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Awassi sheep and Baladi goat milk composition in extensive production systems in Lebanon and cardoon inclusion in sheep diet

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To my family...

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List of Abbreviations and Acronyms

ADF= acid detergent fiber

ADL= acid detergent lignin

asl= above sea level

ANOVA= Analysis of Variance

BCFA= branched-chain fatty acids, sum of iso- and anteiso-FA

BCS= body condition score

BW= body weight

CEF= cardoon extracted flour

CON= control

CP= crude protein

CPE= cardoon polyphenolic extracts

DHA = docosahexaenoic acid

DIM= days in milk

DM= dry matter

DMI= dry matter intake

DPA = docosapentaenoic acid

EE= ether extracts

EPA = eicosapentaenoic acid

FAME= fatty acid methyl ester

GDP= gross domestic product

HD= high dose

LA= linoleic acid

LCFA= long-chain fatty acids

LD= low dose

L.L.= Lebanese Lira

LNA= linolenic acid

MCFA= medium-chain fatty acids

MCP= milk coagulation properties

MoA= ministry of agriculture

MUFA= sum of the individual monounsaturated fatty acids

NDF= neutral detergent fiber

NGO's= non governmental organizations

OBCFA= odd- and branched-chain fatty acids, sum of odd-, iso-, and anteiso-FA

OM= organic matter

PUFA= sum of the individual polyunsaturated fatty acids

RA= rumenic acid

SA= stearic acid

SCC= somatic cell count

SCFA = short-chain fatty acids

SFA = sum of the individual saturated fatty acids

TFA = trans fatty acids

TMR= total mixed ration

TRT= treatment

UFA = sum of the individual unsaturated fatty acids

VA = vaccenic acid

Introduction

Small ruminant productions in Mediterranean region, represent two thirds of the world sheep milk production and more than a quarter of goat milk production. In the Arab countries, livestock resources in terms of species and number are significant: there are 38 breeds of sheep and 54 breeds of goats according to the Arab Center for the Study of Arid Zones and Drylands (ACSAD, 2007). The majority of these breeds are adapted to the dry climatic conditions. Despite the number and breeds diversity, the livestock production in meat and milk is still low compared to other developed countries. The low production is caused by different factors including the lack of programs related to the genetic improvement, the poor production systems and the mismangement of the natural resources in terms of rangelands and water; in fact rangelands are subject to overgrazing and degradation due to the absence of policies and legislations regulating their management (Darwich and Faour, 2008).

Lebanon is a Mediterranean country located in the Middle East between the latitudes 36 º70 to 38º60 North and longitudes 39º00 to 40º66 East. Lebanon's superficie is 10452 km^2, with a shoreline facing the Mediterranean Sea from the North to the South of 210 Km and a width of 25 to 80 km from East to west. The topography of Lebanon is mainly mountainous, composed of a narrow coastal strip in the west, and two mountainous chains: the Mount Lebanon and the Anti Lebanon, separated by the plain of the Bekka (Kharrat, 2010). Mount Lebanon chain have an average height of 1000 m up to the highest peak rising more than 3087 m of altitude and is characterized by a high vegetative density constituted of forests in the western side facing the Mediterranean while the eastern side which face the Bekaa valley is semi arid composed of a limestone soil mainly cultivated with fruit trees, and constitute a good environment for the vertical transhumance system in winter (Srour *et al.,* 2006). The Anti Lebanon mountainous chain, forms the border between Lebanon and Syria, and is parallel to the Mount Lebanon chain, it has the highest peak reaching 2800 m of altitude where few trees and plants can grow. These two mountainous chain, are separated by the historically known Bekaa valley, which is the most fertile plain in the region and represents the most important agricultural area in Lebanon. This valley has an average altitude of 900 m and constitutes the breeding area of half of the Lebanese small ruminant population. Lebanon is characterized by a Mediterranean climate with a dry and mild summer and a cold rainy

winter with snow on the mountains from November to May. The moutainous topography of Lebanon leads to an uneven rainfall distribution between the different regions of the country and thus creates several eco climatic zones between the two mountainous chain: for example the semi-arid to continental climate in the Bekaa region and the sub-desertic climate in the Hermel region. The average annual precipitation ranges from 200 ml in the Hermel region, 650 mm in the Bekka, 1500 ml in the mountain and between 700-800 ml in the coast. At the high altitudes (generally above 800 m), a part of the precipitations occurs in the form of snow. The dry season occurs in summer, and the average monthly temperature is 18-15ºC in winter and 28-29ºC in summer. The evapotranspiration rate of the precipitation ranges between 50% in the coastal areas and 57% in the mountains. The climatic condition in addition to the soil fertility and water richness, are very important factors which permits the cultivation of a wide range of vegetable and fruit crops.

The economy in Lebanon is based on the service and tourism sector, followed by the industry and the agricultural sector. The latter, represents an important part of the social and economic life of the country, and contributes 9% of the Lebanese active population and 5% of the total gross domestic product (GDP), more specifically in rural areas, where it contributes up to 25% of the rural labor force (MoA, 2014). Women represent about 8.6% of the total farmers (MoA, 2012), and their contribution relies on the processing of the dairy products and the production traditional food preserves. Livestock sector in Lebanon is the property of the private sector, and includes cows, sheep and goats raised for meat and milk and other non-food products such as wool, manure and leather.

The small ruminant production systems widespread in Lebanon ranges from sedentary to transhumant and semi nomadic with a majority based on family farms and they vary by their size and composition, and by their use of pastures and natural rangelands (Hamadeh *et al.,* 1996; Hosri and Nehme, 2006; Haddad and Chamoun, 2014 ; Chedid, 2019). The majority of the Lebanese goat population is of the Baladi breed (95%), and 5% of Shami or Damascus breed (Hajj, 1999; Nehme and Abi Saab, 2003) whereas sheep are all of Awassi breed. On the other hand, intensive production systems are using breeds with high milk production like the Shami, Saanen and Alpine breed. According to FAO in 2009, small ruminants contribute up to 25% of the milk and 35% of meat production in Lebanon (Srour *et al.,* 2006). Milk production is used for direct consumption and is also processed into local and traditional dairy products such as Laban, Labneh, Darfiyeh and Aricheh, Kishk, Shankleesh, Serdale and many cheese type (Serhan *et al.,* 2017). Despite the number of animals, the production does not meet the growing demand due to the increase of the population: in fact, it was estimated

that between the period of 1980-2005, the growth rate of the goat and sheep meat demand was 252% and that of milk and dairy products was 243%, while the production growth rate was only 91 and 102% respectively (Srour *et al.,* 2006).

From here, there is a need to develop the small ruminant production in Lebanon in order to meet the local needs, especially that this sector remains among the least developed due to several factors including: the seasonality of the grazed fodder resources and the absence of local fodder stocks, the uncontrolled reproduction of the herd, the manual milking, the inappropriate sanitary treatments and the artisanal processing of the products.

CHAPTER 1

Small ruminants production systems in Lebanon

1- Small ruminants production systems in Lebanon

Small ruminant production systems in the Mediterranean are in majority managed under extensive production modes, and they varry by the level of pasture use (pure pastoral, agro-pastoral ans sylvo-pastoral). These systems can be sedentary, nomadic or transhumant, and are submitted to harsh climatic conditions (arid or semi-arid), roughage defeciency, and are dependent on feed importations (Srour *et al.,* 2006). In Lebanon, extensive production systems are widely widespread, while intensive system are starting to gain importance (Srour *et al.,* 2006). Small ruminant production systems, herd size and breeds in Lebanon vary from region to another. The narrow coastal plains are mainly cultivated by fruit trees and greenhouse vegetation and farming is generally absent. In the two plains of Akkar and Bekaa, where cereals are produced, the production system adopted are transhumant and sedentary system, and feeding the flocks is based on the crop residues (Hamadeh *et al.,* 1996 ; Hosri and Nehme, 2006). While in the slopes of Mount Lebanon and the hills of the north rich in vegetation cover and forests, semi nomadic production system between the littoral and the rangelands in the mountains is the most adopted system, in addition to the sedentary system. In the Northern part of the Bekaa and the Anti Lebanon, characterized by a semi-arid climate and a high proportion of uncultivated area, semi nomadic and transhumant systems are adopted and these areas are considered as agro-pastoral lands for mixed herds of goats and sheep. On the other hand, the subsistence and the intensive production system where herds are raised on supplied diet, are spread in the entire country, and are encouraged by different rural development projects (Hamadeh *et al.,* 1996 ; Srour *et al.,* 2004 ; Hosri and Nehme, 2006).

Topography and relief have also a direct influence on the species of small ruminant used: in the mountains, vertical transhumance is characterized by the abundance of rustic breed of goat known as Baladi, which is adapted to steep terrain and have the ability to reduce their metabolism when the nutritional quality of the courses decrease with the advancement of the season, while in the horizontal transhumance and semi nomadic system, sheep are dominant and herds graze on wheat stubble, crop residues, under fruit trees and on herbaceous plants abundant in the moutain pastures. In addition, the system of production and the flock size are interdependent: the subsistence system is adopted by farmers having small sized flocks while farmers having large size flock practice the semi nomadic system. Breeds used in extensive production system are the Awassi sheep and the Baladi goat, while for the intensive production system, improved breeds are used; this is due to the high adaptation of Awassi sheep and Baladi goat to semi-arid environments and their capacity to withstand drought and

high ambient temperature. Moreover, these two breeds are not suitable to be raised under zero grazing in intensive production systems. The production systems identified in Lebanon are: sedentary, transhumant, semi-nomadic and intensive production system.

1.1- Sedentary system

This system is widespread all over Lebanon especially in the mountainous regions. Farmers have few hectares (cereal crops or vegetables) irrigated based on gravity and animals are housed in or near the village according to the National Institute of Livestock. The herd is of medium size 100-300 head with generally an equal number of sheep and goats. Birth take place mostly in January-February, and can last from November to April. Young suckle their mother for the age of at least one month, and their mothers are not milked during this period. From April to June, the animal graze on communal or public ranges and during this period, ewes and goats are milked in the morning, once a day until the weaning of the young which occurs at the age of 3-4 months. The number of young weaned per adult female does not exceed 0.8 (low prolificacy and loss rate around 10% between the birth and weaning). From the end of June to mid-November, if the herd is in the plain, animal graze on stubble, vegetable residues and the standing crops of the barley and vetch mixture, while if they are in the mountains, they graze on natural rangelands belonging to local communities which vegetation becomes scares due to the advancement of the summer season, or graze on forests located near the village thus allowing the herds to induce a short daily movement and to return every evening in the village. In mid November, herds are fed with concentrate and shopped straw, with about 100 Kg per head. At the end of 4 months of lactation, ewes and goats are dry, and thus this system have a low milk harvest: 55 Kg per ewe, 60 Kg per goat. Most of this milk is consumed by the family and the surplus is processed and sold directly to the villagers or to collectors, in the form of Laban, Labneh or cheese (Srour, 2006 ; Marie, 2009).

1.2- Semi nomadic system

The majority of the farmers of semi nomadic production system are from the Bekaa region and are landless. The movement of this system is of a high amplitude reaching 200-300 Km, where the farmer and all his family move between Mount Lebanon and the Anti Lebanon, through the Bekaa plain, and very low proportion of the farmers use trucks for moving their herds. During summer, the tent is the only mean of accommodation for the family in high mountains, whereas in winter, farmer have usually a home in his village which gives

children the opportunity to go to school. In this system, family members constitute the labors. Herds in this system are large in numbers 300-500 heads and more, with often a greater proportion of sheep than goats. On high moutains ranging between 1500-2500 m, the vegetative development is severly affected by the climatic conditions during winter, and are not accessible to herds only starting from May after the snowmelt until October. On the other hand, in the plains, spring and fall grazing is done on crop residues and wheat stubble. During the grazing season, herds can move between 2-3 Km on a well defined territory generally rented on a surface which varies between 1000-1500 hectar, and herds returns in the evening to the location were the tent is located, near the roads where the milk collector can access. In this system, the seasonality of birth is more marked than other system: there is an important grouping in December- February because all kids must be weaned during the climb to the mountains in May. During summer, no supplement is provided to the herds. In November, herds move, and return to the plain or the coast where diet is based on grazing on land surrounding the villages generally rented, in addition to the distribution of the concentrate feed with the straw. Milk is generally processed in dairy products, while meat production is lower than that in the sedentary farms (0.6-0.7 lamb or kid weaned per mother) (Hamadeh *et al.,* 1996; Srour, 2006; Marie, 2009; Haddad and Chamoun, 2014). This system is completely dependent on rangeland, and is becoming fragile due to several factors such as overgrazing, climate change, urbanization etc., in addition to the rental prices which are independent from the quality of the feeding resulting in the scarcity of rangelands (Hosri and Nehme, 2006; Srour *et al.,* 2006).

1.3- Transhumant farming

This system is intermediate between the sedentary and the semi nomadic system. The use of rangeland is determined by the size of the cultivated land of the breeder and the availability of harvest residues (stubble and cereal, residues of legumes or tubers). Generally, greater fodder resources available in the village, corresponds to a longer lactation period (Kharrat, 2010 ; Haddad and Chamoun 2014). There are two types of transhumance: vertical transhumance system where the herd follow a path between the littoral in winter and the high mountain in summer; and the horizontal transhumance where the herd move between the plains.

1.3.1- The vertical transhumance

In this sytem, herds move between two fixed regions with a distance ranging between 30-100 Km: from the low or medium altitude in winter to the high altitude in summer at an altitude of 1500 m, making advantage of the majority of the seasonal pastures. The seasonality of the transhumance is determined by the climatic conditions: the drought and high temperatures during summer at low altitude, and the snow in high mountains during winter. The main breed of this system is the Baladi goat which graze in forests and woody rangelands, in addition to the Awassi sheep especially in Mount Lebanon region. The majority of the herds in this system graze in private or collective fallow lands, while for the rest, farmers may have a land of 2-15 hectar, planted with fruit trees without irrigation, or neglected without any cultivation. During both seasons, farmers stay near the villages which are the main market of their dairy products.

