stable, protected from rainfall and direct exposure to sunlight. Recorders were set to record each hour. Each month the data from the recorders were downloaded and the condition of the recorder and sensor checked. The downloaded data were compared with the data from the local weather stations belonging to the National Observatory of Athens network and evaluated. In the study area there were 6 weather stations in close proximity with the farms. From the database of the same stations the data regarding rainfall was obtained.

2.8 Analytical methods

2.8.1 Milk samples analysis of chemical composition, Somatic Cell Count and bacterial load.

These analyses were conducted at the facilities of the local state milk quality laboratory in Rethymno. The samples where heated to 25°C and pH measurement was taken (HI 9126V, HANNA Instruments - HANNA NORD EST SRL, Italy). For samples with pH above 6.00 chemical compositions (fat, protein, lactose and non-fat solid content) was assessed by infrared methods (MilkoscanTMFT, FOSS®, Denmark) and by flow cell cytometry the Somatic Cell Count (SCC/ FossomaticTM FC, FOSS®, Denmark) and the Colony Forming Units (CFU/ BactoScanTM FC, FOSS®, Denmark). Samples with pH<6.00, 38 in total, were discarded since they were below instruments' acceptable level for analysis. One vial was used for chemical composition and SCC assessment and a second vial for Colony Forming Units.

2.8.2 Copranological examination.

For faecal samples, parasitic egg counts were carried out according to the modified McMaster technique (MAFF, 1986), using a saturated sodium chloride solution as floatation means, with a sensitivity of 50 eggs per gram of faeces. Faecal cultures of samples with EPG≥20 were carried out for L3 stage larvae identification. Samples were incubated for 12days at 27ºC and afterwards larvae were collected according to Baermann technique (Hendrix and Robinson, 2014).

2.8.3 Mastitis Related Pathogens Detection.

Evaluation of sub-clinical mastitis and microbiological examination of half udder milk samples was carried out when SCC was over 500,000 cells/ml milk (Kiossis *et al.*, 2007). In total **5.432** half udder milk samples were examined.

After thawing, 20 μl of milk was spread on 5% blood sheep agar (TSA base agar - Biokar diagnostics BK028HA - and 5% sheep blood) and on MacConkey agar (Biokar diagnostics BK050HA), incubated at 37°C for 16 to 20h and haemolysis was evaluated and colony forming units (CFU) counted. Samples with less than 5 phenotypically similar colonies were classified as negative. Samples with more than two different types of colonies were considered as contaminated and dismissed from further evaluation. When at least 5 colonies of one or two different phenotypic type were present a typical colony for each type was inoculated on TSA agar (Biokar diagnostics BK028HA) for further biochemical testing.

Oxidase and coagulase tests (Bactident® Coagulase, Merck, 113306) wer used for differentiation of *Staphylococcus Coagulase Negative* (CNS) and *Staphylococcus Coagulase Positive* (CPS). Gram stain was used for microscopy observation with a 100x magnification. Samples with CFU ≥250/ml of one or two mammary pathogens (CNS, CPS, *Corynebacterium spp., Escherichia coli, Streptococcus spp.*, other minor microorganisms and mixed infections of previous) were classified as positive to subclinical mastitis infection.

2.8.4 Milk Fatty Acid Analysis.

For the FA profile analysis of milk samples 37 FA methyl ester mix C4−24, t11C18:1 and C22:5 c7,10,13,16,19 standards, were purchased from Sigma-Aldrich (Gillingham, U.K.). Standards for conjugated linoleic acid (CLA) isomers c9t11 and t10c12 were kindly provided by colleagues in the Faculty of Agricultural Sciences, Aarhus University, Denmark. Milk preparation, fatty acid methylation and gas chromatography analysis for FA milk composition were carried out as described by (Butler *et al.*, 2011).

Using an appropriate Gilson pipette, 2ml of hexane and 2ml of 0.5 sodium methoxide solution purchased from Sigma-Aldrich (Chillingham, UK) were added, mixing the chemicals with the vortex after each addition. The samples were left for 15 minutes on a hot block (Techne, Dri-Block, DB3D) at 50°C. When room temperature was reached, 75 μl of 12N HCl purchased from VWR (Lutterworth, UK) was added, the tubes vortexed and left for other 15 minutes at room temperature. Three ml of hexane and 3 ml of deionised water were added, and samples were vortex again before centrifuging with a Fischer Scientific accuspin 3R centrifuge at 1,160xg at 5°C for 5 minutes. Finally, 400 μl of the upper layer was collected

from each sample and put in appropriate GC vials. All samples were stored at -20°C until analysis by GC.

Milk samples in GC vials were analysed with a GC (Shimadzu, GC-2014, Kyoto, Japan) using a Varian CP-SIL 88 fused silica capillary column (100m x 0.25mmID x 0.2µm film thickness). Carrier gas in the column was helium purified at 109.9 kPa of pressure with 0.39 ml/min of column flow. Identification of individual FA was performed from the retention time by using FA methyl ester standards mix and expressed as a proportion of total peak areas for all quantified FA (g / 100g of total FA). The total area of unidentified peaks (which may or may not have been fatty acid methyl esters) was <6.5% of total peak area. Additionally, for each sample the following groups were calculated:

a) Saturated Fatty Acids (SFA).
   C4:0, C5:0, C6:0, C7:0, C8:0, C9:0, C10:0, C11:0, C12:0, C14:0, C15:0, C16:0, C17:0, C18:0, C20:0, C22:0, C23:0, C24:0
b) Monounsaturated Fatty Acids (MUFA).
   C12:1, C14:1, c10C15:1, t9C16:1, c9C16:1, c10C17 :1, t6C18 :1, t8C18 :1, t9C18 :1, t10C18 :1, t11C18 :1, t12C18 :1, t13C18 :1, c9C18 :1, c11C18 :1, c12C18 :1, c14C18 :1, t16C18 :1, c15C18 :1, C19 :1, c5C20:1, c8C20:1, c11C20:1, C22:1, C24:1)
c) Polyunsaturated Fatty Acids (PUFA),
   t8c13 C18:2, c9t12 C18:2, t9c12 C18:2, t11c15 C18:2, c9t11C18:2, t10c12C18:2 c9c12 C18:2, c9c11c15C18:3, c6c9c12C18:3, C20:2, c8c11c14C20:3, c11c14c17C20:3, c5c8c11c14C20:4, c5c8c11c14c17C20:5, c13c16C22:2, c7c10c13c16C22:4, c7c10c13c16c19C22:5, c4c7c10c13c16c19C22:6
d) omega-3 PUFA (n-3)
   c9c11c15C18:3, c11c14c17C20:3, c5c8c11c14c17C20:5, c7c10c13c16c19C22:5, c4c7c10c13c16c19C22:6
e) omega-6 PUFA (n-6)

c9c12 C18:2, c6c9c12C18:3, C20:2, c8c11c14C20:3, c5c8c11c14C20:4, c13c16C22:2, c7c10c13c16C22:4

2.9 Statistical analysis

All statistical analysis was carried out in the R platform. The significance level for all the statistical tests was defined at 5%. All the analysis was conducted in the R statistical language (R Development core team).

The analysis used to describe the characteristics of each farm and investigate differences between the two different management systems, namely semi-intensive and extensive, was based on descriptive statistics, such as mean, standard deviation, stand error, graphs and t-test or ANOVA. Furthermore, correlations were investigated using Spearman's rank correlation coefficient for continues variables and Pearson chi-square for categorical variables.

The main statistical inference analysis was based on Linear mixed-effects models (Pinheiro and Bates, 2000). Linear mixed-effects (LME) models can account for the repeated measurements at the farm and animal level. These models use two types of explanatory variables: fixed effects, which affect the mean of the response variable; and random effects, which affect the variance of the response. Tukey's honest significant-difference test was used for pairwise comparisons of means where appropriate in order to account for the family-wise error. Residual normality was assessed using the qqnorm plots (Crawley, 2007), with no reported data showing deviation. Furthermore, all the homoscedasticity of all the models was investigated, with no reported data showing heteroscedasticity.

Redundancy analysis (RDA) was also used to extract and summarise the variation in a set of response variables that can be explained by a set of explanatory variables. RDA was performed with the CANOCO package (ter Braak and Smilauer, 2002) using automatic forward selection of variables with significance calculated using the Monte Carlo permutation test. Redundancy analysis (RDA) is a method to extract and summarise the variation in a set of response variables that can be explained by a set of explanatory variables. More accurately, RDA is a direct gradient analysis technique which summarises linear relationships between components of response variables that are "redundant" with (i.e. "explained" by) a set of explanatory variables. To do this, RDA extends multiple linear regression by allowing regression of multiple response variables on multiple explanatory variables. A matrix of the fitted values of all response variables generated through MLR is then subject to principal components analysis.

The total variance of the data set, partitioned into constrained and unconstrained variances, is a standard result of RDA. This result shows how much variation in your response variables was redundant with the variation in your explanatory variables. If the constrained variance is much higher than your unconstrained variance, the analysis suggests that much of the variation in the response data may be accounted for by your explanatory variables. Furthermore, each RDA axis has an eigenvalue associated with it. As the total variance of the solution is equivalent to the sum of all eigenvalues (constrained an

unconstrained), the proportion of variance explained by each axis is simply the quotient of a given eigenvalue with the total variance of the solution.

The RDA ordinations are presented as a biplots on which:

a. Parameters closer together are likely to be associated with or affected by similar conditions or circumstances
b. The closer a response variable is to an explanatory variable (e.g. the smaller the angle between their respective vectors) the higher their (linear) correlation is.
c. The length of the vector of the response variable to axis origin is related to the recorded value of the variable thus is not evaluated
d. The angles of the above vectors are meaningless and are also not evaluated

2.9.1 Farm level statistical analysis

The characteristics of each farm were documented using the "Farm Main questionnaire" and the "Farm surveillance" questionnaires - collecting the following characteristics:

1. Number of animals:
    a. total ewes
    b. multiparous ewes, (lambing either in autumn (early) or winter (late),
    c. primiparous ewes (lambing only in winter (late),
    d. non-milked ewes,
    e. number of rams,
    f. ewes per ram
    g. total goats,
    h. multiparous goats,
    i. primiparous goats and
    j. number of billy goats
    k. female goats per billy goats
2. number of farms with mixed flocks
3. % percentage of:
    a. multiparous ewes lambing in autumn (late),
    b. multiparous ewes lambing in winter (early),
    c. primiparous ewes,
    d. non-milked ewes
4. Facilities available ($m^2 animal^{-1}$)
5. Stable size ($m^2 animal^{-1}$)

6. Storage facilities size ($m^2 animal^{-1}$)
7. Procession of equipment such as milking machine, tractor, harvester generator
8. Water supply source
9. Number of silos available
10. Average volume of silos (in 1000kg)
11. Grazing pastures (in hectares):
    a. owned natural pastures,
    b. rented/ communal natural pastures,
    c. owned cultivated pastures,
    d. rented cultivated pastures
12. Characterization of natural pastures as rocky arid areas, heathland, forest, grassland
13. Natural pasture available ($ha10^{-1}$ $animal^{-1}$)
14. Cultivated pasture available ($ha10^{-1}$ $animal^{-1}$)
15. Concentrate feed bought annually (in 1000kg)
16. Forage hay harvested annually (in 1000kg)
17. Forage hay bought annually (in 1000kg)
18. Concentrate feed per ewe annually (kg ewe^{-1})
19. Forage hay per ewe annually (kg ewe^{-1})
20. % conception rate (number of ewes that lambed compared to the number of ewes exposed to the ram.)
21. Lambs per ewe per birth
22. Lamb mortality the first 48 hours
23. % lambs
24. Total Lamb mortality (% lambs)
25. Lactation period duration
26. Estimated annual ewe milk yield (lt $ewe^{-1} year^{-1}$)
    a. flock average
       (Annual milk production divided by the number of the milked ewes),
    b. as measured from individual animals the 1st year and
    c. as measured from individual animals the 2nd year
       (1st months measured milk yield *days milked within the month) + (2nd month measured milk yield *30) +….+ (last month measured milk yield * days milked within the month)
27. Number of preventive treatments for parasites (GIN specific, broad spectrum)
28. Number of vaccinations against

a. enterotoxaemia,
b. mastitis causing staphylococcus,
c. contagious agalactia and
d. tuberculosis

29. Number of treatments applied to lambs
    a. antibiotics
    b. vaccination against enterotoxaemia and
    c. antiparasitic drugs

The statistical analysis at the farm level also involved 3 multivariate redundancy analyses (RDA) using the following sets of variables:

A. Explanatory variables: i) individual dietary components, ii) environmental conditions, iii) floristic zone and iv) milking system. Response variables: a) milk yield, b) milk composition, c) SCC and CFU, d) content of major FA, and e) FA groups.
   Only the RDA plots for a) milk yield and d) content of major FA, and e) FA groups are presented since the explained variation for b) milk composition, c) SCC and CFU was minor.
B. Explanatory variables: i) individual dietary components, ii) environmental conditions, iii) lambing period and iv) milking system. Response variables: a) SCC and CFU, b) prevalence of the recorded diseases (as indicated by the farmers and the veterinarian of the flock) and c) average EPG count
C. Explanatory variables: i) SCC, ii) CFU, iii) prevalence of the recorded diseases (as indicated by the farmers and the veterinarian of the flock) and iv) average EPG count. Response variables: a) milk yield, b) milk composition, c) content of major FA, and d) FA groups. Though minor variation was explained and the RDA plot is not presented.

Furthermore, three different sets of analysis on the farm level were carried out using linear mixed effects models. In total 116 models were created, all using farm for the random effects.

1st set of analysis. Linear mixed-effects models were used to investigate if a) feeding and b) grazing regimes parameters are influenced by the different systems (semi-intensive, extensive) during the two-year study (2010 and 2011). The influence on feeding and grazing regimes parameters of sampling season (12 months) and year was also investigated.

As feeding regime we considered in our models (response variable):

1. Total concentrate and components of it (g $day^{-1}animal^{-1}$). The components that were investigated were:
    a. Maize
    b. Barley
    c. Soya
    d. Sunflower
    e. Wheat bran
2. Total conserved forage (g $day^{-1}animal^{-1}$)
3. Alfalfa hay (g $day^{-1}animal^{-1}$) and
4. Whole-crop oat (g $day^{-1}animal^{-1}$)

As grazing regime, we considered in our models (response variable):

1. Grazing on natural pastures (ha day^{-1})
2. Grazing on improved pastures (ha day^{-1})

In total eleven linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Sampling Season (SS) and (c) the Sampling Year (Y). In all models the three-way interactions between the fixed effects was investigated. The data from concentrate feed components is not presented since the found effects were like these of total concentrate.

2^{nd} set of analysis. Linear mixed-effects models were used to investigate if a) milk yield, b) milk composition (protein, fat, lactose, NFS content), c) milk somatic cell counts and colony forming units and d) milk fatty acid profile parameters are influenced by the different systems (semi-intensive, extensive) during the two-year study (2010 and 2011). The influence on these parameters of sampling season (January to June) and year was also investigated. Data from all the farms was available only for months January to June, because only these months all the farms had milk production.

The response variables used in the linear mixed effects models were:

1. Milk yield (l day^{-1} ewe^{-1})
2. The concentrations of milk fat, protein, lactose and non-fat solids (g $100ml^{-1}$ milk),
3. The concentrations (1000 cells $100ml^{-1)}$ of SCC (milk Somatic Cell Counts) and CFU (Colony Forming Units) which were transformed to lnSCC and lnCFU, respectively, in order to fulfil the normality assumption of linear mixed effects models.

4. The concentrations (g 100 g^{-1}total FA) of the 77 identified different fatty acids which were calculated as proportions and thus were arcsine transformed in order to follow the normal distribution and fulfil the requirements of the linear mixed effects models.

   Only the results of the ones "considered nutritionally important'' (***see sub-section 1.5.3***). C12:0, C14:0, C16:0, C18:0, C18:1 *trans11*, C18:1 *cis9*, C18:2*cis9.cis12*, C18:3*cis9.cis12.cis15*, CLAcis9.*trans11*, C20:5 n-3, C22:5 n-3, C22:6 n-3) are reported in the thesis.
5. The concentrations (g 100 g^{-1}total FA) of the SFA, MUFA, PUFA, omega-3 PUFA, omega-6 PUFA groups and the omega-6/omega-3 ratio.

In total nineteen linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Sampling Season (SS) and (c) the Sampling Year (Y). In all models the three-way interactions between the fixed effects was investigated.

3rd set of analysis. Linear mixed-effects models were used to investigate if the prevalence of the recorded diseases (as indicated by the farmers and the veterinarian of the flock) is influenced by the different management systems, namely semi-intensive and extensive, for the two different lambing periods (early and late) and for the two different sampling years (2010 and 2011). Specifically, the response variables, measured as percentage of animals within the flock, were:

1. Clinical mastitis,
2. Lameness,
3. Abortions (after 3month),
4. Pregnancy toxaemia,
5. Ruminal acidosis,
6. Bloat,
7. Diarrhoea,
8. Death associated with Clostridium infections,
9. Chronic diseases,
10. Contagious ecthyma,
11. Piroplasmosis,
12. Coenurosis,
13. Ectoparasites (ticks, flees, flies, etc.),
14. Casualties and

15. Percentage of obligatory replacements

Variables calculated as proportions (prevalence of diseases) were arcsine transformed. In total fifteen linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Lambing Periods (LP) and (c) the Sampling Year (Y). In all models the three-way interactions between the fixed effects was investigated.

2.9.2 Animal level statistical analysis

Multivariate redundancy analyses (RDA) was also applied to the animal level analysis for the following set of variables:

A. Explanatory variables: i) individual dietary components, ii) environmental conditions, iii) grazing zone altitude and iv) milking system. Response variables: a) milk yield, b) milk composition, c) content of major FA, and e) FA groups.
   Only the RDA plots for a) milk yield and d) content of major FA, and e) FA groups are presented since explained variation for b) milk composition, was minor.
B. Explanatory variables: i) individual dietary components, ii) environmental conditions, iii) use of anthelmintic drugs. Response variables: a) EPG of GIN, b) FAMACHA record, c) Body Conditions Score, d) EPG of Nematodirus and e) EPG of Monienzia.
   Little variation was explained (3,3% along axis 1) and the RDA plot is not presented in the results.
C. Explanatory variables: i) EPG of Nematodirus and ii) EPG of Nematodirus and iii) EPG of Monienzia. Response variables: a) milk yield and b) milk composition.
   Though minor variation was explained, and the RDA plot is not presented
   Little variation was explained (0,4% along axis 1) and the RDA plot is not presented in the results.

Furthermore, three different sets of analysis were carried out using linear mixed effects models. In total 128 models were created. Animal accounted for the random effects in each model.

1st set of analysis. Linear mixed-effects models were used to investigate if a) EPG counts of GIN, b) body condition score and c) FAMACHA parameters are influenced by the different systems (semi-intensive, extensive) during the two-year study (2010 and 2011). The influence on these parameters of sampling season (12 months or 4 months when only lactation was examined) and lambing period (early lambing and late lambing) was also investigated.

The response variables used in the linear mixed effects models were:

1. Eggs of gastrointestinal nematodes per gram of faeces
2. Body Condition Score and
3. FAMACHA

In total six linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Sampling Season (SS, either throughout the year or only during the 4 months that both lambing periods were milked) and (c) the Lambing Period (LP). In all models the three-way interactions between the fixed effects was investigated.

The results for FAMACHA record are not presented since there were no statistically significant main effects of management system and lambing period and through season FAMACHA score had little variation (from 1.3 to 1.6)

2nd set of analysis. Linear mixed-effects models were used to investigate, for the multiparous ewes only, if a) milk yield, b) milk composition (protein, fat, lactose, NFS content), c) milk somatic cell counts, d) colony forming units, e) feeding and f) grazing regimes parameters are influenced by the different systems (semi-intensive, extensive) during milking period (January to June). The influence of the parameter of sampling season (January to June) was also investigated. Data from all the farms was available only for months January to June, because only these months the ewes from all twenty farms were weaned and had milk production. The analysis was carried out separately for the two different sampling years.

The response variables used in the linear mixed effects models were:

1. Milk yield (l day^{-1} ewe-^{1})
2. The concentrations of milk fat, protein, lactose and non-fat solids (g $100ml^{-1}$ milk),
3. The concentrations (1000 cells $100ml^{-1)}$ of SCC (milk Somatic Cell Counts) and CFU (Colony Forming Units) which were transformed to lnSCC and lnCFU, respectively, in order to fulfil the normality assumption of linear mixed effects models.

As feeding regime we considered in our models (response variable):

4. Total concentrate and components of it (g $day^{-1}animal^{-1}$). The components that were investigated were:
    a. Maize
    b. Barley
    c. Soya
    d. Sunflower

e. Wheat bran

5. Total conserved forage (g day^{-1} $animal^{-1}$)
6. Alfalafa hay (g day^{-1} $animal^{-1}$) and
7. Whole-crop oat (g day^{-1} $animal^{-1}$)

As grazing regime, we considered in our models (response variable):

8. Grazing on natural pastures (ha day^{-1})
9. Grazing on improved pastures (ha day^{-1})

In total eighteen linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS) and (b) the Sampling Season (SS). In all models the two-way interactions between the fixed effects was investigated. The data from concentrate feed components is not presented since the found effects were like these of total concentrate.

3rd set of analysis. Linear mixed-effects models were used to investigate for the two different lambing periods (early and late) if a) milk yield, b) milk composition (protein, fat, lactose, NFS content), c) milk somatic cell counts, d) colony forming units e) feeding regimes and f) grazing regimes are influenced by the different systems (semi-intensive, extensive) during mid-milking period (March, April, May and June). The influence on these parameters of sampling season (March to June) and lambing period were also investigated. Data from all the animals was available only for months March to June, because only these months' ewes of both lambing periods were weaned and had milk production. The analysis was carried out separately for the two different sampling years.

The response variables used in the linear mixed effects models were:

1. Milk yield (l day^{-1} ewe^{-1})
2. The concentrations of milk fat, protein, lactose and non-fat solids (g $100ml^{-1}$ milk),
3. The concentrations (1000 cells $100ml^{-1)}$ of SCC (milk Somatic Cell Counts) and CFU (Colony Forming Units) which were transformed to lnSCC and lnCFU, respectively, in order to fulfil the normality assumption of linear mixed effects models.

As feeding regime we considered in our models (response variable):

4. Total concentrate and components of it (g $day^{-1}animal^{-1}$). The components that were investigated were:
    a. Maize
    b. Barley
    c. Soya

d. Sunflower
e. Wheat bran

5. Total conserved forage (g day^{-1} $animal^{-1}$)
6. Alfalafa hay (g day^{-1} $animal^{-1}$) and
7. Whole-crop oat (g day^{-1} $animal^{-1}$)

As grazing regime we considered in our models (response variable):

8. Grazing on natural pastures (ha day^{-1})
9. Grazing on improved pastures (ha day^{-1})

In total eighteen linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Sampling Season (SS) and (c) lambing period (LP). In all models the three-way interactions between the fixed effects was investigated. The data from concentrate feed components is not presented since the found effects were similar to these of total concentrate.

4^{th} set of analysis Linear mixed-effects models were used to investigate for the two different lambing periods (early and late) if milk fatty acid profile parameters are influenced by the different systems (semi-intensive, extensive) during mid-milking period. The two months that were compared (March and May) were selected based on the criteria mentioned in **section 2.5.** The influence on the aforementioned parameters of sampling season (March, May) and lambing period (early and late) was also investigated.

The response variables used in the linear mixed effects models were:

1. The concentrations (g 100 g^{-1}total FA) of the 77 identified different fatty acids which were calculated as proportions and thus were arcsine transformed in order to follow the normal distribution and fulfil the requirements of the linear mixed effects models.
   Only the results of the ones "considered nutritionally important'' see sub-section **1.5.3.** (C12:0, C14:0, C16:0, C18:0, C18:1 *trans11*, C18:1 *cis9*, C18: 2cis9.cis*12*, C18:3*cis9.cis12.cis15*, CLAcis9.*trans11*, C20:5 n-3, C22:5 n-3, C22:6 n-3) are reported in the thesis.
2. The concentrations (g 100 g^{-1}total FA) of the SFA, MUFA, PUFA, omega-3 PUFA, omega-6 PUFA groups and the omega-6/omega-3 ratio.

In total eighty-three linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Sampling

Season (SS) and (c) the Lambing period (LP). In all models the three-way interactions between the fixed effects was investigated.

5th set of analysis. Linear mixed-effects models were used to investigate for the two different lambing periods (early and late) if a) the % percentage of ewes with SCC> 500,000 (indication of sub-clinical mastitis), b) the % percentage of the examined samples in which GRAM positive pathogens were detected, c) the % percentage of the examined samples in which GRAM negative pathogens d) the % percentage of the examined samples in which no pathogens were detected and e) the % percentage of the examined samples in which both GRAM negative and positive pathogens were detected are influenced by the different systems (semi-intensive, extensive) during the two-year study (2010 and 2011). The aforementioned five parameters were used as response variables.

In total five linear mixed effects models were created, one for each of the response variables, having as fixed effects, (a) the Managements System (MS), (b) the Sampling Season (SS) and (c) Lambing period (LP). In all models the three-way interactions between the fixed effects was investigated.

Chapter 3. Results and Discussion

3.1 Characteristics of the two different management systems and background climatic conditions

This section presents the characteristics of the two management systems, as recorded during the two-year study, reports the management practices applied and finally presents the background climatic conditions during the two-year study.

3.1.1 Flock characteristics

Farms under the two management systems had similar number of ewes (Table 3.1) and similar portions of multiparous and primiparous ewes (Table 3.1). However, in the extensive farms a significantly higher portion of the ewes were categorized as non-milked, estimated after weaning (Table 3.1). This group consisted of ewes that were sick (e.g. progressive pneumonia) or not lactating, mainly due to miscarriage or mastitis.

Although the ewes per ram ratio was similar for the two management systems, the extensive farms tended to have a higher ratio since the practice of borrowing rams was still being applied in some farms and there was also a significant number of castrated rams in the EX flocks, kept purely for guiding the flock.

More than half of the farms in the study had mixed flocks, with the number of goats within the flock varying greatly (Table 3.1). When the number of goats was high (around 20 goats) they were kept as a separate flock while in the farms with fewer, the sheep and goats were kept together.

parameter assessed	semi-intensive *(n=10)*	extensive *(n=10)*	t-test (P-values)
Number of animals			
total ewes	494.6 ± 47.3	416 ± 201.6	ns
multiparous ewes	341.9 ± 35.1	275 ± 39.9	ns
late lambing multiparous ewes	10.1 ±1.0	8.9 ±1.3	ns
primiparous ewes	112.7 ± 10.9	91.2 ± 13.4	ns
non-milked ewes	6.2 ± 0.8	17.1 ±4.5	*
number of rams	24.7 ±3.0	18.3 ±3.4	ns
ewes per ram	20.8 ±1.1	25.9 ±4.1	ns
Number of farms with mixed flocks	7	5	ns
Number of animals			
total number of goats	46.8 ±17.8	47.4 ±23.8	ns
multiparous goats	36.7 ±16.3	24.2 ±14.7	ns
primiparous goats	21.9 ±4.5	10.6 ±5.1	ns
number of billy goats	3.8 ±0.8	9.2 ±7.7	ns
female goats per billy goats	19.4 ±2.8	33.7 ±6.1	*P*

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

Table 3.1. Flock characteristics (means ±standard errors) of the two management systems.

parameter assessed	semi-intensive *(n=10)*	extensive *(n=10)*	t-test (P-values)
% percentage			
multiparous ewes	70.2 ± 9.3	67.3 ±8.2	ns
late lambing multiparous ewes	8.97 ±0.8	9.87 ±1.0	ns
primiparous ewes	23.4 ± 1.9	22.4 ± 1.3	ns
non-milked ewes	1.3 ±0.2	3.9 ±0.6	***

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

Table 3.2. Allocation of ewes within flock for the two management systems (means ± standard errors).

3.1.2 Facilities and pastures

The semi-intensive farms had larger housing facilities with almost double the space available per ewe, as well as more space available for storage (feed and equipment included) and were more likely to invest in equipment (Figure 3-1).

Moreover, the facilities in the semi-intensive farms were in a close proximity to the majority of the grazing lands, while in the extensive farms the facilities were close to the pastures the animals grazed during winter. At the summer grazing areas, which were at higher altitudes, there were only simple structures as paddocks or small barns. The semi-intensive farmers owned more cultivated pastures compared with the extensive farmers which relayed more on natural pastures, owned, rented or communal for grazing (Table 3.4). While both farms utilized natural pastures in the case of the semi-intensive farms these were mainly characterized as grasslands while in the case of the extensive systems these were characterized as rocky arid areas or heathlands (Figure 3-3).

Almost all semi-intensive farmers had different paddocks or areas within their facilities for lambed ewes and sick animals while less than half of extensive farmers had similar practices (Figure 3-4).

parameter assessed	semi-intensive *(n=10)*	extensive *(n=10)*	t-test (P-values)
facilities available (m^2animal^{-1})	2.93 ±0.32	1.74 ±0.28	
stable size (m^2animal^{-1})	2.42 ±0.27	1.51 ±0.25	*
storage facilities size (m^2animal^{-1})	0.51 ±0.1	0.22 ±0.05	*
number of silos available	2.2 ±0.4	1.2 ±0.2	*
average volume of silos (1000kg)	20.6 ±3.4	10.4 ±2.2	*

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

Table 3.3. Facilities size for the two management systems (means ± standard errors).

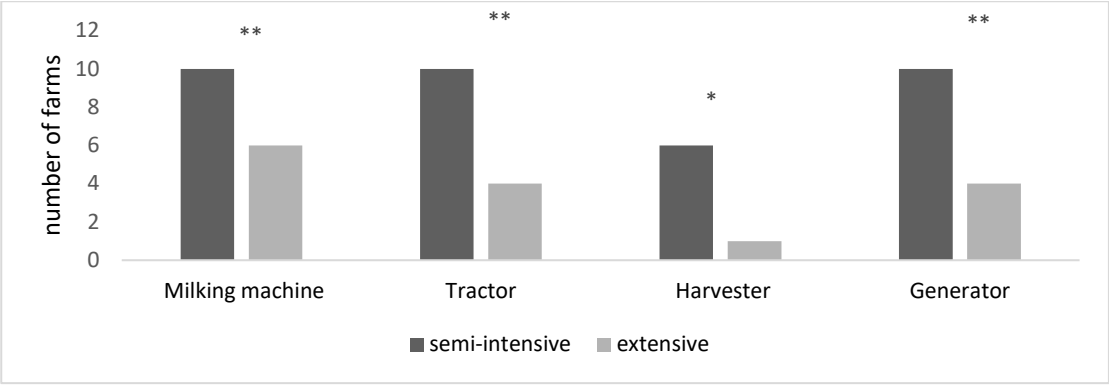

Figure 3-1. Availability of equipment for the two management systems.

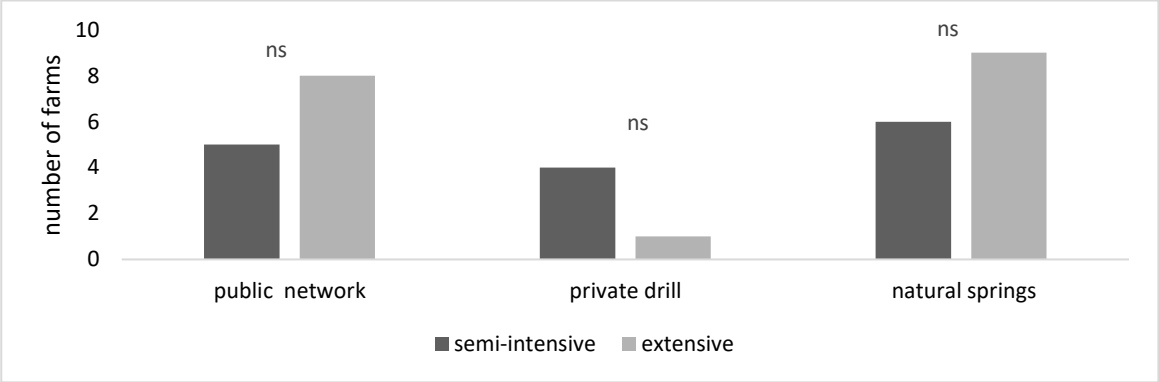

Figure 3-2. Water supply source.

parameter assessed	semi-intensive *(n=10)*	extensive *(n=10)*	t-test (P-values)
grazing pastures (in hectares)			
owned natural pastures	31.8 ±6.4	81.3 ±12.4	***
rented/ communal natural pastures	0.4 ±0.2	38.9 ±5.8	***
owned cultivated pastures	23.6 ±4.7	3.7 ±1.6	***
rented cultivated pastures	3.8 ±3.0	0.3 ±0.3	ns
natural pasture available ($ha10^{-1}$ $animal^{-1}$)	0.96 ±0.01	7.43 ±0.33	***
cultivated pasture available ($ha10^{-1}$ $animal^{-1}$)	0.62 ±0.01	0.08 ±0.00	***

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

Table 3.4. Grazing land area size (means ± standard errors).