1.3.2- The horizontal transhumance

This system is exclusively specific to the plains regions (Bekaa region or the Akkar region in the north). Farmers with their families moves between May and October for 100-150 Km in search for wheat stubble and crop residues which is the main source of feed in the lowland region. Farmers have a large area of land cultivated by the family, one part is cultivated in cereals especially barley and wheat which are directly served to animals or used in the form of seeds after mowing, and the other part is generally cultivated with vegetables or fruit trees. Herds are formed by only sheep or a mix of goats and sheep where goats represents only 10 % of the herd, this is because of the better adaptibility of the sheep grazing in the plain, and goats production is generally used for self consumption.

1.4- The intensive system (Zero grazing)

This system is gradually expending due to the help of the non governmental organizations (NGO's) supported by the European funds and is characterized by the high dairy production and the herd high demand in food. Herds in this type of farms is small in number (15-95 heads), and composed of foreign goat breeds (Alpine, Saanan and Damascus), with only one vocation: milk production. Milk production range between 270-500 Kg of milk per lactation. Intensive system can also be composed of local Baladi breed with the vocation of meat production. In this system, animals do not feed on pastures, and all their feed are purchased, thus large quantities of feed are needed (500 Kg of concentrates/head) and is characterized by the direct sale of its local dairy products (Srour, 2006).

2- Animal numbers and breeds

Small scale herd production systems are the dominant farms in Lebanon, where 70% of sheep and goat farmers have under 50 heads (MoA, 2012). The number of small ruminant herd in Lebanon, have showed many fluctuations throughout the years and was affected by many incidences: sheep number have increased between 2003-2006 and then declined in 2007 after the war in 2006, and resumed growth in 2008-2009 to decrease again in 2010. Also, goat numbers have increased between 2003-2005, and have decreased significantly by 19.35% between 2005-2008, to increase again in 2009 before declining in 2010 (Figure 1) (MoA, 2010; Chedid, 2019). According MoA (2012), the number of sheep and goats have reached 265345 and 403861 respectively compared to 340000 and 430000 head in 2005 (FAO, 2005) and to 330,000 sheep and 450,000 goats in 2009 (FAO, 2009). Nowadays, the livestock population number have increased due to the support of international development agencies (Chedid, 2019), and according to FAO 2020 statistics, sheep and goats population are composed by 431718 and 534497 head, respectively. Goat herd is composed mainly of the local population named Baladi which represent about 95% of the herd in extensive systems, while the remaining 5% are formed by the Damascus breed. Baladi goat is characterized by a high capacity of adaptation in harsh environment in order to ensure the survival of the specie by regulating its nutrient partitioning towards the reconstitution of body reserves and thus guaranteeing a new reproduction and lactation cycle: at the early stage of lactation and when the feed supply is high, Baladi goat have a tendency to divert the nutrient flow towards milk production, which increase milk yield and by the end of lactation the extra feed is progressively oriented towards body reserves (Kharrat, 2010). On the other hand, sheep herd is composed by the fat tailed Awassi breed. These breeds, are highly adapted to semi-arid condition, the mountainous topography of Lebanon and the extensive production systems (Hamadeh *et al.,* 1997; Jaber *et al.,* 2004): sedentary and vertical transhumance systems are common for goats which are raised for meat and milk production, while semi nomadic and horizontal transhumance production system are more common for sheep generally raised with triple vocation (meat, milk and wool) (Srour, 2006). The livestock farming is widespread all over Lebanon, with 15800 farmers of which 10% are handled by women. The herd composition differs from region to another, for example in the South, Nabatiyeh, Akkar and Mount Lebanon, goats represents the majority of the herd followed by sheep, whereas in Baalbeck-Hermel region and in North, sheep represents the majority of the herds, and in the Bekaa the number of sheep and goats in the herds are approximately the same (Figure 2-3). These differences are caused by the production system adopted and by the consumer

preferences. Baalbeck-Hermel region have the highest numbers of sheep and goats farm followed by the Bekaa region representing 71% and 51% of the sheep and goat national number respectively (190422 sheep head and 206305 goat head) (FAO, 2009; MoA, 2012).

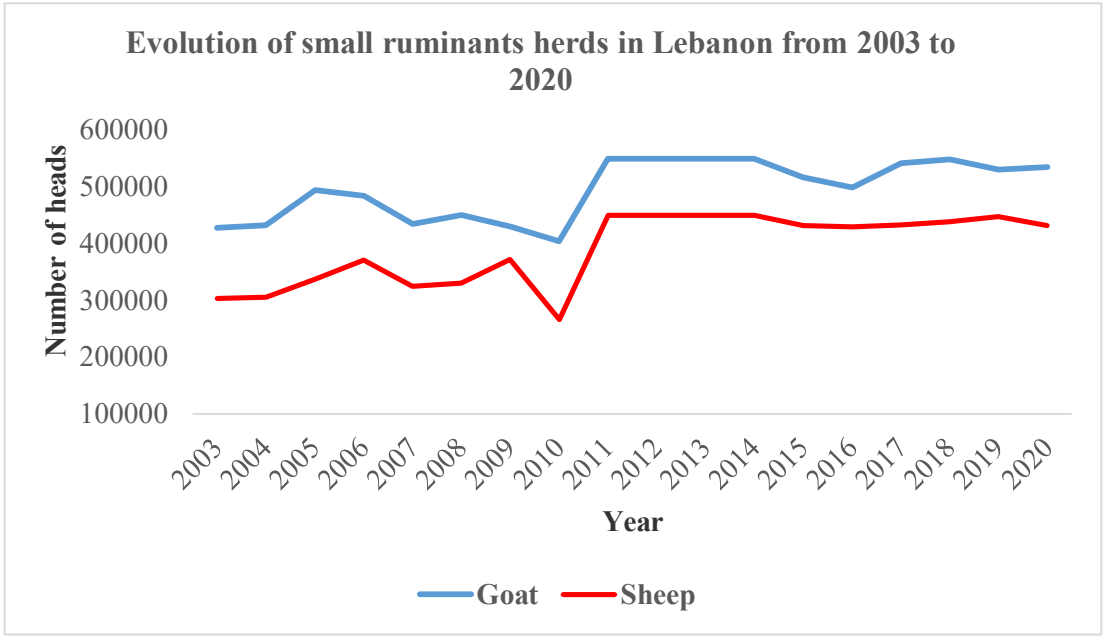

Figure 1: Evolution of small ruminant herds in Lebanon from 2003 to 2020 (thousands of heads) (FAOSTAT, 2020).

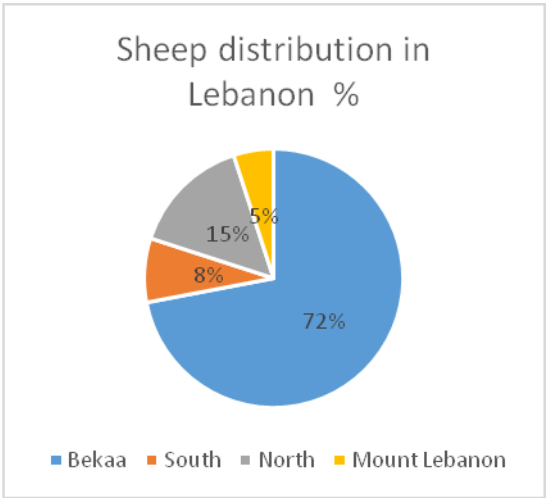

Figure 3: Distribution of sheep population in Lebanon (MoA, 2012)

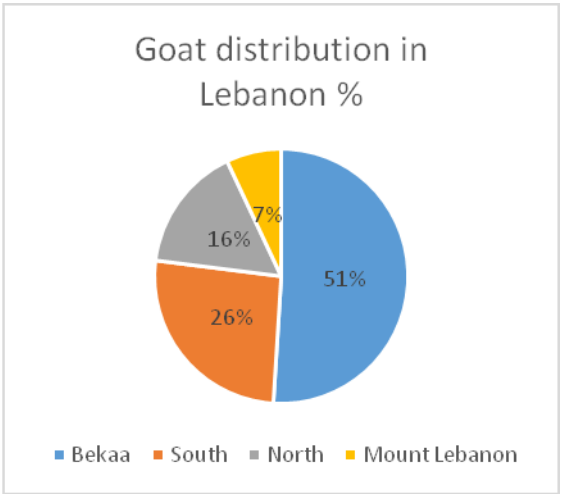

Figure 2: Distribution of goat population in Lebanon (MoA, 2012)

3- Flock and farms management

Sheep and goat herds in Lebanon are characterized by the seasonality of reproduction, with the highest conception rate taking place in August and September and the kidding period occurs between January and March (Hamadeh *et al.,* 1996; Hosri *et al.,*2016). Herd prolificacy rate varies between 1-1.75 with an average of 1.26, depending on the production system (intensive system 1.56; sedentary 1.27; vertical transhumance 1.23, horizontal transhumance 1.28 and semi nomadic 1.21), while the fertility rate of the herd, varies between 61-100%. Weaned lambs and kid mortality rate (40 and 45% respectively) in extensive system is higher than that in intensive systems, and the adult average mortality is about 7%. This is caused by the raising and management conditions in the farms, in addition to diseases and the severe climatic conditions in some years especially during calving period (Abi Saab, 2001; Hosri *et al.,* 2016). The duration of the lactation period of Awassi sheep is 150 days with an average milk production of 100-200 liters per year, whereas Baladi goats produce 120-140 liters of milk with a lactation period of 180 days (National Livestock Institute, 2003; Kharrat, 2005). The roughage quality and rangelands affected by climate change and overgrazing, in addition to production system adopted and the feed type used, affects enormously milk production in Lebanon. In extensive systems, rearing sheep and goats is for two vocations: milk and meat production, while in intensive systems where other productive breeds are used, milk production is the principal source of income (Hosri and Nehme, 2006). Weaning of kids and lambs is done at the age of 70 days, and kids are generally sold at an average age of 1-2 years with a weight of 40-60 Kg while lambs are sold at the age of 1 year with a live weight of 55 Kg. Goat meat price is sold with an average price of 1.25-1.50 €/Kg of live weight, while sheep meat price is 1.50-1.75 €/Kg of live weight, with a carcass yield of 45% for kids and 53% for lambs (Hamadeh *et al.,* 2001; Hosri and Nehme, 2006; Srour *et al.,* 2006). In addition to the milk and meat revenues which represent about 90% of the farms income, wool and manure have a minor contribution to the farmer's revenue: manure is sold at a price of 0.011 €/Kg dried while wool is sold at a price of 0.37 €/Kg. Prices of milk varies according to several factors including the market, while the meat price is relatively homogenous at the national level (Srour *et al.,* 2006).

4- Fodder and forage cultivation

Agricultural sector is an important sector in Lebanon, and is characterized by a wide range of agricultural crops due to its climate and fertile soil. Nevertheless, fodder and cereal cultivation does not constitute a significant part of the total cultivated area, compared to other

crops: only 1-2% (1620 ha) of the cultivated land in Lebanon are cultivated by fodder aimed to be used as animal feed, and 20% (44924 ha) are cultivated by cereals, while fruit trees, olives, vegetables (36776 ha), pulses (9452 ha) and industrial crops (9699 ha) represents 32, 23, 16, 4 and 4% respectively of the total cultivated land. Barley, corn silage, alfalfa and vetch are the most cultivated fodder crops in Lebanon and represent respectively 4.60%, 0.86%, 0.35% and 0.25% of the total agricultural land (MoA, 2012; Haddad and Chamoun, 2014). Fodder cultivation varies depending on regions: Bekaa valley represent 73% of alfalfa cultivated area, followed by Akkar (15%) and Baalbeck-Hermel (10%). Whereas, 38% of the cultivated vetch land is in the Bekaa, 26% in Akkar, 20% in Nabatiyeh and 10% of the acreage in Baalbeck-Hermel region. Most of barley acreage (81%) are in Baalbeck-Hermel, and most of it is left to fallow land in order to be grazed by small ruminants, while 57% of the total acreage of maize fodder are located in Bekaa, followed by Akkar (37%) and Balbeck-Hermel (6%) (MoA,2012). The cultivation of vetch and sorghum was in continual decline in Lebanon, while the cultivation of oats, barley and maize fluctuates depending on the years, and alfalfa cultivation was stable from 2003 till 2011 (FAO, 2011). All the production of alfalfa and vetch is processed as feed for animals, barley is used as fodder, and corn production is used as sweet or silage corn, whereas the oat and sorghum production is used for both human nutrition and animal feed (Haddad and Chamoun, 2014). This evolution is due to the climatic factors which affect the production in non-irrigated regions, but also due to the crop rotation practiced by the farmers.

5- Small ruminant source of feed

One of the most important problem facing the small ruminant sector in Lebanon, is the fodder deficit: the national production is very low and insufficient, and the cost of the imported supplements and feed ensuring the supply of livestock, is high representing 90% of the feed cost value (175 million $). As a consequence to imported feed high cost, and to the limited forage cultivated areas, feeding sheep and goats in extensive systems, is mainly based on rangeland resources and on rented crop residues land (Hosri and Nehme, 2006). Seasonal grasslands are unevenly distributed in Lebanon, they cover 16000 ha representing about 15% of the total country surface (Chedid, 2019). In Akkar, rangelands cover 50% of the land making it an important pasture area (Srour, 2006), and in the Bekaa valley, rangelands and the surrounding mountains of Mount Lebanon and the Anti Lebanon represents 40% of the Lebanese grazing land (Hamadeh, 2002). In Mount Lebanon, rangelands are formed by the terraced hills of the mountain (Abi Said, 2004), and the pastures land in the South are

composed of the Anti Lebanon and Mount Lebanon southern prolongation. Grasslands areas are affected by the precipitation rate and rainfall distribution in addition to the climate and overgrazing and are not being enough to support livestock on a year round basis, thus forcing farmers to use feed supplements and concentrates as hay and barley or woodlands and forests especially in winter (Srour *et al.,* 2007; Asmar, 2011). In addition, pasture lands, are becoming less available for small ruminants because of their use for agricultural production. The quantities of concentrates used varies depending on the farm size. Cereals such as barley and wheat represents 89-100% of the ration used by farmers, while protein crops and commercial feed are not widely used (Srour *et al.,* 2006). This explains why shepherds in Lebanon, rely on feeding and grazing during the autumn and winter months, and on grazing of rangelands and crop residues during spring and summer to feed their animals (Hamadeh *et al.,* 1996; Srour, 2006).