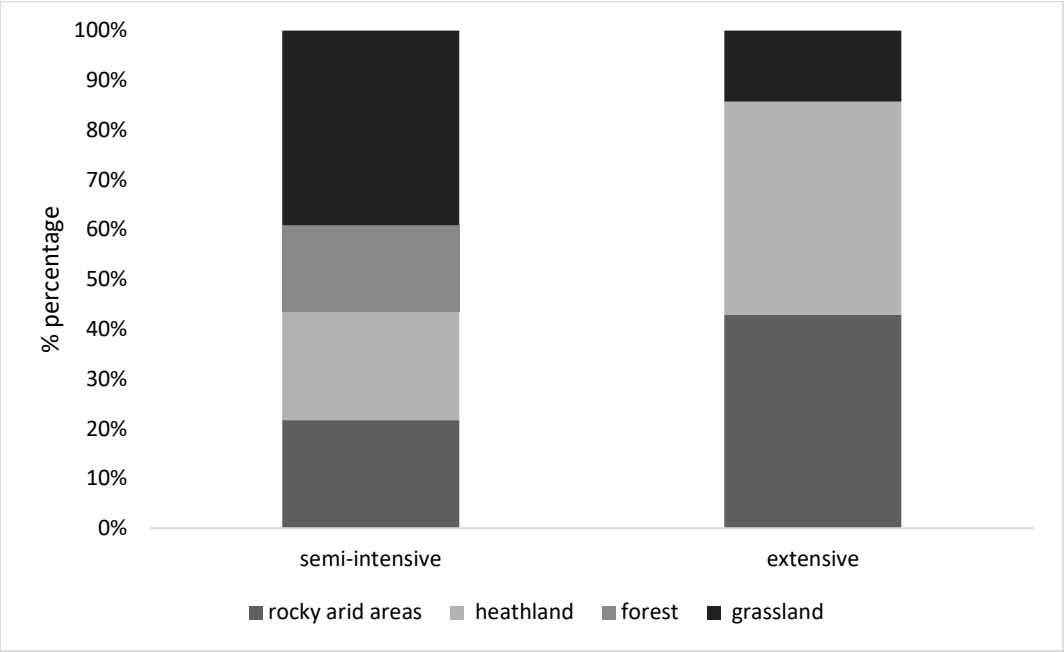

Figure 3-3. Characterization of natural pastures.

3.1.3 Management practices – feeding and grazing regimes

On annual base the semi-intensive farmers would feed more concentrate feed (~1.6 times more) and hay to their ewes compared with the extensive farmers, though the difference is statistically significant only for the concentrate feed (Table 3.5). The seasonal changes in diet composition for flocks under the two management systems (averaged over the 2 years) are presented in (Figure 3-5). Both systems fed similar amounts of concentrate feed during winter (Figure 3-5) but later the semi-intensive farmers constantly provided more concentrate to their ewes compared with the extensive farmers.

parameter assessed	semi-intensive *(n=10)*	extensive *(n=10)*	t-test (P-values)
concentrate feed bought annually (in 1000kg)	148 ±21	28 ±8.8	**
forage hay harvested annually (in 1000kg)	24.0 ±6.7	0.3 ±0.2	**
forage hay bought annually (in 1000kg)	19.7 ±7.7	24.1 ±4.4	ns
concentrate feed per ewe annually (kg ewe^{-1})	270 ±23	162 ±18	**
forage hay per ewe annually (kg ewe^{-1})	82 ±6	60 ±10	P

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

Table 3.5. Feeding regimes average values of flocks for the two systems (means ± standard errors).

In the case of forage, both farmers provided most hay to the ewes during the lambing period. That was in October and November for the semi-intensive farms and in December and January for the extensive farms (Figure 3-5). During lactation, the SI farmers depended on grazing cultivated lands and on home-produced whole crop oat, supplementing with bought alfalfa hay when needed. In contrast, the EX farmers utilized natural pastures all year around and, since cultivated land is minimal, they fed more hay (mainly bought alfalfa) to compliment the needs of the ewes (Figure 3-5 and Table A.4.3, appendix). After spring and until the subsequent lambing, both systems relied almost solely to the natural pastures for grazing.

Between the two lambing groups the collected data was analysed only for the milking period when both groups co-existed within the same flock and the results for this period are presented in Table 3.6. The main effect of lambing period on concentrate intake, based on LME models, was statistically significant only during the second year of the study, when 10% less concentrate was fed to the older ewes of the early lambing period (Table 3.6).

Prior to lambing, as reported by the farmers, primiparous ewes were kept as a separate flock in all farms. In detail, after weaning the lambs were kept in the main facilities and were fed with concentrates and hay (1 to 1 concentrate to hay ratio, starting from 200 gr mixed ratio ending to around 1,000 g mixed when they are 20kg live weight). When the replacement lambs were selected, from the lambs born during early lambing period, they would be transferred in separate pastures where were kept with the rams until a month prior to lambing. During lambing, the yearlings would be given concentrate and hay, around 10% less compared with the amounts provided for the multiparous ewes, mainly due to differences in body weight.

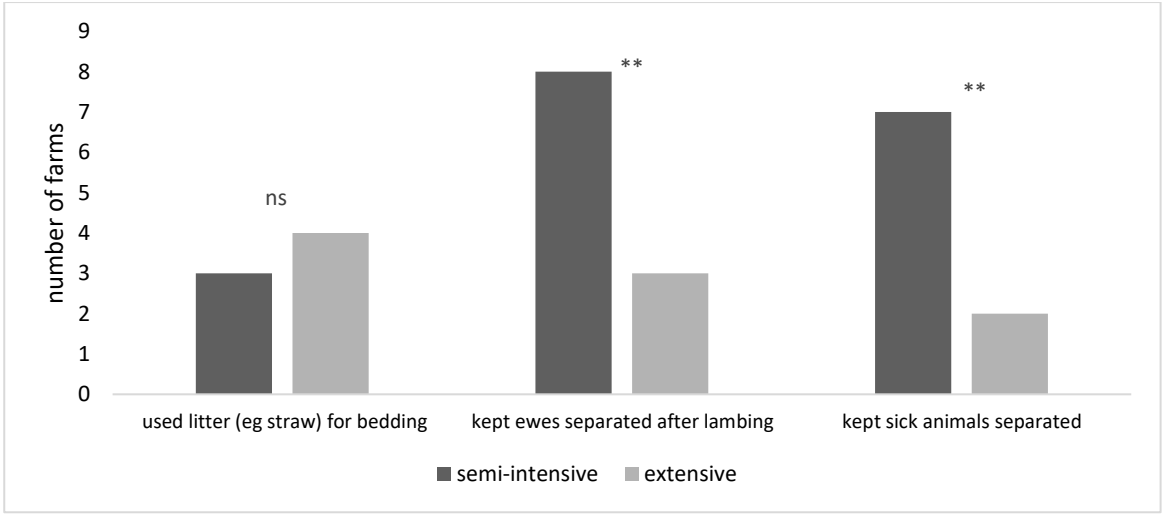

Figure 3-4. Animal handling practices for the two management systems

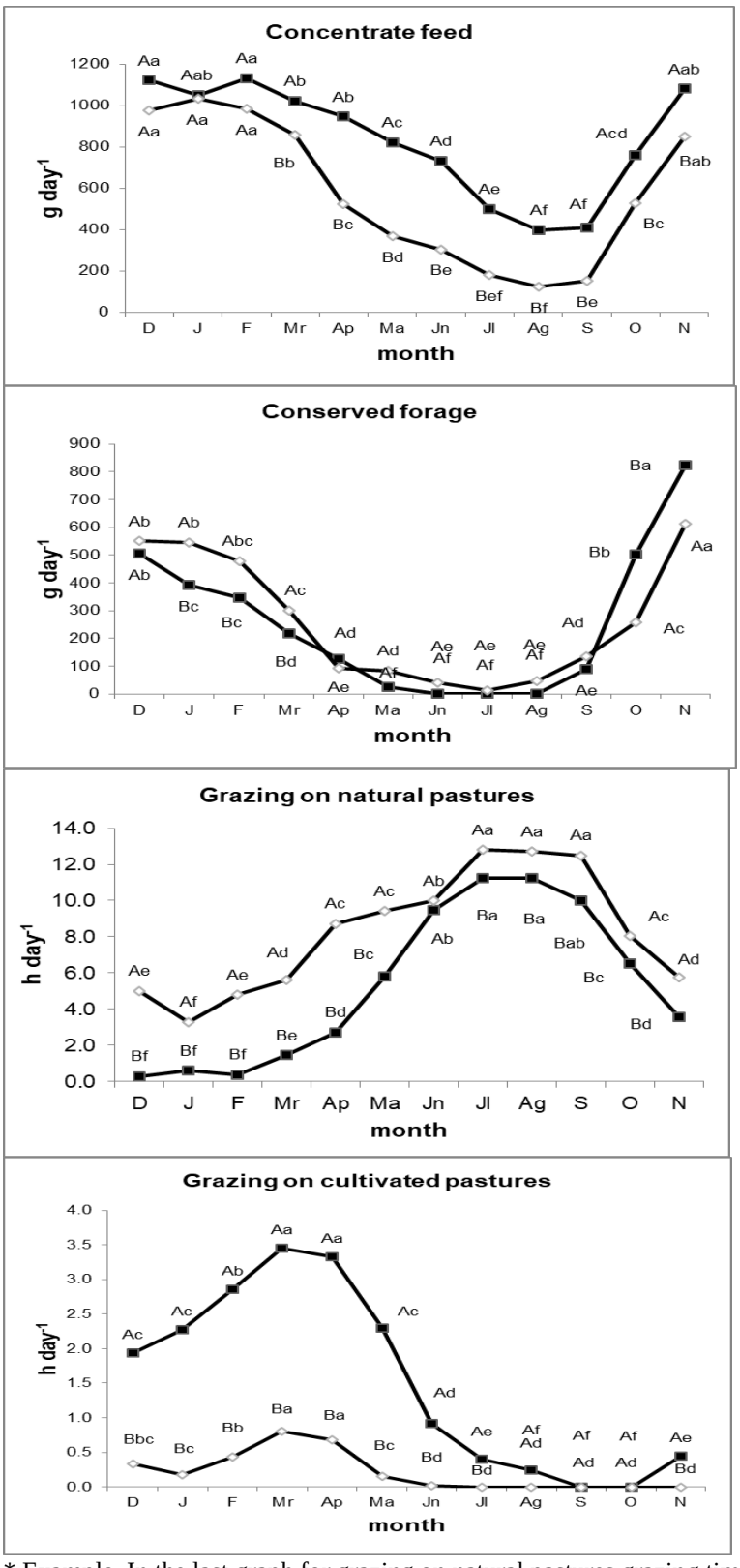

Figure 3-5. Interaction means for feeding and grazing regimes for different management systems and sampling season.

Semi-intensive management system is represented by (♦) and extensive management system by (◊).J:January, F:February, Mr: March, Ap: April, Ma: May, Jn: June, Jl: July, Ag: August, S: September, O:October, N:November, D: December Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase letters represent statistically significant differences between months within the same system (P-value<0.05).*

* Example. In the last graph for grazing on natural pastures grazing time in Decemeber (D) was statistically significanly higher for the extensive flocks (capitalized letter A) compared to the semi-intensive flocks (capitalized letter B). Contrary in June (Jn) both flocks spent similar times grazing natural pastures (Capitallized letter A for both systems). Regarding within system variations, for the extensive farms the ewes spent similar amount of time in July (Jl), August (Ag) and Septmeber (S) grazing in natural pastures (for all average values there is a lowercase 'a'). Afterwards, the time spent grzazing decreased from September (lowercase letter a), to Ocotober (lowercase letter c) and from October to Novemeber (lowecase letter d).

parameter assessed	management system (MS)		lamping period (LP)		sampling season (SS)				LME (P-values)						
									Main effects				Interactions		
	semi-intensive *(n=10)*	extensive *(n=10)*	early *(n=20)*	late *(n=20)*	March	April	May	June	MS	LP	SM	MS x SS	MS x LP	SS x LP	MS x SS x LP
Sampling Year 1															
feed intake (g animal^{-1}day^{-1})															
total concentrate	946 ±11	620 ±14	768 ±13	861 ±13	1067 ±15^{a}	872 ±19^{b}	771 ±23^{c}	708 ±19^{d}	***	ns	***	***[1]	ns	ns	ns
conserved forage	127 ±7	176 ±9	147 ±8	156 ±8	388 ±13^{a}	196 ±11^{b}	107 ±14^{c}	42 ±6^{d}	***	ns	***	***[1]	ns	ns	*
grazing (h day^{-1})															
cultivated pastures	2.2 ±0.09	0.3 ±0.0	1.3 ±0.1	1.3 ±0.1	2.2 ±0.1^{a}	1.8 ±0.1^{b}	1.4 ±0.1^{c}	0.8 ±0.1^{d}	***	ns	***	***[1]	ns	ns	ns
natural pastures	5.2 ±0.2	8.7±0.1	7.0 ±0.2	7.0 ±0.1	3.0 ±0.2^{d}	5.1 ±0.2^{c}	7.0 ±0.2^{b}	8.9 ±0.2^{a}	***	ns	***	***[1]	ns	ns	ns
Sampling Year 2															
feed intake (g animal^{-1} day^{-1})															
total concentrate	666 ±11	310 ±11	468 ±12	515 ±13	809 ±17^{a}	598 ±20^{b}	415 ±18^{c}	343 ±17^{d}	***	***	***	***[1]	*[2]	P	ns
conserved forage	21 ±2	41 ±4	28 ±3	33 ±3	129 ±8^{a}	20 ±4^{b}	0 ±0^{c}	0 ±0^{c}	***	ns	***	***[1]	ns	ns	ns
grazing (h day^{-1})															
cultivated pastures	2.0 ±0.1	0.4 ±0.0	1.2 ±0.1	1.2 ±0.1	2.1 ±0.1^{a}	2.2 ±0.1^{a}	1.1 ±0.1^{b}	0.2 ±0.02^{c}	***	ns	***	***[1]	ns	ns	ns
natural pastures	6.9 ±0.1	9.3 ±0.1	8.1 ±0.1	8.1 ±0.1	4.1 ±0.1^{d}	6.3 ±0.2^{c}	8.3 ±0.2^{b}	10.5 ±0.1^{a}	***	ns	***	***[1]	ns	ns	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (non significant).
[a-d] Means within a raw with different superscripts letter are significantly different according to Tukey's honestly significant difference test (*P*-values < 0.05).
[1] see **Figure A3** for interaction means/SE. [2] see **Table A5** for interaction means/ standard errors.

Table 3.6. Grazing and feeding regimes (means ± standard errors) used in different management systems (production intensity), lambing periods and months both sampling years.

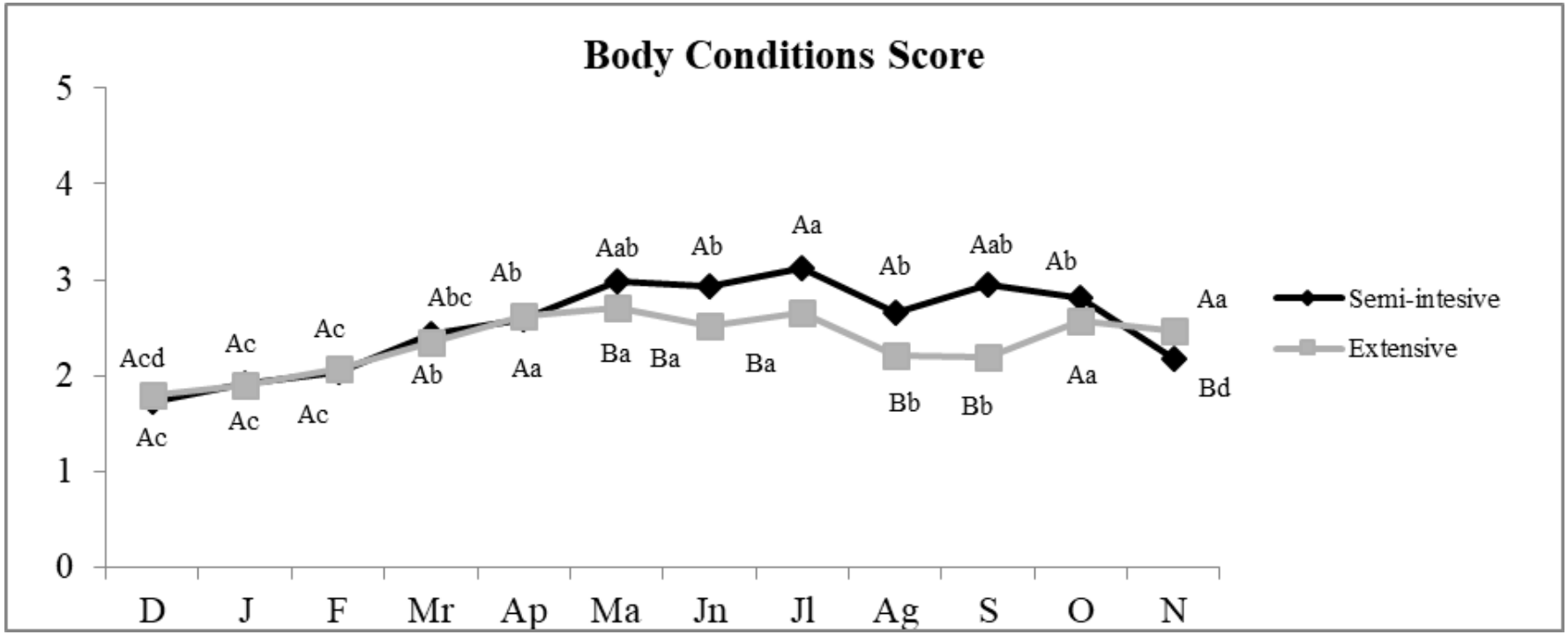

Figure 3-6. Interaction means for Body Condition Score (BCS) for different management systems and sampling season.

Semi-intensive management system is represented by (♦) and extensive management system by (■).J:January, F:February, Mr:March, Ap:April, Ma:May, Jn:June, Jl:July, Ag:August, S:September, O:October, N:November, D: December. Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase letters represent statistically significant differences between months within the same system (P-value<0.05).

3.1.4 Body condition score

As an index of nutritional and general health status Body Condition Score was recorded each month for the 20 animals selected in each farm. The seasonal changes in BCS for animals under the two management systems (averaged over the 2 years) are presented in (Figure 3-6). In both systems ewes' body condition score declined in August and after lambing while afterwards it steadily increased during milking reaching it highest values at the end of spring (Figure 3-6).

In order to focus on feeding and grazing regimes effect during lactation, the main effects of management system and sampling season on BCS were analysed in two sets. In the first only the late lambing period ewes were included, and the sampling season was narrowed from January to June (ewes from both systems are milked on daily basis) and the 2nd included ewes from both lambing periods and the sampling season was narrowed to March to June (ewes from both systems and both lambing periods are milked on daily basis). Based on the results from the LME models, the main effects of management system and sampling season on BCS were statistically significant in both sets of analysis, both years of the study (Table 3.7). In the second set of analysis the main effect of lambing period on BCS was also statistically significant. The ewes of the SI systems had higher average BCS values in both years compared with the EX ewes, in both analyses. Moreover, the older, EL ewes had higher BCS compared with the LL ewes, in both years. Furthermore, BCS increased in both years as lactation progressed until May and while no change was found in June in year 1, the 2nd year a drop was observed.

Furthermore, the interaction effect between season and management system for BCS was also statistically significant (Table 3.7). The 1st year SI ewes improved in condition in May but not the EX ewes. In year 2 BCS increased from March to April for the SI ewes while EX ewes increased in BCS later, in May (Figure A.4-5, appendix).

	only early lambing group (data from January to June)		both lambing groups (data from March to June)	
	1st year *(n=200)*	2nd year *(n=200)*	1st year *(n=400)*	2nd year *(n=400)*
management system				
semi-intensive	2.39 ±0.02	2.37 ±0.02	2.51 ±0.03	2.50 ±0.03
extensive	2.26 ±0.02	2.25 ±0.02	2.30 ±0.02	2.29 ±0.03
lamping period				
early	-	-	2.68 ±0.03	2.79 ±0.03
late	-	-	2.13 ±0.02	2.01 ±0.02
Sampling Months				
January	1.91 ±0.03[d]	2.43 ±0.05[d]	-	-
February	2.05 ±0.04[d]	2.36 ±0.05[d]	-	-
March	2.39 ±0.05[c]	2.62 ±0.06 [c]	2.15 ±0.03[c]	2.27 ±0.04[c]
April	2.61 ±0.05[b]	2.88 ±0.07 [b]	2.32 ±0.03[b]	2.46 ±0.05[b]
May	2.83±0.05[a]	3.05 ±0.07 [a]	2.52 ±0.04[a]	2.62 ±0.05[a]
June	2.73±0.06 [a]	2.72 ±0.06[c]	2.47 ±0.04[a]	2.30 ±0.04[c]
LME (P-values)				
Main effects				
management systems (MS)	***	*	***	***
lamping period (LP)	-	-	***	***
sampling season (SS)	***	***	***	***
Interactions				
MS x SS	***[1]	***[1]	ns	ns
MS x LP	-	-	P	***[2]
LP x SS	-	-	ns	ns
Ms x LP x SS	-	-	ns	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant); -, not investigated

[a-d] Means within a raw with different superscripts letter are significantly different according to Tukey's honestly significant difference test (*P*-values < 0.05).

[1] see **Table A.5.5** for interaction means/SE. [2] see **Table A.5.6** for interaction means/SE.

Table 3.7. Effect of management systems (production intensity), sampling season and lambing period on body condition score (means ± standard errors).

3.1.5 Veterinary regimes

In both management systems there was nominal use of antibiotics and antiparasitic drugs. Necessary proactive vaccinations against Enterotoxaemia were applied in both systems, at least once a year, before lambing, with some farmers (5 SI farms and 2 EX farms) applying a booster before mating. Other vaccinations (Figure 3-7) were non-systematic, selected by individual farmers to counter regional outbreaks or threats.

Mastitis prevention treatments in both systems were specific to each farm's background and only 3 farms (2 SI farms and 1 EX farms) used vaccines against clinical mastitis once over the two years. No dry period antibiotics were used on any farm. Most farms used systemic antibiotics to treat mastitis incidences and 4 farms (3 SI farms and 1 EX farms) would administrate a combination of intramammary and intramuscular antibiotics (Figure 3-7).

The farmers used anthelmintic drugs mainly once a year, before lambing to deal with the periparturient rise. All SI farmers changed the drug of choice each year and 6 of them used a broad spectrum antiparasitic drug (mainly *Ivermectin*) at least once every two years. This practice was applied by only 1 EX farmer. The most common substances used were *Albendazole* and *Fenbendazole.* Sprayed and pour on drugs against ectoparasites were applied systematically by the farmers before dry period (Figure 3-7).

All farmers vaccinated the weaned lambs against Enterotoxaemia and treated them with oral antiparasitic drugs. Additionally, more than half farmers in both systems preventively treated the new-born lambs with antibiotics (Figure 3-7).

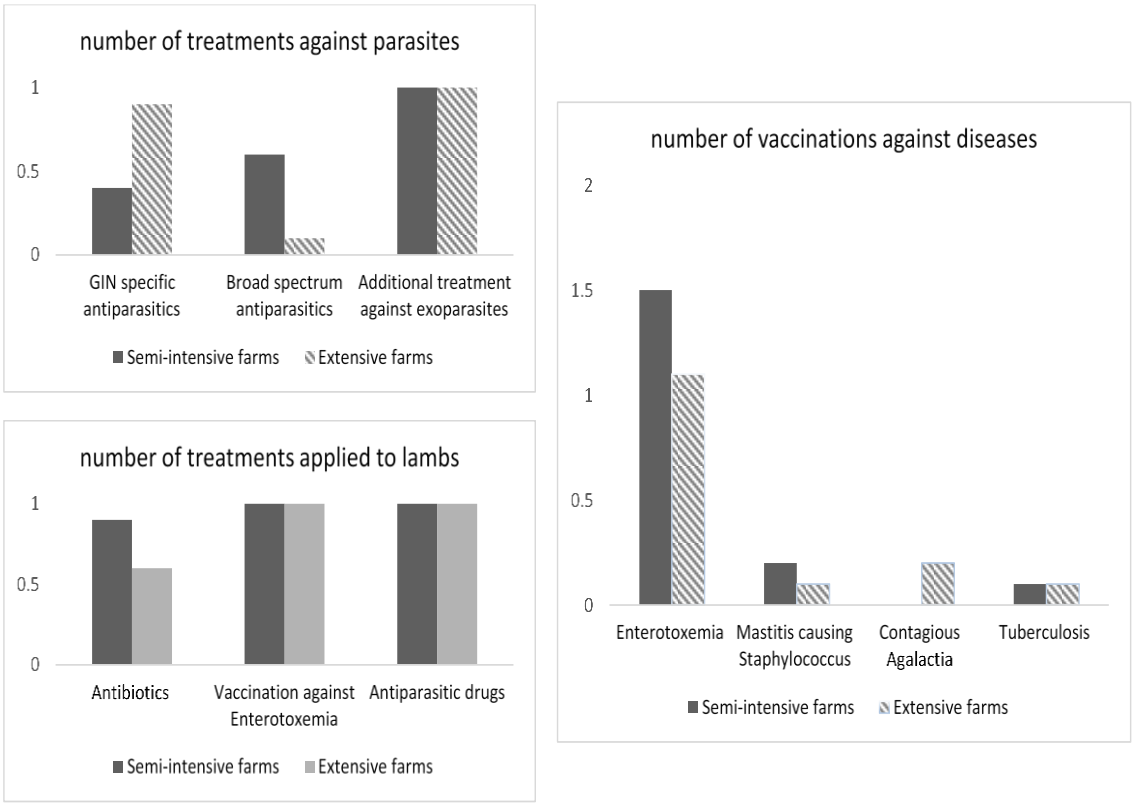

Figure 3-7. Average number of a) vaccinations for ewes (against Enterotoxaemia and other Clostridium infections, Mastitis causing Staphylococcus, Contagious Agalactia and Tuberculosis), b) treatments applied against endo-, and ectoparasites and c) treatments and vaccinations applied to the new born lambs by the farmers of the two different management system.

3.1.6 Production characteristics

Higher conception rates were recorded for the semi-intensive flocks compared with the extensive flocks although in both systems the percentage was high. In both systems ewes had on average approximately 1.2 lambs and while lamb mortality within the first 48 hours was

higher for the extensive flocks, overall lamb mortality was similar for the two systems (Table 3.8). Concerning milk production, the ewes in the semi-intensive systems were milked for longer (about a month more) and produced around 100 kg more milk on annual bases. This difference was similar when annual milk yield was estimated using flock average and when annual milk yield was estimated using the milk yield measurement from the individual animals (Table 3.8).

parameter assessed	semi-intensive (*n=10)*	extensive *(n=10)*	t-test (P-values)
% conception rate	98.7 ±0.46	96.1 ±1.8	***
lambs per ewe per birth	1.19 ±0.11	1.24 ±0.05	ns
lamb mortality the first 48 hours (% lambs)	1.71 ±0.39	2.59 ±0.72	***
total Lamb mortality (% lambs)	9.03 ±2.72	10.22 ±2.50	ns
lactation period duration	231 ±24.7	192±15.5	***
estimated annual ewe milk yield (lt $ewe^{-1}year^{-1}$)			
flock average*	192.7 ±29.9	98.0 ±18.9	***
as measured from individual animals the 1st year**	245.3 ±22.7	127.5 ±20.4	***
as measured from individual animals the 2nd year**	237.3 ±28.1	116.8 ±24.7	***

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

* annual milk production to the number of the milked ewes

** [(1st month measured milk yield *days milked within the month) + (2nd month measured milk yield *30) +…. +(last month measured milk yield * days milked within the month)

Table 3.8. Production characteristics of flocks for the two management systems. (means ± standard errors)

3.1.7 Background climatic conditions the two years of the study

The average monthly temperatures and rainfall for the two production years are shown in Table 3.9. The first year was characterized as drier/more arid with rain occurring mostly during December and February and relatively high temperatures during January and March. In the second year, average temperatures in the winter-months (December to March) were lower; it was wetter in spring especially during January and March, but summer temperatures were higher than in year 1.

parameter assessed [1]	1st sampling year (2009-2010)		2nd sampling year (2010 -2011)	
	temperature (°C)	rainfall (mm)	temperature (°C)	rainfall (mm)
December	16.2 ±2.0	4.1 ±1.4	16.2 ±3.6	2.7 ±1.8
January	15.7 ±2.9	2.1 ±1.4	12.9 ±4.1	3.8 ±1.6
February	11.2 ±2.7	5.7 ±3.4	11.2 ±2.5	5.0 ±1.7
March	14.7 ±2.0	1.0 ±0.8	12.2 ±2.8	4.7 ±2.9
April	14.1 ±2.2	0.4 ±0.5	14.6 ±2.6	1.7 ±0.7
May	16.3 ±2.0	0.3 ±0.1	18.6 ±2.7	1.3 ±0.7
June	17.3 ±5.2	0.9 ±0.9	22.0 ±2.7	0.8 ±0.6
July	23.7 ±2.7	0.0 ±0.0	24.4 ±1.8	1.4 ±5.7
August	25.2 ±2.5	0.0 ±0.0	26.0 ±1.2	0.0 ±0.0
September	25.5 ±1.9	0.0 ±0.0	23.6 ±2.8	0.8 ±1.4
October	22.1 ±3.1	1.2 ±2.1	13.7 ±3.4	4.2 ±2.4
November	17.6 ±2.3	4.7 ±2.1		

[1]Average values ± standard deviation from onsite records

Table 3.9. Average monthly temperature and rainfall during the study

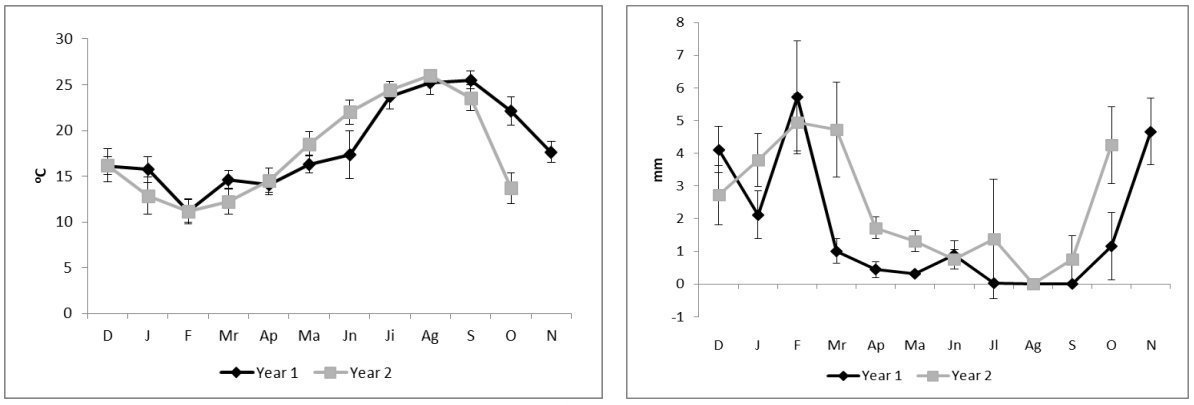

Figure 3-8. Average monthly temperatures (°C) and rainfall (mm) the two years the study lasted.

3.1.8 Discussion

As expected, the results from comparing the characteristics of the two management systems were in accordance to the criteria used to classify them (**section 2.4**). The semi-intensive farms had more and larger infrastructures for animal handling and feed storage and were more likely to invest in machinery, compared with the extensive farms. Furthermore, in

the semi-intensive systems higher quality pastures were utilized, and stocking rates were higher. Moreover, a higher portion of these lands were owned by the semi-intensive farmers where the extensive farmers relayed more on communal or rented pastures. It should also be noted that in our study, there was no difference in flock size between the two management systems and similar replacement rates were recorded. This is of no surprise since as it has been reported that subsidies and labor demands are defying factors determining flock size (Hadjigeorgiou *et al.*, 2002).

Regarding nutrition, the semi-intensive ewes were overall fed more concentrates over a year, when compared with the extensive ewes, and the difference was also reflected to the monthly concentrate intake with the exception of winter months (December to February). At that time most vegetation is still in dormant and not suitable for grazing while the low temperatures and frequent rainfalls (section 3.1.7) make grazing even more difficult. Simultaneously ewes have higher demands in energy and nutrients due to milk production, thus supplementation with concentrates are essential to maintain health in both systems (Tzanidakis *et al.*, 2014). Conserved forages were fed *ad libitum* in both systems during lambing but afterwards the EX farmers provided more hay (mainly bought alfalfa) compared with the SI farmers mainly due to low quality pasture available for grazing. These practices are similar to those observed for other regions in the Mediterranean basin such as the Pyrenees and Castilla-La Mancha region in Spain, Sardinia, Sicily and Tuscany in Italy or Peloponnesus and Epirus in Greece (Parente, 2011). The differences between the two management systems in nutrition were also reflected in BCS. The stable provision of concentrate in the semi-intensive farms during lactation along with grazing of higher quality pastures resulted in ewes having a higher BCS at the end of lactation and during dry period (**sub-section 3.1.4**).