Natural rangelands in Lebanon are characterized by a rich biodiversity and are composed of different trees such as prune trees, storax and wild pear trees (*Prunus amygdalus (L.), Styrax officinalis L., Pyrus syriaca Boiss*), and different type of shrubs such as thorny burnet (*Poterium spinosum L.*), berberis (*Berberis libanotica*), spiny broom (*Calicotome villosa*) and hawthorn (*Cratageus azarulus*). In addition other plants from different families are present in rangelands as wild oat (*Avena sterilis L.*) and bulbous Barley (*Hordeum bulbosum L*), legumes like Alfalfa (*Medicago sativa L.),* blue vetch (*Vicia cracca L.*), white and red clover *Trifolium pratense L., Medicago orbicularis,* wild lentils species (Hamadeh *et al.,* 1996), and the asteracea family which inculde *Hypochoeris glabra L., Centaurea scabiosa L., Xanthium spinosum L., Cirsium vulgare (Savi) Ten., Anthemis rascheyana Boiss* and others. Also other types of plants from other families are found on the natural rangelands such as wild rocket (*Diplotaxis tenuifolia (L.) DC*), *Rosmarinus officinalis L., Tulipa agenensis DC., Ranunculus arvensis L.*

On the other hand the vegetative cover in agricultural lands differ from that in natural rangelands. It is also composed of trees such as the Syrian mapple, Carob, Black poplar, grape vine and shrubs such as blackberry (*Rubus fruticosus).* Agricultural land are rich in: cereal plants such as the wild oat (*Avena fatua L.*), common barley (*Hordeum vulgare L*), bermuda grass (*Cynodon dactylon (L.),* johnson grass (*Sorghum halepense (L.) Pers.*), *Festuca arundinacea Schreb,* durum wheat (*Triticum durum Desf.)* and common wild sorghom (*Sorghum bicolor (L.) Moench),* legume plants such as alfalfa, red clover (*Trifolium pratense L.*), chick pea (*Cicer arietinum L*) and common vetch (*Vicea Sativa*). In addition to plants from the asteracea family such as lettuce (*Lactuca sativa L.),* creeping thistle (*Cirsium*

arvense (L.) Scopoli), and plants from different families such as stinging nettle, bindweed, giant horstail, potato, duckweed, wild radish etc. (Kharrat, 2010).

The annual grazing period of the herd is between 11-12 months depending on the farming system (Srour, 2006). Season and altitude affect the botanical diversity of the natural rangelands : with the advancement of seasons and altitude, the availability of herbaceous species decrease and lignified bushes increase. The nutritive value of natural rangelands also decrease as the summer advance: the mean of dry matter increase from 26 to 45% as well as ADF from 21 to 37% and NDF 35 to 53%, while the crude protein content decrease from 13 to 10% as reported by Kharrat (2010). On the other hand, the chemical characteristics of agricultural residues are more stable compared to the natural rangeland with a dry matter mean 24-45%, crude protein 10-16%, ADF 20-29% and NDF 30-46%. This show that the pastures in the Mediterranean region are subject to dietary and quality decline as the summer advance, and thus herds needs to move long distances searching for feed (7-20 Km). In fact, in Lebanon, the daily reached altitude for feed searching is comprised between 1200 and 1295 meters and can reach 1340 m, especially for Baladi goats which are characterized by their capacity of adaptation in harsh environment being able to recover their body reserve at the end of lactation in order to increase the success of reproduction (Kharrat, 2010).

6- Milk, Dairy products and meat production

Lebanese agricultural production value amounted in 2009 for 2.115 $ billion, and the animal production value for 638.6 $ million, from which milk production represented around 20% and 6% respectively (MoA, 2010). Small ruminant milk production is characterized by a strong seasonality, in fact, goat production lasts from May to November: the rustic Baladi goat lactation period ranges from 140-180 days with an average production of 90-150 Kg/head/year, while sheep milk production lasts from April to July (around 150 days) with an average production of 100-200 liters per year (Abi Saab, 2001; Haddad and Chamoun, 2014). Goat and sheep milk production varies based on the production system adopted, with the highest milk production observed in intensive systems due to the use of foreign goat breed (Chami, Saanen and Alpine) with an average milk yield per lactation per head of 420 liters in intensive systems, 137 liters in sedentary, 125 L in vertical transhumance, 128 L in horizontal transhumance and 118 L in semi nomadic system (Srour *et al.,* 2006). Baladi goats milk production quantities are low compared to other European and Shami breeds: in general, Shami breed produce more than 500 Kg of milk per year, but in Lebanese condition produce

about 220 Kg/year, a prolificacy rate of 1.8 weaned kids/year, and a faster growth rate of the kids while the Baladi goat prolificacy rate is 1.3 weaned kids/year. The quality of the milk produced and collected is initially affected by the health and safety conditions of the animals, by the milking hygiene and by the transport conditions: generally, milk from different farms is delivered to the collection centers as a milk mix produced under different conditions and thus resulting in an unstable microbiological quality, with higher possibility of milk contamination by Brucella, antibiotics and somatic cells (Touma, 2002). Few dairies analyze milk for microbiological and physical features and regards semi modern and modern dairies, while the traditional dairy farms rely on olfactory and visual examination of the raw milk and on trust. Milk production in the extensive systems is directly sold to consumer as raw milk or dairy products at the household of the farmer or in local market, or can be sold to dairy industries by a collector.

The major production is sold as whole milk (92.4% of sheep milk and 87.6% of goat milk), and a small part is processed into dairy products (Chedid, 2019). Milk processing into dairy products varies from region to another and on the production system: for example, cheese production is high in the South of Lebanon and low in the Bekaa, also, it is the highest in intensive systems (93%) and the lowest in semi nomadic and horizontal transhumance systems (16.5% and 1.8%, respectively). The dairy products transformation in Lebanon can be divided into three categories: the first category with a short shelf life (up to 10 days) includes milk and fermented milk as yogurt or Laban commonly known, or Labneh which is a spreadable fresh cheese with a lower water content compared to yoghurt. The second category includes cheese such as Halloumi and Akkawi with their serum byproducts such as Double cream which could be preserved for few months in a refrigerated environment in addition to the Kariche which cannot be preserved for long periods. The dairy products that can be preserved for longer period are: the powdery mixture of dehydrated milk mashed with wheat known as Kechek and might be consumed as soup or spread as pizza, in addition to Chanklich which is a highly fermented cheese covered with spices and thyme (El Balaa and Marie, 2008). Halloumi cheese and kechek are the most preferred products by the Lebanese consumer, followed by Double cream cheese, Akkawi and Chanklich (El Balaa and Marie., 2004).

In extensive systems, milk is sold at an average price of 0.4 $/Kg for sheep milk and 0.5 $/Kg for goat milk, and milk is generally collected without refrigeration by a collector or by the farmer. In intensive system, goat milk is sold at 0.63 $/Kg and 0.5 $/Kg for sheep milk (Hosri and Nehme,2006). Due to the absence of shepherd cooperatives and quasi absence of milk

collecting centers across Lebanon, milk sale strategies differ depending on the production system, milk yield and the seasonality (Haddad and Chamoun, 2014). Cow milk price is determined by the Ministry of Agriculture whereas the goat and sheep milk price is determined by the market based on the quantity and the season. Price is also affected by the fat content and by the farm hygiene and transportation. Milk price also differ from region to region: it is the highest in Mount Lebanon and the lowest in the Bekaa region. Lebanon's milk value chain suffers from the lack of investment and marginalization and need an efficient marketing strategies regarding the quality of the products, infrastructure rehabilitation and the production systems innovation including the packaging, marketing, diversification of new products (El Balaa and Marie, 2008).

The consumption of dairy products in Lebanon is about 189 Kg per capita yearly compared to 190 Kg per capita dairy intake in Spain and 207 Kg per capita in Greece (Serhan *et al.,* 2017). However, the local production covers 28% of the market demands and 63-78% of the market needs are imported each year (Asmar, 2011; MoA, 2005; Kharrat et al., 2010; Haddad and Chamoun, 2014). The 72% of the Lebanese national diet, is consisted of meat, milk and dairy products, and demands far exceeds the local domestic production (FAO, 2006). Only one third of the Lebanese milk consumption needs (fresh milk equivalent) are covered by the local milk production. In 2004, 610,000 tons of milk were consumed in Lebanon, and the quantity locally produced was about 244,000 tons, with goat products representing 14.8% (MoA, 2004). The evolution of milk production in Lebanon (FAO, 2020) in the last decade is reported in Figure 4. It is evident the increase of sheep and goat milk until the 2016 and then a stability in the production until 2020. In fact, the amount of milk annually produced is equal to 19759 tonnes for sheep milk and 29157 tonnes for goat milk (FAO, 2020). Milk production in 2014 reached 391,800 tons per year but still not in position to satisfy the demand, both in terms of quantity and consumer quality expectations (FAO, 2014). In 2002, a total of 32,000 tons of cheese were imported while the total export was about 420 tons especially for Halloumi and Akkawi cheese in addition to the transit and re-export of dairy products to Arab countries, while cheese imports where about 32000 tons in 2002 (MoA, 2005; Serhan *et al.,* 2017). The production of cheese in the last decade was summarized in Table 1 (FAO, 2020). The production of small ruminant meat in Lebanon (FAO, 2020) is about 4500 tons for sheep (155000 head) and 3000 tons for goats (135000 head), with an average weight of sheep about 39 Kg and 37 Kg for goats. The meat production in the last years is stable for both species (Figure 5).

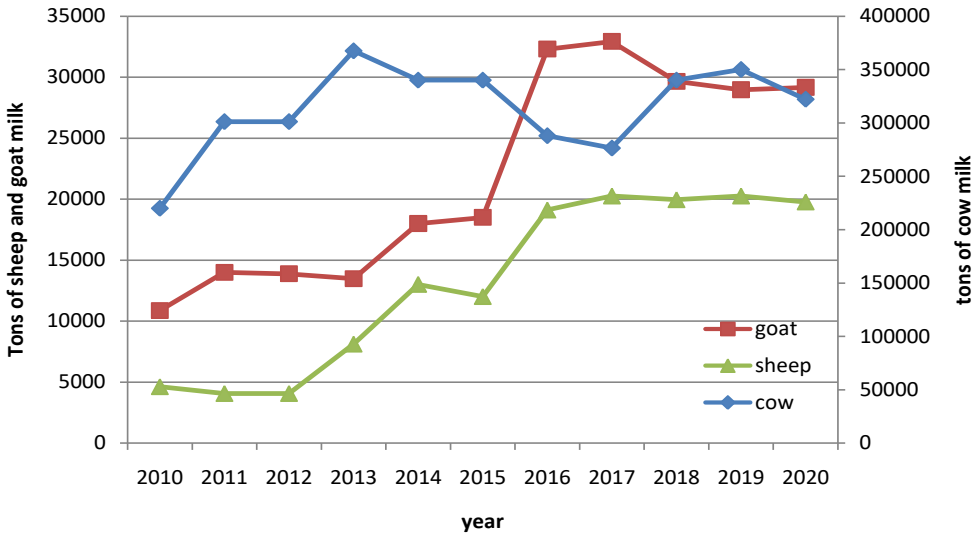

Figure 4: The evolution of milk production in Lebanon between 2010 and 2020 (FAO, 2020).

Table 1: Amount of total cheese and cheese from sheep and goats (in tonnes) produced in Lebanon in the last 10 years (FAO, 2022).

Year	All type cheese	Goat cheese	Sheep cheese
2010	17378	652	461
2011	23108	839	405
2012	23154	833	405
2013	28331	809	810
2014	27265	1080	1300
2015	27184	1110	1200
2016	28778	1938	1910
2017	27402	1110	1350
2018	27177	1064	1062
2019	29154	1718	2059

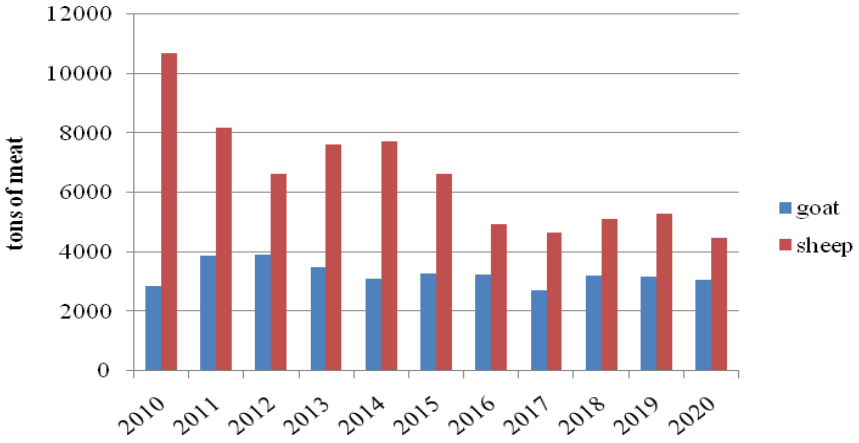

Figure 5: Meat produced by sheep and goats in the last decade in Lebanon (FAOSTAT 2022).

7- Strenght and constraints of small ruminant sector in Lebanon

Lebanese small ruminant production systems are characterized by their traditional aspect and their authentic dairy products transformed by the farmer families are very appreciated by the consumers, and represent an integrated part in the Lebanese economy, tradition and culture. Also, the Lebanese topography, climate and richness in water, plays a strength role for the small ruminant sector making rangelands rich in fresh vegetation from the end of winter to mid-summer, in addition to the high adaptability of Awassi sheep and Baladi goat.