Contrary to expectations and to what has been reported in other production systems (Lean *et al.*, 2008; Ridler, 2008) there were no differences in veterinary regimes. However, when the mean treatment rates were examined, most farmers in both systems relied in what were "perceived" by them and other farmers as appropriate treatment (rather than what was actually necessary) and only sought guidance from a veterinarian when problems occurred.

Regarding productivity, as expected the semi-intensive flocks had higher annual milk yields as a result of intensification of production (Morand-Fehr *et al.*, 2007), however the size of the impact was higher than expected based on previous studies in the area (Stefanakis *et al.*, 2007; Volanis *et al.*, 2007). With milking period being similar to those of previous studies, annual harvested milk for the extensive farmers was around 100 kg/ewe per year,

similar to what has been reported previously (Volanis *et al.*, 2007) and close to those described for the breed (Volanis *et al.*, 2002), while the annual harvested milk for the semi-intensive farms was around 190 kg/ewe per year, 40 kilos higher than the previously reported average of the semi-intensive farms on Crete (Stefanakis *et al.*, 2007). Finally, conception rates was high in both systems and slightly higher for the semi-intensive flocks that also had lower rates of lamb mortality the first 48 hours after birth. These differences were expected, since there are aspects of farm management related to fertility and lamb mortality that had been previously reported as unsatisfactory for the extensive farms in Crete. These include non-programmed distribution of mating, poor care of ewes and new-born lambs during parturition and lack of preventive measures for animal diseases (Volanis *et al.*, 2007).

3.2 Effect of feeding regimes on flock productivity and bulk milk quality parameters in extensive and semi-intensive dairy sheep production systems

This section presents and discusses the results relating the effect of management system and background climatic conditions on flock productivity and bulk milk quality.

3.2.1 Milk yield, basic composition.

The main effect of management system on milk yield, milk fat and milk lactose content, based on LME models, was statistically significant (Table 3.10). On the other hand, it was not statistically significant for protein and non-fat solid (NFS) concentrations, somatic cell count (SCC), and colony forming units (CFU) of bacteria in milk. As for milk yield and fat concentration is concerned, extensive flocks had a 37% lower milk yield and 11% higher fat content compared with semi-intensive flocks (Table 3.10).

Sampling season had a statistically significant main effect on all examined parameters apart from milk protein content (Table 3.10). Milk yield per ewe gradually decreased as lactation progressed between January and June, while fat content sharply decreased (around 17%) in March and then remained consistent between April and June (Table 3.10). For lactose, NFS, SCC and CFU a significant change was observed in May and June when lactose and NFS concentrations were lower and CFU were higher than in the previous months. SCCs were lower in April and May compared with other months (Table 3.10).

Sampling year had a statistically significant main effect on all examined parameters apart from and milk protein content and non-fat solids (Table 3.10). The average milk yield was 18% lower, whilst the SCC and CFU were 48% and 60% higher respectively in the second production year (Table 3.10). Furthermore, the interaction effect between management system and sampling season was statistically significant, based on LME models, for lactose content meaning that lactose was influenced by the management system differently for the sampling seasons (Table 3.10). While the lactose content was similar between the two systems between January and April, in May and June milk from the extensive flocks was significantly lower in lactose (Table A.4.4, appendix).

parameter assessed	milk yield	fat	protein	lactose	non-fat solids	somatic cell count	colony forming units
	l day^{-1} ewe^{-1}	g 100ml^{-1}	g 100ml^{-1}	g 100ml^{-1}	g 100ml^{-1}	1000 cells 100ml^{-1}	1000 units 100ml^{-1}
management system							
semi-intensive *(n=10)*	1.00 ±0.03	5.43 ±0.11	5.17 ±0.03	4.78 ±0.02	10.76 ±0.04	98 ±6	477 ±54
extensive *(n=10)*	0.63 ±0.02	6.05 ±0.11	5.24 ±0.03	4.73 ±0.03	10.79 ±0.04	108 ±8	537 ±60
sampling season							
January	1.02 ±0.06^{a}	6.22 ±0.17^{a}	5.17 ±0.06	4.78 ±0.03^{ab}	10.80 ±0.06^{a}	107 ±10^{abc}	331 ±74^{b}
February	0.92 ±0.05^{ab}	6.53 ±0.16^{a}	5.28 ±0.06	4.77 ±0.04^{ab}	10.84 ±0.06^{a}	126 ±15^{a}	435 ±93^{b}
March	0.83 ±0.04^{bc}	5.41 ±0.16^{b}	5.24 ±0.06	4.88 ±0.03^{a}	10.91 ±0.06^{a}	97 ±12^{abc}	423 ±90^{b}
April	0.79 ±0.04^{cd}	5.33 ±0.21^{b}	5.24 ±0.06	4.85 ±0.03^{ab}	10.87 ±0.06^{a}	91 ±13^{bc}	387 ±88^{b}
May	0.71 ±0.05^{de}	5.21 ±0.20^{b}	5.23 ±0.05	4.76 ±0.05^{b}	10.79 ±0.07^{a}	76 ±12^{c}	786±114^{a}
June	0.60 ±0.05^{e}	5.68 ±0.17^{b}	5.06 ±0.05	4.47 ±0.08^{c}	10.39 ±0.07^{b}	121 ±14^{ab}	714 ±117^{a}
sampling year							
first	0.90 ±0.03	5.41 ±0.11	5.16 ±0.03	4.85 ±0.03	10.78 ±0.04	83 ±6	389 ±50
second	0.74 ±0.03	6.06 ±0.11	5.24 ±0.03	4.66 ±0.03	10.76 ±0.04	123 ±9	623 ±61
LME (*P*-values)							
main effects							
management system (MS)	<0.001	<0.001	ns	0.048	ns	ns	ns
sampling season (SS)	<0.001	<0.001	ns	<0.001	<0.001	0.019	<0.001
sampling year (Y)	<0.001	<0.001	ns	<0.001	ns	<0.001	0.001
Interactions							
MS x SS	ns	ns	ns	<0.001[1]	ns	ns	ns
MS x Y	ns	ns	ns	ns	ns	ns	ns
SS x Y	ns	ns	ns	ns	ns	ns	ns
MS x SS x Y	ns	ns	ns	ns	ns	ns	ns

[a-e] Means within a column with different superscripts are significantly different (*P*-values < 0.05); ns, P > 0.10 (nonsignificant).
[1]see **Table A.5.2** for interaction means/SE.

Table 3.10. Effect of management systems (production intensity), sampling season, and sampling year on milk yield and quality parameters (means ±standard errors)

3.2.2 Milk fatty acid profile.

For all 3 factors (management system, sampling season and sampling year) the main effects on the concentration of many individual FAs and a wide range of FA groups based on LME models were statistically significant (Table 3.11, Table 3.12 and Table 3.13).

Major saturated and mono-unsaturated fatty acids. The main effect of management system, sampling season and sampling year on the concentrations of all major SFAs [lauric (C12:0), myristic (C14:0), palmitic (C16:0) and stearic (C18:0) acid] and MUFAs (oleic, c9C18:1) and vaccenic, (t11C18:1 acid) found in milk was statistically significant (Table 3.12). Milk from extensive systems had 17%, 9% and 12%, less lauric, myristic and vaccenic acids respectively, but 2%, 10%, and 10% more palmitic, stearic and oleic acids respectively (Table 3.12). Concentrations of: (a) lauric acid decreased over time, being lowest in June, (b) myristic acid were highest in March and lowest in June, (c) palmitic, stearic, and oleic acid were lower in January to April than May and June, and (d) vaccenic acid was lower in May and June than between January and April (Table 3.12). In the first sampling year, concentrations of lauric, myristic and palmitic acids in milk were 26%, 16% and 9% higher, while levels of stearic, oleic and vaccenic acids were 18%, 13% and 74% lower than in the 2nd sampling year (Table 3.12).

Furthermore, the interaction effect between sampling season and management system was statistically significant based on LME models for all major SFAs (lauric, myristic, palmitic and stearic acid) and MUFAs (oleic and vaccenic acid) found in milk (Table 3.12). Significant differences between management systems were detected in some, but not all months during the milking period (Figure A.4-1 and Figure A.4-2, appendix). Milk from extensive systems had significantly more myristic acid in January, but less in May and less oleic acid in January, but more in May and June (Figure A.4-1 and Figure A.4-2, appendix).

Additional statistically significant interaction effects: (a) between sampling year and management systems was found for lauric, palmitic and oleic acid, and (b) between sampling season and sampling year was found for lauric and palmitic acid (Table 3.12). For lauric acid, the relative difference between extensive and semi-intensive was larger in the first than the second sampling year (Table A.4.5, appendix). For palmitic and oleic acid, significantly higher concentrations were found in milk from extensive systems in the first sampling year, while similar concentrations were found in milk from the 2 systems in the second sampling year (Table A.4.5,appendix). Significant differences in lauric acid concentrations between sampling year were found in some, but not all sampling months (Table A.4.6, appendix).

parameter assessed	SFA	MUFA	PUFA	omega-3 PUFA	omega-6 PUFA	omega-6/ omega-3
	g 100g^{-1}of total FA					
management system						
semi-intensive *(n=10)*	69.75 ±0.40	24.52 ±0.36	5.73 ±0.09	0.97 ±0.04	3.06 ±0.07	3.87 ±0.20
extensive *(n=10)*	67.64 ±0.49	26.25 ±0.36	5.88 ±0.14	1.18 ±0.07	3.03 ±0.05	3.71 ±0.25
sampling season						
January	69.83 ±0.61^{a}	24.77 ±0.54^{a}	5.40 ±0.15^{c}	0.85 ±0.06^{d}	3.06 ±0.10	4.55 ±0.39^{ab}
February	69.37 ±0.63^{a}	24.91 ±0.53^{a}	5.72 ±0.19^{bc}	0.86 ±0.08^{cd}	3.21 ±0.12	4.90 ±0.56^{a}
March	70.11 ±0.75^{a}	24.25 ±0.65^{a}	5.64 ±0.17^{bc}	0.94 ±0.06^{cd}	2.95 ±0.09	3.74 ±0.28^{bc}
April	68.84 ±0.96^{a}	24.25 ±0.52^{a}	6.28 ±0.26^{a}	1.40 ±0.12^{a}	2.94 ±0.11	2.71 ±0.25^{c}
May	68.21 ±0.79^{a}	25.70 ±0.64^{a}	6.08 ±0.22^{ab}	1.31 ±0.11^{ab}	3.00 ±0.10	3.00 ±0.29^{c}
June	65.60 ±0.81^{b}	28.66 ±0.70^{b}	5.74 ±0.18^{abc}	1.08 ±0.09b^{c}	3.10 ±0.09	3.76 ±0.40^{bc}
sampling year						
first	71.63 ±0.31	23.30 ±0.28	5.07 ±0.09	0.78 ±0.04	2.93 ±0.07	4.93 ±0.28
second	65.92 ±0.42	27.36 ±0.35	6.51 ±0.10	1.35 ±0.05	3.15 ±0.05	2.70 ±0.10
LME (*P*-values)						
main effects						
management system (MS)	<0.001	<0.001	ns	<0.001	ns	ns
sampling season (SS)	<0.001	<0.001	0.0002	<0.001	ns	<0.001
sampling year (Y)	<0.001	<0.001	<0.001	<0.001	0.0057	<0.001
Interactions						
MS x SS	<0.001[1]	0.001[1]	<0.001[1]	<0.001[1]	ns	<0.001[1]
MS x Y	ns	ns	0.028[2]	0.027[2]	ns	ns
SS x Y	ns	ns	ns	ns	ns	ns
MS x SS x Y	ns	ns	ns	ns	ns	ns

[a-c] Means within a column with different superscripts are significantly different (*P*-values < 0.05); ns, P > 0.10 (nonsignificant).

[1] see **Figures 3.9, A.5.1 and A.5.2** for interaction means/SE.

[2] **Table A.5.3** reports interaction means/SE

Table 3.11. Effect of management systems (production intensity), sampling season, and sampling year on concentrations of major fatty acid groups (SFA, MUFA, PUFA, omega-3 PUFA, omega-6 PUFA) and the omega-6 PUFA to omega-3 PUFA ratio (omega-6/omega-3). Means ±standard errors.

parameter assessed	lauric acid C12:0	myristic acid C14:0	palmitic acid C16:0	stearic acid C18:0	oleic acid C18:1 *cis9*	vaccenic acid C18:1 *trans11*
	g 100 g^{-1}total FA					
management system						
semi-intensive *(n=10)*	4.92 ±0.13	12.40 ±0.15	27.17 ±0.20	8.72 ±0.19	19.28 ±0.33	1.15 ±0.05
extensive *(n=10)*	4.06 ±0.12	11.34 ±0.15	27.62 ±0.24	9.60 ±0.18	21.28 ±0.35	1.01 ±0.05
sampling season						
January	4.93 ±0.18^{ab}	11.65 ±0.21^{ab}	26.87 ±0.27^{b}	9.13 ±0.30^{bc}	19.68 ±0.51^{bc}	1.18 ±0.08^{ab}
February	5.14 ±0.19^{a}	12.04 ±0.24^{a}	26.65 ±0.36^{b}	8.83 ±0.32^{bc}	19.53 ±0.48^{bc}	1.19 ±0.09^{a}
March	5.06 ±0.21^{ab}	12.27 ±0.26^{a}	27.05 ±0.30^{b}	8.34 ±0.33^{c}	19.07 ±0.60^{c}	1.14 ±0.07^{ab}
April	4.56 ±0.22^{b}	12.07 ±0.30^{a}	26.76 ±0.36^{b}	9.19 ±0.35^{bc}	18.99 ±0.49^{c}	1.18 ±0.09^{ab}
May	3.97 ±0.22^{c}	12.00 ±0.31^{a}	28.49 ±0.46^{a}	9.36 ±0.34^{ab}	20.64 ±0.61^{a}	1.00 ±0.07^{b}
June	3.19 ±0.17^{d}	11.20 ±0.30^{b}	28.72 ±0.41^{a}	10.15 ±0.29^{a}	24.03 ±0.60^{a}	0.76 ±0.07^{c}
sampling year						
first	5.18 ±0.13	12.96 ±0.12	28.74 ±0.20	8.38 ±0.18	19.00 ±0.33	0.78 ±0.04
second	3.85 ±0.11	10.84 ±0.13	26.10 ±0.16	9.89 ±0.18	21.48 ±0.34	1.36 ±0.04
LME (*P*-values)						
main effects						
management system (MS)	<0.001	<0.001	0.010	<0.001	<0.001	<0.001
sampling season (SS)	<0.001	0.001	<0.001	<0.001	<0.001	<0.001
sampling year (Y)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
Interactions						
MS x SS	0.001[1]	<0.001[1]	0.027[1]	0.023[1]	0.009[1]	0.015[1]
MS x Y	0.031[2]	ns	0.023[2]	ns	0.038[2]	ns
SS x Y	ns	ns	0.005[3]	ns	ns	ns
MS x SS x Y	0.022	ns	ns	ns	0.018	ns

[a-e] Means within a column with different superscripts are significantly different (*P*-values < 0.05); ns, P > 0.10 (nonsignificant).
[1] see **Figures A.5.1 and A.5.2** for interaction means/SE.
[2] **Table A.5.3** reports interaction means/SE.
[3]**Table A.5.4** reports interaction means/SE.

Table 3.12. Effect of management systems (production intensity), sampling season, and sampling year on concentrations of major SFA and MUFA in milk fat. Means ±standard errors.

Major polyunsaturated fatty acids. Statistically significant main effects of management system were detected, based on LME models, on the concentrations of α-linoleic acid (c9c12c14C18:3), EPA (C20:5 n-3), DPA (C22:5 n-3), but not linoleic acid (c9c12C18:2), rumenic acid (c9t11 CLA) and DHA (C22:2 n-3), with higher concentrations found in extensive compared with semi-intensive systems (Table 3.13). The main effect of sampling season on the concentrations of α-linoleic acid, rumenic acid, EPA and DPA was statistically significant, but not on LA or DHA. Concentrations of α-linoleic acid and rumenic acid increased between January and April and then decreased again, while concentrations of EPA and DPA were lower in the winter (January – March) compared with the spring period (April – June) (Table 3.13). The main effects of sampling year were also statistically significant on α-linoleic acid, rumenic acid, EPA, DPA and DHA, but not on linoleic acid. Concentrations of all six PUFAs were higher in the second sampling year, but the difference was not significant for linoleic acid (Table 3.13).

There were statistically significant interaction effects, based on LME models, (a) between management systems and sampling season on α-linoleic acid, rumenic acid , EPA and DPA and (b) between management system and sampling year on α-linoleic acid and EPA (Table 3.13). a-linoleic acid and DPA concentrations in milk from extensive farms were significantly higher in February, April, and June, but not in January, March and May (Figure 3-9). Except for January, EPA concentrations in milk from extensive system were significantly higher and increased between January and June; EPA concentrations in milk from semi-intensive system were similar throughout the milking season (Figure 3-9). a-linoleic acid concentrations were higher in milk from the extensive system in both years, but in the first year the relative differences in α-linoleic acid concentrations between extensive and semi-intensive systems were smaller than in the second year (Table A.4.5, appendix). EPA concentrations were significantly higher in milk from extensive farms only in the first sampling year (Table A.4.5, appendix).

parameter assessed	linoleic acid C18:2*cis9.cis12*	a-linolenic acid C18:3 *cis9.cis12. cis15*	rumenic acid CLA *cis9.trans11*	eicosapentaenoic acid C20:5 n-3	docosapentaenoic acid C22:5 n-3	docosahexaenoic acid C22:6 n-3
	g 100 g^{-1}total FA					
management system						
semi-intensive *(n=10)*	2.62 ±0.06	0.55 ±0.02	1.04 ±0.02	0.046 ±0.003	0.075±0.004	0.019 ±0.002
extensive *(n=10)*	2.58 ±0.05	0.75 ±0.05	0.99 ±0.03	0.061 ±0.003	0.092 ±0.005	0.020 ±0.002
sampling season						
January	2.64 ±0.10	0.47 ±0.03^{d}	0.87 ±0.04^{c}	0.041 ±0.003^{c}	0.076 ±0.007^{bc}	0.019 ±0.003
February	2.77 ±0.11	0.48 ±0.05^{d}	0.98 ±0.05^{bc}	0.037 ±0.003^{c}	0.074 ±0.007^{c}	0.019±0.003
March	2.52 ±0.09	0.54 ±0.03^{cd}	1.07 ±0.04^{ab}	0.039 ±0.004^{c}	0.068 ±0.005^{c}	0.017 ±0.003
April	2.47 ±0.10	0.88 ±0.09^{a}	1.13 ±0.05^{a}	0.056 ±0.005^{b}	0.099 ±0.008^{a}	0.020 ±0.003
May	2.55 ±0.09	0.84 ±0.08^{ab}	1.08 ±0.05^{ab}	0.066 ±0.006^{b}	0.095 ±0.009^{ab}	0.023 ±0.003
June	2.66 ±0.09	0.68 ±0.06^{bc}	0.96 ±0.04^{bc}	0.084 ±0.005^{a}	0.088 ±0.008^{abc}	0.020 ±0.003
sampling year						
first	2.55 ±0.05	0.53 ±0.03	0.90 ±0.03	0.044 ±0.003	0.049±0.002	0.003±0.000
second	2.65 ±0.06	0.75 ±0.04	1.12 ±0.03	0.062 ±0.003	0.116±0.004	0.036±0.001
LME (*P*-values)						
main effects						
management system (MS)	ns	<0.001	ns	<0.001	<0.001	ns
sampling season (SS)	ns	<0.001	<0.001	<0.001	<0.001	ns
sampling year (Y)	ns	<0.001	<0.001	<0.001	<0.001	<0.001
Interactions						
MS x SS	ns	<0.001[1]	0.019[1]	<0.001[1]	<0.001[1]	0.034[1]
MS x Y	ns	0.017[2]	ns	0.039[2]	ns	ns
SS x Y	ns	ns	0.036[3]	ns	ns	ns
MS x SM x Y	ns	ns	ns	ns	ns	ns

[a-c] Means within a column with different superscripts are significantly different (*P*-values < 0.05); ns, P > 0.10 (nonsignificant).

[1] see **Figure 3.9** for interaction means/SE.

[2] **Table A.5.3** reports interaction means/SE.[3]**Table A.5.4** reports interaction means/SE

Table 3.13. Effect of management systems (production intensity), sampling season and sampling year on concentrations of major PUFAs in milk fat. Means ±standard error.

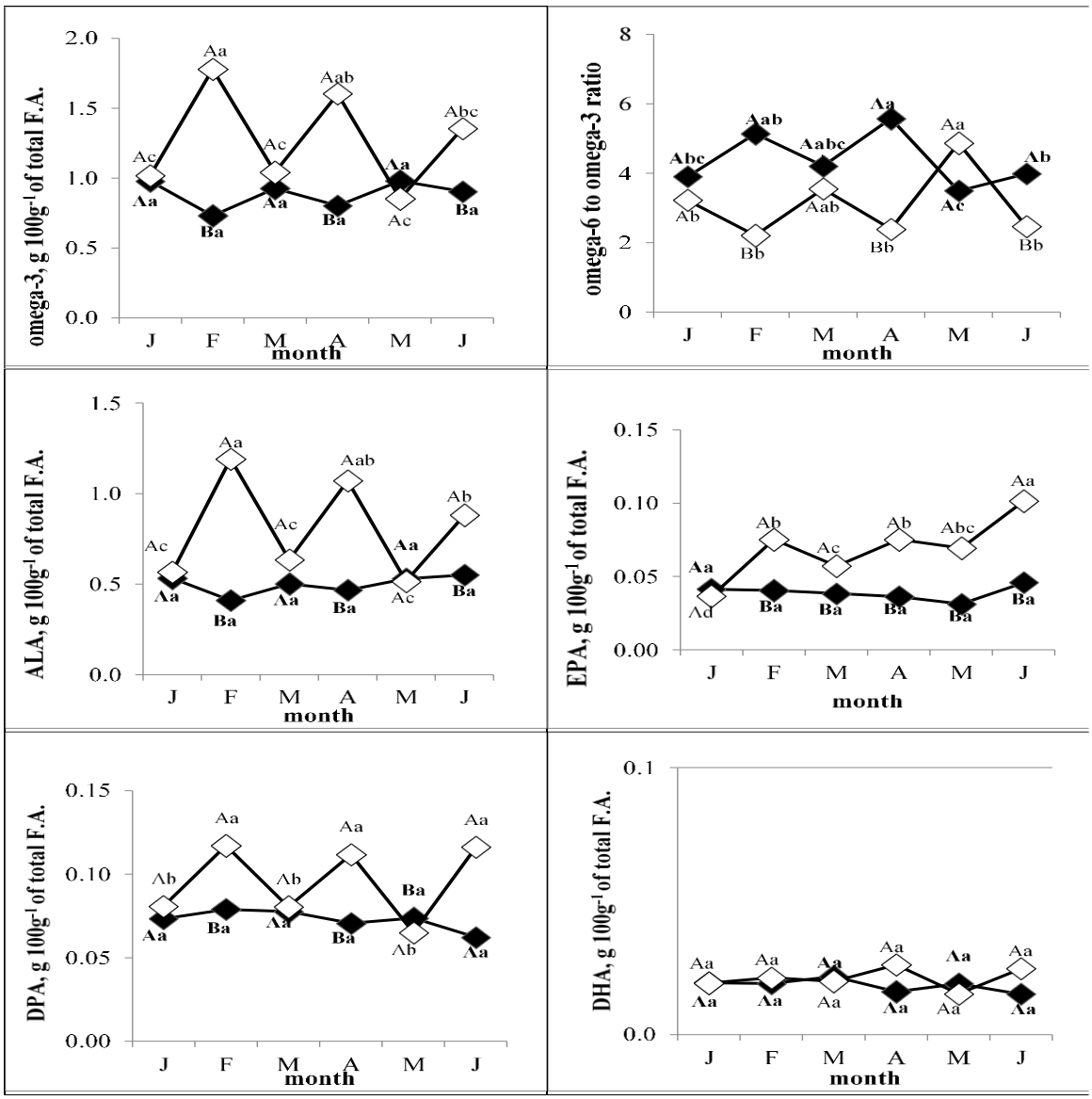

Figure 3-9. Interaction means for omega-3 PUFA (omega-3), omega-3 to omega-6 ratio, a-Linoleic acid (C18:3cis9.cis12.cis15; ALA), Eicosapentaenoic acid (C20:5omega-3; EPA), Docosapentaenoic acid (C22:5omega-3; DPA) and Docosahexaenoic acid (C22:6omega-3; DHA) for the different management systems and sampling seasons.

Semi-intensive management system is represented by (♦) and extensive management system by (◊). J:January, F:February, M:March, A:April, M:May, J:June.Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system (P-value<0.05).

Fatty acid groups and PUFA omega-6/omega-3 ratio. The main effect of management system, based on LME models, were statistically significant on total SFA, MUFA and omega-3PUFA concentrations, but on total PUFA, omega-6 PUFA, and the ratio of omega-6/omega-3 PUFA in milk (Table 3.11). Concentrations of SFA were 3% lower, while MUFA and omega-3 PUFA were 7% and 22% higher respectively in milk from extensively managed flocks (Table 3.11).

The main effect of sampling season on total SFA, MUFA, PUFA, omega-3 PUFA concentrations and the omega-6/omega-3PUFA ratio was statistically significant, but not on omega-6 PUFA levels in milk (Table 3.11). Concentrations of SFA were lower, and MUFA were higher in June, compared with all other months (Table 3.11). Concentrations of PUFA and omega-3 PUFA were lower and the omega-6/omega-3PUFA ratio was higher in winter milk (January to March) compared with milk collected in spring/early summer (April to June) (Table 3.11).

The main effect of sampling year on all FA-groups and the omega-6/omega-3 PUFA ratio was based on LME models statistically significant (Table 3.11). Concentrations of SFA were 8% higher in the first year, while concentrations of MUFA, PUFA, omega-3 PUFA, and omega-6 PUFA were 17%, 28%, 73% and 8% higher in the second sampling year (Table 3.11). Also, the omega-6/omega-3PUFA ratio was higher in the first sampling year.

Moreover, the interaction effect between sampling season and management systems on SFA, MUFA, PUFA, and omega-3 PUFA concentrations and the omega-6/omega-3PUFA ratio was also statistically significant (Table 3.11). The magnitude of difference between management systems varied between sampling season. Also for many parameters significant differences could be detected in some, but not all months (Figure 3-9, Figure A.4-1 and Figure A.4-2, appendix). For the omega-6/omega-3PUFA ratio, higher values were recorded in the semi-intensive system in most months except for May (Figure 3-9).

Finally, the interaction effect between sampling year and managements systems on PUFA and omega-3 PUFA was also statistically significant, but not on SFA, MUFA, omega-6 PUFA or the omega-6/omega-3PUFA ratio (Table 3.11). In the first year, no significant differences in total PUFA omega-3 PUFA were detected between production systems. In contrast, in the second sampling year, concentrations of total PUFA and omega-3 PUFA were higher in milk from the extensive flocks (Table A.4.5, appendix).

3.2.3 Redundancy analysis. Environmental and agronomic parameters on milk yield and quality.

In the RDA 35.0% of variation in milk yield, the concentrations of the milk FA and FA groups included was explained by all axis, mainly by axis 1 (27.5%).

The strongest driver identified by the RDA was supplementary concentrate intake (CON, explaining 26.3% of variation, P-value 0.002). Other strong drivers included were (a) time spent grazing on cultivated pastures (GC, explaining 3.2% of variation, P-value 0.002), (b) oat hay intake (OH, explaining 4.0 % of residual variation, P-value 0.012), (c) alfalfa hay intake (AH, explaining 3.8% of residual variation, P-value 0.008), (d) stage of lactation/days in milk (DIM, explaining 3.5% of residual variation, P-value 0.008), (e) milking system used (ML explaining 3.0% of residual variation, P-value 0.02) and (f) time spent grazing on natural pastures (GN, explaining 2.9% of residual variation, P-value 0.034).

The RDA biplot showed positive associations (along the negative axis 1) between grazing time on natural pastures, number of days in milk, grazing at high altitude, (and to a lesser extent hand milking rather than machine milking), and concentrations of total n-3 and VLC n-3 and to a lesser extent total PUFA and MUFA (Figure 3-10).

In contrast, there were positive association (along positive axis 1) between concentrate, alfalfa hay and oat hay intake (and to some extent with time spend grazing cultivated pastures), and milk yield, concentration of lauric acid (and to a lesser extent myristic acid) and the n-6/n-3 ratio (Figure 3-10).

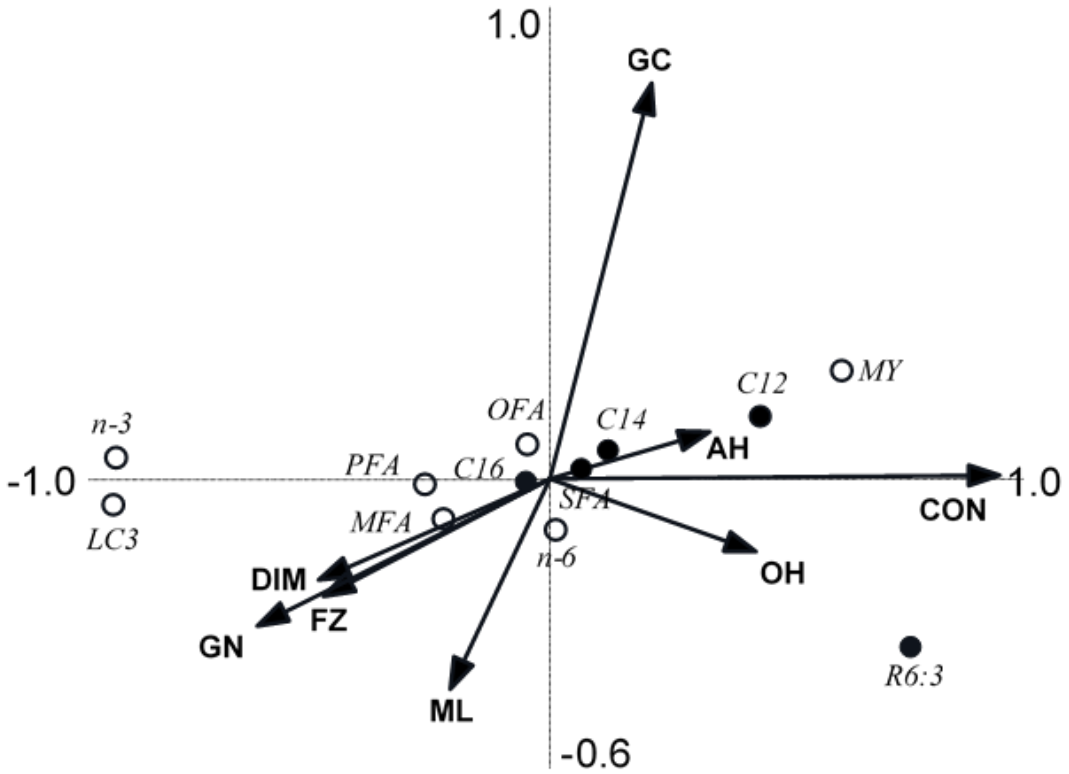

Figure 3-10. Biplot derived from redundancy analyses showing the relationship between milk yield, the content of fatty acid groups in milk fat, the content of major fatty acids in milk fat, the omega-6 to omega-3 ratio and nutritional variables, the days in milk and the milking system.

MY: milk yield; SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PFA: polyunsaturated fatty acids; OFA: odd chain fatty acids; n-3: omega-3 fatty acids; n-6: omega-6 fatty acids; LC3: very-long omega-3 fatty acids-EPA,DPA,DHA; R6:3: omega-6 to omega-3 ratio; C12: lauric; C14: myristic acid; C16: palmitic acid; GC: grazing time on cultivated pastures; GN: grazing time on natural pastures; FZ: floristic zone; CON: total supplementary concentrate; OH: oat hay; AH: alfalfa hay; DIM: days in milk; ML: milking system.

3.2.4 Discussion

Over the last 20 years many traditional, grazing-based sheep producers have intensified management to increase milk yields. Intensification was brought about by:(a) changing grazing systems (e.g. remaining at lower altitudes close to the farm, use of improved pastures, longer periods in corals), (b) using more concentrates, and (c) replacing hand-milking with semi-automatic or automatic milking systems. Similar intensification approaches have been shown to reduce bovine milk quality as a result of decreased concentrations of

desirable PUFAs (omega-3 PUFA and CLA) and vitamins (α-tocopherol and carotenoids) (Slots *et al.*, 2009). As a result, there has also been concern about potential negative effects of intensification on ovine milk quality, especially FA profiles.

In our study ewes in extensive systems produced 37% less milk, had 11% more milk fat content, and there were no differences in protein or NFS content, SCC and CFU and only a small difference in lactose content between milk from semi-intensive and extensive systems. Thus, the intensification of feeding regimes of Sfakiano dairy sheep on Crete increased productivity without altering the processing and microbial quality to a level that can be noted by farmers and dairy industry (Marques and Belo, 2001; Fox *et al.*, 2017).

However, the lower milk fat content for the semi-intensive flocks is a concern to be addressed since milk fat has an important role in the development of ripened cheese flavours (Skeie, 2010). The difference can be attributed to the dilution effect (Morand-Fehr *et al.*, 2007) where while milk yield for the ewe increases fat yield does not change resulting in lower concentrations. Moreover, regarding milk FA profile this study clearly documented a significant negative effect of intensification for sheep milk from traditional Sfakiano production systems, similar to that noted for bovine production (Stergiadis *et al.*, 2012). The 22% lower omega-3 PUFA concentrations may have the greatest nutritional impact, since typical Western diets are known to be deficient, especially in the very long chain (VLC) omega-3 PUFA (EPA, DPA and DHA) (Hibbeln *et al.*, 2006). The European Food Standards Agency (EFSA) therefore recommends that average, daily intakes of VLC omega-3 PUFA are at least doubled, with even higher intakes for pregnant and breast-feeding women (EFSA NDA Panel, 2010). Although EPA, DPA and DHA content in sheep milk is small, dairy products are consumed in high rates in many developed countries (Sanchez-Villegas *et al.*, 2003) thus the overall added impact could benefit these populations.

The 18% and 9% higher concentrations of the saturated lauric and, especially, myristic acid respectively found in milk from semi-intensive systems are also of concern, since lauric, myristic and palmitic acid have been the main SFAs linked to inflammation and increased cardiovascular disease risk, with the strongest connection for myristic acid (Lichtenstein *et al.*, 2006).These findings are in agreement with previous studies reporting that higher dairy animal feed concentrate use does increase productivity, but also has negative effects on milk quality, especially FA profiles (Marques and Belo, 2001; Min *et al.*, 2005).

It should also be noted that, unlike to bovine dairy production (Srednicka-Tober *et al.*, 2016), intensification of Sfakiano dairy sheep production in Crete did not result in significant

differences in Rumenic acid and that the magnitude of difference in nutritionally desirable n-3 and undesirable SFA did vary within and between years season/stage of lactation.

Redundancy analysis identified high levels of grazing on natural pastures, pasture composition (represented by floristic zone), and low concentrate use as the main drivers for enhanced milk quality. This agrees with previous studies in both bovine and ovine production systems. For example, several recent studies/reviews concluded that milk from ruminants with high fresh-forage intakes has more PUFA and omega-3 PUFA and/or lower myristic and lauric acid content (Dewhurst *et al.*, 2006; Chilliard *et al.*, 2007; Tsiplakou *et al.*, 2008; Tsiplakou *et al.*, 2010), compared with ruminants fed diets high in concentrate. Differences on the impact between our findings and those for bovine milk can be attributed to the different concentrations of the various FA in the ovine milk compared with the bovine (Chilliard *et al.*, 2007). Moreover, since many FA such as rumenic acid are formed in the rumen some variations are expected due to the differences in ruminal biohydrogenation species (Chilliard *et al.*, 2007).

The botanical composition of grazing swards may also have been at least partially responsible for differences in milk quality between semi-intensive and extensive systems. Although not assessed in this study, the semi-natural vegetation grazed by extensive flocks is highly likely to be species-rich (Parente, 2011) including grasses, legumes, flowers and woody shrubs. In contrast, the improved or sown swards grazed in the semi-intensive systems consisted primarily of oats (at a vegetative growth stage). In such homogenous pastures, feeding behaviour is controlled by accessibility and sheep will graze the planted crop, in contrast to mixed pastures which allow sheep to exhibit "opportunist" selective behaviour with strong preferential grazing of legumes and plants in vegetative stage (Molle *et al.*, 2004), which is also likely to contribute to differences in milk quality. This view is also supported by previous studies reporting higher PUFA in sheep milk when ewes graze pastures rich in legumes in comparison to pure grass swards (Cabiddu *et al.*, 2005; Nudda *et al.*, 2014).

Extensive flocks were also fed more conserved forage in their diet, mainly alfalfa hay. Although the cutting, drying, and baling of hay reduces the concentration of FAs compared with fresh herbage (Dervishi *et al.*, 2012), there is evidence from studies with cows showing that conserved forages from legumes increase the concentration of PUFA and omega-3 PUFA in milk compared with conserved forages from pure grass swards (Dewhurst *et al.*, 2006).

The finding of substantial seasonal variation in milk fatty acid profiles (especially in the extensive system) is also in agreement with previous studies (Tsiplakou *et al.*, 2008; Carloni *et al.*, 2009; de La Fuente *et al.*, 2009) and can be attributed to changes in sward

quality, feeding regimes, and stage of lactation (Molle *et al.*, 2004; Cabiddu *et al.*, 2005). Also, the increased concentration of FAs, such as palmitic, stearic, and oleic in May and June may be due to the mobilization of body fat as a response to the decreased energy intake as reported previously by Chilliard *et al.* (2003).

The survey was repeated in two production years characterized by markedly different climatic conditions. Both examined management systems are grazing based and grazing regimes are determined significantly by environmental conditions, as recorded in the study. Especially, in a semi-arid area such as Crete, environmental conditions determine the productivity of pasture and fodder crop plants (Parente, 2011). Hence, weather-related differences likely affected forage composition, availability, intake and quality. Results demonstrated the strong influence of climatic conditions on both milk productivity and milk product quality, either directly or via impacts on animal diets, as reported by previous studies (Virto *et al.*, 2012).

The strong effect of nutrition on milk quality, as represented by the effect of management system on sheep milk composition and milk fat FA profile, and the interactions identified of nutrition with background climatic condition (between year differences) on this effect, highlights the need for a holistic approach when feeding regimes are planned for grazing based management systems, like the ones in Crete. Such programs should aim not only to cover animal needs and increase productivity, but also to reduce potential negative effects of changes in nutrition on milk quality and should consider the varying effect of weather conditions. For example, before planning feeding and grazing regimes specific targets for milk production, milk fat content and milk fat FA profile should be established based on the intended use of milk and the demands of the cheese makers (Nudda *et al.*, 2014). Afterwards, the appropriate target concentrate to forage ratios should be determined for the different stages of the reproductive cycle (e.g. early pregnancy, mid pregnancy, late pregnancy, lambing, and lactation) (Chilliard *et al.*, 2007). The next step would be the quantification of concentrate and forage intake based on the utilized breed, the reproductive stage, lambing period, availability of fresh or conserved forage and the price of concentrates, hay and milk along with other factors the veterinarian in charge may consider important. Following, the planned feeding and grazing regimes will be implemented and evaluated on ongoing basis by applying a predetermined monitoring and recording schemes (de la Fuente *et al.*, 1997). The recording scheme should be focused on the established targets for production (e.g. milk yield, fat content and composition) and include all factors that may affect them including nutrition, incidences of diseases, parity and prolificacy.

3.3 Effect of intensification practices and environmental parameters on animal milk yield and quality of ewes from the two different lambing periods in dairy sheep production systems on Crete

This section presents and discusses the results related to the effect of management system and background climatic conditions on milk yield and composition of ewes (individual animals) belonging in two different lambing groups, the early lambing group comprising the mature ewes and the late lambing group comprising the yearlings. Moreover, the results are compared with the results from the bulk tank samples.

3.3.1 Milk Yield, Composition

Data from early lambing group only. Statistical analyses showed that the main effect of management system on milk yield for both years of the study was statistically significant, based on LME models (Table 3.14). In both years, ewes of the SI flocks had 25-35% higher milk yield in comparison to EX ewes. Regarding milk composition, the main effect of management system on milk fat content and milk protein content was statistically significant only the 2nd year of the study, when the EX ewes had 7.3% higher concentration of fat in milk and 2% higher concentrations of protein in milk compared with the SI ewes (Table 3.14). No statistically significant main effects of management system on milk lactose content and NFS were found both years of the study (Table 3.14).

The main effect of sampling season or month on milk yield, milk fat, protein, lactose and non-fat solids content, based on LME models, was also statistically significant (Table 3.14). Milk yield steadily declined from January to June, but the 2nd year when lower yields were observed the drop was smoother (58% total drop the 1st year and 54% the second year). In both years, milk fat increased from January to February (20.6% the 1st year and a non-significant 2.7% the 2nd year) only to decrease back in March and April (6.4% the 1st year and 24.3% the 2nd). Afterwards it sharply decreased in May (11.0% the 1st year and 7.6% the 2nd year) and only to increase back in June (1.0% the 1st year and 25.7% the 2nd year). Protein content was less variable although, in both years, did increase from January to April (7.9% the 1st year and 6.4% the 2nd year) when a decline started (~3.8% both years). Lactose milk content followed an opposite trend with the concentration steadily decreasing from January to June (14.7% total drop the 1st year and 13.4% total drop the 2nd year). NFS milk content increased from January to March (0.4% the 1st year and 2.4% the 2nd year) and afterwards steadily decreased until June (6.4% and 8.6% less in June compared with March the 1st year and 2nd year respectively).

Furthermore, the interaction effect between management system and sampling season was statistically significant, based on LME models, for milk yield, fat, protein, lactose and non-fat solids concentrations (Table 3.14). Meaning that, all parameters are influenced by the management system differently for the sampling seasons. The observed between systems differences in seasonal variation were more intense for fat and protein milk content compared with the ones observed for milk lactose and non-fat solids content (Figure A. 4-3).

Data from both lambing groups. The main effect of management system on milk yield and all basic milk components, based on LME models, was statistically significant (Table 3.15) with the exception for protein content the 1st year of the study. In both years the ewes of the SI flocks had 30-40% higher milk yield compared with EX ewes, but the milk fat content was 7% higher and milk lactose content 3% lower for the EX ewes compared with the SI ewes. Milk protein content differed between the two systems only in the 2nd year when it was 4% higher for the EX ewes (Table 3.15).

The main effect of sampling season on milk yield, milk fat, protein, lactose and non-fat solids content, based on LME models, was also statistically significant, either the 1st or the 2nd year of the study. Exceptions were the main effect on non-fat solids milk content the 1st year of the study and the main effect on protein milk content the 2nd year of the study (Table 3.15). Milk yield declined by around 30% between March and June when milk fat content increased 5% the 1st year and 28% the 2nd while lactose content decreased around 14% both years. Milk protein content increased from March to May in the 1st year, while in the 2nd year it was similar in March and April then declined (Table 3.15).

Furthermore, the interaction effect between management system and sampling season was statistically significant, based on LME models, for milk yield, fat, protein and lactose concentrations (Table 3.15). In the 1st year of the study rate of milk yield decline was smoother for the SI flocks, compared with the EX flocks, but similar rates were observed for the two systems in the 2nd year. The pattern of change for the three constituents differed for the two systems as lactation progressed and these changes were not the same in the two years (Figure A.4-7, appendix).

The interaction effect between lambing period and management system was also statistically significant, based on LME models, for milk fat content the 1st year and for milk lactose content and NFS the 2nd year of the study (Table 3.15). In the SI flocks there were greater differences in milk fat content between EL and LL compared with the EX flocks. Lactose was higher for the LL ewes in the SI systems but not for the LL of the EX systems. Non-fat solids

were similar for the two lambing periods in the EX flocks but lower for the EL ewes in the SI flocks (Table A.4.8, appendix).

Finally, the interaction effect between lambing periods and sampling season was also statistically significant, based on LME models, for milk yield, milk fat, protein and NFS content both years (Table 3.15). Although in the 1st year of the study the LL ewes retained similar yields as lactation progressed the 2nd year of the study their milk yields steadily dropped, as was the case for the EL ewes both years. All three of milk components changed differently for the two lambing periods as lactation progressed and similar changes were observed the two years (Figure A.4-8, appendix).

parameter assessed	management system (MS)		samplings (SS)						LME (P-values)		
									Main effects		Interaction
	semi-intensive *(n=100)*	extensive *(n=100)*	January	February	March	April	May	June	MS	SS	MS x SS
Sampling Year 1											
milk yield ($l\ day^{-1}\ ewe^{-1}$)	0.94 ±0.02	0.71 ±0.02	1.28 ±0.03^{a}	0.94 ±0.03^{b}	0.86 ±0.03^{c}	0.69 ±0.02^{d}	0.59 ±0.02^{e}	0.54 ±0.02^{e}	***	***	***[1]
milk composition ($g\ 100ml^{-1}milk$)											
fat	6.08 ±0.06	6.24 ±0.07	5.73 ±0.11^{d}	6.91 ±0.11^{a}	6.23 ±0.09^{c}	6.47 ±0.09^{b}	5.76 ±0.10^{d}	5.82 ±0.08^{d}	P	***	***[1]
protein	5.35 ±0.02	5.31 ±0.02	5.07 ±0.04^{c}	5.40 ±0.04^{ab}	5.36 ±0.04^{a}	5.47 ±0.04^{a}	5.45 ±0.05^{a}	5.24 ±0.05^{b}	ns	***	***[1]
lactose	4.57 ±0.03	4.57 ±0.03	4.82 ±0.03^{a}	4.75 ±0.03^{a}	4.72 ±0.04^{a}	4.60 ±0.05^{b}	4.41 ±0.07^{c}	4.11 ±0.08^{d}	ns	***	***[1]
non-fat solids	10.70 ±0.03	10.62 ±0.04	10.77 ±0.04^{a}	10.88 ±0.04^{a}	10.81 ±0.04^{a}	10.73 ±0.06^{ab}	10.59 ±0.08^{b}	10.12 ±0.08^{c}	ns	***	ns
Sampling Year 2											
milk yield ($l\ day^{-1}\ ewe^{-1}$)	0.73 ±0.01	0.47 ±0.01	0.87 ±0.02^{a}	0.71 ±0.02^{b}	0.66 ±0.02^{b}	0.55 ±0.02^{c}	0.44 ±0.01^{d}	0.40 ±0.02^{d}	***	***	***[1]
milk composition ($g\ 100ml^{-1}milk$)											
fat	5.67 ±0.07	6.12 ±0.07	6.78 ±0.11^{a}	6.96 ±0.11^{a}	5.32 ±0.11^{c}	5.27 ±0.13^{c}	4.87 ±0.12^{d}	6.12 ±0.12^{b}	***	***	***[1]
protein	5.38 ±0.03	5.49 ±0.03	5.31 ±0.04^{b}	5.43 ±0.05^{b}	5.61 ±0.04^{a}	5.65 ±0.05^{a}	5.40 ±0.04^{b}	5.20 ±0.05^{d}	***	**	***[1]
lactose	4.45 ±0.03	4.46 ±0.03	4.70 ±0.03^{a}	4.48 ±0.04^{b}	4.62 ±0.04^{a}	4.47 ±0.05^{b}	4.37 ±0.06^{b}	4.07 ±0.07^{c}	ns	***	ns
non-fat solids	10.68 ±0.04	10.79 ±0.04	10.81 ±0.04^{bc}	10.77 ±0.05^{c}	11.07 ±0.05^{a}	10.96 ±0.07^{ab}	10.66 ±0.08^{d}	10.12 ±1.0^{e}	P	***	**[1]

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant). CFU: Colony Forming Units. SCC: Somatic Cell Count

[1] see **Figure A.5.3** for interaction means/SE.

Table 3.14. Effect of management systems (production intensity), sampling season and lambing periods, on milk yield and quality parameters for early lambing periods. Means ±standard errors

parameter assessed	management system (MS)		lamping period (LP)		sampling season (SS)				LME (P-values)						
									Main effects			Interactions			
	semi-intensive *(n=200)*	extensive *(n=200)*	early *(n=200)*	late *(n=200)*	March	April	May	June	MS	LP	SS	MS x SS	MS x LP	SS x LP	MS x SM x LP
Sampling Year 1															
milk yield (l day^{-1} ewe^{-1})	0.89 ±0.01	0.58 ±0.01	0.65 ±0.01	0.82 ±0.02	0.87 ±0.02^{a}	0.77 ±0.02^{b}	0.72 ±0.02^{b}	0.66 ±0.03^{c}	***	***	***	***[1]	ns	***[3]	P
milk composition (g 100ml^{-1}milk)															
fat	5.51 ±0.04	5.97 ±0.05	6.03 ±0.04	5.45 ±0.05	5.58 ±0.08^{b}	5.92 ±0.07^{a}	5.47 ±0.07^{b}	5.85 ±0.08^{a}	***	***	***	***[1]	***[2]	***[3]	**
protein	5.14 ±0.02	5.18 ±0.03	5.36 ±0.02	4.97 ±0.02	5.02 ±0.03^{c}	5.16 ±0.03^{b}	5.23 ±0.04^{a}	5.20 ±0.04^{a}	ns	***	***	***[1]	ns	***[3]	ns
lactose	4.70 ±0.02	4.52 ±0.03	4.40 ±0.03	4.81 ±0.02	4.93 ±0.03^{a}	4.80 ±0.03^{b}	4.67 ±0.04^{c}	4.31 ±0.05^{d}	***	***	***	***[1]	ns	ns	*
non-fat solids	10.60 ±0.03	10.43 ±0.03	10.49 ±0.03	10.54 ±0.02	10.67 ±0.03^{a}	10.66 ±0.04^{a}	10.62 ±0.05^{a}	10.29 ±0.06^{b}	***	P	ns	ns	ns	***[3]	ns
Sampling Year 2															
milk yield (l day^{-1} ewe^{-1})	0.67 ±0.01	0.44 ±0.01	0.50 ±0.01	0.62 ±0.01	0.80 ±0.02^{a}	0.66 ±0.02^{b}	0.48 ±0.01^{c}	0.41 ±0.01^{d}	***	***	***	*[1]	P	***[3]	ns
milk composition (g 100ml^{-1}milk)															
fat	5.22 ±0.06	5.58 ±0.06	6.57 ±0.06	5.20 ±0.06	4.80 ±0.08^{b}	4.85 ±0.08^{b}	4.80 ±0.09^{b}	6.12 ±0.08^{a}	***	***	***	***[1]	P	***[3]	ns
protein	5.25 ±0.02	5.49 ±0.03	5.49 ±0.03	5.23 ±0.03	5.38 ±0.04^{ab}	5.40 ±0.03^{a}	5.29 ±0.03^{b}	5.17 ±0.04^{c}	**	***	P	***[1]	ns	***[3]	ns
lactose	4.53 ±0.03	4.44 ±0.03	4.25 ±0.03	4.71 ±0.03	4.90 ±0.03^{a}	4.70 ±0.03^{b}	4.55 ±0.04^{c}	4.32 ±0.05^{d}	*	***	***	***[1]	***[2]	ns	ns
non-fat solids	10.62 ±0.03	10.76 ±0.04	10.61 ±0.05	10.76 ±0.03	11.04 ±0.04^{a}	10.95 ±0.04^{a}	10.72 ±0.05^{b}	10.34 ±0.06^{c}	***	***	***	***[1]	***[2]	***[3]	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).
[1] see **Table A.5.6** for interaction means/SE. [2] see **Table A.5.7** for interaction means/SE. [3]see **Table A.5.8** for interaction means/SE.

Table 3.15. Effect of management systems (production intensity), sampling season and lambing periods on milk yield and quality parameters for both lambing periods. Means± standard errors

3.3.2 Microbiological quality of milk.

Data from early lambing group only. The 1st year of the study the main effect of management system on CFU and milk pH, based on LME models, was statistically significant (Table 3.16). The 2nd year of the study the main effect of management system was statistically significant only for CFU (Table 3.18). Regarding CFU, the 1st year of the trials saw the SI ewes with higher values (~19%) in comparison to the EX ewes while the 2nd year the SI had lower values (~9%) compared with the EX ewes. Milk pH was lower for the extensive farms the first year of the study (Table 3.16).

The main effect of sampling season on milk pH was statistically significant for both years. No statistically significant main effect of sampling season was found for CFU (Table 3.16). The differences in milk pH were generally lower however statistically significant and only the 2nd year a steady trend was observed when it steadily increased over the months.

The interaction effect between management system and sampling season was statistically significant based on LME models, for CFU for both years of the study whereas for milk pH only for the 2nd year of the study (Table 3.16). Regarding, CFU the 1st year of the study SI ewes had higher numbers of CFU in milk compared with the EX ewes all months except February and June. The 2nd year of the study, the two systems had similar numbers apart from April and June when the EX ewes had higher values (Table A.4.11).

Data from both lambing groups .The main effects of management system or lambing period on milk pH was, based on LME models, statistically significant for both years, but the main effect on CFU was only statistically significant only the 1st year of the study (Table 3.17). Contrary the main effect of sampling season on milk pH and CFU was statistically significant for both years of the study (Table 3.17).

Concerning CFU, the 1st year of the trials saw the SI ewes with higher values (~19%) in comparison to the EX ewes and the 2nd year of the study CFU values significantly decreased from March to April (~21%) and from April to May (~30%) only to increase slightly in June (Table 3.23). The differences in milk pH were generally small however statistically significant. The interaction effects were statistically significant, based on LME models, : (a) between management systems and sampling season for milk pH (Both years milk pH rapidly increased from May to June for the EX ewes but not for the SI ewes (Figure A.4-5 appendix), (b) between management system and lambing period for milk pH the 1st year of the study (the LL ewes had lower values in the semi-intensively managed flocks but

not in the extensive flock.(Table A.4.8 appendix) and (c) between lambing period and season for milk pH and for CFU only the 2nd year of the study (Figure A.4-6, appendix).

parameter assessed	management system (MS)		sampling season (SS)						LME (P-values)		
									Main effects		Interaction
	semi-intensive *(n=100)*	extensive *(n=100)*	January	February	March	April	May	June	MS	SS	MS x SS
Sampling Year 1											
SCC ($10^3$100ml^{-1}milk)	234 ±1	145 ±1	141 ±1 ^{c}	178 ±1 ^{b}	166 ±1^{bc}	186 ±1 ^{b}	200 ±1 ^{ab}	257 ±1 ^{a}	***	*	ns
CFU ($10^3$100ml^{-1}milk)	16.6 ±0.1	13.2 ±0.1	14.5 ±0.1	14.1 ±0.1	15.5 ±0.1	13.8 ±0.1	15.9 ±0.1	15.5 ±0.1	***	ns	***[1]
milk pH	6.70 ±0.01	6.67 ±0.01	6.65 ±0.01^{b}	6.69 ±0.01^{b}	6.66 ±0.01^{b}	6.73 ±0.01^{a}	6.65 ±0.01^{b}	6.73 ±0.01^{a}	***	***	***[1]
Sampling Year 2											
SCC ($10^3$100ml^{-1}milk)	182 ±1	170 ±1	132 ±1^{b}	186 ±1^{b}	141 ±1^{b}	195 ±1^{a}	162 ±1^{b}	282 ±1^{a}	ns	***	**[1]
CFU ($10^3$100ml^{-1}milk)	14.1 ±1.0	15.9 ±1.0	13.8 ±1.1^{c}	17.8 ±1.1^{b}	13.5 ±1.1^{c}	20.0 ±1.1^{a}	11.5 ±1.0^{d}	15.1 ±1.1^{bc}	***	ns	*[1]
milk pH	6.75 ±0.01	6.78 ±0.01	6.69 ±0.01^{c}	6.73 ±0.01^{c}	6.73 ±0.01^{c}	6.79 ±0.01^{b}	6.81 ±0.02^{a}	6.85 ±0.04^{a}	ns	***	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).
CFU: Colony Forming Units. SCC: Somatic Cell Count
[a-d] Means within a raw with different superscripts letter are significantly different according to Tukey's honestly significant difference test (*P*-values < 0.05).
[1] see **Table A.5.9** for interaction means/SE

Table 3.16. Effect of management systems (production intensity), sampling season and lambing periods on milk microbiological quality for early lambing ewes only. Means ±stand and error

parameter assessed	management system (MS)		lamping period (LP)		sampling season (SS)				LME (P-values)						
									Main effects			Interactions			
	semi-intensive *(n=200)*	extensive *(n=200)*	early *(n=200)*	late *(n=200)*	March	April	May	June	MS	LP	SS	MS x SS	MS x LP	SS x LP	MS x SM x LP
Sampling Year 1															
SCC ($10^3$100ml^{-1}milk)	182 ±5	138 ±4	209 ±6	123 ±4	135 ±6^{b}	148 ±4^{b}	151 ±7^{b}	186 ±4^{a}	***	***	**	***[1]	ns	ns	ns
CFU ($10^3$100ml^{-1}milk)	15.5 ±0.1	12.6 ±0.1	15.1 ±0.1	12.9 ±0.1	13.8 ±0.1^{bc}	12.3 ±0.1^{c}	13.8 ±0.1^{bc}	14.8 ±0.1^{a}	***	***	P	P	ns	ns	ns
milk pH	6.68 ±0.01	6.67 ±0.01	6.70 ±0.01	6.68 ±0.01	6.67 ±0.01^{b}	6.73 ±0.01^{a}	6.63 ±0.01^{c}	6.71 ±0.01^{a}	*	*	***	***[1]	*[2]	P	ns
Sampling Year 2															
SCC ($10^3$100ml^{-1}milk)	162 ±6	191 ±6	214 ±6	145 ±4	140 ±5^{b}	145 ±4^{b}	135 ±2^{b}	309 ±6^{a}	*	***	***	*[1]	ns	**[3]	ns
CFU ($10^3$100ml^{-1}milk)	14.1 ±1.1	15.8 ±1.0	15.1 ±1.0	14.5 ±1.1	20.0 ±1.3^{a}	15.8 ±1.0^{b}	11.0 ±1.0^{c}	13.8 ±1.1^{bc}	ns	ns	**	*[1]	ns	***[3]	ns
milk pH	6.78 ±0.01	6.83 ±0.01	6.82 ±0.01	6.79 ±0.01	6.77 ±0.01^{b}	6.76 ±0.01^{b}	6.79 ±0.01^{b}	6.85 ±0.01^{a}	***	**	***	***[1]	ns	***[3]	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant). CFU: Colony Forming Units. SCC: Somatic Cell Count. [a-d] Means within a raw with different superscripts letter are significantly different according to Tukey's honestly significant difference test (*P*-values < 0.05).
[1] see **Figure A.5.5** for interaction means/SE. [2] see **Table A.5.6** for interaction means/SE. [3]see **Figure A.5.6** for interaction means/SE.

Table 3.17. Effect of management systems (production intensity), sampling season and lambing period on milk microbiological quality for both lambing periods. Means ±standard error

3.3.3 Milk fatty acid profile.

For all 3 fixed effect variables (management system, lambing period and sampling season – March vs May) the main effects on the concentration of many individual FAs and a wide range of FA groups based on LME models were statistically significant (Table 3.18 and Table 3.19).

Fatty acid groups and n-6/omega-3 ratio. The main effect of management system on all groups was statistically significant, either in the first or the second year (Table 3.18 and Table 3.19). Overall ewes in the EX systems had significantly lower quantities of SFA by around 4%, compared with the SI ewes. On the other hand, they had more total MUFA (11% in the 1st year and 5% in the 2nd), total PUFA (4% in the 1st year and 16.5% in the 2nd), omega-3 PUFA (18% in the 1st year and 41% in the 2nd) and omega-6 PUFA only in the 2nd year (11% higher). Additionally, the EX ewes had a lower ratio of omega-6 to omega-3 but the difference only reached significance in the 1st year (Table 3.18 and Table 3.19).

The main effect of lambing period on all FA groups and the omega-6 to omega-3 ratio was also statistically significant either in the first or second year (Table 3.18 and Table 3.19). In both years the LL ewes had higher concentrations of PUFA (8% in 1st year and 5.5% the 2nd) and omega-6 PUFA (10% in 1st year and 3% the 2nd). They also had higher concentrations of total MUFA and omega-3 PUFA but the differences were only statistically significant in the 1st year. Similarly, total SFA concentration was 3% lower for the LL ewes, compared with the EL ewes, the 1st year while no difference was observed the 2ndyear. As for the omega-6 to omega-3 PUFA ratio it was higher for the EL ewes but the difference was statistically significant only in the 1st year (Table 3.18 and Table 3.19).

The main effect of sampling season on FA groups and the omega-6 to omega-3 ratio was also statistically significant either in the first or second year (Table 3.18 and Table 3.19). Total SFA concentration was 5% higher in March, compared with the concentrations in May, while total MUFA was 10% lower, omega-3 was 30% lower and total PUFA were 5% and 11.5% lower, the 1st and the 2nd year respectively. Omega-6 PUFA concentration was lower in March the 1st year with no differences found the 2nd. Both years the omega-6 to omega-3 PUFA ratio was higher in March (Table 3.18 and Table 3.19).

The interaction effect between management systems and lambing period was statistically significant for the omega-6 to omega-3 ratio the 1st year and for the omega-3 PUFA group the 2nd year of the study (Table 3.18 and Table 3.19). In the SI flocks there was no difference between the two lambing periods for omega-6 to omega-3 ratio but in the EX

flocks the ratio was higher for the EL ewes compared with the LL ewes. The LL ewes had higher concentration of omega-3 PUFA in comparison to the EL in the SI systems while no difference was found for the extensive systems (Table A.4.8, appendix).

The interaction effect between management systems and sampling season was statistically significant for omega-3 PUFA and for omega-6 to omega-3 ratio both years and for total PUFA and MUFA the 2nd year (Table 3.18 and Table 3.19). Omega-3 PUFA concentrations drastically increase in May for the EX flocks (and, as a consequence, decreased the n-6:n-3 ratio) while contrary in the SI flocks omega-3 PUFA concentration slightly decreased (Table A.4.9, appendix). As for total PUFA the increase in the concentration between March and May was only significant for the EX ewes. (Table A.4.9, appendix).

Finally, the interaction effect between lambing period and sampling month was statistically significant for all FA groups and omega-6 to omega-3 PUFA ratio (Table 3.18 and Table 3.19). Both years saw the differences between the two lambing groups only reaching significance in March (Table A.4.10, appendix).

parameter assessed	management system (MS)		lamping period (LP)		sampling season (SS)		LME (P-values)						
							Main effects			Interactions			
	semi-intensive *(n=200)*	extensive *(n=200)*	early *(n=200)*	late *(n=200)*	March	May	MS	LP	SS	MS x SS	MS x LP	SS x LP	MS x SS x LP
fatty acid (g $100g^{-1}$ of total FA)													
C12:0	5.79 ±0.08	4.30 ±0.07	5.20 ±0.08	4.91 ±0.08	5.46 ±0.09	4.63 ±0.08	***	**	***	ns	ns	*[3]	ns
C14:0	12.97 ±0.10	11.32 ±0.10	12.80 ±0.10	11.51 ±0.11	11.99 ±0.11	12.32 ±0.11	***	***	**	*[1]	ns	***[3]	ns
C16:0	26.23 ±0.17	25.96 ±0.15	26.65 ±0.16	25.54 ±0.16	25.74 ±0.17	26.46 ±0.15	ns	***	**	ns	ns	*[3]	ns
C18:0	6.67 ±0.13	8.68 ±0.15	7.35 ±0.14	7.98 ±0.16	7.08 ±0.15	8.27 ±0.14	***	**	***	ns	ns	ns	ns
C18:1 trans11	1.48 ±0.04	1.60 ±0.05	1.42 ±0.04	1.65 ±0.05	1.46 ±0.05	1.62 ±0.04	P	***	*	***[1]	***[2]	*[3]	ns
C18:1 *cis9*	16.40 ±0.19	19.09 ±0.23	17.38 ±0.21	18.09 ±0.23	16.93 ±0.21	18.56 ±0.23	***	*	***	**[1]	ns	**[3]	ns
C18:2 cis9.cis12	2.95 ±0.05	2.95 ±0.04	2.79 ±0.05	3.11 ±0.05	2.90 ±0.05	3.00 ±0.04	ns	***	ns	ns	ns	P	ns
C18:3 cis9.cis12.cis15	0.57 ±0.01	0.80 ±0.02	0.65 ±0.02	0.72 ±0.02	0.58 ±0.01	0.79 ±0.02	***	**	***	***[1]	ns	***[3]	**
CLAcis9.trans11	1.07 ±0.03	1.09 ±0.02	1.09 ±0.03	1.07 ±0.03	1.02 ±0.02	1.14 ±0.03	ns	ns	**	**[1]	*[2]	ns	ns
C20:5 n-3 (EPA)	0.054 ±0.002	0.069 ±0.003	0.059 ±0.002	0.064 ±0.002	0.048 ±0.001	0.075 ±0.003	***	ns	***	**[1]	ns	ns	ns
C22:5 n-3 (DPA)	0.126 ±0.003	0.129 ±0.004	0.120 ±0.004	0.135 ±0.004	0.090 ±0.002	0.166 ±0.004	ns	**	***	ns	ns	*[3]	ns
C22:6 n-3 (DHA)	0.044 ±0.002	0.049 ±0.002	0.043 ±0.001	0.051 ±0.002	0.038 ±0.001	0.056 ±0.002	*	***	***	ns	ns	ns	ns
SFA	68.98 ±0.25	66.02 ±0.30	68.45 ±0.29	66.57 ±0.27	69.15 ±0.26	65.83 ±0.28	***	***	***	ns	ns	**[3]	ns
MUFA	24.27 ±0.21	26.95 ±0.23	24.91 ±0.23	26.28 ±0.23	24.33 ±0.22	26.9 ±0.22	***	***	***	ns	ns	***[3]	ns
PUFA	6.76 ±0.08	7.03 ±0.09	6.63 ±0.09	7.15 ±0.08	6.52 ±0.08	7.27 ±0.09	*	***	***	ns	ns	**[3]	ns
omega-3 PUFA	1.20 ±0.02	1.42 ±0.04	1.23 ±0.03	1.39 ±0.03	1.13 ±0.02	1.50 ±0.04	***	***	***	***[1]	ns	**[3]	**
omega-6 PUFA	3.85 ±0.06	3.82 ±0.05	3.66 ±0.05	4.01 ±0.05	3.67 ±0.05	4.00 ±0.05	ns	***	**	ns	ns	*[3]	ns
omega-6/omega-3 ratio	3.63 ±0.08	3.38 ±0.10	3.67 ±0.11	3.37 ±0.07	3.79 ±0.10	3.22 ±0.08	*	*	**	***[1]	***[2]	*[3]	**

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (no-significant).
[1] see **Table A.5.6** for interaction means/SE.
[2] see **Table A.5.7** for interaction means/SE.
[3]see **Table A,5,8** for interaction means/SE.