On the other hand, this sector suffers from different problems which affect its sustainability. Herd health is among the most important problems due to the lack of governmental support for animal health, the absence of health and veterinary extension services and the limited surveillance of transboundary animal diseases in addition to the animal vaccination programs and quarantine facilities (Hamadeh *et al.,* 1996; Curtis *et al.,* 2013). Moreover, due to the Syrian war, and the high number of refuges entry, a high number of animals have entered Lebanon and have subjected the local flocks to a high risk of contagious disease outbreak (FAO, 2014). The availability and the quality of pastures land for extensive system have been decreasing due to climate change, urbanization, early drought, expansion of agricultural lands especially orchards and vineyards and to overgrazing (Hamadeh *et al.,* 2006). In addition, the high pasture land rental fees are causing an additional cost to the farmers added to the feed costs, in absence of any governmental support for feed subsidies (Hosri and Nehme, 2006). In fact, natural pastures are grazed from the end of winter till the beginning of summer, due to

the lack of fodder and cereal residues during the dry season. Thus, at the end of lactation, animals under goes a dietary imbalance leading to a decrease in milk production and in milk fat and protein contents (Kharrat, 2010). Price fluctuation of milk and the high price of feeds are also affecting this sector adding to this, the competitive price of illegal animal products smuggled since the war in Syria through the uncontrolled borders, which have a lower price by 75-50% than that of local animal products. This economic situation, has led farmers to sell animals in order to cover the feed, health and labors expenses (Hamadeh *et al.,* 1999; Hamadeh *et al.,* 2001). Market structure should be developed by an efficient strategy which enhance the marketing and valorize the quality of local products (Srour, 2006). This sector is an important economic source especially in rural areas despite its low production and the absence of governmental aids and supports. An effective plan concerning the crop rotation and increasing the fodder cultivated areas can improve the sustainability of the small ruminant sector (Srour *et al.,* 2007).

8- General factors influencing the fertility and reproduction in small ruminants

8.1- Nutrition, body weight and body condition score

A balanced diet is essential to prevent body weight loss of the animal and problems during lambing. Poor nutrition can affect the regularity of the cycles in females, reduce the ovulation, and leads to weak offspring, reduction in twinning rate and enhance pregnancy toxemia in females, whereas in males it affects the sperm quality and quantity. The amount of feed during reproduction period increases ovulation (flushing) and increase the number of twin birth. In addition, the amount of feed to pregnant mother have a direct effect on the weight of the offspring at birth, and thus increase the survival of the offspring, whereas undersize new born are not able to maintain their body temperature and have less chance of surviving. Also, overfeeding pregnant animals can cause difficulties in birth and lead to the death of the offspring and mother (Petrovic *et al.,* 2012). A study has showed that ewes fed with barley and wheat bran (700-900g and 250-300g per head respectively) and grazing on the residues of vegetable have about 4 times higher twin rate than ewes fed with 600g barley and 200g wheat bran per head per day. Fetal loss increases by 13 time in ewes pregnant with twins compared to single fetus, in addition more fetal loss occur in semi nomadic flocks compared to stationary flocks, due to under-nutrition particularly during the dry seasons (Lafi *et al.*, 2009). Also, supplements containing vitamins (A, D3, E) prior to mating, have showed higher lambing and twinning rate compared to non-supplemented ewes, because vitamin A

increase the rate of conception, pregnancy and lambing in sheep (Lafi *et al.,* 2009). Ewes suitably fed and with good BCS have better respond to the onset of breeding season and show an increase in ovulation. In the semi-arid regions of the Mediterranean area where feed supply is low (Talafha and Ababneh, 2011) lambing and twinning rates are boosted after nutritional flushing and when the BW of the ewes is high. In fact, BCS in stationary flock is higher than that in the semi nomadic ones due to the use of supplements and grazing on vegetable residues (Santolaria *et al.,* 2011).

8.2- Farm management, season and animal age

Different management practices in the farm have an impact on the fertility. The right proportion of male to female in the farm can increase the conception and the lambing rate (Lafi *et al.,* 2009). The selection of animals adapted to the environment condition, and providing a correct and efficient nutrition during the reproduction period of the animals is much important. In addition, health surveillance of the flock and a suitable vaccination program with a good reproductive planning (season, intervals between lambing, age) can greatly affect the fertility (Petrovic *et al.,* 2012).

Season mediated by photoperiod have an effect on small ruminant reproduction by modifying the hormonal balance: photoperiod cause variations in the melatonin secretion which affects the secretion of luteinizing hormone-releasing hormone (GnRH), follicle stimulating hormone (FSH), and luteinizing hormone (LH), and as an effect decrease the reproductive activity of the animals during long days (Santolaria *et al.,* 2011).

Age affects the reproduction of ewes, because more the ewes are young, their fertility decrease compared to adult due to the lower mucus production in the cervical canals during oestrus and to the impaired sperm transport (Santolaria *et al.,* 2011).

Overall objectives of the thesis

The overall objectives of this thesis were to evaluate the farming system of sheep and goats in Lebanon and to improve knowledge on their milk composition. In addition, the effect of inclusion in the diet of cardoon byproducts, a Mediterranean plant widespread in Lebanon on milk production traits of dairy sheep has been studied.

The specific contributions were:

1- First contribution is the survey on farming systems of goats and sheep in Lebanon with focus on the extensive production systems.

2- Second contribution: study the of milk fatty acid composition of Awassi sheep and Baladi goat in extensive production systems in Lebanon.

3- Third contribution regards the experimental trial: effect of Cardoon (*Cynara cardunculus*) byproduct on the production, composition, and fatty acid profile of sheep milk.

4- Fourth experimental contribution: Effect of polyphenolic extracts of cardoon (*Cynara cardunculus*) on the production, composition and coagulation properties of sheep milk.

CHAPTER 2

Survey to study the socio-economic conditions of extensive small ruminant production systems in Lebanon

1- Objectives

A survey was carried out to classify the extensive production systems of small ruminants, specifically Baladi goat and Awassi sheep, in Lebanon and to collect data from farmers with reference to the flock and farm management, feeding resources, production, livelihood strategies and the different grazing routes.

2- Material and Methods

2.1- Data collection in the survey

The primary data was collected through a survey, which was composed of seven sections with open-ended and closed-ended questions (35 questions) in order to collect as much data as possible to quantify the number of Baladi goats and Awassi sheep in the farms, and to understand the farm production system and composition, the herd composition and its reproductive traits, herd management in the farms and its production in addition to the farmer source of income and challenges. Each section includes the following questions:

- Section 1, general information about the farm (location, altitude, farming system and management);
- Section 2, goat husbandry, including herd composition and reproductive performance;
- Section 3, sheep husbandry, including herd composition and reproductive performance;
- Section 4, information about the feeding strategies (feed type and quantity) and pasture use;
- Section 5, information about milk production, milk transformation, price and selling routes;
- Section 6, small ruminant diseases and veterinary service;
- Section 7, economics of farmers, their income and constraints.

The study relied on quantitative and qualitative data obtained from the survey which have covered farms from different regions and at different altitudes in Lebanon. Contacts of the farmers were obtained by the help of the Ministry of Agriculture, the Lebanese University Professors and colleagues, the Chouf Biosphere Reserve and the Agricultural COOP. In addition, snowball sampling was used: some interviewed farmers helped us in identifying and locating other farmers from the region. The questionnaire was filled through direct

interview with the farmers in their farms or was sent as Google form to educated farmers or by phone interview. A total of 43 farms have been interviewed from 30 different regions in Lebanon: 22 from Mount Lebanon, 4 from the Bekaa, 3 from North and 1 farm from the South (Figure 1). The altitude of the farms and the production system adopted was used as factors to analyze the collected data. Farms altitudes was classified into 4 altitude ranges: below 500 m above sea level (asl), between 500-1000 m, between 1000-1500 m and above 1500m asl, whereas 3 production systems were identified: sedentary, semi nomadic and transhumant system (Figure 2,3,4 and 5). It is worth to mention that it was difficult to obtain accurate and specific numbers and data from the farmers.

Figure 1: Distribution of the selected farms in Lebanon were the survey on small ruminant was carried out (google map)

Figure 2-3: Baladi goat herds in extensive systems in Lebanon

Figure 4-5: Awassi sheep herds in extensive systems in Lebanon

2.1- Statistical analysis

Descriptive statistic of collected data from interviewer were carried out. One-way ANOVA statistical analysis was performed using MINITAB® software (Version 16.1.0, Minitab, State College, PA, USA) to detect differences between species.

3- Results and Discussion

3.1. Flock consistency

In total, 43 farmers were interviewed. The total number of animals was 11348 head, with a mean of 265±230 (mean ± SD) head per farm. Goats represented 64% of the total animal number while sheep represented 36% (7277 and 4071 head, respectively). Some of the interviewed farms were mixed composed both from goats and sheep. The breeds raised were Awassi for sheep, and Baladi goats represented 95% of the goats while the rest 5% were from Shami (Damascus) breed.

Table 1: Sheep and goat numbers in the interviewed farms and their percentage (%).

	Total number	Mean	STDEV	Minimum	Maximum	Percentage (%)
Farm animals	11348	265	230	5	1000	
Awassi Sheep	4071	95	191	0	1000	35.9
Goats	7277	169	170	0	700	64.1
Baladi goat	6916	161	171	0	700	60.9

3.2- Animal consistency, flock composition and reproductive performance based on the altitudes of the farms.

The animal number in the different farms varies based on the altitude (Table 2). Also, the distribution of the two species varied with the altitude, with the prevalence of sheep at altitudes lower than 1000 m and goats at higher altitudes (Table 3). This is due to the high adaptation of these breeds especially the Baladi goat to the mountainous topography of Lebanon and the extensive systems widely used (Hamdeh *et al.,* 1997; Jaber *et al.,* 2004). In fact, the rustic Baladi goat is the dominant breed with lower numbers of Shami goats, characterized by higher production level but lower adaptation to extensive system. Awassi sheep is the only breed of sheep used in Lebanon because of its excellent adaptation to semi-dry environmental condition. The results observed in this survey are in accordance with previous studies which states that Awassi sheep, Baladi goats and Shami goats represents 100%, 95% and 5% respectively of the small ruminants in the Lebanese extensive systems (Hajj, 1999; Nehme and Abi Saab, 2003).

Table 2: Breed consistency at different altitudes (m asl) of the interviewed farms.

	<500m			**500-1000m**			**1000-1500m**			**>1500m**		
	Total	Mean	Stdev	Total	Mean	Stdev	Total	Mean	Stdev	Total	Mean	Stdev
Total Animal number	672	91	111	2247	375	425	6266	313	183	2163	240	170
Awassi sheep number	394	49	67	1793	299	439	1119	56	74	765	85	128
Baladi goat number	252	32	40	436	73	115	5060	253	188	1168	130	123

Table 3: Number of total animals and percentage distribution (%) at different altitudes of the interviewed farms

	<500m	**500-1000m**	**1000-1500m**	**>1500m**
Total Animal, number	672	2247	6266	2163
Awassi sheep, %	58.6	80	18	35.4
Baladi goats, %	37.5	19	81	54.0
Shami goats, %	3.9	1	1	10.6

The flock composition of Baladi goats is represented by 72% of adults and 28% of young. The females represent 87% and males 13% of the flock. However, the composition varies with the altitudes, as detailed in table 4: at altitude lower than 500 m is present the highest male and young animals number used for meat production, probably due to the consumer preference of goat meat in regions such as the South of Lebanon, Nabatiyeh and Akkar located <1000 m of altitude. The percentage of young animals reached the highest value (34.5%) above 1500m (Table 4) due to the highest necessity of replacement animals. The reproductive performance of Baladi goat vary greatly with the altitudes. The high fertility and technical conception rate at low altitudes, is probably related to the better nutrition condition of animals as evidenced by the highest quantity of feed given per animal per day (Table 11-12). Fecundity and prolificity rate were also the highest at lower altitude, also because of the better nutrition condition of the flocks compared to the high altitude (Table 11-12). The

twining rate and the survival rate increase with better nutrition and health status of the mother and kids, associated with the highest vaccination and veterinary consultancy observed in farms located at lower altitude (Table 11-12).

Table 4: Baladi goat flock composition and reproductive performance based on the different altitude categories (m asl) of the interviewed farms (%).

	<500m	**500-1000m**	**1000-1500m**	**>1500m**
Baladi goat number	252	436	5060	1168
Adults, %	70	78	76.4	65.5
Young, %	30	22	23.5	34.5
Male, %	17	11	11.1	12
Female, %	83	89	88.4	87
Fertility rate, %	82.9	81.6	77.6	74.3
Technical conception rate, %	100	86.9	96.1	96.2
Fecundity rate, %	98	120.5	83.7	77.5
Prolificity rate, %	1.15	1.5	1.07	1.03
Survival rate, %	91	72.5	68.7	75.3

Fertility rate= (number of female kidded/number of female exposed) *100; Technical conception rate= ((number of female kidded+ number of observed female aborted)/number of female exposed) *100; Fecundity rate= (kid born number/ female exposed number) *100; Prolificity rate=kids number/ female kidded number; Survival rate= kids alive till weaning number/ kids born number.

The reproductive performance for Awassi sheep are reported in Table 5. Substantially, as goats, also the sheep are present in very low number below 500 m. In these conditions the farms are very small, and this explains the lower reproductive performance but also the better health status of the animals, which in small numbers can be kept in better conditions. This is supported by the highest percentage of vaccination, veterinary services (Table 11-12) and the lowest percentage of Enterotoxaemia and foot and mouth diseases in the farms located below 500m (Figure 11).

Table 5: Awassi sheep flock composition and reproductive performance based on the different altitude categories (m) of the interviewed farms (%)

	<500m	**500-1000m**	**1000-1500m**	**>1500m**
Awassi sheep number	394	1793	1119	765
Adults, %	81	76	73	78
Young, %	19	24	27	22
Male, %	27	15	12	10
Female, %	73	85	88	88.4
Fertility rate, %	79.5	90.5	83.2	84.1
Technical conception rate, %	97.7	95.8	98	98.4
Fecundity rate, %	88.8	96.7	87.1	91.8
Prolificity rate, %	1.14	1.07	1.1	1.09
Survival rate, %	86.9	68.2	77.1	81.5

Fertility rate= (number of female kidded/number of female exposed) *100; Technical conception rate= ((number of female kidded+ number of observed female aborted)/number of female exposed) *100; Fecundity rate= (kid born number/ female exposed number) *100; Prolificity rate=kids number/ female kidded number; Survival rate= kids alive till weaning number/ kids born number.

3.3- Animal consistency, flock composition and reproductive performance based on the production systems of the farms.

Extensive production systems are the most widespread small ruminant systems in Lebanon. The most important systems are the sedentary, semi nomadic and transhumant systems where only Awassi sheep, Baladi goats and small percentage of Shami goats are raised. On the other hand, intensive systems are being more widespread nowadays using other types of breeds generally more productive like the Shami and Alpine goats. More than 50% of animals are bred in the semi nomadic system with average 426±275 animal per farm (Table 6). In all system goats represent the prevalent specie with the highest number in the transhumant system, followed by the sedentary system and the lowest in the semi-nomadic. Also, the highest number of sheep are bred in the semi nomadic system (41%; Table 6-7). These results are in accordance with previous surveys (Hamadeh *et al.,* 1996; Srour, 2006; Marie, 2009; Haddad and Chamoun, 2014).

Table 6: Animal consistency based on the different production systems of the interviewed farms.

	Sedentary			Semi nomadic			Transhumant		
	Total	Mean	Stdev	Total	Mean	Stdev	Total	Mean	Stdev
Total Animal number	1594	118	144	6384	426	275	3370	241	124
Sheep number	530	38	61	2603	174	300	938	67	74
Goats number	1064	76	134	3781	252	204	2432	174	114
Awassi number	530	38	61	2603	174	300	938	67	74
Baladi number	1014	72	135	3701	247	204	2201	157	121

Table 7: Animal percentage (%) based on the different production systems of the interviewed farms.

	Sedentary	Semi nomadic	Transhumant
Total Animal number	1594	6384	3370
Sheep, %	33.2	41	27.8
Goats, %	66.7	59	72.2
Awassi, %	33.2	41	27.8
Baladi, %	63.61	58	65.3
Others (Shami Goats), %	3.2	1	6.9

The composition of Baladi goat herds for the three productive systems is reported in Table 8. Compared to the sedentary system, the others evidence lower reproduction parameters, as evidenced by the fertility rates, the technical conception rate, fecundity and survival rates (Table 8), due to differences in dietary supplements and the higher use of veterinary services which performs the highest number of vaccination per year (Table 10- 13). On the other hand, the highest percentage of small ruminant disease found in the transhumant system could explain the low reproduction performance (Figure 8-9).

Table 8: Baladi goat flock composition and percentage (%) based on the different production systems of the interviewed farms.

	Sedentary	Semi nomadic	Transhumant
Baladi goat number	1014	3701	2201
Adults, %	71	76.2	72.6
Young, %	29	23.5	27.4
Male, %	14	11	11.3
Female, %	86	89	87.6
Fertility rate, %	84.5	78.4	73.1
Technical conception rate, %	98	94.9	94.3
Fecundity rate, %	104.5	90.7	76.6
Prolificity rate, %	1.2	1.16	1.05
Survival rate, %	88	64.7	74.4

Fertility rate= (number of female kidded/number of female exposed) *100; Technical conception rate= ((number of female kidded+ number of observed female aborted)/number of female exposed) *100; Fecundity rate= (kid born number/ female exposed number) *100; Prolificity rate=kids number/ female kidded number; Survival rate= kids alive till weaning number/ kids born number.

Awassi adult number was the highest in the sedentary system and the lowest was found in the transhumant system (81% and 75.9% respectively). In the latter system, the female number of Awassi sheep was the highest and the lowest in the sedentary (86.8 and 76% respectively). The reproductive parameters are less variables among the different production systems compared to goats. However, Awassi fertility and fecundity rate were the highest in the transhumant system and the lowest in the sedentary system (Table 9), probably due to the higher use of concentrate in the diet in the former (Figure 6). In fact, the 100% of the transhumant farms use hay and 57% use concentrates whereas only 36% and 13% of farms use concentrate in sedentary and semi nomadic system, respectively (Figure 6).

Table 9: Awassi sheep flock composition and percentage (%) based on the different production systems of the interviewed farms.

	Sedentary	Semi nomadic	Transhumant
Awassi sheep number	530	2603	938
Adults, %	81	76	75.9
Young, %	19	24	24.1
Male, %	24	13.1	13.2
Female, %	76	86.4	86.8
Fertility rate, %	80.6	81.2	83.3
Technical conception rate, %	98.2	97.9	97.5
Fecundity rate, %	88	90.2	90.3
Prolificity rate, %	1.1	1.1	1.08
Survival rate, %	84.8	76.9	77.7

Fertility rate= (number of female kidded/number of female exposed) *100; Technical conception rate= ((number of female kidded+ number of observed female aborted)/number of female exposed) *100; Fecundity rate= (kid born number/ female exposed number) *100; Prolificity rate=kids number/ female kidded number; Survival rate= kids alive till weaning number/ kids born number.

3.4- Managerial aspect of the farms based on the production systems and the altitude of the farms.

The managerial aspects of the farms are reported in Table 10 based on the production system and in Table 11 based on the different altitudes. The highest percentage of farms which use supplements, byproducts, and ask for veterinary services are the sedentary system while the lowest are the semi nomadic because this system suffer the most from the lack of budget (Figure 13). The semi-nomadic, is the system which make more analysis on milk quality because of the high number of animals, production level (Figure 8-9). All farms use supplement in the diets of animals at all altitudes, especially by-products at low altitude represented by residues of cottonseed and peanut peels (Figure 7).

Table 10: Percentage of farms that use supplements, byproducts, antibiotic in the feed of goat and sheep, make quality analysis of milk and dairy products, ask for veterinary services, make vaccinations and treat for ticks based on the production system adopted (%).

	Sedentary	**Semi nomadic**	**Transhumant**
Use supplement in the feed, %	92.8	80	92.8
Use byproducts in the feed, %	35.7	26.6	28.6
Make quality analysis, %	28.6	53.3	28.6
Ask for veterinary services, %	64.3	20	28.6
Make vaccination, %	85.7	93.3	100
Treat for ticks, %	85.7	86.7	100

Table 11: Percentage of farms that use supplements and byproducts, make quality analysis of milk and dairy products, ask for veterinary services, make vaccinations and treat for ticks based on the altitude of the farm (%).

	<500m	**500-1000m**	**1000-1500m**	**>1500m**
Use supplement in the feed, %	100	100	80	100
Use byproducts in the feed, %	50	16.7	30	22.2
Make quality analysis, %	25	50	45	22.2
Ask for veterinary services, %	100	50	15	22.2
Make vaccination, %	100	83.3	90	100
Treat for ticks, %	87.5	83.3	90	100

Milk production traits at different altitudes are summarized in Table 12. The kids suckled milk for about 2.5 months at <1000 m and almost for one month more at >1000 m (3 months). Similar pattern has been observed for lambs. These differences are explained by the slaughtering of young of both species to produce meat below 1000 m and the rearing of animal for animal replacement above 1000 m.

Based on the production systems of the farms (Table 13) kids suckled milk for about 4 months in the semi nomadic system, 3 months in the transhumant and less than 3 months in the sedentary systems. Similar pattern can be observed for the lambs even if they show a shorter duration of suckling period compared to goats. The daily average milk production in Awassi was 50% lower in the sedentary compared to the others systems (Table-13).

Table 12: Mean and standard deviation of the number of day milk feeding for kids and lambs (days), Awassi and Baladi milk production in the farms (L/head/day), price of Awassi and Baladi milk (L.L.), the quantity of feed offered per day per animal (Kg/day/animal) and the number of vaccination done per year in the farms based on the altitudes categories.

	<500m		**500-1000m**		**1000-1500m**		**>1500m**	
	Mean	Stdev	Mean	Stdev	Mean	Stdev	Mean	Stdev
Kids suckling duration (days)	79	32	71.3	32	102	55.5	100	30
Lambs suckling duration (days)	64	49	41.3	43	76.5	65.7	76.6	47.7
Sheep milk yield (L/head/day)	0.7	0.75	0.75	0.9	1.15	0.9	0.94	0.63
Goat milk yield (L/head/day)	1.8	0.65	1.8	1.3	1.725	0.7	1.6	0.4
Goat milk price (L.L.)	3900	223	3000	2449	2444	1580	3642	475
Sheep milk price (L.L.)	3312	221	3050	1155	2697	944	3111	485
Quantity of feed per animal per day (Kg/day/animal)	2.7	0.7	1.7	0.4	2.24	0.7	2.43	1.2
Number of vaccination per year	3.9	3.5	2.1	2	2.05	1	2.9	1.16

[1]L.L.: Lebanese lira

Table 13: Mean and standard deviation of the number of day milk feeding for kids and lambs (days), Awassi and Baladi milk production in the farms (L/head/day), price of Awassi and Baladi milk (L.L.), the quantity of feed offered per day and per animal (Kg/day/animal) and the number of vaccination done per year in the farms based on the production system of the farm.

	Sedentary		**Semi nomadic**		**Transhumant**	
	Mean	Stdev	Mean	Stdev	Mean	Stdev
Kids suckling duration (days)	75.6	33	112	47.5	90	47
Lambs suckling duration (days)	54	47	86	63.1	66.4	55.4
Sheep milk yield (L/head/day)	0.60	0.76	1.16	0.81	1.10	0.8
Goat milk yield (L/head/day)	1.78	0.95	1.66	0.61	1.8	0.67
Goat milk price (L.L.)	3000	1715	2285	1528	3900	516
Sheep milk price (L.L.)	3061	480	2733	820	3089	1045
Quantity of feed per animal per day (Kg/day/animal)	2.3	0.85	2.16	1	2.42	0.67
Number of vaccination per year	2.8	3	2.4	1.5	2.6	0.9

3.5- Feed and diet components

The feed and diet components most used in the different farms are mainly barley, hay, mais and wheat bran (88, 68, 59 and 51 % of farms, respectively), followed by soya and wheat that are used by 24 and 20% of the farms, respectively. Very small percentage of farms use alfalfa, medicago, and other types of byproducts such as cotton seeds and peanut peal due to their high cost. Hay, concentrates and forage leaves are the types of supplements mostly used by the farmers in Lebanon. Hay is the most used in all the three types of production systems (Figure 6) and in all altitudes (Figure 7).

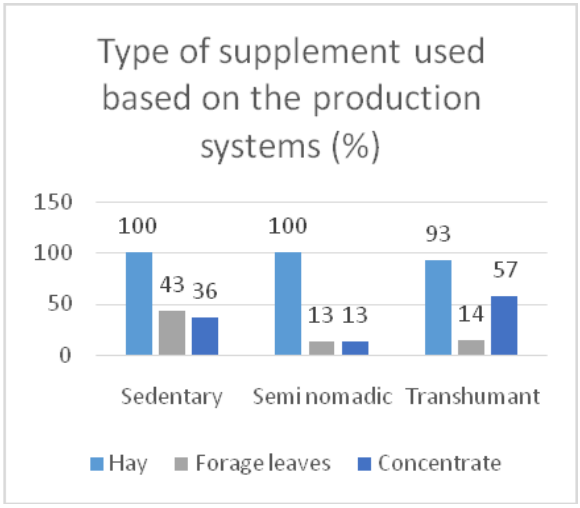

Figure 6: Type of supplement used in the feed based on the production system (%).

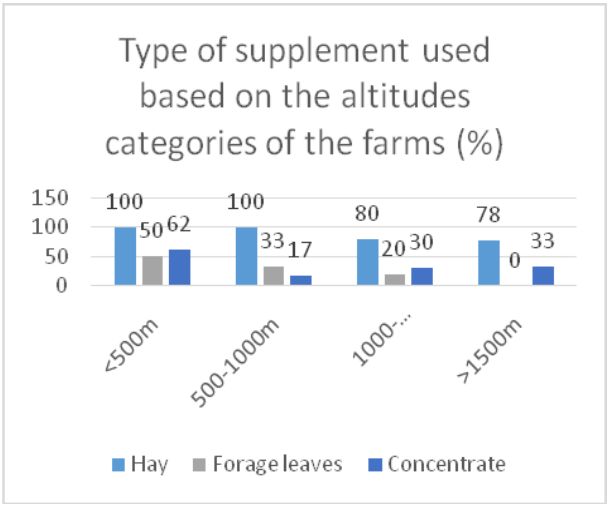

Figure 7: Type of supplement used in the feed based the altitude categories of the farms (%).

3.6- Livestock disease

The main disease that are present in the farms include internal and external parasites and infections. Based on the production system (Figure 8-9), the highest percentage of farms suffering from animal diseases are found in the transhumant systems where enterotoxemia, pasteurella and foot and mouth disease, sheep scab and goat plague were more widespread. Variations on animal disease frequency are observed based at different altitudes (Figure 10 - 11). These results could be explained by the different management in the farms and the movement of the flock for different distances searching for feeds especially in the transhumant system. In addition, the weather conditions that may increase the risk of disease (hot and dry in summer at low altitude, with high humidity at high altitude), and the illigal

entry of Syrian flocks to Lebanon without any surveillance increase the risk of transboundary diseases.