Table 3.18. Effect of management systems (production intensity), sampling season and lambing periods on Fatty acid profile. The 1st sampling year. Means ± standard errors

parameter assessed	management system (MS)		lamping period (LP)		sampling season (SS)		LME (P-values)						
							Main effects			Interactions			
	semi-intensive *(n=200)*	extensive *(n=200)*	early *(n=200)*	late *(n=200)*	March	May	MS	LP	SM	MS x SM	MS x LP	SM x LP	MS x SM x LP
fatty acid (g $100g^{-1}$ of total FA)													
C12:0	4.35 ±0.07	3.71 ±0.06	4.07 ±0.07	4.00 ±0.06	4.75±0.07	3.30 ±0.04	***	ns	***	ns	P	ns	ns
C14:0	11.94 ±0.08	10.73 ±0.08	11.72 ±0.09	10.96 ±0.08	11.71±0.10	10.95 ±0.07	***	***	***	P	ns	**[3]	ns
C16:0	25.95 ±0.11	26.02 ±0.11	26.20 ±0.11	25.77 ±0.12	25.73±0.14	26.25 ±0.09	ns	**	**	*[2]	ns	P	ns
C18:0	9.00 ±0.14	9.16 ±0.11	8.97 ±0.12	9.18 ±0.13	8.01±0.13	10.17 ±0.10	ns	ns	***	**[2]	ns	ns	ns
C18:1 trans11	1.79 ±0.04	1.95 ±0.05	1.62 ±0.03	2.11 ±0.04	1.95±0.05	1.78 ±0.03	*	***	*	ns	ns	*[3]	ns
C18:1 *cis9*	18.79 ±0.18	19.70 ±0.17	19.63 ±0.19	18.86 ±0.17	17.65±0.17	20.87 ±0.15	***	***	***	*[2]	ns	ns	P
C18:2 cis9.cis12	2.57 ±0.03	2.85 ±0.04	2.65 ±0.04	2.76 ±0.03	2.74±0.04	2.67 ±0.03	***	*	ns	***[2]	ns	***[3]	P
C18:3 cis9.cis12.cis15	0.73 ±0.01	1.15 ±0.03	0.93 ±0.03	0.95 ±0.02	0.76±0.02	1.13 ±0.03	***	ns	***	***[2]	***[1]	***[3]	ns
CLAcis9.trans11	1.22 ±0.02	1.29 ±0.02	1.17 ±0.02	1.34 ±0.02	1.29±0.02	1.22 ±0.02	*	***	*	ns	ns	ns	ns
C20:5 n-3 (EPA)	0.049 ±0.001	0.065 ±0.001	0.055 ±0.001	0.059 ±0.001	0.051 ±0.001	0.063 ±0.001	***	**	***	***[2]	ns	ns	ns
C22:5 n-3 (DPA)	0.109 ±0.002	0.143 ±0.003	0.122 ±0.003	0.129 ±0.003	0.105 ±0.002	0.147 ±0.003	***	*	***	***[2]	ns	**[3]	ns
C22:6 n-3 (DHA)	0.031 ±0.001	0.042 ±0.001	0.036 ±0.001	0.037 ±0.001	0.031 ±0.001	0.043 ±0.001	***	ns	***	***[2]	ns	***[3]	P
SFA	66.05 ±0.20	63.55 ±0.25	65.03 ±0.25	64.58 ±0.22	66.35 ±0.24	63.22 ±0.20	***	ns	***	ns	ns	**[3]	ns
MUFA	27.19 ±0.18	28.58 ±0.18	27.86 ±0.19	27.91 ±0.18	26.52 ±0.18	29.28 ±0.15	***	ns	***	*[2]	ns	P	ns
PUFA	6.75 ±0.06	7.87 ±0.10	7.11 ±0.09	7.51 ±0.08	7.13 ±0.09	7.50 ±0.08	***	***	**	***[2]	ns	***[3]	ns
omega-3 PUFA	1.38 ±0.03	1.94 ±0.05	1.61 ±0.04	1.70 ±0.04	1.45 ±0.03	1.87 ±0.04	***	P	***	***[2]	**[1]	***[3]	ns
omega-6 PUFA	3.48 ±0.04	3.88 ±0.04	3.62 ±0.04	3.74 ±0.04	3.69 ±0.05	3.67 ±0.03	***	*	ns	ns	ns	***[3]	ns
omega-6/omega-3 ratio	2.80 ±0.05	2.68 ±0.09	2.81 ±0.08	2.67 ±0.07	3.12 ±0.09	2.35 ±0.05	ns	ns	***	***[2]	ns	ns	*

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (non-significant).
[1] see **Table A.5.6** for interaction means/SE.
[2] see **Table A.5.7** for interaction means/SE.
[3]see **Table A,5,8** for interaction means/SE.

Table 3.19. Effect of management systems (production intensity), sampling months and lambing periods on Fatty acid profile. The 2nd sampling year. Means ± standard errors

Major fatty acids. For all 3 fixed effect variables of LME models (management system, lambing period and sampling season – March vs May) the main effects on the concentration of many major SFA (lauric, myristic, palmitic and stearic acid), MUFA (oleic and vaccenic acid) and PUFA (linoleic acid, α-linoleic acid, rumenic acid, EPA, DPA and DHA) in milk either in the 1st or the 2nd year (Table 3.18 and Table 3.19), were statistically significant.

The EX ewes had lower concentrations of lauric acid (26% in 1st year and 15% the 2nd) and myristic acid (13% in 1st year and 10% the 2nd) and higher concentrations of oleic acid (16% the 1st year and 5% the 2nd), linoleic acid (~50%), EPA (~30%) and DHA (~20%) compared with the SI ewes (Table 3.18 and Table 3.19), both years. Additionally, the EX had, compared with the SI ewes, higher concentrations of stearic acid (~30%) in the 1st year of the study and higher concentrations of vaccenic acid (~9%), linoleic acid (~10%), rumenic acid (~6%) and DPA (32%) in the 2nd year of the study (Table 3.18 and Table 3.19).

The LL ewes had both years less myristic acid (10% in 1st year and 6.5% the 2nd) and palmitic acid (4% in 1st year and 2% the 2nd) and higher concentrations of vaccenic acid (16% 1st year and 31% the 2nd), linoleic acid and DPA when in comparison to the EL ewes (Table 3.18 and Table 3.19). Additionally, in the 1st year of the study lauric acid concentrations were lower (6%) while stearic acid, α-linoleic acid and DHA were 8.5%, 11.5% and 30%, higher in the milk of the LL ewes, which was also higher in rumenic acid (14%) and EPA (7%) in the 2nd year. Regarding oleic acid the concentration was 4% higher for the LL ewes the 1st year but 4% lower the 2nd, compared with the EL ewes (Table 3.18 and Table 3.19).

Between the two sampling seasons, lauric acid was lower in May (15% in 1st year and 31% the 2nd) and higher concentrations for palmitic (~2.5 % both years), stearic (17% in 1st year and 27% the 2nd), oleic acid (10% in 1st year and 18% the 2nd), a-linolenic, EPA, DPA and DHA (ranging from 25 to 83% depending on the FA and the year) were observed in May (Table 3.18 and Table 3.19). Myristic and vaccenic acids concentrations were respectively 3% and 11% higher in May than in March the 1st year of the study and 6.5% and 9% higher the 2nd year. As for CLA, while the concentrations was fairly static throughout the 1st year, it decreased from March to May in the 2ndyear of the trials (Table 3.18 and Table 3.19)

The interaction effect between management system and lambing period, based on LME models, was statistically significant for vaccenic and rumenic acids the 1st year of the study and for a-linolenic acid in the 2nd year (Table 3.18 and Table 3.19). The concentration of vaccenic acid was similar for the two lambing periods in the SI flocks while in the EX flocks, higher concentrations were found in the milk of the LL ewes. (Table A.4.8, appendix).

Contrary, for both PUFA while differences were observed between the two lambing groups in the SI flocks, none were found for the ewes in the EX flocks (Table A.4.8, appendix).

Moreover, the interaction effect between management system and sampling season was statistically significant for myristic, vaccenic and oleic acid the 1st year, for palmitic, stearic and oleic acid the 2nd year and for all major PUFA either in the 1st or the 2nd year (Table 3.18 and Table 3.19). In both years EPA concentration was similar between the two management systems in March, but it was higher in the milk of the EX ewes in May. ALA concentration increased from March to May in the milk of the EX ewes but did not change in the milk of the SI ewes. A similar trend was observed for DPA and DHA but only in the 2nd year of the trials. An increase in CLA concentration from March to May was observed for the SI ewes but only in the 1styear of the study (Table A.4.9, appendix).

Finally, the interaction effect between lambing period and management system was, based on LME models, statistically significant for lauric, myristic, palmitic, vaccenic, oleic, linoleic, a-linolenic, DPA and DHA acid (Table 3.18 and Table 3.19). Regarding the PUFA, while the concentrations in milk were higher for the LL ewes in March compared with EL ewes' milk, they were similar between the two lambing groups in May. These differences were statistically important both years for DPA, but only reached significance in the 1st year for ALA and only in the 2nd year for LA and DHA (Table A.4.10, appendix).

3.3.4 Redundancy analysis. Environmental and agronomic parameters on milk yield and quality.

In the RDA 24.7% of variation in milk yield, the concentrations of the milk FA and FA groups included was explained by all axis, mainly by axis 1 (23.3%).

Strong drivers identified by the RDA included (a) time spend grazing on natural pastures (GN, explaining 18.8% of residual variation, P-value 0.002), (b) supplementary concentrate intake (CON, explaining 4.0% of variation, P-value 0.002), (c) time spend grazing on cultivated pastures (GC, explaining 0.6% of residual variation, P-value 0.002), (d) conserved forage intake (HAY, explaining 0.5% of residual variation, P-value 0.002), (e) average altitude of grazing pastures (ALT, explaining 0.4% of variation, P-value 0.002), (f) the average environmental temperature (AT, explaining 0.2% of residual variation, P-value 0.03) and (f) the average rainfall (AR, explaining 0.2% of residual variation, P-value 0.05) (Figure 3-11). The milking system used did not participate in the explanation of the variation.

The RDA biplot shows positive associations (along the positive axis 1) between grazing time on natural pastures and average altitude of grazing pastures, grazing at high

altitude, (and to a lesser extent hand milking rather than machine milking), and concentrations of total MUFA, total n-3 PUFA, a-linolenic acid, DPA and EPA and to a lesser extent total PUFA, DHA and Rumenic acid (Figure 3-11).

In contrast, there were positive association (along negative axis 1) between concentrate intake, hay intake and time spend grazing cultivated pastures), and milk yield, concertation of SFA and the n-6/n-3 ratio (Figure 3-11).

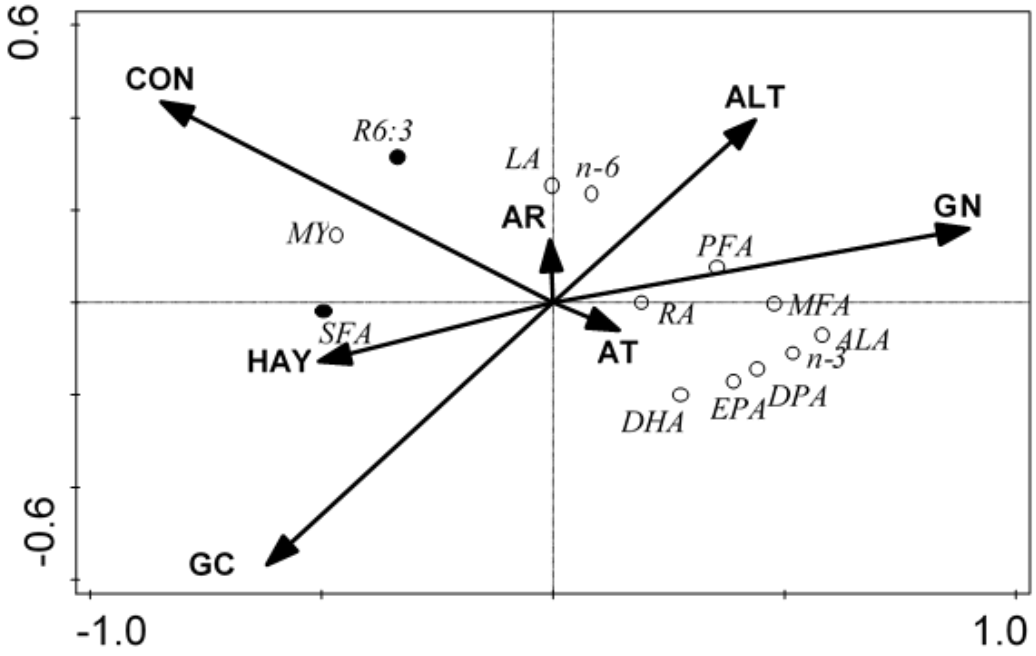

Figure 3-11. Biplot derived from redundancy analyses showing the relationship between milk yield, the content of fatty acid groups in milk fat, the omega-6 to omega-3 ratio, the content of major fatty acids in milk fat and agronomic and environmental parameters.

MY: milk yield; SFA: saturated fatty acids; MFA: monounsaturated fatty acids; PFA: polyunsaturated fatty acids; n-3: omega-3 fatty acids; n-6: omega-6 fatty acids; R6:3: the omega-6 to omega-3 ratio; LA: linoleic aicd; ALA: a-linolenic acid; RA: rumenic acid; EPA: eicosapentaenoic acid; DPA: docosapentaenoic acid; DHA: docosahexaenoic acid; GC: grazing time on cultivated pastures; GN: grazing time on natural pastures; ALT: average altitude of grazing pastures; CON: total supplementary concentrate intake; HAY: preserved forage intake.

3.3.5 Discussion

Similarly to the results in the previous section (**1.1**) on and from previous studies on ewes (Addis *et al.*, 2005; Tsiplakou *et al.*, 2008; Nudda *et al.*, 2014), management system had a significant main effect on most of the examined parameters. The ewes of the semi-intensive systems had 30 to 50% higher milk yield and 7% lower milk fat content, compared with the EX ewes. Moreover, the SI ewes had higher SFA concentration in milk fat and especially of the nutritionally undesired lauric acid and myristic acid, and lower concentrations for total PUFA and omega-3 PUFA. Redundancy analysis similarly to the bulk milk results identified high levels of grazing on natural pastures and concentrate intake, as main drivers for milk yield and composition [in opposite directions] confirming previous findings.

Of great interest are the recorded differences between the two lambing periods. The EL ewes, which were at the second or third lactation, had a significant higher milk protein and fat content in both years. These findings are in agreement with Sevi *et al.* (2004) who reported an increase in milk protein and casein content as lactation number advances. A reason for the found differences can be the increased body weight leading to a higher intakes and availability of body reserves for synthesis of milk components along with an increasing activity of udder tissue with age (Sevi *et al.*, 2004).

The lower concentrations of myristic and palmitic acid in the milk of the LL ewes and the higher concentrations of linoleic acid and PUFA, both years of the study, indicate a nutritionally more preferable FA profile. Since, the two lambing groups had similar feeding and grazing regimes these differences may be due to animal status (age/lactation stage). Only, during the 2nd year of the study an increased concentrate intake was recorded and that for the LL ewes, though farmers tended to also let the LL ewes graze higher quality pastures. Soják *et al.* (2013) in an experimental flock of 328 ewes of different breeds, reported an increase of Short- and Medium -chain SFA, CLA and ALA from first to third parity with a following decrease at older animals.Tsiplakou *et al.* (2006) however in a similar study in an experimental flock of 237 ewe found no relationship between CLA milk content and parity or DIM (Days in Milk) and similar results have been reported for cows (Kelsey *et al.*, 2003).

Considering the interactions between management systems, sampling season and lambing period, as well as the different results between the two experimental years, it is indicated that any effect lambing period may have on milk fat FA composition is greatly affected by nutrition and other environmental factors. For example, during the first year of the trials when feeding rations appeared similar between the two lambing groups, MUFA, oleic acid and omega-3 FA were higher in the milk of the late lambed ewes. However, the second

year of the trials, when higher quantities of supplementary concentrate were fed to the late lambed ewes, these differences were not statistically important, Additionally, many differences between the two lambing periods were only statistically significant only during the first sampling date in March, not long after lambing in the LL group. Thus, it is possible to exploit the potential of the winter lambed ewes to provide milk with higher nutritionally quality by altering the ration provided and the grazing practices. The quality characteristics of milk are affected by the interaction of these different factors (nutrition, individual animal characteristics, lactation stage, and background environmental conditions) and no single factor can have a fixed result.

By comparing the results from this section which focused on the effects relating to individual ewes and the previous section which focused on the effects relating to the flock (**section 1.1**), it can be summarized that there was a systematic statistically significant main effect of management system on milk yield, milk fat content (though only a trend in one case), and the concentration of SFA, MUFA, omega-3 PUFA, lauric acid, myristic acid, oleic acid, a-linoleic acid and eicosapentaenoic acid in milk (Table 3.20). The consistency between the findings of the different analysis further enhances the confidence that these effects can be unmistakably expensed to management systems and thus can be quantified and altered through different management practices.

Similarly, in both cases sampling season had a statistically significant main effect on milk yield, milk fat content, milk lactose content and the concentration of SFA, MUFA, PUFA, omega-3 PUFA and most of the presented Fatty Acids in milk (excluding linoleic acid and docosahexaenoic acid). The variations of these parameters throughout lactation though is affected by a plethora of other factors (nutrition, individual animal characteristics, lactation stage, and background environmental conditions) and may reveal weaknesses (inability to alter) and opportunities (points in time we can intervene), which are significant information when planning a production system.

factors	management system					sampling season					year	lambing period	
	bulk tank samples	**Individual samples**				**bulk tank samples**	**Individual samples**				**bulk tank samples**	**Individual samples**	
		early lambing only		both lambing periods			early lambing only		both lambing periods			both lambing periods	
parameter assessed		Year 1	Year 2	Year 1	Year 2		Year 2	Year 1	Year 1	Year 2		Year 1	Year 2
milk yield	***	***	***	***	***	***	***	***	***	***	***	***	***
fat content	***	P	***	***	***	***	***	***	***	***	***	***	***
protein content	ns	ns	***	ns	**	ns	***	**	***	P	ns	***	***
lactose content	*	ns	ns	***	*	***	***	***	***	***	***	***	***
NFS	ns	ns	P	***	***	***	***	***	***	ns	ns	***	P
SCC	ns	***	ns	***	*	*	*	***	***	***	***	***	***
CFU	ns	***	***	***	ns	***	ns	ns	P	**	***	***	ns
SFA	***	-	-	***	***	***	-	-	***	***	***	***	ns
MUFA	***	-	-	***	***	***	-	-	***	***	***	***	ns
PUFA	ns	-	-	*	*	***	-	-	***	**	***	***	***
omega-3 PUFA	***	-	-	***	***	***	-	-	***	***	***	***	P
omega-6 PUFA	ns	-	-	ns	***	ns	-	-	**	ns	***	**	*
omega-6/ omega-3	ns	-	-	*	ns	***	-	-	**	***	***	**	ns
C12:0	***	-	-	***	***	***	-	-	***	***	***	**	ns
C14:0	***	-	-	***	***	***	-	-	**	***	***	***	***
C16:0	*	-	-	ns	ns	***	-	-	**	**	***	***	**
C18:0	***	-	-	***	ns	***	-	-	***	***	***	**	ns
C18:1 cis9	***	-	-	***	***	***	-	-	***	***	***	*	***
C18:1 *trans11*	***	-	-	P	*	***	-	-	*	*	***	**	*
C18:2*cis9.cis12*	ns	-	-	ns	***	ns	-	-	ns	ns	ns	***	*
C18:3 *is9.cis12.cis15*	***	-	-	***	***	***	-	-	***	***	***	**	ns
CLA*cis9.trans11*	ns	-	-	ns	*	***	-	-	**	*	***	ns	*
C20:5 n-3	***	-	-	***	***	***	-	-	***	***	***	***	**
C22:5 n-3	***	-	-	ns	***	***	-	-	***	***	***	***	*
C22:6 n-3	ns	-	-	*	***	ns	-	-	***	***	***	***	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).

Table 3.20 Summary of p-values of main effects examined in sections 3.3 and 3.4.

3.4 Effect of intensification practices and environmental parameters on animal health, of ewes from the two different lambing periods in dairy sheep production systems on Crete.

This section presents and discusses the results related to the effect of management system and background climatic conditions on animal health for ewes (individual animals) belonging in two different lambing groups, the early lambing group comprising the mature ewes and the late lambing group comprising the yearlings. Subclinical –mastitis and gastrointestinal nematode infections are examined on animal level while other diseases and treatments were examined on flock level.

3.4.1 Somatic cell count.

Data from early lambing group only. The 1st year of the study the main effect of management system on SCC, based on LME models, was statistically significant (Table 3.16) but not for the 2nd year of the study. While both years the SI farms had higher values, these differences were only significant the 1st year (Table 3.16). The main effect of sampling season on SCC in both years was statistically significant. In both years an increased was observed from January to February and the highest values were measured in June (Table 3.16).

The interaction effect between management system and sampling season was statistically significant based on LME models, for SCC only the 1st year of the study, meaning that SCC was influenced by the management system differently for the sampling seasons the 1st year. Specifically, the differences between the two systems were statistically significant only in January and February (Table A.4.11).

Data from both lambing groups. The main effects of management system, lambing period and sampling season on SCC were, based on LME models, statistically significant both years of the study (Table 3.17). SCC were higher in milk from the SI ewes in year 1 in comparison to the values for the EX ewes, but the reverse was found the 2nd year. In both years the EL ewes had 30-40% higher SCC compared with the LL ewes. Moreover, in both years SCC were similar from March to May and significantly increased in June (Table 3.17).

The interaction effect between management systems and sampling month was both years statistically significant for SCC (Table 3.17). The 1st year of the study SCC were higher for the SI ewes between March and May but not in June compared with the EX ewes. Contrary, the 2nd year the SI ewes had lower SCC in April and June. (Figure A.4-6, appendix).Finally, the interaction effect between lambing period and month was statistically significant for SCC only in the 2nd year of the study (Table 3.17). While SCC was similar for

the two lambing periods in March the LL had lower values later in the year (Figure A.4-6, appendix).

3.4.2 Incidences of sub-clinical mastitis and mastitis related pathogens in milk.

No statically significant main effect of management system was found for the examined parameters. The percentage of animals with sub-clinical mastitis (SCC >500,000 from half udder testing and a pathogenic microorganism present) was similar for the two management systems and over both years of the study (~16%, Table 3.21).

Contrary, the main of effect of lambing period was statistically significant for all examined parameters except for the percentage (%) of examined samples that had mixed infections (Table 3.21). Subclinical mastitis was more frequent (~10%) for EL compared with the LL ewes. Both gram positive and negative bacteria were more frequent in the EL ewes compared with the LL ewes (1.7% and 2.1% respectively), while it was more common not to isolate pathogen in the LL ewes.

Finally, the main effect of sampling year was statistically significant for a) the percentage (%) of examined samples with GRAM positive pathogens, b) the percentage (%) of examined samples with GRAM negative pathogens and c) the percentage (%) of examined samples that had mixed infections. In the 1st year it was more common to identify Gram positive bacteria (57% of the examined samples) while the 2nd, in most cases, no pathogen was detected (49.8% of the examined samples). Moreover, the 2nd year Gram negative bacteria were isolated with higher frequencies and mixed infections were more common (Table 3.21).

As for the pathogens detected, the most common was *Staphylococcus* coagulase negative (52%), followed by *Corynebacterium spp. (10%), Staphylococcus* coagulase positive (8%), *E. coli (4%)* and *Streptococcus spp. (4%).* Other microbes identified that are not related with mastitis were *Klebsiella, Pseudomonas, Tetracocci, Dicocci* accounting for around 10% of the examined samples.

parameter assessed	management system (MS)		lamping period (LP)		sampling year (Y)		LME (P-values)						
							Main effects			Interactions			
	semi-intensive *(n=10)*	extensive *(n=10)*	early *(n=20)*	late *(n=20)*	1st	2nd	MS	LP	Y	MS x LP	MS x Y	LP x Y	MS x SM x LP
average % of ewes with SCC> 500,000	30.0 ±2.8	31.1 ±2.9	38.9 ±2.6	22.3 ±2.4	31.5 ±3.4	29.7 ±2.2	ns	***	ns	ns	*[2]	ns	ns
pathogens detected average % within the examined samples													
no pathogenic microorganism	45.3 ±4.0	41.8 ±4.3	42.7 ±2.9	44.4 ±5.1	37.3 ±4.3	49.8 ±3.7	ns	***	P	ns	ns	ns	ns
GRAM positive (animal related) pathogens [a]	34.3 ±4.5	41.8 ±4.8	38.9 ±4.2	37.2 ±5.2	57.0 ±4.3	19.1 ±2.7	ns	***	***	ns	P	P	ns
GRAM negative (environmental) pathogens [b]	13.4 ±2.3	11.4 ±1.9	13.5 ±1.8	11.4 ±2.4	4.1 ±1.3	20.7 ±2.0	ns	***	***	ns	ns	*[3]	ns
both GRAM negative and positive pathogens	4.9 ±1.3	4.0 ±1.0	4.6 ±1.1	4.3 ±1.2	1.3 ±0.6	7.7 ±1.4	ns	ns	***	ns	ns	ns	*

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).
[3]see **Table A.5.7** for interaction means/SE.
[a] *Staphylococcus* coagulase negative and positive, *Corynebacterium*, *Streptococcus spp.*,
[b] Escherichia coli. (*Klebsiella spp.*, *Serratia spp.* and *Pseudomonas spp.* were recorded as other Gram- pathogens)

Table 3.21. Prevalence of subclinical mastitis (SCC > 500,000) and mastitis related pathogens in different management systems (production intensity and lambing period) and sampling years. Means ± standard errors

3.4.3 Faecal egg counts, gastrointestinal parasites and FAMACH record.

In both years gastrointestinal nematodes (GIN) were identified in over 50% of the animals (Figure 3-12) and the extensive flocks tended to have higher infection rates in comparison to the semi-intensive flocks, although this difference was not significant (61 ±4% vs 51 ±4%, p-value 0.62). Moreover, the yearling had higher infection rates compared with the older ewes (61 ±3% vs 49 ±3%, p-value 0.02). However, the parasitic burden was in general low with EPG counts being below100 (eggs per grams of faeces) in most cases, numbers that do not indicate an active infection. High EPG number(> 500) were found only during lambing, characteristic of the periparturient rise (Niezen j.H., 1996).

The EPG counts tended to be higher the 2nd year of the experiment, especially during parturition when EPG counts were twice those observed in the first year (Figure 3-13). In both years, no statistically significant main effect was found of management system on EPG counts of GIN (Table 3.22). However, the main effect of lambing period on EPG counts of GIN was both years statistically significant and the yearlings had higher average numbers of EPG (twice as high) compared with the older ewes (Table 3.22). This difference was steady throughout the year but more profound during the lambing period (Year 1: 399 ±38 vs 256 ±22, $p<0.02$; Year 2: 581±57 vs 295 ±25, $p<0.001$). During milking period recorded statistically significant differences, based on LME models, were also found between the two management systems and the two lambing periods and these may represent existing trends (Table 3.23).

Additionally, the interaction effect between lambing period and management system was statistically significant when the data from only the milking period was examined (Table 3.23). Between the primiparous ewes, the extensive flocks had lower EPG numbers compared with the semi-intensive flocks (Year 1: 54 ±5 vs 77 ±5, $p<0.05$ and Year 2: 66 ±15 vs 153 ±19, $p<0.05$) while no difference was found for the multiparous ewes.

FAMACHA records were significantly affected only by season, increasing from March to April only to drop again the following months (Table 3.23).

In both years the most common species identified were *Trichostrongylus spp.* (65%), Teladorsagia spp. (23%) and *H. contortus* (12%). RDA analysis show little correlation between management characteristics and background climatic conditions with EPG counts, and no correlation between EPG counts and milk composition.

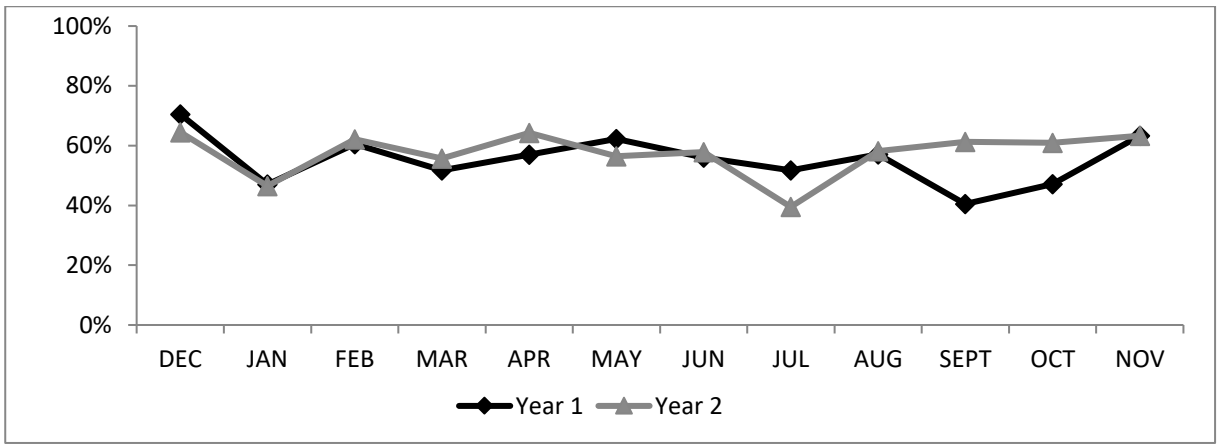

Figure 3-12. Average monthly % percentage of animals with GIN identified* in their faeces the two production years.

*Methods sensitivity is 50 eggs per gram of faeces

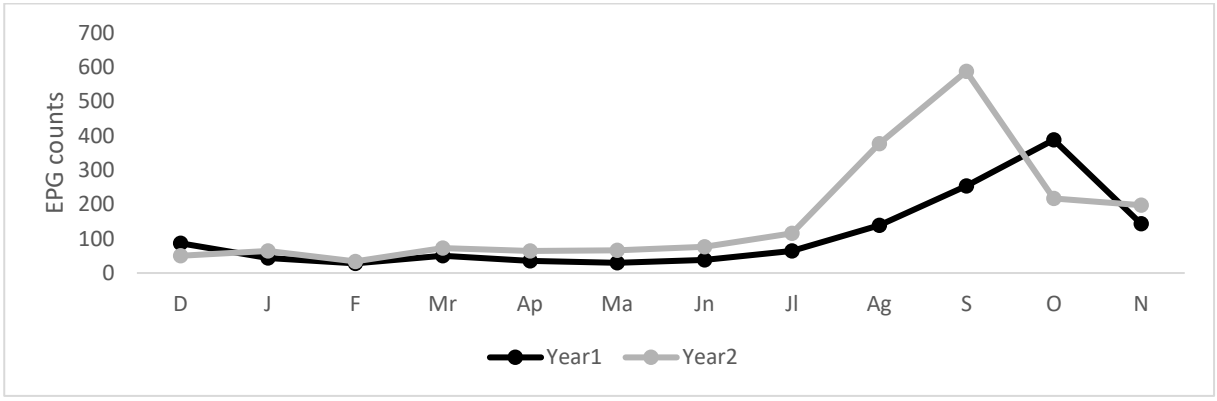

Figure 3-13. Average monthly EPG counts for the two years of the study.

	management system (MS)		lamping period (LP)		LME (P-values)		
					Main effects		Interactions
parameter assessed	semi-intensive *(n=200)*	extensive *(n=200)*	early *(n=200)*	late *(n=200)*	MS	LP	MS x LP
Sampling Year 1							
GI Nematodes (eggs per gram/faeces)	104 ±6	96 ±5	68 ±4	142 ±7	ns	***	ns
Sampling Year 2							
GI Nematodes (eggs per gram/faeces)	144 ±8	122 ±10	88 ±6	196 ±11	P	***	**

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and †, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant)

Table 3.22. Effect of management systems (production intensity) and lambing period on GIN burden. Means ± standard errors

parameter assessed	management system (MS)		lamping period (LP)		sampling season (SS)				ANOVA (P-values)						
									Main effects			Interactions			
	semi-intensive *(n=200)*	extensive *(n=200)*	early *(n=200)*	late *(n=200)*	March	April	May	June	MS	LP	SM	MS x SS	MS x LP	SS x LP	MS x SS x LP
Year 1															
FAMACHA records	1.39 ±0.02	1.39 ±0.02	1.41 ±0.02	1.37 ±0.02	1.35 ±0.03^{b}	1.51 ±0.03^{a}	1.35 ±0.03^{b}	1.32 ±0.03^{c}	ns	ns	***	ns	**[2]	ns	ns
GI Nematodes (eggs per gram/faeces)	49 ±3	38 ±3	21 ±2	66 ±4	51 ±6^{a}	36 ±4^{ab}	29 ±4^{b}	38 ±4^{a}	***	***	***	ns	***	ns	ns
Year 2															
FAMACHA records	1.68 ±0.03	1.71 ±0.03	1.68 ±0.03	1.71 ±0.03	1.52 ±0.04^{c}	1.84 ±0.05^{a}	1.84 ±0.05^{a}	1.66 ±0.05^{b}	ns	ns	***	ns	ns	ns	ns
GI Nematodes (eggs per gram/faeces)	101 ±8	54 ±5	34 ±3	121 ±9	73 ±8^{a}	64 ±10^{b}	66 ±8^{ab}	76 ±14^{a}	***	***	**	*[1]	***[2]	ns	ns

Means differ significantly at ***, P < 0.001; **, P < 0.01; *, P < 0.05; and P, 0.05 < P < 0.10 (trend); ns, P > 0.10 (nonsignificant).
[a-d] Means within a raw with different superscripts letter are significantly different according to Tukey's honestly significant difference test (*P*-values < 0.05).
[1] see **Table A.5.6** for interaction means/SE. [2] see **Table A.5.7** for interaction means/SE.