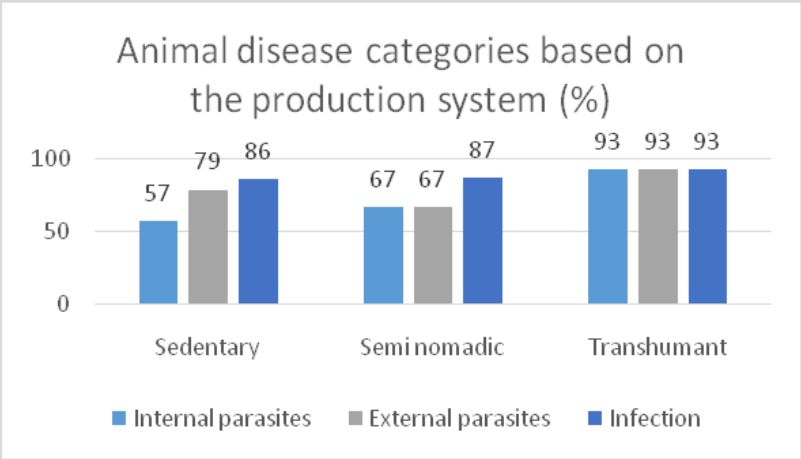

Figure 8: Animal disease categories based on the production system (%).

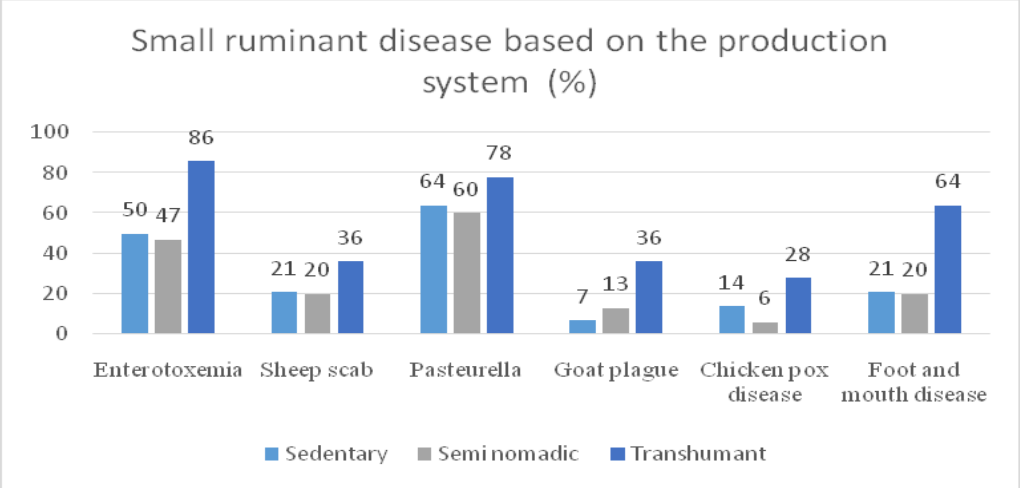

Figure 9: Most important small ruminant diseases based on the production system.

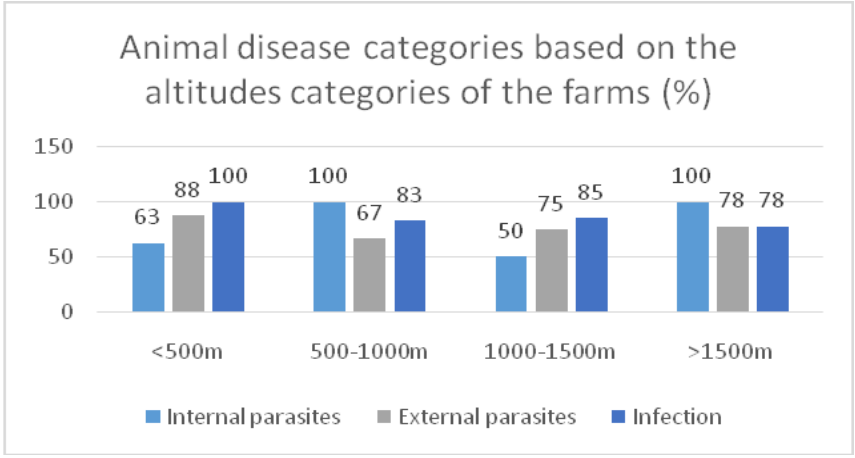

Figure 10: Animal disease categories based on the altitude categories of the farms (%).

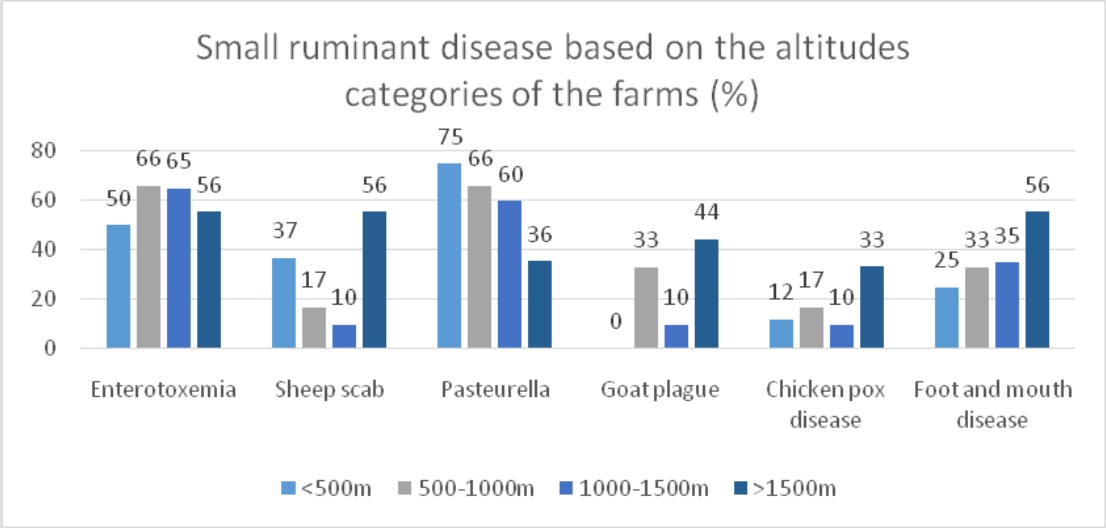

Figure 11: Most important small ruminant diseases based on the altitude categories of the farms (%).

3.7- Incomes and contrains of the interviwed farms

In all production systems and at different altitudes, milk is partly sold fresh and part is transformed at farm level into the principal typical dairy products as yoghurt, labneh and different types of traditional cheese. The amount of milk processed into dairy products vary among regions based on the consumer preference. Based on the production systems (Figure 12), milk was the principal source of income for farmers, followed by selling kids and lambs. The selling of manure contribute also to the income of farms, followed by the selling of wool (Figure 12).

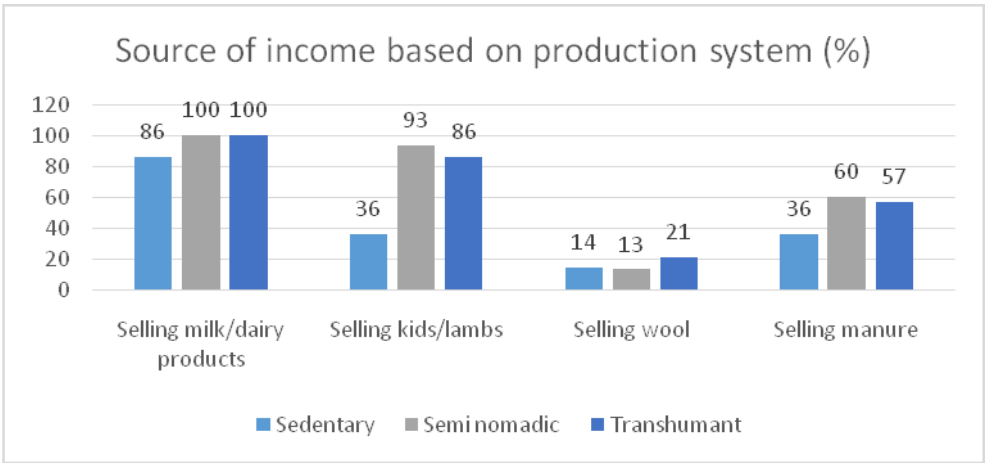

Figure 12: Source of income based on the production system (%).

The major constraints facing the farmers in all the three production systems studied and in all altitudes are the market price, feed availability and budget. The low pasture land, the high rental fees of land and the high costs of feeds could be the main reasons of these constraints. On the other hand, transport and predators constraints were faced in the semi nomadic and transhumant system and at high altitudes (>1000 m) (Figure 13-14) because of the movement from region to another.

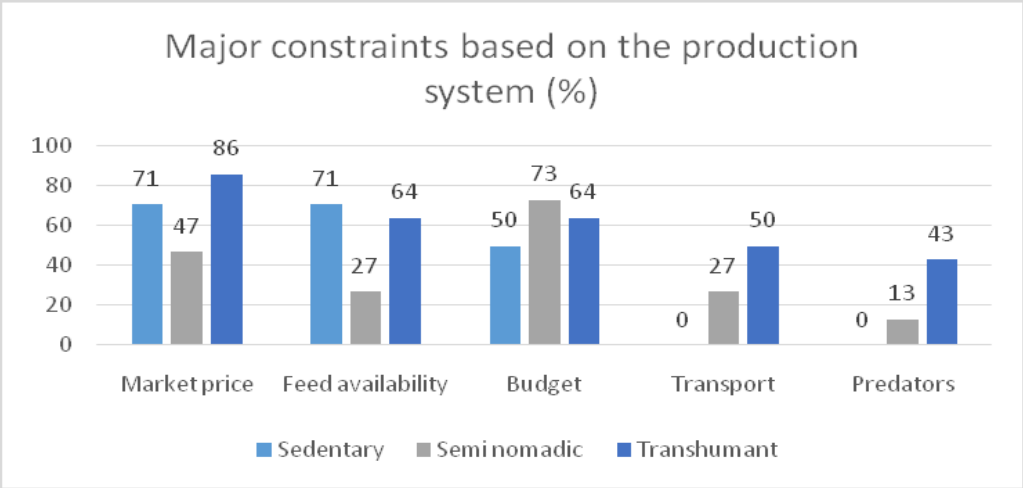

Figure 13: Major constraints faced by the farmers based on the production system (%).

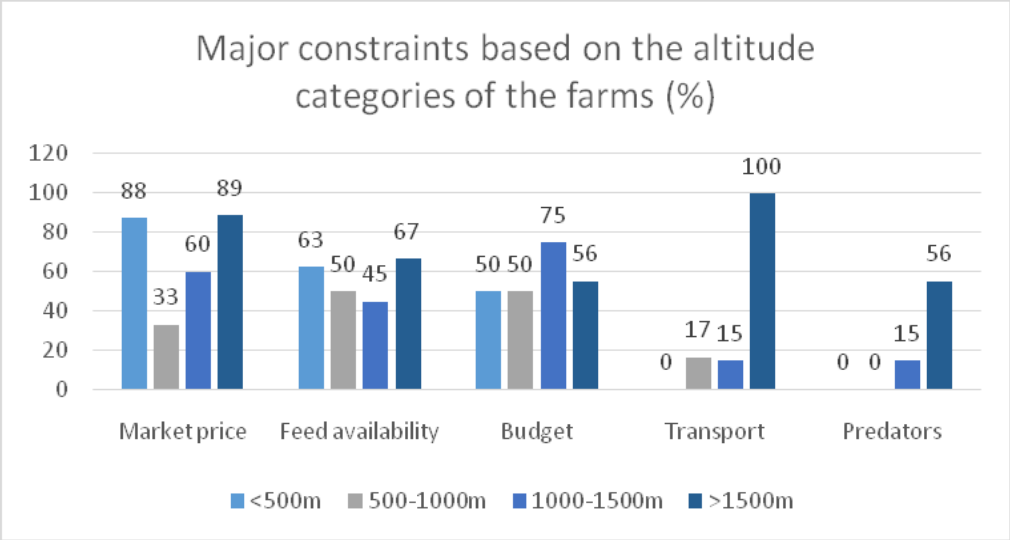

Figure 14: Major constraints faced by the farmers based on the altitude categories of the farms (%).

4- Conclusion

In Lebanon, small ruminant production has a socio-economic importance in rural regions by providing income to a large part of families and by providing typical dairy products to consumer. The type of breeds used, the farm management, the availability of feeds, in addition to the climatic factor, affects the productive performance of the herds. From this survey was possible to understand that sheep and goats of Lebanon are mainly raised in the semi nomadic and transhumant farming system which suffer from animal diseases, pasture availability and feeds costs. Lebanese small ruminant system can be more efficient if actions as better nutrition management, improvement of pasture management, and screening of diseases are implemented. Grazing on mountainous rangelands is efficient during the early lactation stage where the quality of grazed forage is enough and help to reduce the negative energy balance. At the end of spring, and at mid lactation, due to the decline in the quality and quantity of rangeland because the dry season, grazing on residual crops guarantee the maintenance of milk yield and the success of reproduction. The productivity of local breeds can be improved by selection, and by conserving their capacity of adaptation, and by adopting a surveillance program for the diseases of small ruminants to improve the health status of flocks. Moreover, the use of byproducts and increasing fodder cultivated land can reduce the feed cost and improve their quality. At the marketing level, products should be more valorized by the use of new technologies, labels and attractive packaging. Obviously, the implementation of these strategies at farm level requires technical assistance and support.

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CHAPTER 3

Composition and fatty acid profile of milk from Baladi goat and Awassi sheep milk in different farms in Lebanon

1- Objectives

The aim of this study was to determine the milk fatty acid profile of Awassi sheep and Baladi goat reared in different extensive production system (sedentary, semi nomadic and transhumant) and at different altitudes in Lebanon.

2- Material and Methods

2.1- Milk samples collection

Milk samples of Baladi goat and Awassi sheep were collected from 10 farms. Farms were from different regions, and were composed of different ratio of sheep/goat (Figure 1) bred with different extensive production system (sedentary, semi nomadic or transhumant). On the basis of altitude above sea level (asl), the farms were classified into three categories: <1000m asl, 1000-1500m and >1500m. Pasture was the principal source of feed, with additional supplements and/or concentrates especially in the sedentary farms. Animals were at their final stage of lactation (in August) and were selected randomly. Different number of samples were collected from each farm based on the animal consistency. Each farm was visited twice and a total of 64 milk samples of Baladi goat and Awassi sheep were collected (60 ml). Milk samples were stored in freezer and then were transported to Italy using dry ice, and analyzed for their protein and fat content and their fatty acid composition (Figure 2-3-4). Samples of dietary ingredients were also collected and analyzed.