T**able 3.23.** Effect of management systems (production intensity), sampling season and lambing period on animal health parameters. Means ± standard errors

3.4.4 General health status of the flocks.

The main effect of lambing group (reflecting mainly ewe age) on all recorded diseases was, based on LME models, statistically significant. The LL ewes had lower prevalence of clinical mastitis (~42%), lameness (~51%), pregnancy toxaemia (~43%), ruminal acidosis (~28%), bloat (~16%), diarrhoea (~13%), chronic diseases (~91%) and lower number of causalities (~34%) and obligatory replacement needs (~37%). In contrast, the LL ewes had higher percentages of abortions (~34%), enterotoxaemia and other deaths connected to clostridium infections (~13%), piroplasmosis (~23%), Coenurosis (~102%), ectoparasite infestations (~34%) and contagious ecthyma was almost exclusively a problem for the LL (30.9% infection rate for the LL ewes vs 1.7%% for the EL ewes) (Table 3.24).

Moreover, the main effect of management system was also statistically significant for most recorded diseases. EX ewes had less lameness (~54%), abortions (~43%), pregnancy toxaemia (~64%), ruminal acidosis (~43%), bloat (~56%), diarrhoea (~49%), enterotoxaemia and other deaths connected to clostridium infections (~34%) and lower number of causalities (~14%) and obligatory replacement needs (~14%). On the other hand, ectoparasites and piroplasmosis were higher in the EX systems (46% and 54% respectively) (Table 3.24).

Finally, the main effects of sampling year (reflecting the environmental conditions) on lameness, ruminal acidosis, bloat, diarrhoea, piroplasmosis and ectoparasite infections were, based on LME models, also statistically significant. The recorded differences between year 2 and year 1 were for lameness 9% higher, ruminal acidosis 10% lower, bloat 4% lower, diarrhoea 5% lower, piroplasmosis 13% lower and ectoparasite infections 8% lower. (Table 3.24).

The interaction effects between management system and lambing period were also statistically significant for lameness, pregnancy toxaemia, ruminal acidosis, bloat, diarrhoea, chronic diseases and ectoparasites (Table 3.24).

Additionally, the interaction effects between management system and production year (reflecting the environmental conditions) were statistically significant for clinical mastitis, Coenurosis and ectoparasites (Table 3.24). However, the interaction effect between lambing period and production year was statistically significant only for pregnancy toxaemia (Table 3.24).

parameter assessed	management system (MS)		lamping period (LP)		sampling Year (Y)		ANOVA (P-values)						
							Main effects			Interactions			
	semi-intensive *(n=10)*	extensive *(n=10)*	early *(n=20)*	late *(n=20)*	1st	2nd	MS	LP	Y	MS x LP	MS x Y	LP x Y	MS x SM x LP
Diseases from farm records													
Clinical mastitis	5.2 ±0.43	4.5 ±0.34	6.2 ±0.43	3.6 ±0.19	5.1 ±0.47	4.7 ±0.30	ns	***	ns	ns	ns	*	ns
Lameness	5.6 ±0.27	2.6 ±0.23	5.5 ±0.28	2.7 ±0.25	3.9 ±0.33	4.3 ±0.36	***	***	*	***	ns	ns	ns
Abortions (after 3month)	6.6 ±0.24	3.8 ±0.15	4.4 ±0.24	5.9 ±0.31	5.1 ±0.29	5.2 ±0.31	***	***	ns	P	ns	ns	ns
Pregnancy toxaemia	4.1 ±0.23	1.5 ±0.08	3.6 ±0.31	2.0 ±0.17	2.7 ±0.25	2.9 ±0.30	***	***	ns	***	*	ns	ns
Ruminal acidosis	5.4 ±0.2	3.1 ±0.14	4.9 ±0.26	3.6 ±0.2	4.4 ±0.25	4.0 ±0.25	***	***	**	*[1]	ns	ns	ns
Bloat	7.5 ±0.17	3.3 ±0.14	5.9 ±0.36	5.0 ±0.37	5.3 ±0.37	5.5 ±0.37	***	***	*	*[1]	ns	ns	ns
Diarrhoea	8.5 ±0.16	4.4 ±0.15	6.9 ±0.35	6.0 ±0.37	6.3 ±0.36	6.6 ±0.37	***	***	*	*[1]	ns	ns	ns
Death associated with Clostridium infections	4.0 ±0.15	2.7 ±0.15	3.1 ±0.15	3.6 ±0.21	3.2 ±0.18	3.5 ±0.19	**	**	P	ns	ns	ns	ns
Chronic diseases[a]	2.1 ±0.3	2.8 ±0.4	4.5 ±0.19	0.4 ±0.05	2.4 ±0.35	2.5 ±0.37	ns	***	ns	**[1]	ns	ns	ns
Contagious ecthyma	18.0 ±3.94	14.7 ±2.89	10.7 ±0.45	30.9 ±3.59	16.9 ±3.69	15.8 ±3.23	ns	***	ns	ns	ns	ns	ns
Piroplasmosis	3.0 ±0.17	4.4 ±0.18	3.3 ±0.19	4.0 ±0.21	3.9 ±0.19	3.4 ±0.21	**	***	ns	ns	ns	ns	ns
Coenurosis	1.2 ±0.15	1.8 ±0.17	1.0 ±0.11	2.0 ±0.18	1.4 ±0.17	1.5 ±0.17	P	***	**	ns	*	P	ns
Ectoparasites (ticks, flees, flies etc.)	43.9 ±2.52	67.6 ±1.99	47.7 ±2.43	63.8 ±2.87	53.7 ±2.98	57.8 ±2.89	***	***	ns	*	ns	*	ns
Casualties [b]	12.2 ±0.45	10.4 ±0.46	13.6 ±0.29	9.0 ±0.3	11.2 ±0.48	11.4 ±0.47	*	***	*	**	ns	**	ns
Percentage of obligatory replacements [c]	17.4 ±0.72	15.0 ±0.72	19.8 ±0.55	12.6 ±0.35	16.3 ±0.81	16.1 ±0.67	*	***	ns	ns	ns	ns	ns

Means differ significantly at ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; and †, $0.05 < P < 0.10$ (trend); ns, $P > 0.10$ (nonsignificant).

[a] Progressive Pneumonia (Maedi-Visna), chronic piroplasmosis, paratuberculosis, endoparasites and other diseases connected with gradual weight loss and cachexia

[b] % percentage of ewes of the flock that died due to diseases or injuries

[c] % percentage of ewes of the flock that farmer had to replace with new animals (due to death or severe illness)

Table 3.24. Prevalence of diseases (farm records) in different management systems (production intensity and lambing period) and sampling year. Means ± standard errors

3.4.5 Redundancy analysis. Environmental and agronomic parameters on diseases prevalence, CFU and SCC in milk and EPG of GIN in faeces

In the RDA 28.4% of variation in CFU, SCC and disease prevalence were explained by axis 1 (Figure 3-14).

The strongest drivers identified by the RDA included (a) supplementary concentrate intake (CON, explaining 12.7% of variation, P-value 0.004), (b) time spend grazing on cultivated pastures (GC, explaining 2.8% of residual variation, P-value 0.126), (c) the use of automatic machine milking (AM, explaining 5.1% of residual variation, P-value 0.028), (d) being in the late lambing group (LL, explaining 5.8% of residual variation, P-value 0.012), (e) being in the early lambing group (EL, explaining 5.8% of residual variation, P-value 0.012).

Other drivers included were (a) conserved forage intake (HAY, explaining 1.8% of residual variation, P-value 0.16), (b) hand milking (HM, explaining 0.8% of residual variation, P-value 0.428), (c) the use of semi-automatic machine milking (AM, explaining 0.8% of residual variation, P-value 0.428), (d) the average rainfall (AR, explaining 0.1% of residual variation, P-value 0.734), (e) average altitude of grazing pastures (ALT, explaining <0.1% of variation, P-value 0.89) and (f) the average rainfall (AR, explaining <0.1% of residual variation, P-value 0.808) (Figure 3-14).

The RDA biplot shows positive associations (along the negative axis 1) between concentrate intake and clinical mastitis, CFU in milk, casualties, obligatory replacements and to a lesser extent with lameness, ruminal acidosis and pregnancy toxaemia. Additionally, (along the negative axis 1) there was a positive association between belonging to the early lambing group and chronic diseases (Figure 3-14).

Along positive axis 1 there were positive associations between grazing time on cultivated pastures and belonging to the late lambing group and ectoparasite infections, the average EPG of GIN in faeces, deaths associated with Clostridium infections of gastrointestinal track and contagious ecthyma (Figure 3-14).

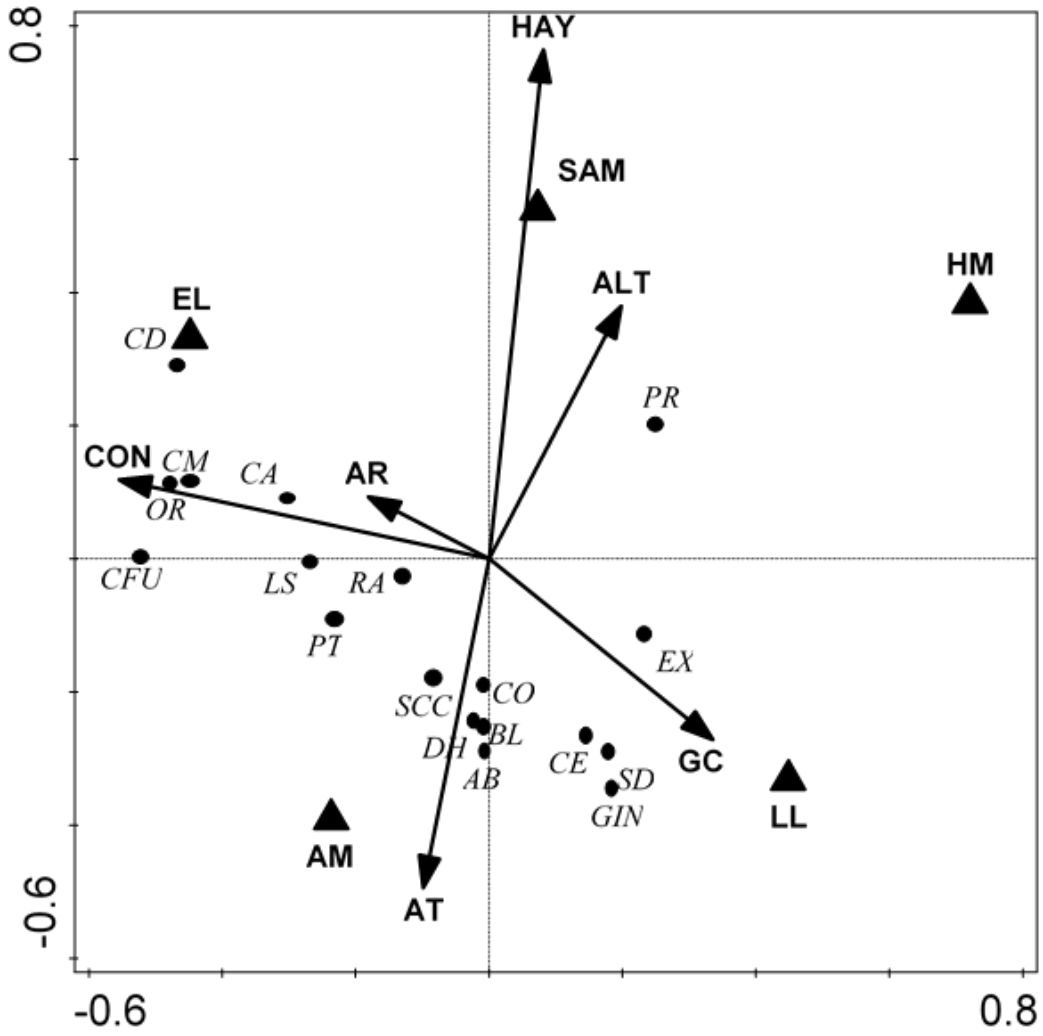

Figure 3-14. Biplot derived from redundancy analyses showing the relationship between the prevalence of diseases the average bacterial load of milk (CFU), the average Somatic Cell Counts in milk and the average number of egg per grams of gastrointestinal nematodes in faeces, lambing period of ewe and agronomic and environmental parameters.

CM: Clinical Mastitis; LS: Lameness;AB: Abortions after the 3rd month; PT: Pregnancy Toxaemia; RA: Ruminal Acidosis; BL: Bloat; DR: Diarrhoea; SD: deaths associated with Clostridium infections of gastrointestinal track; CD: chronic diseases; CE: contagious ecthyma; PR: piroplasmosis; CO: Coenurosis; EX: ectoparasite infections; CA: casualties; OR: obligatory replacement of ewes; CFU: the average bacterial load of milk; SCC: the average Somatic Cell Counts in milk; GIN: the average number of egg per grams of gastrointestinal nematodes in faeces; EL: early lambing; LL: late lambing; GC: grazing time on cultivated pastures; GN: grazing time on natural pastures; ALT: average altitude of grazing pastures; CON: total supplementary concentrate intake; HAY: preserved forage intake; HM: hand milking; SAM: Semi-automatic milking machine; AM: automatic milking machine.

3.4.6 Discussion

Though the technological progress was easily adapted by farmers in their effort to increase productivity, their management practices were not always adapted accordingly and many problems have been met (Stefanakis *et al.*, 2007). Many farmers increased the invested capital (facilities, milking machines), increased feed intake, improved the available pastures, increased flock size and selected high yield animals for breeding while trying to maintain the extensive characteristics of their farm (Hadjigeorgiou *et al.*, 2002). However, the lack of knowledge on the implications these changes have on animal health/welfare and on milk quality has been one of the main chalenges during this transition. Additionally, correct ewe lamb management is regularly ignored and not adapted to the specific needs of an intensified production (Thomas, 2016).

While feeding and grazing regimes clearly differ between the two management systems, the farmers in both systems had similar health management practices. All farmers removed the manure and disinfected the facilities at least once a year, vaccinated against clostridium infections and treated against ectoparasites and gastrointestinal parasites although frequency and drug selection differentiated. Additionally, subclinical mastitis and GI infections, diseases considered by the farmers (as recorded before the study) and the EU (Discontools, acessed 21/04/2017) as significant problems that affect production, did not differ considerably between the two management systems, in contrast to what was anticipated based on the risk factors identified by previous studies (Contreras *et al.*, 2007; Ridler, 2008; Casasús *et al.*, 2012; Taylor, 2013). The prevention measures applied by the SI farmers and the lack of efficient prevention measures from the EX farmers are most likely the reason why recorded infection levels were similar.

In the case of subclinical mastitis around a third of the milk samples were examined for potential active sub-clinical mastitis and for 43% of these samples, mastitis related pathogen was detected. These percentages are similar to those reported in other Mediterranean regions (Lafi, 2006; Contreras *et al.*, 2007; Giadinis *et al.*, 2012). However, while the 1st year of the study the SI ewes had higher SCC and CFU in milk the 2nd year there was no difference for CFU, and SCC were higher in the milk of the EX ewes. Considering the differences in the pathogens prevalence between the two years it is safe to assume that background climatic conditions (rather than management) are the main factor that affects the sub-clinical mastitis (Bergonier *et al.*, 2003; Contreras *et al.*, 2007) and thus the farmers in both system should adapt flock health management each year depending on the annual environmental conditions as done for feeding and grazing regimes.

In contrast to what was anticipated very low EPG of gastrointestinal nematodes were found. Moreover, *Haemonchus contortus*, a significant problem for the flocks in arid regions and an arising crisis in Europe (Jackson and Coop, 2008), was rarely identified in the examined faeces (12%). FAMCHA scores were also generally low (<2), and the recorded seasonal variations can be attributed to changes in ewes nutritional status (Cannas, 2004). In the extensive systems parasitism of GIN is low probably due to the low stocking density and the unfavourable environmental for GIN in the arid natural pastures the animal graze. As for the semi-intensive systems, pastures are mostly fenced, and rotational grazing is a common practice. Furthermore, given the semi-arid conditions the pastures are self-sterilized during summer (Torres-Acosta and Hoste, 2008). These relatively low levels of exposure, justifies the minimal use of anthelmintic drugs by the farmers and make Crete a fitting area for the application of target selective treatment as a way to reduce use of anthelmintic drugs and the rising problem of resistance to them (Cringoli *et al.*, 2009). To our knowledge, our study is the first to report that out of these two expected production limiting diseases, only sub-clinical mastitis is a significant problem for the sheep dairy sector on Crete.

The records obtained from the questionnaires and farm monitoring showed a clear connection between management system and general health status of the flock. The SI farms had higher percentages of causalities among the ewes in comparison to the EX farms, likely due to the greater challenge of diseases that impair animal health and productivity (Stafford and Gregory, 2008). In the SI systems there were more incidences of problems that are predisposed by the combination of intensification of production, intensification of feeding and higher stocking rates. In contrast in the EX systems the predominate diseases- problems are associated with greater exposure of animals to pathogens, less rigorous monitoring and control of risk factors. Specifically, the higher stocking density and the increased time the animals spent inside the stables may be the cause of the more lameness (Raadsma and Egerton, 2013), abortions (after the 3rd month of pregnancy) and contagious ecthyma (Nandi *et al.*, 2011) for the SI flocks while the higher percentage of multiple-pregnancies on the SI farms may have led to higher percentages of pregnancy toxaemia cases (Brozos *et al.*, 2011)

Regarding the differences between the two systems for nutritionally related diseases, more cases of ruminal acidosis and diarrhoea of adult ewes were recorded for the SI farms compared with the EX farms - conditions related to increased concentrates in the ration (Commun *et al.*, 2009; Krehbiel, 2014). Moreover, the ewes in the SI farms grazed more forage on cultivated fields and, in many cases, the natural pastures they used were located inside olive orchards. The growth stage of plants and the consumption of specific plants (eg

sorrel) can explain diarrhoea and bloat in ruminants (Colvin and Backus, 1988; Westendarp, 2006). Some acute problems with ruminal acidosis, bloats or diarrhoea observed in some flocks under both systems were caused by mistakes in feeding that disrupted the balance between forage and concentrate intake and the unilateral use of supplementary feeds (e.g. cereals). Similarly, high rates of feed supplementation along with high production intensity correlate with enterotoxaemia and other Clostridium infections of the gastrointestinal track in sheep (Lewis, 2011), possibly explaining why the SI flocks had more of such infections in this study.

On the other hand the EX flocks had higher percentages of animals with chronic diseases such as progressive pneumonia, chronic piroplasmosis, paratuberculosis and endoparasites, possibly attributed to the less efficient ewe inspection during milking and feeding (Sitzia and Ruiz, 2016). Additionally, the ewes in the EX farms were more exposed to ectoparasites, since they spent more time grazing in natural pastures and less time inside the facilities, thus higher infection rates and incidences of vector borne diseases such as coenurosis (Oryan *et al.*, 2014) and piroplasmosis (Friedhoff, 1997) were recorded. Exposure and animal inspection play an important role in the control of ectoparasites (Plant and Lewis, 2011).

Regarding the two different lambing periods, a clear difference was found between the two groups for most animal health parameters and the prevalence of diseases, possibly due to the age difference between these 2 groups. The EL ewes had higher SCC in the milk and more cases of clinical mastitis. Moreover, more milk samples from this group were examined for sub-clinical mastitis (SCC>500,000) which were more likely to prove positive for pathogens. The age of the ewe is most likely the cause of these differences as recorded and for other studies (de la Fuente *et al.*, 1997; Sevi *et al.*, 2000) although lambing month might also affect the occurrence of sub-clinical mastitis (Stefanakis *et al.*, 1995; Albenzio *et al.*, 2004). Specifically, wrongly balanced feeding regimes high in concentrates that do not take into account ewes' nutritional status (which is different for the two lambing groups) are linked to higher incidences of diseases such mastitis (Contreras *et al.*, 2007). This is also supported by redundancy analysis results, where high level of concentrate intake was identified as a driver correlated with clinical mastitis incidences and CFU in milk.

Differences were also recorded for lameness which was more common for the EL ewes, probably because of the increased time the animals spent inside the stables (Raadsma and Egerton, 2013). Additionally, the older EL ewes tended to have more multiple births resulting higher incidence of pregnancy toxaemia along with the nutritional stress from the

previous lactation (Brozos *et al.*, 2011). Furthermore, lambing (a period nutrient intake is required) of LL ewes coincided with the sprouting of pastures in the spring, resulting in a more higher quality forage and a more suitable forage to concentrate ratio after lambing, thus ruminal acidosis, bloats and diarrhoea were less frequent for the LL ewes (Colvin and Backus, 1988; Westendarp, 2006; Commun *et al.*, 2009).

It was also observed that farmers tended to graze LL ewes on the high-quality pastures (plant density) though the time they accessed the pastures did not differ between the two groups. These practices could be the cause of the slightly higher frequency of enterotoxaemia for the LL ewes, along with an incorrect vaccination practices (time of vaccination) observed for some farms. Imbalances in nutrition of grazing ewes have been linked to intestinal tract clostridium infection (Lewis, 2011) and this is supported by redundancy analysis results where grazing of cultivated pastures and belonging to the late lambing group were correlated with deaths associated with Clostridium infections of the gastrointestinal tract.

Abortions were also more common for the LL ewes, probably caused by the underdeveloped immune protection as has been reported for ewe lambs (Carson, 2017). The underdeveloped immune protection was probably likewise the reason for the relatively high percentages (31%) of contagious ecthyma for the LL ewes, while the problem in older EL ewes was minimal (1.7%), (Nandi *et al.*, 2011).The same would apply for the higher percentages of piroplasmosis and ectoparasite infections found for the LL ewes (Plant and Lewis, 2011). However, it should be considered that LL ewes had been reared purely extensively before lambing with minimal or no time spent in protected facilities, thus the exposure to ectoparasites would be greater. The higher percentage of clinical mastitis along with chronic diseases that predominate the EL ewes result in higher number of casualties and higher replacement rates among these older ewes.

Chapter 4. General Discussion

4.1 Summary of main results

Dairy sheep rearing has been and will continue to be an important economic activity for many countries, especially around the Mediterranean basin, where the ability of small ruminants to utilise low quality forage on marginal areas, gave the population a suitable economic activity and a source of high quality protein (OECD, 2016). While, many efforts have been made to increase productivity sustainability of dairy sheep sector is questioned if production aims are not adjusted accordingly (de Rancourt *et al.*, 2006; Stefanakis *et al.*, 2007).

By comparing the data from paragraphs 3.2.1 & 3.3.1 it can be summarized that intensification achieves its primary goal to increase productivity. In fact the average annual production was double for the semi-intensive farms (**sub-section 3.1.6**) compared with EX and on average the ewes of the SI systems had from 32 to 55% higher milk yield, in both years, regardless lambing period (**sub-section 3.1.6**). Moreover, the recorded annual milk yield for the Sfakiano breed in this study is far higher than that previously reported (Kominakis *et al.*, 2001; Volanis *et al.*, 2002). This was achieved by increasing input (feed) but also through correct management practices, utilizing suitable facilities that promote animal welfare (section 3.1). At the same time the increase in productivity resulted in lower milk fat content for the semi-intensive farms and ewes compared with EX farms and ewes respectively (Morand-Fehr *et al.*, 2007) and the decrease was relevant to the increase in milk yield (Table 4.1).

parameter assessed (EX – SI)	Bulk tank samples	Animal samples			
		early lambing only		both lambing periods	
		1st sampling year	2nd sampling year	1st sampling year	2nd sampling year
Milk yield	-58.7%	-32.4%	-55.3%	-53.4%	-52.3%
Fat content	+10.2%	+2.6%	+7.4%	+7.7%	+6.5%

Table 4.1. Percent difference between the average milk yield and milk fat content of the extensive farms and ewes compared with the semi-intensive farms and ewes

However, other factors related to cheese production such as bacterial counts, milk protein and NFS content (Lucas *et al.*, 2005) were not drastically changed with intensification when the bulk tank milk samples were examined and their seasonal variations were similar for the two systems (section 1.1). That was not the case when the milk samples from individual ewe were examined. The effect of management system on milk compositions and the

observed seasonal changes differed between the two sampling years and the two lambing periods (section 3.3), highlighting the importance that background climatic condition and lambing period have on milk quality traits (Sevi *et al.*, 2004). Moreover, this is an indication that milk measurements at a flock level are not only a practical but also a safer choice to monitor and adjust management practices on daily basis, since there are many more factors to consider when record programs for ewes are planned (de la Fuente *et al.*, 1997).

Additionally, the results indicate that proper management and feeding practices can successfully alleviate the effect of environmental factors on milk quality. For example, the SI ewes tended to have less variations in milk protein and fat content (Figure A. 4 1) compared with the EX ewes, attributed to a more stable diet throughout the season, thus providing a better defence against changes in the environmental conditions for the SI farms (Pulina et al., 2006b). Furthermore, milk microbial load and microbial profile were almost exclusively affected by the background environmental conditions. Thus, good hygiene practices during milking and animal sheltering should be sufficient in order to fortify milk hygiene (Oliver et al., 2005).

Concerning "nutritional quality" (**sub-sections 3.2.2 and 3.3.3**) intensification did affect milk FA profile, increasing total and individual SFA (lauric acid and myristic acid) and decreasing total MUFA, total omega-3 PUFA along with some of the nutritionally desired MUFA (oleic acid) and PUFA (a-linolenic acid and eicosapentaenoic acid). This effect is most likely due to the increased portion of concentrate feed in feeding regimes and due to the composition of the grazed pasture (Addis *et al.*, 2005; Tsiplakou *et al.*, 2008; Nudda *et al.*, 2014; (Cabiddu *et al.*, 2005). Additioannly, the results were consistent between bulk milk samples and ewe milk samples and over the two sampling years (Table 4.2) supporting further the importance that feeding and grazing regimes have for milk F.A. profile manipulation.

However, environmental and climatic conditions had a major contribution to milk FA profile variation making it difficult to quantify and standardize the effect. As a result, for both management systems, enhancement of "nutritionally desired" fatty acids in milk is foremost linked to the proper management of pastures, protecting their sustainability (e.g. protection against overgrazing) and their biodiversity (Morand-Fehr *et al.*, 2007). Additional strategies that may increase total and individual PUFA concentration in sheep milk is the addition of plant or marine oils, vegetable oilseeds or rumen protected or inert lipids in the diet. These are considered established practices for bovine milk (Dewhurst *et al.*, 2006) and have also been successfully applied for milking ewes (Mele *et al.*, 2011; Tsiplakou and Zervas, 2013).

parameter assessed	Bulk tank samples	Animal samples	
		1st Sampling	2nd sampling year
SFA	-3.1%	-4.5%	-3.9%
MUFA	+6.6%	+9.9%	+4.9%
PUFA	ns	+3.8%	+14.2%
omega-3 PUFA	+17.8%	+15.5%	+28.9%
omega-6 PUFA	ns	ns	+10.3%
omega-6/omega-3	ns	-0.25	ns
C12:0	-17%	-34.7%	-17.3%
C14:0	-9%	-14.6%	-11.3%
C16:0	+2%	ns	ns
C18:0	+10%	+23.2%	-
C18:1 cis9	+10%	+14.1%	+4.6%
C18:1 *trans11*	-12%	+7.5%	+8.2%
C18:2*cis9.cis12*	ns	ns	+9.8%
C18:3*cis9.cis12.cis15*	+73%	+28.8%	+36.5%
CLA*cis9.trans11*	ns	ns	+5.4%
C20:5 n-3	+24.6%	+21.7%	+24.6%
C22:5 n-3	+18.5%	ns	+23.8%
C22:6 n-3	ns	+10.2%	+26.2%

Table 4.2. Percent difference between the concentration of individual fatty acids in milk fat of the extensive farms and ewes compared with the semi-intensive farms and ewes

As regards to animal health and flock general health status (**sub-section 3.4.4**) there were diseases that pose a similar threat to both systems (e.g. clostridium infections) and diseases that each system had different exposure levels. It should be noted that the SI flocks tended to have higher prevalence for most of the examined diseases. Any veterinary regime designed should take into consideration these differences and the greater risk from intensification and farmers should protect their animals from the specific hazards (DEFRA, 2013). In our study the farmers of both systems followed similar veterinary regimes that were "perceived" as appropriate for Crete and their region without taking into consideration possible differences with system. Guidance from a veterinarian was inquired mainly on outbreaks or severe problems. Though background environmental conditions (year to year differences) did not affect the prevalence of most of the recorded diseases or treatments, in contrast to what was found for subclinical mastitis and GIN infections, disease related to feeding regimes (e.g. bloat, diarrhoea) or facilities hygiene (Lameness) should also identify climate as a potential risk factor (Nieuwhof and Bishop, 2005)

In the case of mastitis (clinical and subclinical) (**sub-sections 3.4.1, 3.4.2, 3.4.4**) both systems had similar infection levels. However, the underlying causes vary between the systems (mainly due to poor feeding practices) and from year to year (e.g. exposure to different pathogens, impact on animal welfare from extreme conditions) (Contreras *et al.*, 2007). Unlike the current situation, each system should establish appropriate veterinary regimes and management practices (e.g. regular animal inspection, udder disinfection, facilities disinfection, isolation of sick animals) for mastitis prevention and treatment

measures. This will minimize loses (yield drop, sick animals) and also reduce the use of antibiotics, a significant food hazard (DEFRA, 2013).

Regarding gastrointestinal nematode infections (**sub-section 3.4.3**), though the problem is well handled by the famers in Crete and the burden is low, the fact that background environmental conditions and age of the ewe significantly affects the burden favours the application of practices such as target selective treatment (Hoste *et al.*, 2002) combined with simple parasitological examinations to minimize the use of anthelmintic, thus lessening the expenses and fortifying against resistance development. Special consideration should be taken for periparturient rise of EPG counts (Niezen j.H., 1996), which our study confirmed, and with the application of preventive practices (treatment, use of natural anthelmintic) the low burden will be further protected (Torres-Acosta *et al.*, 2012).

Finally, examining differences between the two lambing periods (**sections 3.3 and 3.4**) it was clear that age of ewe in combination with the different lambing season (and hence diets) result in the production of milk with different composition. Some of these effects can be attributed to age (e.g. milk lactose content, linoleic acid concentration in milk) and some to the fact that the animals are in a preliminary stage in lactation (e.g. milk protein and fat content) (Sevi *et al.*, 2000). However, in the case of diseases the greater susceptibility to sub-clinical mastitis and GIN infections was clearly demonstrated and the farmers should differentiate their management accordingly (Sevi *et al.*, 2000; Taylor, 2013). For example, younger animlas are likely to require control exposure to parasites and boosted feeding regimes in order to develop immunity. This can be achieved by grazing of contaminated pastures together with the use of feed such as sainfoin that is known to help control GIN burden (Hoste *et al.*, 2015). On the other hand, the older ewes are more susceptible to chronic diseases and diseases that are related to time exposure to hazard (clinical mastitis, lameness).

4.2 Conclusions

By increasing milk yield and stabilizing milk composition, thus adding process quality, the SI farms provide a more preferable products for the larger cheese factories where production optimization and product quality are the main demands (Raynal-Ljutovac *et al.*, 2005). However, dependence on imported feeds is a great concern regarding the sustainability (economic and environmental) of the SI flocks (Stefanakis *et al.*, 2007). Self-production of forage and access to high quality pastures is a starting point but other cheap resources of nutrients, preferably locally produced, should be incorporated to the feeding regimes. If carefully selected, these feeds can also provide additional benefits to counter the unwanted effects of intensification.