Figure 1: Distribution of the farms from which Baladi goat and Awassi sheep milk samples were collected in Lebanon (google map).

Figure 2-3-4: Milking and Milk samples collection

2.2- Laboratory analysis

Feed

The samples of the administered feed collected from 6 different farms in Lebanon, were grinded and analyzed for their dry matter content, protein, NDF, ADF, ADL and ash content as detailed in Chapter 7 paragraph 1.

Milk fat and protein contents

Milk fat content was determined according to Gerber method (ISO, 1975) and the total protein (N × 6.38) was analyzed by the Kjeldahl method. The Rose-Gottlieb method (AOAC, 1990) was performed to determine the milk fat extraction, with modifications as described by Nudda *et al.,* (2013) as detailed in Chapter 7 paragraph 2.1.

Milk fatty acid profile

The fatty acid methyl esters (FAME) were analyzed with a base-catalyzed transesterification according to FIL-IDF standard procedure (1999) as detailed in chapter 7 paragraph 2.3, and the nutritional properties were calculated as detailed in Chapter 7 paragraph 2.4.

2.3- Statistical analysis

One-way ANOVA was performed using MINITAB® software (Version 16.1.0, Minitab, State College, PA, USA) to detect differences between the fatty acid composition of the two species, and their variation between the production systems and the altitude of the farms used as factors.

3- Results and Discussion

3.1- Diet and chemical composition

The chemical composition of feeds used in different farming systems is reported in Table 1. The concentration of protein varied from 13 to 18% of DM, whereas the NDF ranged 36.3-33.8% of DM from semi nomadic to sedentary system.

3.2- Fatty acid profile and nutritional indices of fat in Baladi goat and Awassi sheep milk

The milk fat content was 9.17±1.65% in Awassi milk and 4.08±1.43% in Baladi goats. The high content in fat in both species could be attributed to the advanced stage of lactation of animals since milk sampling was performed during August. The FA profile of sheep and goats milk fat is reported in Table 2. The content of SCFA was higher in goat than sheep milk (+38%) due to a higher concentration of C6:0, C8:0 and C10:0 ($P<0.001$). In fact, it is known that goat milk contains higher concentration of SCFA such as caproic, caprylic and capric acids (C6:0, C8:0 and C10:0, respectively), and these SCFA constitute about 20% of the total fatty acid in goat milk (Jandal, 1996). The MCFA were significantly higher in sheep compared to goat, mainly due to the higher content of C14:0 ($P < 0.001$). No differences between the two species were observed for the LCFA. The total saturated FA (SFA) was higher in goat than in sheep milk ($P <0.01$) mainly for the higher contents of saturated SCFA (from C6:0 to C10:0) and the higher content in C18:0 ($P <0.01$). The total MUFA content did not showed any difference between the two species ($P > 0.05$), whereas among individual MUFA, palmitoleic acid (C16:1 cis9) and oleic acid (C18:1 cis9) content were higher in sheep milk ($P<0.001$). These results are in accordance with previous reports which states that in comparison to the milk of goat and cow, sheep milk has the lowest content in SFA (especially myristic, palmitic, and lauric acids) and that the amount of MUFA is similar among the species (Djordjevic *et al.,* 2019). On the other hand, PUFA content was higher in sheep milk ($P<0.001$) due to the higher content of both PUFA n-6 and PUFA n-3, and especially related to the higher content of linoleic acid ($P = 0.029$), conjugated linoleic acid ($P<0.001$), alpha-linolenic acids ($P<0.01$) and their longer derivatives EPA, DPA and DHA. Studies with sheep and goats maintained in fresh pasture showed that sheep have higher content of PUFA n-3 than goat (Nudda *et al.,* 2020), probably due to different feeding behavior of the two species. The content of BCFA were higher in sheep milk ($P=0.001$) due to the higher content of isoC15:0, anteisoC15:0, isoC16:0, *anteiso*C17:0 and *iso*C18:0,

whereas the odd chain FA (OCFA) content were similar between species and in line with recent observation in other breeds (Nudda *et al.,* 2021). The variations in OCFA could be related to the sheep and goat differences in feeding behavior since these FA change with relative abundance of bacterial population in the rumen; other reason could be the differences in intestinal absorption efficiency and the release of OBCFA after their storage in the adipose tissues, and also to the feeding regime of goats that pasture also shrubs containing tannins. The n6: n3 ratio was higher in goat than in sheep milk ($P<0.05$) due to the PUFA n-6 higher content as mentioned previously. The content of rumenic acid, which in sheep and goat accounted for the 75% and 70%, respectively of total CLA, was higher in sheep compared to goat milk ($P<0.001$). The preference of goats to woody plants and shrubs and its behavior in consuming the feed using smaller meals quantity and more frequent meals compared to sheep, could have done a more regular rumen pH and bolus outflow which affected the bio-hydrogenation of UFA, reducing the escape of VA and RA toward the mammary gland (Nudda *et al.,* 2020). In addition, the higher expression of delta9 desaturase in the mammary gland of sheep could have increased the desaturation of VA to RA (Tsiplakou *et al.,* 2010). The rumenic and vaccenic acid (RA and VA) are two of the important intermediate products resulting from the biohydrogenation of PUFA by the rumen bacteria (Kepler *et al.,* 1966). If a typical forage diet fed to small ruminants, VA represents the major biohydrogenation intermediate in the rumen (Szumacher-Strabel *et al.,* 2009). Part of the VA which escape from the rumen, is transported to the mammary gland and is used for CLA endogenous synthesis by the enzymatic reduction operated by the delta-9 desaturase (Griinari *et al.,* 2000; Piperova *et al.,* 2004). Therefore, the [cis-9, trans-11 CLA] / [cis-9, rans11 CLA + VA] ratio is used as indirect index of Δ9-desaturase activity in endogenous synthesis of CLA. The estimated desaturation, was higher in sheep (Figure 5) than in goats (Figure 6), as previously mentioned by Nudda *et al.* (2021), related to the different feeding behavior during grazing. Also the other desaturase indexes of C10, C14, C16 and C18 were higher in sheep than goat milk (Table 2). The nutritional indices AI, TI and h/H ratio did not show differences between the two species, in line with previous results evidencing similar nutritional indexes among sheep, goat and cows (Markiewicz-Keszycka, M., *et al,* 2013). Our results are interesting because the differences between the species have been revealed in the same environmental and feeding conditions.

Table 1: Chemical composition of feeds supplied in the different farm systems.

	Farm system		
Item	**Semi Nomadic**	**Transhumant**	**Sedentary**
Dry matter DM, %	90.10	89.81	89.58
Protein, % of DM	12.98	13.90	17.96
NDF, % of DM	36.29	34.94	33.76
ADF, % of DM	18.52	11.89	16.12
ADL, % of DM	4.65	9.84	3.17
Ash, % of DM	5.92	4.12	6.51

[1]Supplemented diet: in the semi nomadic system composed of: barley, hay and wheat bran; in the transhumant system: barley, hay, wheat bran, wheat and maize; and in the sedentary system: barley, hay and soya or hay, wheat bran, wheat and maize.

[2]DM, NDF, ADF and ADL: dry matter, neutral detergent fiber; acid detergent fiber: acid detergent lignin respectively.

Table 2: Milk fatty acid profile and nutritional indices of Baladi goat and Awassi sheep from different farms in Lebanon.

	Specie			
Fatty acid (g/100g of FAME)	**Baladi goat**	**Awassi sheep**	**SEM**	**P value**
Fat content, %	4.08	9.17		
C4:0	1.43	1.84	0.048	<0.001
C6:0	1.44	1.14	0.049	<0.001
C7:0	0.03	0.03	0.001	0.153
C8:0	1.57	1.01	0.070	<0.001
C9:0	0.10	0.07	0.006	0.003
C10:0	6.07	3.61	0.305	<0.001
C10:1	0.12	0.11	0.008	0.608
C11:0	0.20	0.21	0.020	0.648
C12:0	2.84	2.63	0.167	0.376
isoC13:0	0.03	0.04	0.002	0.005
anteisoC13:0	0.04	0.05	0.004	0.089
isoC14:0	0.11	0.17	0.008	<0.001
C14:0	9.32	10.62	0.248	<0.001
isoC15:0	0.25	0.40	0.018	<0.001
anteisoC15:0	0.43	0.60	0.024	<0.001
C14:1c9	0.18	0.28	0.027	0.010
C15:0	1.20	1.24	0.034	0.380
C15:1	0.07	0.09	0.006	0.010
isoC16:0	0.27	0.41	0.015	<0.001
C16:0	29.24	30.16	0.660	0.328
C16:1trans-4	0.03	0.03	0.002	0.031
C16:1trans-5	0.02	0.03	0.001	0.036

Fatty acid (g/100g of FAME)	Baladi goat	Awassi sheep	SEM	P value
isoC17:0	0.40	0.49	0.019	0.002
C16:1trans-9	0.06	0.07	0.004	0.066
C16:1trans-10	0.02	0.02	0.001	0.071
C16:1trans-11 + trans-12	0.06	0.07	0.005	0.030
C16:1cis-7	0.29	0.31	0.010	0.353
anteisoC17:0	0.44	0.56	0.021	<0.001
C16:1cis-9	0.63	1.32	0.070	<0.001
C16:1cis-10	0.06	0.09	0.007	0.005
C16:1cis-11	0.02	0.02	0.001	0.100
C17:0	1.03	0.91	0.023	<0.001
isoC18:0	0.07	0.12	0.006	<0.001
C17:1 cis-6 + cis-7	0.03	0.04	0.002	<0.001
C17:1cis-8	0.02	0.03	0.002	0.002
C17:1cis-9	0.32	0.36	0.013	0.023
C18:0 (SA)	12.59	9.42	0.562	<0.001
C18:1trans-4	0.02	0.02	0.001	0.152
C18:1trans-5	0.02	0.02	0.001	0.635
C18:1trans-6 +trans-8	0.18	0.18	0.011	0.894
C18:1trans-9	0.20	0.23	0.008	0.031
C18:1trans-10	0.27	0.28	0.031	0.766
C18:1trans-11 (VA)	0.95	1.10	0.068	0.107
C18:1trans-12	0.24	0.27	0.016	0.164
C18:1trans-13+ trans-14	0.58	0.61	0.043	0.679
C18:1cis-9	19.46	19.51	0.540	0.944
C18:1cis-11	0.52	0.56	0.016	0.095
C18:1cis-12	0.22	0.23	0.012	0.794
C18:1cis-13	0.06	0.07	0.003	0.031
C18:1trans-16 + cis-14	0.36	0.37	0.027	0.693
C19:0/C18:1cis-15	0.26	0.31	0.017	0.039
C18:2trans-10,trans-14	0.02	0.04	0.002	<0.001
C18:2trans-11,trans-15	0.02	0.03	0.003	0.023
C18:2trans-9,trans12	0.02	0.02	0.001	0.004
C18:2cis-9,trans-13	0.24	0.36	0.018	<0.001
C18:2trans-8,cis-13	0.12	0.17	0.009	<0.001
C18:2cis-9,trans-12	0.09	0.13	0.006	<0.001
C18:1cis-16	0.02	0.04	0.002	<0.001
C18:2trans-9,cis-12	0.00	0.00	0.002	0.162
C18:2trans-11,cis-15	0.13	0.27	0.024	<0.001
C18:2n6 (LA)	1.72	1.92	0.064	0.029
C18:2trans-12,cis-15	0.06	0.06	0.003	0.104
C18:2cis-12,cis-15	0.02	0.03	0.002	0.050

Fatty acid (g/100g of FAME)	Baladi goat	Awassi sheep	SEM	P value
C20:0	0.48	0.62	0.032	0.003
C18:3n6	0.02	0.04	0.003	<0.001
C20:1cis-9	0.04	0.03	0.003	0.042
C20:1cis-11	0.06	0.08	0.003	<0.001
C18:3n3 (LNA)	0.54	0.80	0.055	0.001
CLAcis-9,trans-11 (RA)	0.47	0.75	0.031	<0.001
C20:1cis-15	0.02	0.03	0.001	<0.001
CLAtrans-9,cis-11/C21:0	0.11	0.14	0.008	0.041
CLAtrans-10,cis-12	0.02	0.03	0.002	0.002
CLAtrans-12,trans-14	0.02	0.02	0.001	0.874
CLAtrans-11,trans-13	0.03	0.04	0.002	0.004
C20:2n9	0.01	0.02	0.001	<0.001
CLAtrans-9,trans-11	0.01	0.03	0.001	<0.001
C18:4n-3	0.01	0.02	0.001	0.085
C20:2n-6	0.01	0.02	0.001	0.001
C20:3n-9	0.04	0.07	0.002	<0.001
C22:0	0.23	0.33	0.019	<0.001
C20:3n-6	0.03	0.03	0.002	0.013
C22:1n-9	0.02	0.06	0.004	<0.001
C20:3n-3	0.02	0.02	0.001	<0.001
C20:4n-6	0.12	0.15	0.008	0.010
C23:0	0.09	0.14	0.008	<0.001
C20:4n-3	0.01	0.01	0.001	0.007
C22:2n-6	0.02	0.05	0.002	<0.001
C22:5n-3 (EPA)	0.05	0.09	0.004	<0.001
C24:0	0.12	0.16	0.010	0.015
C22:3n-6	0.01	0.01	0.001	0.287
C24:1cis-15	0.02	0.05	0.002	<0.001
C22:4n-6	0.02	0.02	0.002	0.709
C25:0	0.03	0.03	0.002	0.049
C26:0	0.06	0.08	0.007	0.030
C22:5n-3 (DPA)	0.13	0.19	0.008	<0.001
C22:6n-3 (DHA)	0.04	0.08	0.004	<0.001
SCFA	10.77	7.82	0.442	<0.001
MCFA	47.77	51.47	0.976	0.009
LCFA	41.46	40.71	1.248	0.671
SFA	70.24	67.25	0.771	0.008
MUFA	25.51	26.99	0.651	0.114
PUFA	4.25	5.75	0.156	<0.001
UFA	29.76	32.73	0.771	0.008
OCFA	2.68	2.63	0.052	0.499