Such an example are some agriculture by products e.g. olive leaves which further than providing nutrients they also enhance beneficial for human health FA (Zervas and Tsiplakou, 2011). Another example are the locally produced carob fruits which also have a protective effect against GIN (Hoste and Torres-Acosta, 2011). Finally, there are also by-products of food industries such as orange pulp and milk whey that have been previously successfully incorporated to sheep rations, are cheap reassures of carbohydrates (energy) and their use as feed provide also an environmental benefit, since otherwise these products would have been disposed to the environment (Vasta *et al.*, 2008) . Furthermore, the SI farms should adapt or maintain pasture management practices that were traditionally in place such as rotational grazing and revegetation protection (Kairis *et al.*, 2015). Proper understanding and utilization of the available pastures is the first step in targeting for a more preferable milk FA profile (Dewhurst *et al.*, 2006).

On the other hand the EX farmers produce milk which is already perceived by the consumers in Greece and other countries as of higher "nutritional quality" with stronger aromas and flavours (though cheese processing and especially ripening has an also a great effect to these characteristics) (Sanchez-Villegas *et al.*, 2003). These beliefs can be further enhanced by adding the clear effect that intensification has in milk FA profile. That will give another advantage to the EX farmers for claiming an "added value" to their milk (Zervas and Tsiplakou, 2011). However, since environmental conditions, pasture availability and compositions have a major effect to milk FA profile, any claim is difficult to be supported with consistent evidences. Thus a clearly defined and predetermined management protocol (e.g. lambing season, concentrate intake, forage intake, grazing time) and season specifications (e.g. milk only from May and June when animals graze on the mountains) are required to back it up (Volanis *et al.*, 2007). Such practices will also help farm sustainability since now background environmental conditions have a major effect on overall productivity (annual milk production may vary greatly from year to year).

In cases where an increase of grazing time is necessary (as portion in energy intake) and the use of concentrate feed and bought forage will subsequently decrease, the production of milk and milk products with low energy fingerprint will also be achieved. This is an additional potential claim, above a social, legal and ethical obligation for protection against climatic change (Allen *et al.*, 2014).

The correct management practices for both systems will combine a) animal breeding in facilities that promote animal welfare, (the appropriate animal in the appropriate facilities) b) feeding regimes with a balanced in concentrate to forage ratio and c) adequate grazing

time, utilizing the available pastures (relatively inexpensive source of energy and nutrients) in the best possible way, through a holistic management approach. This will be achieved by examining first in depth a) the characteristics of the natural resources available (pasture, water, climatic conditions), b) the characteristics of the animal that will utilize these resources (breed, age, needs in nutrients, production traits), the facilities that are necessary for production optimization (sheltering, milking, handling, storage) d) the culture and the potential of the people that will have to apply these practices and e) the expectations of the consumer.

Considering pasture management, in semi-arid environments, such the one in Crete and the other para-Mediterranean areas, where rainfalls are limited to few days a year coexistence of sheep and pastures should evolve to an symbiosis that will provide benefits for the farmer (energy, nutrients), the consumer (bioactive compounds in milk) and the local society (environmental protection) (Hadjigeorgiou *et al.*, 2005). In particular in these areas grazing of pastures is a necessary protective measure against fires that provides the farmers with a cheap source of natural resources (energy, nutrients, water) (Molle *et al.*, 2004) and also adds "nutritional value" to milk and milk products, by enhancing substances that promote or protect human health (Cabiddu *et al.*, 2005).

Prevalence of diseases in farms, as recorded in the questionnaires, was a affected by a combination of factors (including feeding regimes, grazing regimes, background environmental conditions, age of ewe) thus in order for a successful prevention management protocol to be established all factors should be taken into account. For some diseases preventive vaccination (e.g. for clostridium infections) in a suitable solution for all flocks(Lewis, 2011). For other target selective preventive treatment (e.g. against parasites or lamb mortality) is most likely the best solution (Hoste and Torres-Acosta, 2011) and an almost obligatory practice for protection against anthelmintic resistance. This will go along with management practices (rotational grazing, low stocking rates) which were applied by farmers in our studies and resulted in low EPG counts.

However, for mastitis and other diseases where farm management has an important role, management planning and a basic risk analysis is the preferable solution (Contreras *et al.*, 2007). For example in the case of subclinical mastitis (a major problem for milk producing ewes) the farmer should first take into account the environmental conditions (higher rainfalls will increase the risk of infection with environmental pathogens such as *E.coli*). Afterwards they should adapt feeding regimes accordingly to keep a suitable concentrate to forage ratio (higher ratios as predispose factor for subclinical mastitis). Furthermore, sick animals should be kept separately to prevent the dispersion of the pathogen,

applying proper treatment protocols and when there are indications of subclinical mastitis (e.g. milk drop, fever, high SCC in milk) preventive treatments such as udder disinfection can protect the flock a further spread of the problem.

Moreover, depending on the management systems applied each farmer should be aware of the different diseases their animals are exposed to (EFSA Panel on Animal Health and Welfare, 2014). For example, the SI farms tended to have higher prevalence diseases related with inappropriate feeding practices, resulting in more casualties and need for replacement ewes. Thus, the farmers should be more alert when choosing their feeding and grazing regimes, taking into consideration the background climatic conditions in order to avoid digestive disorders. On the other hand, the EX farmer should be aware that the lack of facilities (e.g. stables, milking facilities) makes it difficult to identify, monitor and treat sick animals, thus correct application of preventive treatments and vaccinations is necessary.

Considering our results, the application of training programs targeting farmers, veterinarians, animal technicians and state agencies is a necessity. This will help all those involved in sheep rearing to consider in their practices the predisposing factors for each system in order to have an optimum result in animal welfare/health and productivity (Stefanakis *et al.*, 2007).

Our results showed a diversion of the produced milk from the two different lambing periods over both years, caused either the different stage of lactation or the age of the animal (Kelsey *et al.*, 2003; Sevi *et al.*, 2004). These findings strengthen the importance of having two lambing periods where 65-70% of the ewe lambs in autumn and 30-35% of the flock lambs in winter. As a result, the milk produced from the flock has a relative stable composition (process quality) throughout the milking season. This is demanded by the cheese factories since a stable composition will help to the production of milk products with stable quality (and yield). Only when process quality is stabilized can any measure be taken for nutritionally quality fortification, by applying grazing strategies. By keeping the two lambing groups separately and applying different feeding and veterinary regimes the farmers a) decrease the prevalence of diseases to their flocks (mastitis and GIN) and b) provided as more stable milk yield and milk composition which is of great significance since after March yearlings produce a great portion of flocks' milk.

APPENDIX A – Additional tables and figures

parameter assessed	grazing time on:		total concentrate	conserved forage used per animal:		
	natural pasture	improved pasture		total	whole-crop oat	alfalfa
	ha day^{-1}	ha day^{-1}	g day^{-1}animal^{-1}	g day^{-1}	g day^{-1}	g day^{-1}
management system						
semi-intensive *(n=10)*	3.5 ±0.4	2.6 ±0.1	934 ±31	183 ±21	68 ±13	115 ±17
extensive *(n=10)*	6.6 ±0.4	0.4 ±0.1	712 ±40	267 ±29	25 ±9	242 ±28
sampling season						
January	2.0 ±0.4^{e}	1.2 ±0.2^{cd}	1036 ±40^{a}	473 ±37^{a}	126 ±27^{a}	347 ±45^{a}
February	2.4 ±0.5^{de}	1.7 ±0.3^{abc}	1017 ±42^{a}	423 ±4 ^{a}	104 ±26^{a}	319 ±45^{ab}
March	3.4 ±0.5^{d}	2.2 ±0.3 ^{a}	942 ±52^{a}	263 ±39^{b}	23 ±17^{b}	241 ±38^{b}
April	5.5 ±0.6^{c}	2.0 ±0.3^{ab}	738 ±61^{b}	103 ±26^{c}	21 ±13^{b}	82 ±24^{c}
May	7.7 ±0.6^{b}	1.3 ±0.3^{bcd}	584 ±68^{c}	58 ±34^{d}	0 ±0^{b}	58 ±34^{c}
June	9.6 ±0.6^{a}	0.6 ±0.2^{d}	551 ±66^{c}	10 ±7^{e}	3 ±3^{b}	7 ±7^{c}
sampling year						
first	4.6 ±0.4	1.5 ±0.2	952 ±34	290 ±26	79 ±14	212 ±25
second	5.5 ±0.4	1.5 ±0.2	698 ±36	159 ±23	14 ±6	144 ±22
LME (*P*-values)						
main effects						
management system (MS)	<0.001	<0.001	<0.001	0.002	<0.001	<0.001
sampling season (SS)	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001
sampling year (Y)	0.005	ns	<0.001	<0.001	<0.001	0.009
Interactions						
MS x SS	<0.001[1]	0.018[1]	<0.001[1]	ns[1]	0.008	0.004
MS x Y	ns	ns	ns	ns	0.037[2]	ns
SS x Y	ns	ns	ns	ns	<0.001[3]	0.013[3]
MS x SS x Y	ns	ns	ns	ns	0.028	ns

[a-e] Means within a column with different superscripts are significantly different (*P*-values < 0.05).
[1] see **Figure3.1** for interaction means/SE
[2] **Table A3** reports interaction means/SE.
[3]**Table A4** reports interaction means/SE.

Table A.4.3. Grazing regimes, supplementary concentrate, and conserved forages used in different management systems (production intensity), by sampling season and sampling year**.** Means ± standard errors

sampling season factors	January	February	March	April	May	June
lactose (g 100ml^{-1} milk)						
semi-intensive *(n=10)*	4.77 ±0.04A^{ab}	4.79 ±0.03A^{ab}	4.72 ±0.07A^{b}	4.82 ±0.05A^{ab}	4.91 ±0.04A^{a}	4.85 ±0.04A^{ab}
extensive *(n=10)*	4.86 ±0.05A^{a}	4.83 ±0.05A^{a}	4.78 ±0.06A^{ab}	4.74 ±0.08A^{ab}	4.65 ±0.05B^{b}	4.25 ±0.15B^{c}

$^{a-c}$ Means within a row with different superscripts are significantly different (*P*-values < 0.05).
A-B Means within a column with different superscripts are significantly different (*P*-values < 0.05).

Table A.4.4. Interaction means and standard errors for milk lactose content for the different management systems and sampling month. Means ± standard errors

	Sampling Year 1		*Sampling Year 2*	
parameter assessed	semi-intensive *(n=10)*	extensive *(n=10)*	semi-intensive *(n=10)*	extensive *(n=10)*
	1	2	3	4
whole-crop oat (g day^{-1})	112 ±22^{a}	45 ±16^{b}	24 ±10^{b}	4 ±4^{b}
FA (g 100ml^{-1} of total FA)				
PUFA	5.13 ±0.11^{c}	5.00 ±0.13^{c}	6.33 ±0.09^{b}	6.69 ±0.19^{a}
n-3PUFA	0.74 ±0.04^{c}	0.83 ±0.07^{c}	1.20 ±0.04^{b}	1.50 ±0.09^{a}
C12:0	5.70 ±0.15^{a}	4.62 ±0.18^{b}	4.15 ±0.16^{c}	3.55 ±0.13^{d}
C16:0	28.28 ±0.26^{b}	29.24 ±0.31^{a}	26.06 ±0.24^{c}	26.14 ±0.22^{c}
C18:1 *cis 9*	17.69 ±0.32^{c}	20.41 ±0.53^{b}	20.87 ±0.51^{ab}	22.08 ±0.44^{a}
C18:3*cis9.cis12.cis15*	0.48 ±0.03^{d}	0.59 ±0.06^{c}	0.61 ±0.03^{b}	0.89 ±0.07^{a}
EPA	0.03 ±0.00^{b}	0.06 ±0.00^{a}	0.06 ±0.00^{a}	0.07 ±0.00^{a}

$^{a-c}$ Means within a row with different superscripts are significantly different (*P*-values < 0.05).

Table A.4.5. Interaction means and standard errors for total PUFA, omega-3 PUFA, lauric acid (C12:0), palmitic acid (C16:0), oleic acid (C18:1 cis 9), a-Linolenic acid (C18:3cis9.cis12.cis15), and eicosapentaenoic acid (EPA) in milk fat from two different management systems and two contrasting sampling years. Means ± standard errors

sampling season parameter assessed	Average of both management systems (n=20) January	February	March	April	May	June
whole-crop oat (g day^{-1})						
Sampling Year 1	196 ±40A^{a}	183 ±45A^{a}	45 ±33A^{b}	43 ±25A^{b}	0 ±0B^{c}	6 ±6B^{c}
Sampling Year 2	55 ±27B^{a}	30 ±17B^{a}	0 ±0B^{b}	0 ±0B^{b}	0 ±0B^{b}	0 ±0B^{b}
alfalfa (g day^{-1})						
Sampling Year 1	326 ±60A^{a}	299 ±66A^{a}	353 ±56A^{a}	148 ±43A^{b}	115 ±67A^{bc}	14 ±14A^{c}
Sampling Year 2	369 ±69A^{a}	338 ±63A^{a}	129 ±38B^{b}	15 ±10B^{c}	0 ±0B^{c}	0 ±0A^{c}
C16:0						
Sampling Year 1	27.67 ±0.32A^{b}	27.66 ±0.41A^{b}	28.24 ±0.32A^{b}	27.97 ±0.31A^{b}	30.43 ±0.66A^{a}	30.87 ±0.37A^{a}
Sampling Year 2	26.14 ±0.36B^{ab}	25.65 ±0.50B^{ab}	25.87 ±0.34B^{ab}	25.55 ±0.52B^{b}	26.74 ±0.31B^{a}	26.68 ±0.24B^{a}
CLA*cis9.trans11*						
Sampling Year 1	0.79 ±0.06B^{c}	0.79 ±0.06B^{c}	0.96 ±0.05B^{ab}	0.97 ±0.06B^{ab}	1.06 ±0.10A^{a}	0.86 ±0.04B^{bc}
Sampling Year 2	0.95 ±0.07A^{c}	1.17 ±0.07A^{ab}	1.17 ±0.06A^{ab}	1.29 ±0.07A^{a}	1.09 ±0.05A^{bc}	1.06 ±0.05A^{bc}

$^{a-c}$ Means within a row with different superscripts are significantly different (*P*-values < 0.05).
A-B Means within a column with different superscripts are significantly different (*P*-values < 0.05).

Table A.4.6. Interaction means and standard errors for whole-crop oat and alfalfa feed fed and palmitic acid (C16:0) and rummenic acid (CLA*cis9.trans11*) concentrations in milk fat from two different milking season and different sampling season. Means ± standard errors

sampling season parameter assessed	January	February	March	April	May	June
body condition score						
semi-intensive *(n=200)*	2.15 ±0.05 A^{d}	2.11 ±0.04 B^{d}	2.57 ±0.06 A^{c}	2.91 ±0.07 A^{b}	3.10 ±0.06 A^{a}	2.90 ±0.07 A^{b}
extensive *(n=200)*	2.19 ±0.04 A^{d}	2.31 ±0.05 A^{cd}	2.44 ±0.06 A^{c}	2.58 ±0.06 B^{b}	2.77 ±0.0 B^{a}	2.54 ±0.06 B^{b}

$^{a-c}$ Means within a row with different superscripts are significantly different (*P*-values < 0.05).
A-B Means within a column with different superscripts are significantly different (*P*-values < 0.05).

Table A.4.7. Interaction means for the effects of management system and sampling months on Body condition score. Means ± standard errors

parameter assessed	semi-intensive		extensive	
	early lambing *(n=100)*	late lambing *(n=100)*	early lambing *(n=100)*	late lambing *(n=100)*
			Sampling Year 1	
FAMACHA records	1.45 ±0.03[a]	1.33 ±0.02[c]	1.36 ±0.02[b,c]	1.41 ±0.03[a,b]
milk pH	6.70 ±0.01[a]	6.66 ±0.01[b]	6.70 ±0.01 [a]	6.70 ±0.01[a]
milk fat (g 100ml^{-1})	5.93 ±0.05 [b]	5.10 ±0.06 [c]	6.14 ±0.06 [a]	5.80 ±0.08 [b]
fatty acids (g 100 g^{-1}of total FA)				
C18:1 *trans11*	1.46 ±0.06[b]	1.50 ±0.06[b]	1.39 ±0.06[b]	1.80 ±0.08[a]
CLA *cis9.trans11*	1.12 ±0.04[a]	1.02 ±0.03[b]	1.06 ±0.03[a,b]	1.12 ±0.04[a]
omega-6/ omega-3 ratio	3.62 ±0.10[a]	3.65 ±0.11[a]	3.67 ±0.19[a]	3.10 ±0.09[b]
			Sampling Year 2	
total concentrate (g animal^{-1} day^{-1})	726 ±21[a]	822 ±26[b]	457 ±28[c]	447 ±28[c]
body condition score	2.98 ±0.04[a]	2.03 ±0.03[c]	2.59 ±0.04[b]	1.98 ±0.03[c]
milk lactose (g 100ml^{-1})	4.24 ±0.04 [c]	4.81 ±0.03 [a]	4.29 ±0.05 [c]	4.58 ±0.04 [b]
milk non-fat solids (g 100ml^{-1})	10.49 ±0.05 [b]	10.74 ±0.04 [a]	10.75 ±0.05 [a]	10.78 ±0.05 [a]
fatty acids (g 100 g^{-1}of total FA)				
C18:3*cis9.cis12.cis15*	0.69 ±0.01[c]	0.78 ±0.02[b]	1.19 ±0.05[a]	1.12 ±0.04[a]
omega-3 PUFA	1.27 ±0.03[c]	1.48 ±0.04[b]	1.95 ±0.07[a]	1.93 ±0.06[a]

[a-c] Means within a row with different superscripts differ (*P*-values < 0.05).

Table A.4.8. Interaction means of feeding regimes, animal health parameters and milk compositions for the two different management systems and the two lambing periods. Means ± standard errors

	March		May	
parameter assessed	semi-intensive *(n=100)*	extensive *(n=100)*	semi-intensive *(n=100)*	extensive *(n=100)*
			Sampling Year 1	
fatty acids (g 100 g^{-1}oftotal FA)				
C14:0	12.64 ±0.16^{b}	11.33 ±0.14^{c}	13.30 ±0.13^{a}	11.31 ±0.15^{c}
C18:1 *trans11*	1.22 ±0.05^{c}	1.70 ±0.08^{b}	1.73 ±0.06^{a}	1.50 ±0.06^{b}
C18:1 *cis9*	16.08 ±0.32^{c}	17.77 ±0.25^{b}	16.72 ±0.21^{c}	20.47 ±0.36^{a}
C18:3*cis9.cis12.cis15*	0.56 ±0.02^{b}	0.59 ±0.02^{b}	0.57 ±0.02^{b}	1.02 ±0.04^{a}
CLA *cis9.trans11*	0.94 ±0.03^{c}	1.11 ±0.04^{b}	1.20 ±0.04^{a}	1.07 ±0.03^{b}
EPA	0.046 ±0.002^{c}	0.050 ±0.002^{c}	0.061 ±0.003^{b}	0.088 ±0.004^{a}
omega-3 PUFA	1.15 ±0.03^{c}	1.11 ±0.04^{c}	1.26 ±0.04^{b}	1.75 ±0.06^{a}
omega-6/omega-3 ratio	3.54 ±0.09^{b}	4.04 ±0.18^{a}	3.72 ±0.12^{ab}	2.69 ±0.08^{c}
			Sampling Year 2	
fatty acids (g 100 g^{-1}oftotal FA)				
C16:0	25.87 ±0.19^{bc}	25.59 ±0.19^{c}	26.03 ±0.12^{ab}	26.47 ±0.12^{b}
C18:0	7.66 ±0.19^{c}	8.35 ±0.16^{b}	10.34 ±0.17^{a}	10.00 ±0.13^{a}
C18:1 *cis9*	16.87 ±0.23^{c}	18.42 ±0.23^{b}	20.70 ±0.21^{a}	21.04 ±0.21^{a}
C18:2 *cis9.cis12*	2.50 ±0.05^{c}	2.98 ±0.06^{a}	2.63 ±0.04^{b}	2.70 ±0.04^{b}
C18:3*cis9.cis12.cis15*	0.75 ±0.02^{b}	0.77 ±0.03^{b}	0.72 ±0.01^{b}	1.55 ±0.04^{a}
EPA	0.052 ±0.002^{c}	0.049 ±0.001^{bc}	0.046 ±0.001^{b}	0.081 ±0.001^{a}
DPA	0.107 ±0.003^{b}	0.103 ±0.003^{c}	0.110 ±0.002^{b}	0.185 ±0.003^{a}
DHA	0.030 ±0.001^{b}	0.032 ±0.001^{b}	0.033 ±0.001^{b}	0.053 ±0.001^{a}
MUFA	25.53 ± 0.24^{d}	27.50 ±0.26^{c}	28.86 ±0.21^{b}	29.72 ±0.23^{a}
PUFA	6.98 ± 0.10^{b}	7.27 ±0.14^{b}	6.53 ±0.05^{c}	8.50 ±0.12^{a}
omega-3 PUFA	1.51 ± 0.04^{b}	1.39 ±0.05^{c}	1.24 ±0.02^{c}	2.52 ±0.06^{a}
omega-6/ omega-3 ratio	2.60 ± 0.07^{c}	3.64 ±0.15^{a}	3.00 ±0.06^{b}	1.68 ±0.04^{d}

$^{a-c}$ Means within a row with different superscripts differ (*P*-values < 0.05).

Table A.4.9. Interaction means for the two different management systems and the two sampling months. Means ± standard errors

parameter assessed	March		May	
	early lambing *(n=100)*	late lambing *(n=100)*	early lambing *(n=100)*	late lambing *(n=100)*
	Sampling Year 1			
fatty acids (g 100 g^{-1}oftotal FA)				
C12:0	5.72 ±0.11[a]	5.20 ±0.12[b]	4.66 ±0.11[c]	4.61 ±0.11[c]
C14:0	12.85 ±0.14[a]	11.11 ±0.15[c]	12.74 ±0.15[a]	11.91 ±0.15[b]
C16:0	26.54 ±0.24[ab]	24.94 ±0.22[c]	26.77 ±0.20[a]	26.15 ±0.22[b]
C18:1 *trans11*	1.28 ±0.06[b]	1.65 ±0.07[a]	1.58 ±0.05[a]	1.66 ±0.07[a]
C18:1 *cis9*	16.23 ±0.27[c]	17.63 ±0.31[b]	18.58 ±0.31[a]	18.55 ±0.34[a]
C18:3*cis9.cis12.cis15*	0.50 ±0.01[c]	0.65 ±0.02[b]	0.80 ±0.03[a]	0.79 ±0.03[a]
DPA	0.079 ±0.003 [c]	0.102 ±0.004 [b]	0.163 ±0.006 [a]	0.168 ±0.005 [a]
SFA	70.77 ±0.34[a]	67.53 ±0.37[b]	66.06 ±0.40**[c]**	65.60 ±0.40**c**
MUFA	23.13 ±0.28[c]	25.53 ±0.32[b]	26.76 ±0.31[a]	27.04 ±0.32[a]
PUFA	6.10 ±0.11[c]	6.94 ±0.10[b]	7.18 ±0.13[ab]	7.35 ±0.12[a]
omega-3 PUFA	0.99 ±0.03[c]	1.26 ±0.04[b]	1.48 ±0.06[a]	1.51 ±0.05[a]
omega-6 PUFA	3.42 ±0.07[b]	3.92 ±0.07[a]	3.90 ±0.07[a]	4.10 ±0.07[a]
omega-6/ omega-3 ratio	4.06 ±0.17[a]	3.52 ±0.10[b]	3.21 ±0.11[b]	3.22 ±0.11[b]
	Sampling Year 2			
fatty acids (g 100 g^{-1}oftotal FA)				
C14:0	12.27 ±0.14[a]	11.16 ±0.13[b]	11.15 ±0.11[b]	10.76 ±0.09[c]
C18:1 *trans11*	1.65 ±0.06[c]	2.26 ±0.07[a]	1.59 ±0.03[c]	1.97 ±0.05[b]
C18:2 *cis9.cis12*	2.60 ±0.06[b]	2.89 ±0.05[a]	2.70 ±0.04[b]	2.64 ±0.04[b]
C18:3*cis9.cis12.cis15*	0.70 ±0.02[d]	0.82 ±0.03[c]	1.18 ±0.04[a]	1.08 ±0.04[b]
DPA	0.097 ±0.003[c]	0.112 ±0.003[b]	0.148 ±0.004[a]	0.146 ±0.004[a]
DHA	0.028 ±0.001[c]	0.034 ±0.001[b]	0.044 ±0.001[a]	0.041 ±0.001[a]
SFA	66.99 ±0.35[a]	65.72 ±0.32[b]	62.98 ±0.28[c]	63.45 ±0.27[c]
PUFA	6.73 ±0.14[b]	7.52 ±0.11[a]	7.51 ±0.12[a]	7.49 ±0.12[a]
omega-3 PUFA	1.32 ±0.04[c]	1.58 ±0.05[b]	1.91 ±0.07[a]	1.82 ±0.06[a]
omega-6 PUFA	3.55 ±0.07[b]	3.84 ±0.06[a]	3.71 ±0.05[a,b]	3.63 ±0.05[b]

[a-c] Means within a row with different superscripts differ (*P*-values < 0.05).

Table A.4.10. Interaction means for the different lamping periods and the two sampling months. Means ± standard errors

sampling season parameter assessed	January	February	March	April	May	June
			Sampling year 1			
milk pH						
semi-intensive *(n=100)*	6.69 ±0.01Ac	6.71 ±0.01Ab	6.72 ±0.01Ab	6.75 ±0.01Ba	6.75 ±0.02Aa	6.74 ±0.02Ba
extensive *(n=100)*	6.65 ±0.01Be	6.70 ±0.01Ac	6.67 ±0.01Bd	6.78 ±0.02Ab	6.71 ±0.02Bc	6.89 ±0.03Aa
CFU in milk						
semi-intensive *(n=100)*	17.1 ±0.1Aa	12.6 ±0.1Aa	18.0 ±0.1Aa	16.7±0.1Aa	21.1 ±0.1Aa	15.1 ±0.1Aa
extensive *(n=100)*	12.3 ±0.1Bb	16.0 ±0.1Ab	13.2 ±0.1Bb	11.5 ±0.1Bb	11.8 ±0.1Bb	15.9 ±0.1Aa
			Sampling year 2			
CFU in milk						
semi-intensive *(n=100)*	14.0 ±0.1Ab	20.8 ±0.1Aa	12.9 ±0.1Ab	16.6 ±0.1Bb	10.7 ±0.1Ab	12.8 ±0.1Bb
extensive *(n=100)*	13.9 ±0.1Ab	15.5 ±0.1Ab	14.1 ±0.1Ab	24.7 ±0.1Aa	11.7 ±0.1Ab	19.8 ±0.1Aa
milk SCC						
semi-intensive *(n=100)*	179±1Ab	244 ±4Aa	176 ±1Ab	199 ±1Aab	196 ±2Aab	245 ±2Aa
extensive *(n=100)*	104±1Bc	137 ±1Bc	134 ±1Ac	183 ±2Ab	159 ±1Ab	262 ±A4a

[a-c] Means within a row with different superscripts are significantly different (*P*-values < 0.05).
A-B Means within a column with different superscripts are significantly different (*P*-values < 0.05).
CFU: Colony Forming Units. SCC: Somatic Cell Count

Table A.4.11. Interaction means and standard errors for milk pH, SCC and CFU content for the different management systems and sampling month. Means ± standard errors

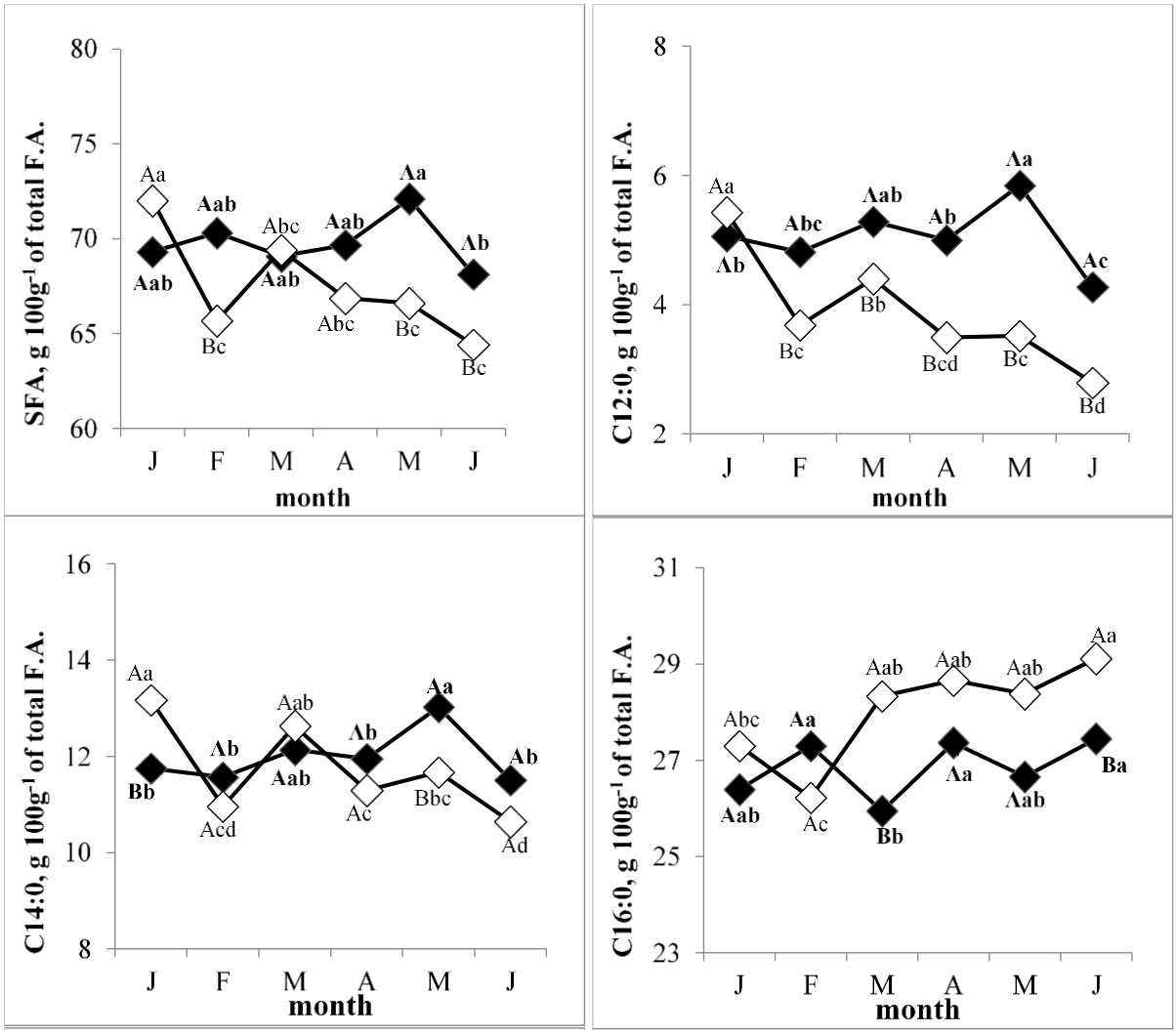

Figure A.4-1. Interaction means for the concentration of saturated fatty acids (SFA), lauric acid (C12:0), myristic acid (C14:0), palmitic acid (C16:0) in milk fat of milk from different management systems and sampling months.

Semi-intensive management system is represented by (♦) and extensive management system by (◊). J:January, F:February, M:March, A:April, M:May, J:June.Values with different, capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system

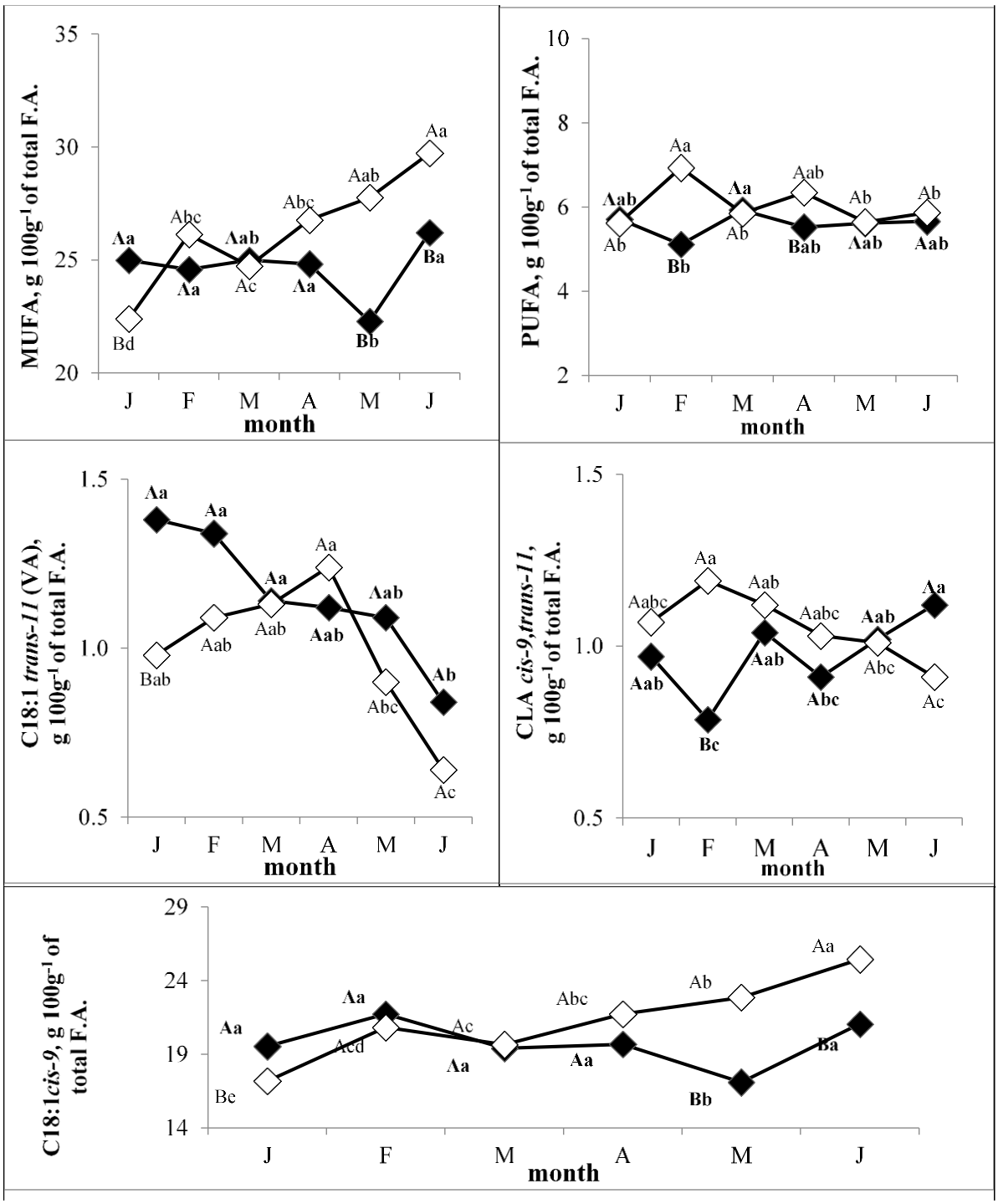

Figure A.4-2. Interaction means the concentration of monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA), vaccenic acid (C18:1trans-11), rumenic acid (CLA*cis9trans11*) and oleic acid (C18:1 cis9) in milk fat for the different management systems and sampling months.

Semi-intensive management system is represented by (♦) and extensive management system by (◊). J:January, F:February, M:March, A:April, M:May, J:June.Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system.

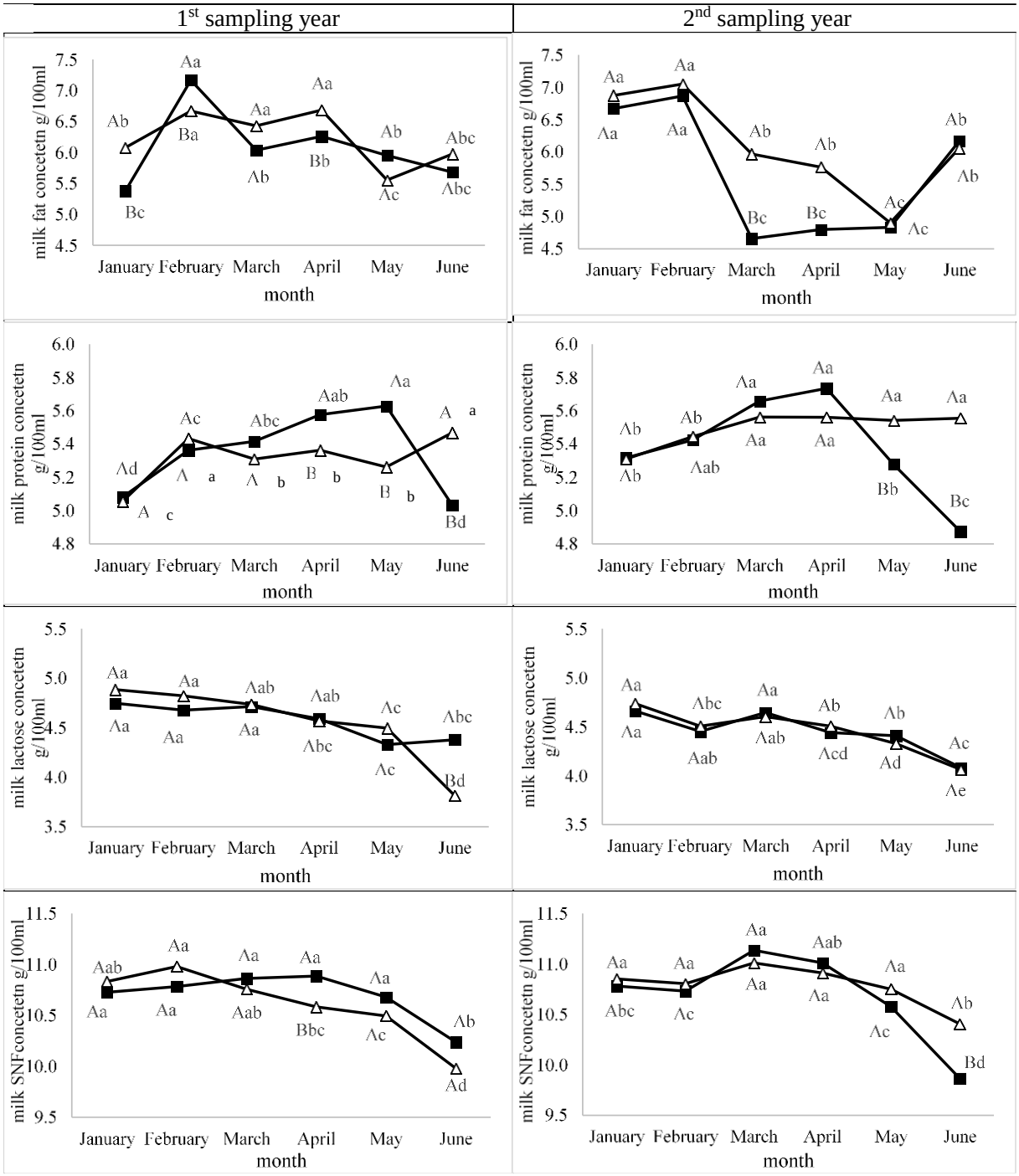

Figure A. 4-3. Interaction means for concentration of fat, protein, lactose and non-fat solids in milk for the different management systems and sampling months.

Semi-intensive management system is represented by (■) and extensive management system by (Δ). Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system (P-value<0.05).

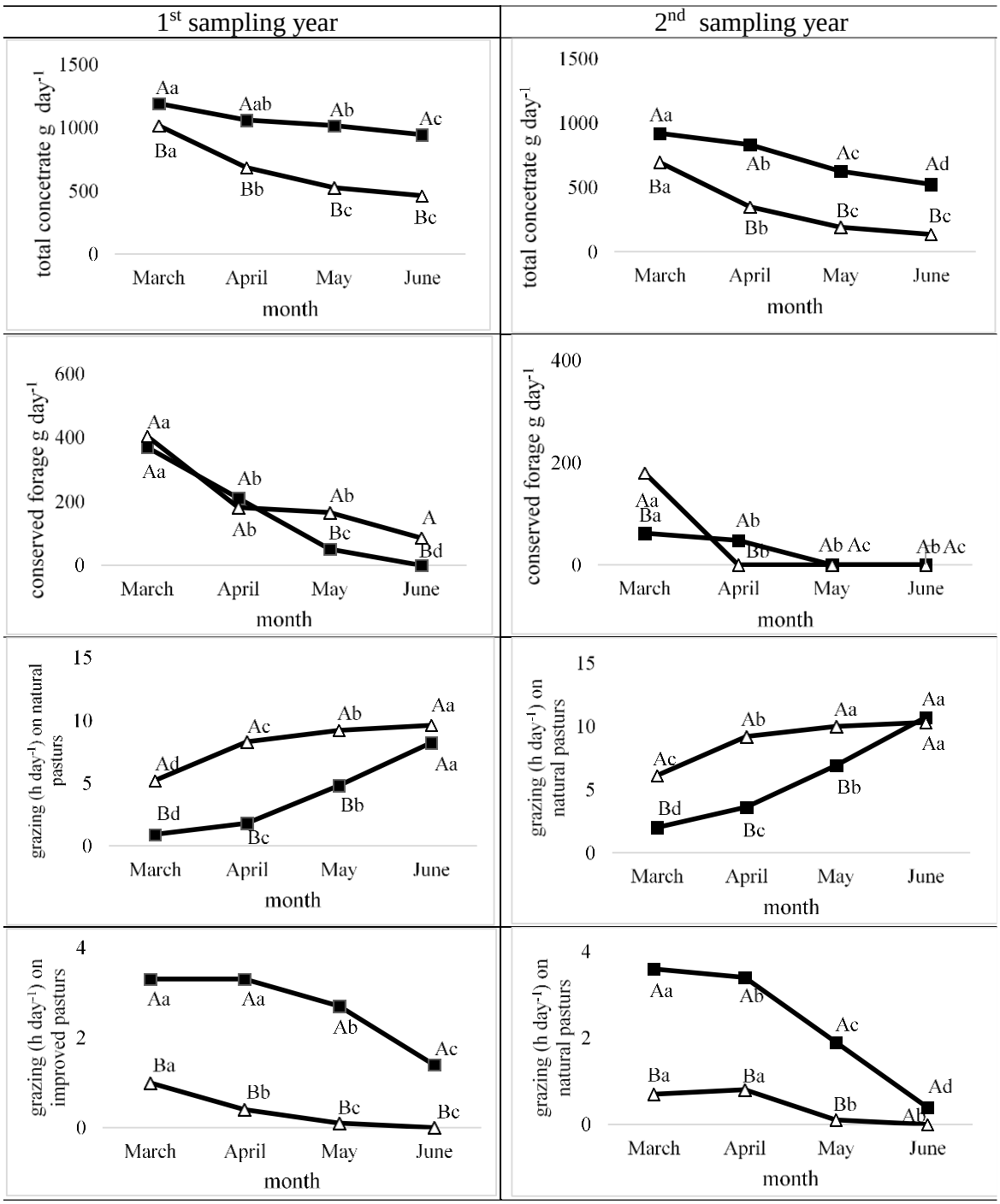

Figure A.4-4. Interaction means for feeding and grazing regimes for the different management systems and sampling months.

Semi-intensive management system is represented by (■) and extensive management system by (Δ). Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system (P-value<0.05).

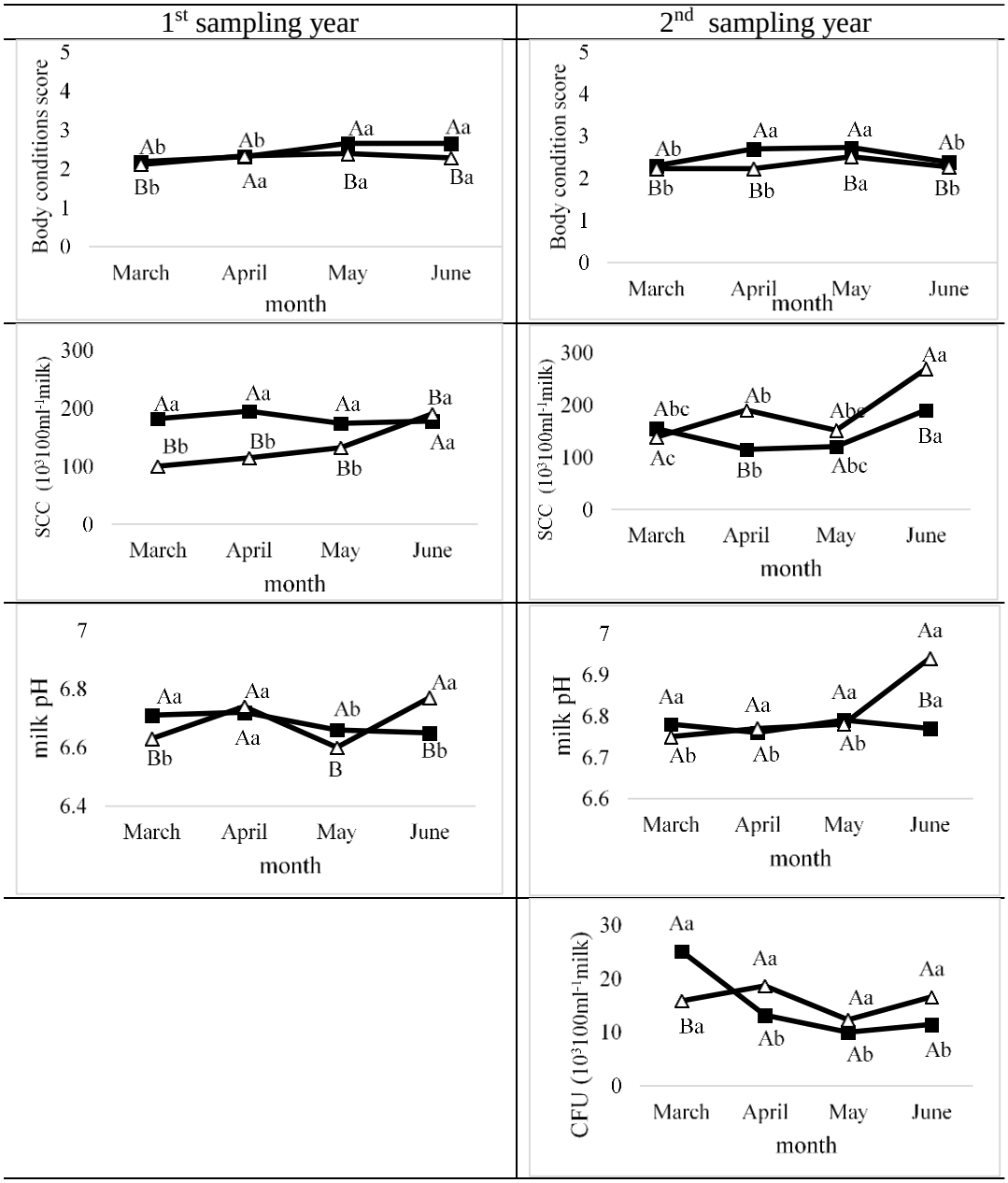

Figure A.4-5. Interaction means for Body Condition Score, milk Somatic Cell Counts (SCC), milk Colony Forming Units (CFU) and milk pH for the different management systems and sampling months.

Semi-intensive management system is represented by (■) and extensive management system by (Δ). Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system (P-value<0.05).

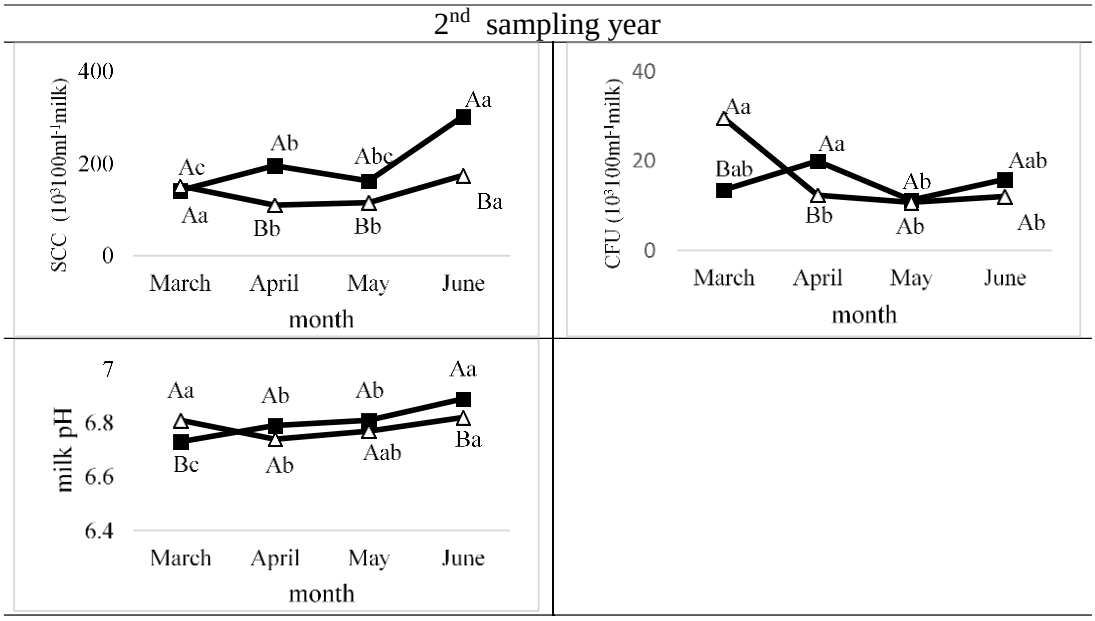

Figure A.4-6. Interaction means for milk Somatic Cell Counts (SCC), milk Colony Forming Units (CFU) and milk pH for the different lambing periods and sampling months.

Early lambing period is represented by (■) and late lambing period by (Δ). Values with different capitilized letters represent statistically significant differences between the two lambing groups (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same lambing group (P-value<0.05).

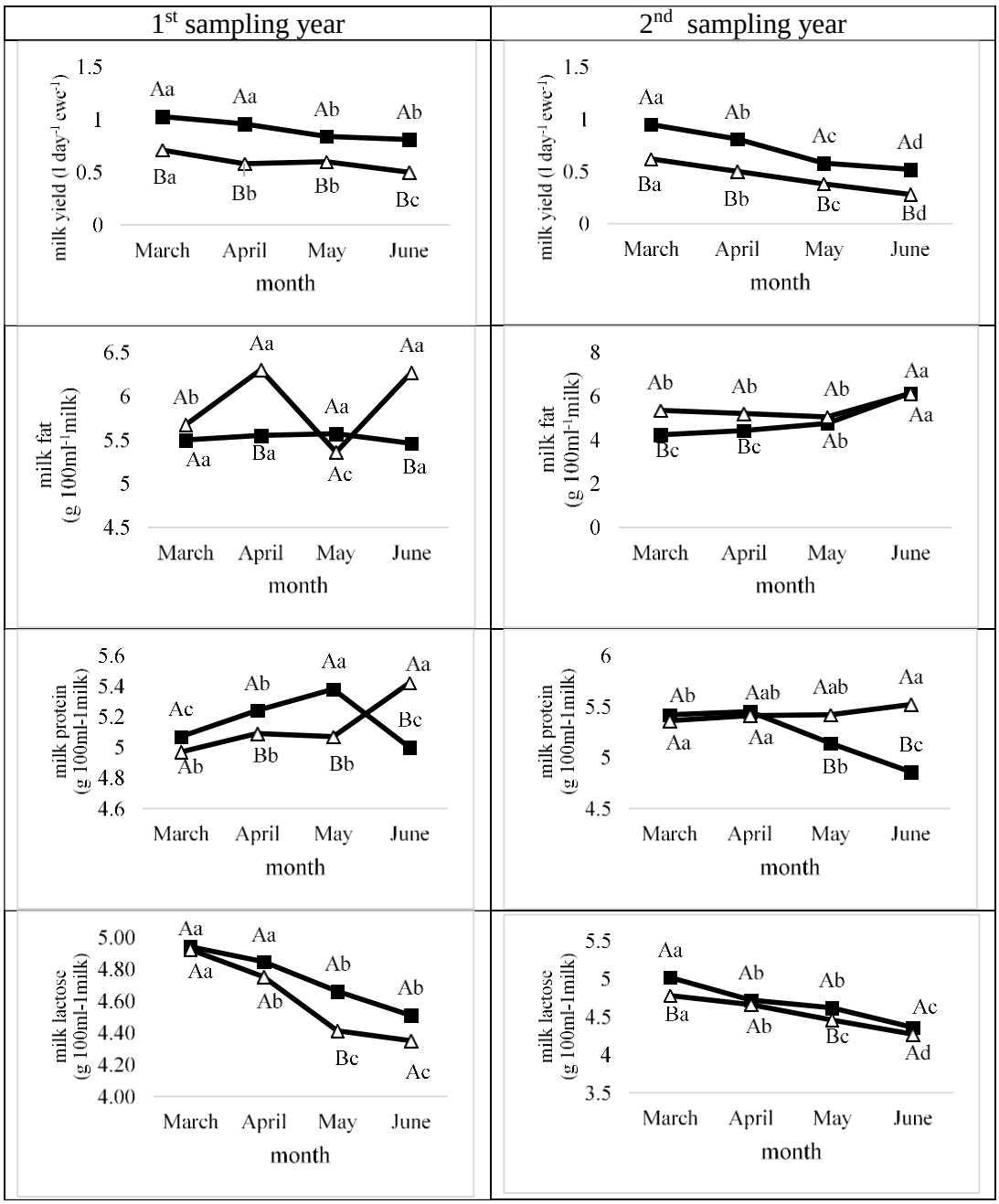

Figure A.4-7. Interaction means for milk yield and milk fat, protein and lactose content for the different management systems and sampling months.

Semi-intensive management system is represented by (■) and extensive management system by (Δ). Values with different capitalized letters represent statistically significant differences between the two management systems (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same system.

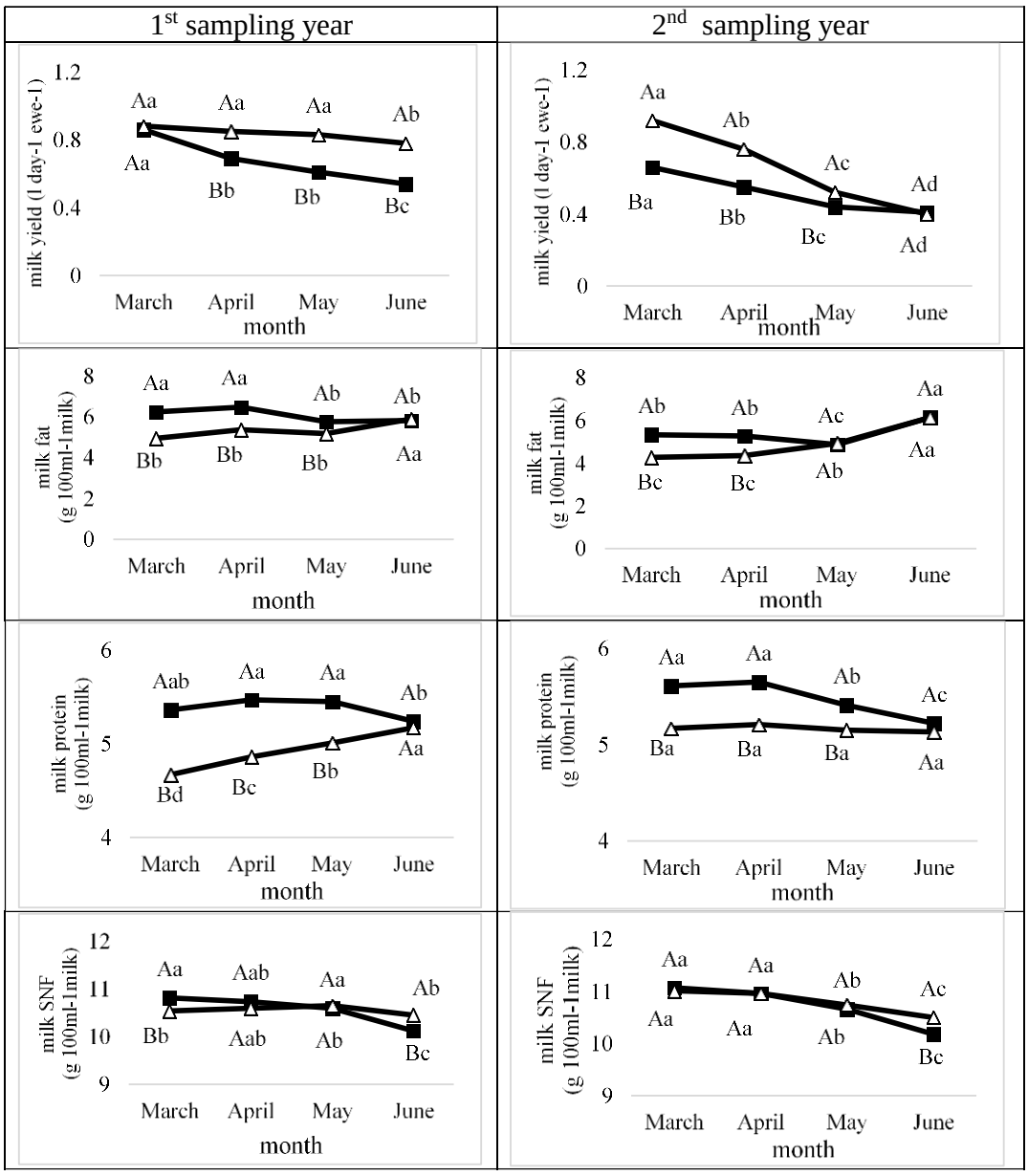

Figure A.4-8. Interaction means for milk yield and milk fat, protein and non-fat solids content for the different Lambing periods and sampling months.

Early lambing period is represented by (■) and late lambing period by (Δ). Values with different capitalized letters represent statistically significant differences between the two lambing groups (P-value<0.05). Values with different lowercase represent statistically significant differences between months within the same lambing group (P-value<0.05).

APPENDIX B - Farm main questionnaire

Figure B.4-9. Farm main questionnaire

FARM MAIN QUESTIONNAIRE – LOW INPUT BREEDS

A. FARM ID

Farm (name / code number):		
Management:	0. Semi-intensive	1. Extensive
Owner:		
Contact details:		
Village:		
Regions:		

B. FLOCK & FARM CHARACTERISTICS

Total number of	a. sheep	
	b. goat	
Milked	a. sheep	
	b. goat	
Yearling	a. sheep	
	b. goat	
Replacement animals	a. lambs	
	b. kids	
Rams:		
Billy goats:		
Breeds:		
Estimated live weight of each group:		

Management system:

Semi-intensive:	0. NO	1. YES
Extensive:	0. NO	1. YES
Extensive – transhumance:	0. NO	1. YES
Location (GPS-altitude);		

Replacement animals

Animal (species/breed):

Female:	0. NO	1. YES
Male:	0. NO	1. YES
Bought animals (number):		
From where;		

Infrastructures

Housing facilities (m^2):	
Storage facilities (m^2):	
Silos (number):	
Silos (volume):	

Engineering equipment:

Milking parlour	0. NO	1. YES
Tractor	0. NO	1. YES
Harvester	0. NO	1. YES
Other:		

Bedding:

Soil	0. NO	1. YES
Straw	0. NO	1. YES

Other:	0. NO	1. YES
Separate cells for lambing	0. NO	1. YES
Weaning unit	0. NO	1. YES
Separate cells for sick animals:	0. NO	1. YES
Water network		
Public network:	0. NO	1. YES
Private drill;	0. NO	1. YES
Natural springs:	0. NO	1. YES
Other:	0. NO	1. YES
Is the property fenced	0. NO	1. YES
Are the pastures fenced	0. NO	1. YES
Estimate %, and explain differences		

Cultivated Pastures

Own (stremma/ 0.1ha):	
Rented (stremma/ 0.1ha):	

Natural pastures

Own (stremma/ 0.1ha):		
Rented (stremma/ 0.1ha):		
Communal (stremma/ 0.1ha):	0. NO	1. YES
Is rotational grazing applied	0. NO	1. YES

Natural pastures characterization

1. rocky-arid areas	0. NO	1. YES
2. heathland	0. NO	1. YES
3. forest	0. NO	1. YES
4. grassland	0. NO	1. YES
5. Other:		

Feeding and grazing regimes

Grazing:	0. NO										1. YES	
Which months;	1	2	3	4	5	6	7	8	9	10	11	12
Average hours per day grazing each month;												
Veterinary plants cultivation:	0. NO										1. YES	
species:												
Harvested forage (bales/ kg)												
Bought forage (bales/ kg)												
Concentrate feed (100kg):												
Average Body conditions Score of flock (ΔΘΚ):												

C. VETERINARY REGIMES

Vaccinations

Enterotoxemia :	0. NO	1. YES
Drug:		
When;		
Contagious Agalactia:	0. NO	1. YES
Drug:		
When;		
Mastitis:	0. NO	1. YES
Drug:		
When;		

Lameness:	0. NO	1. YES
Drug:		
When;		
Chalmydia:	0. NO	1. YES
Drug:		
When;		
Other:	0. NO	1. YES
Against what;		
Drug:		
When;		

Treatments Against endo-parasites

Is applied:	0. NO	1. YES
Frequency:	1. once a year	
	2. twice a year	
	3. trice a year	
When;		
Dosage:		
Drug:		

Treatments Against ecto-parasites

Is applied:	0. NO	1. YES
Frequency:	1. once a year	
	2. twice a year	
	3. trice a year	
When;		
Dosage:		
Drug:		
Is there contact with other animals:	0. NO	1. YES

If yes specify:

1. Dog:	0. NO	1. YES
2. Cat:	0. NO	1. YES
3. Pig:	0. NO	1. YES
4. Rabbits:	0. NO	1. YES
5. Poultry:	0. NO	1. YES
6. Other:	0. NO	1. YES
Disinfection of facilities;	0. NO	1. YES
When;		
With what;		

D.HEALTH STATUS

Most frequent health issues encountered:

1. infectious diseases	0. NO	1. YES
2. respiratory diseases	0. NO	1. YES
3. Foot problems (eg lameness)	0. NO	1. YES
4. Miscarriages	0. NO	1. YES
5. Fertility problems / return to estrus	0. NO	1. YES
6. mastitis and similar diseases	0. NO	1. YES
7. enteritis (lambs)	0. NO	1. YES
8. metabolic diseases	0. NO	1. YES
9. Other		

E. MATING

Number of ewes:	
Number of rams:	
Wes to ram ratio:	
Ram entry date :	
BCS of ewes during entry date:	
Flushing before entry;	
Early births:	
Ram removal date:	
return to oestrus, number of ewes	
Fertility (number of ewes):	
Miscarriages (number of ewes):	
Month of Miscarriage;	
Lambing period (duration in days):	
Problems associated with pregnancy:	
Comments:	
Steaming before lambing:	

F. LAMBING

BCS during lambing:	
Feeding regimes during lambing;	
Number of ewes that gave birth:	
Crunches (number of ewes):	
Number of deaths during lambing /early lactation:	
Lambs born:	
Number of abnormal – stillborn lambs:	
Lambs deaths during lambing	
Lambs deaths the 1st 48 hours:	
Adopted lambs:	
Main problems with lambs:	
Lamb deaths during suckling:	
Weaning system:	
Weaning date:	
Weaning age:	
Lambs Weaned:	
Lambs average body weight at weaning:	
Deaths after weaning	
Treatments applied to the lambs before/at weaning	

G. MILKING / LACTATION

Data regarding previous lactation

Total milk production (kg):	
Weaned lambs:	
Sold lambs:	
Replacement lambs:	
Major issues:	
Milking period finished at.	
Concentrate feed was provided until	
Duration of lactation (month):	

Data regarding current lactation

Ewes milked:		
Ewes BCS during weaning:		
Feeding regimes:		
Date milking started:		
Date milking stopped:		
Duration of lactation (month):		
Total milk production (kg):		
Milking practices – during suckling		
Suckling period (days):		
Are the ewes milked during suckling?		
How many days after birth?		
How often in a day?		
Milking practices – during main milking period		
Milking's per day (number):		
Number of people milking:		
Milking system (hand/ automatic/ semi-automatic etc):		
If a milking parlour exist		
Type:		
Number of spaces:		
Feeding during milking:	0. NO	1. YES
Vacuum:		
Pulses / minute:		
Inflation milk tube type:		
Inflation milk tube storage:		
maintenance of milking parlour every … months/ specify:		
Disinfection of inflation milk tube. Frequency in days:		
Replacement of inflation milk tube every … months/ specify:		
Cleaning and disinfection of milking parlour (eg CIP). Chemicals used:		
Dry Wipe Teats and similar practice:	0. NO	1. YES
Post-dip Teats:	0. NO	1. YES
Substance:		
Cleanness and sanitary conditions of the milking facilities:		
H. UDDER HEALTH / MASTITIS		
Clinical mastitis most often occurs at:	Specify %:	
Yearling		
After the 1st lactation		
During suckling		
At the beginning of milking period		
At the mid of milking period		
At the end of milking period		
At dry season		
Early lambing		
Late lambing		

High yield ewes	
Low yield ewes	
Most common mastitis symptoms:	
Treatment	1. only intramammary drug
	2. only systemic therapy
	3. both
Drugs:	
Duration of treatment: (days)	
Outcome of treatment (in %):	
Withdraw of clinical symptoms and complete loss of milk production.	
Withdraw of clinical symptoms and lower milk yields.	
Withdraw of clinical symptoms and improvement of both milk yield and quality.	
Temporary withdrawal of clinical symptoms.	
Deaths / slaughters:	
Is self-healing observed?	
Number of ewes that	
Produce milk from one udder only:	
Both udders do not produce milk	
Number of animals slaughtered because of mastitis:	
Death rates because of mastitis (%) :	
Are dry period antibiotics used?	0. NO 1. YES
If yes, how many days prior to lambing	
Udder issues (number of animals with)	1. papilloma
	2. injuries
	3. Abscesses
	4. other

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