Fatty acid (g/100g of FAME)	Baladi goat	Awassi sheep	SEM	P value
BCFA	2.05	2.82	0.100	<0.001
OBCFA	4.73	5.45	0.142	0.001
PUFA n-6	1.95	2.24	0.068	0.004
PUFA n-3	0.79	1.22	0.065	<0.001
n6:n3	3.20	2.19	0.303	0.022
n3:n6	0.41	0.56	0.033	0.002
TOTAL CLA	0.67	1.01	0.035	<0.001
TFA	3.76	4.45	0.207	0.022
TFA_no_VA	2.81	3.34	0.155	0.018
AI	2.43	2.37	0.115	0.744
TI	2.43	2.26	0.117	0.290
h/H	0.60	0.59	0.026	0.699
DI C10:1	2.11	3.39	0.271	0.001
DI C14:1	1.83	2.54	0.233	0.037
DIC16:1	2.04	4.14	0.164	<0.001
DI C18:1	61.33	67.85	1.123	<0.001

1SA, VA, LA, LNA = stearic acid; vaccenic acid; linoleic acid and linolenic acid respectively; RA, EPA, DPA and DHA= rumenic acid; eicosapentaenoic acid; docosapentaenoic acid and docosahexaenoic acid respectively; SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; TFA (trans fatty acids), BCFA (branched chain fatty acids), OBCFA (odd- and branched-chain fatty acids) =sum of the individual trans fatty acids, except CLA isomers; sum of iso- and anteiso-FA; sum of odd-, iso-, and anteiso-FA respectively; SCFA (short-chain fatty acids), MCFA (medium-chain fatty acids) and LCFA (long-chain fatty acids) =sum of the individual fatty acids from C4:0 to C10:0; sum of the individual fatty acids from C11:0 to C17:0; sum of the individual fatty acids from C18:0 to DHA respectively; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids, respectively; CLA, TI, AI and h:H = sum of individual conjugated linoleic acids, thrombogenic index; atherogenic index; hypocholesterolemic to hypercholesterolemic ratio respectively. Desaturase indexes = Δ9-desaturase product / Σ Δ9-desaturaseproduct and substrate as described in the Materials and Methods. For example, the desaturase index of cis-9 14:1 is (cis-9 14:1)/ (cis-9 14:1+14:0). To improve the readability, the value was multiplied by 100.

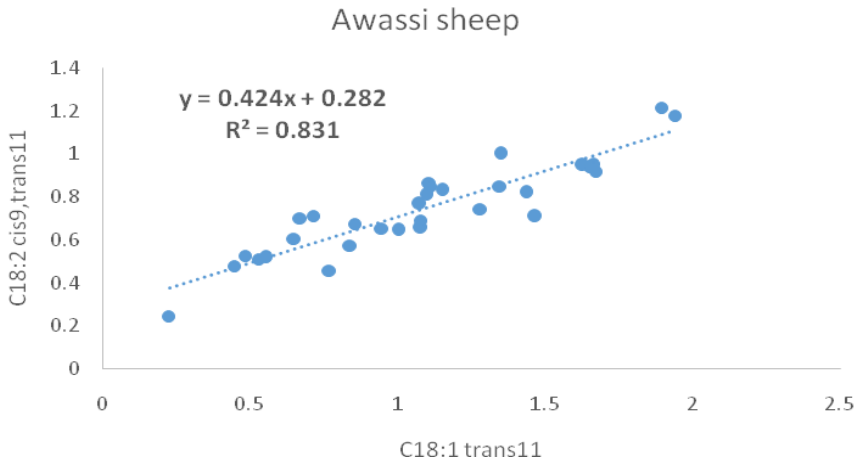

Figure 5: Relationship between the vaccenic acid (trans-11 C18:1) and conjugated linoleic acid (CLA, cis-9, trans-11 C18:2) concentration in sheep milk.

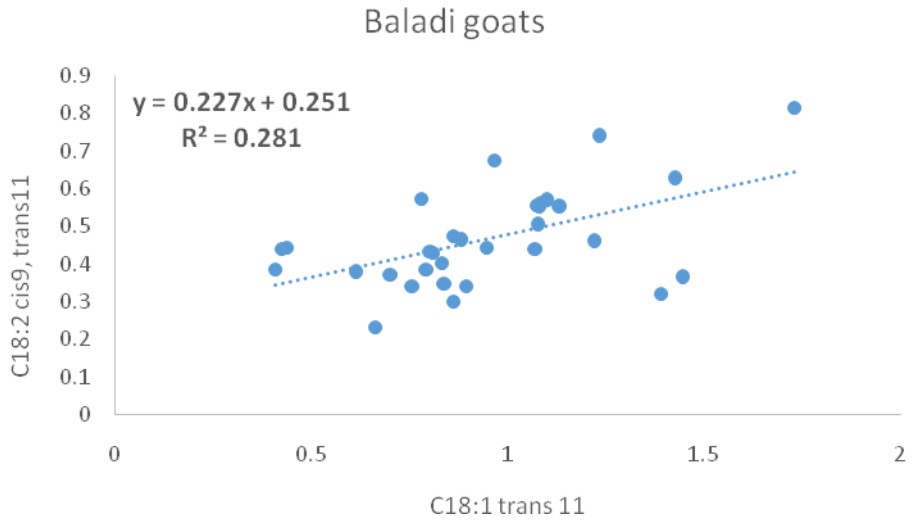

Figure 6: Relationship between the vaccenic acid (trans-11 C18:1) and conjugated linoleic acid (CLA, cis-9, trans-11 C18:2) concentration in goat milk.

3.3- Fatty acid profile and nutritional indices of fatty acids of Baladi goat milk based on the production system of the farm (sedentary, semi nomadic and transhumant system)

The fatty acid profile of goat milk based on the production systems of the farms (sedentary, semi nomadic and transhumant) is reported in Table 3. The content of SCFA, especially C6:0, C8:0, C10:0 and C12:0 were higher in sedentary compared to semi nomadic and transhumant systems. On the other hand, in the sedentary system was markedly lower the content of C18:0 (stearic acid), C18:1c9 ($P<0.001$), C18:3n3 (LNA) ($P = 0.01$) and RA ($P = 0.04$) in comparison to the other systems. The PUFAn-3 content reach the highest value in the semi nomadic system due to higher alpha-linolenic, EPA, DPA and DHA contents. These differences are explained by the feeding regime differences between the three systems: the sedentary system use the highest amount of supplements, concentrates and byproducts in the diets whereas, the semi nomadic and transhumant systems involved highest level of grazing on pastures and shrubs (see Chapter 2). The higher content of UFA in the pasture and shrubs has probably increased PUFA and MUFA content in milk and may have inhibited the de novo synthesis of FA in the mammary gland which is responsible of the decrease in SCFA in the semi nomadic and transhumant system (Tsiplakou *et al.,* 2010; Nudda *et al.,* 2020). No significant difference was observed between the three systems for OCFA and BCFA. The lack of difference between the three systems concerning the OBCFA content could be

explained by the sampling period which was done in the late stage of lactation. According to Nudda *et al.,* 2021, the content of OBCFA increases at the end of the lactation period partly due to the general decrease in PUFA in pasture during summer (hot season), which could have reduced the toxic effect of unsaturated lipids on the microbial growth. Another additional explanation, is the positive association between OBCFA and the dietary fiber, because with the progress of lactation, there is an increase in NDF and ADL in pastures. The higher content of CLA in the transhumant system could be due to the inhibition of rumen bio-hydrogenation and consequently rumenic acid formation (cis9-trans11-CLA) due to the intake of shrub rich in tannins. The nutritional TI and AI indexes were markedly lower in transhumant and semi nomadic system ($P<0.01$), while the hypocholesterolemic to hypercholesterolemic ratio (h/H) was the lowest in the sedentary system. No differences were observed for the desaturase indexes of C14, C16, C18 and CLA except for the desaturase index of C10 which was the highest in the semi nomadic system ($P=0.02$) (Table 4).

Table 3: Fatty acid profile of Baladi goat milk based on the production system of the farm (sedentary, semi nomadic and transhumant system).

	Production systems				
Fatty acid (g/100g of FAME)	**Sedentary**	**Semi nomadic**	**Transhumant**	**SEM**	**P value**
C4:0	1.55	1.48	1.37	0.05	0.26
C6:0	1.78^{a}	1.24^{b}	1.38^{b}	0.05	0.00
C7:0	0.04^{a}	0.03^{ab}	0.03^{b}	0.00	0.04
C8:0	2.22^{a}	1.22^{b}	1.44^{b}	0.08	0.00
C9:0	0.14^{a}	0.08^{b}	0.08^{b}	0.01	0.00
C10:0	9.14^{a}	4.44^{b}	5.46^{b}	0.36	0.00
C10:1	0.14	0.14	0.10	0.01	0.16
C11:0	0.32^{a}	0.12^{b}	0.18^{b}	0.02	0.00
C12:0	4.40^{a}	2.01^{b}	2.53^{b}	0.18	0.00
isoC13:0	0.03	0.03	0.03	0.00	0.25
anteisoC13:0	0.05	0.03	0.03	0.00	0.07
isoC14:0	0.10	0.11	0.12	0.01	0.24
C14:0	10.97^{a}	8.25^{b}	9.04^{b}	0.25	0.00
isoC15:0	0.22	0.25	0.25	0.01	0.44
anteisoC15:0	0.34^{b}	0.43^{ab}	0.46^{a}	0.02	0.02
C14:1c9	0.17	0.08	0.22	0.03	0.23
C15:0	1.20	1.13	1.22	0.03	0.62
C15:1	0.05	0.08	0.07	0.00	0.09
isoC16:0	0.22	0.29	0.29	0.01	0.11
C16:0	30.99^{a}	25.70^{b}	29.71^{a}	0.65	0.02
C16:1trans-4-12	0,22	0,27	0,22	0.00	0.21
isoC17:0	0.36	0.38	0.43	0.01	0.08
C16:1cis-7	0.28^{ab}	0.35^{a}	0.28^{b}	0.01	0.01
anteisoC17:0	0.39	0.44	0.46	0.02	0.30

Fatty acid (g/100g of FAME)	Sedentary	Semi nomadic	Transhumant	SEM	P value
C16:1cis-9	0.63	0.41	0.69	0.06	0.19
C16:1cis-10	0.05	0.06	0.07	0.00	0.12
C16:1cis-11	0.02	0.01	0.02	0.00	0.26
C17:0	0.94^{b}	1.00^{ab}	1.07^{a}	0.02	0.04
isoC18:0	0.05^{b}	0.09^{a}	0.07^{b}	0.00	0.00
C17:1 cis-6 + cis-7	0.03	0.04	0.04	0.00	0.44
C17:1cis-8	0.01^{b}	0.03^{a}	0.02^{b}	0.00	0.00
C17:1cis-9	0.25^{b}	0.31^{ab}	0.34^{a}	0.01	0.01
C18:0 (SA)	10.27^{b}	16.52^{a}	12.20^{b}	0.66	0.01
C18:1trans-4-8	0,17^{b}	0,29^{a}	0,22^{b}	0,01	0,02
C18:1trans-9	0.16^{c}	0.25^{a}	0.20^{b}	0.01	0.00
C18:1trans-10	0.16	0.22	0.32	0.04	0.18
C18:1trans-11 (VA)	0.83	0.96	0.99	0.05	0.51
C18:1trans-12	0.17^{b}	0.30^{a}	0.25^{ab}	0.02	0.01
C18:1trans-13+ tans-14	0.36	0.67	0.64	0.05	0.05
C18:1cis-9	15.07^{c}	22.63^{a}	20.08^{b}	0.58	0.00
C18:1cis-11	0.49	0.49	0.54	0.02	0.48
C18:1cis-12	0.18^{b}	0.18^{b}	0.26^{a}	0.01	0.01
C18:1cis-13	0.04^{b}	0.06^{ab}	0.07^{a}	0.00	0.00
C18:1trans-16 +cis-14	0.20^{b}	0.45^{a}	0.39^{a}	0.03	0.01
C18:2trans-10 +trans+14	0.02	0.02	0.02	0.00	0.25
C18:2trans-11 + trans-15	0.01	0.03	0.03	0.00	0.07
C18:2trans-9 + trans12	0.02	0.02	0.02	0.00	0.84
C18:2cis-9 + trans-13	0.13^{b}	0.28^{a}	0.27^{a}	0.02	0.00
C17cyclo	0.05^{b}	0.09^{a}	0.09^{a}	0.00	0.00
C18:2trans-8 + cis-13	0.07^{b}	0.15^{a}	0.14^{a}	0.01	0.00
C18:2cis-9 + trans-12	0.06^{b}	0.11^{a}	0.10^{a}	0.01	0.00
C18:1cis-16	0.02	0.03	0.02	0.00	0.09
C18:2trans-11 + cis-15	0.06^{b}	0.18^{a}	0.15^{a}	0.01	0.01
C18:2n6 (LA)	1.74	1.67	1.73	0.05	0.88
C18:2trans-12 + cis-15	0.05	0.06	0.06	0.00	0.16
C18:2cis-12 + cis-15	0.01^{b}	0.03^{a}	0.02^{ab}	0.00	0.01
C20:0	0.29^{b}	0.60^{a}	0.51^{a}	0.03	0.00
Δ7,9 17:2	0.02^{b}	0.03^{a}	0.03^{a}	0.00	0.00
C18:3n6	0.03^{a}	0.02^{ab}	0.02^{b}	0.00	0.03
C20:1cis-9	0.02^{b}	0.04^{ab}	0.05^{a}	0.00	0.02
C20:1cis-11	0.05	0.06	0.06	0.00	0.33
C18:3n3 (LNA)	0.33^{b}	0.76^{a}	0.54^{ab}	0.05	0.01
CLAcis-9 + trans-11 (RA)	0.37^{b}	0.44^{ab}	0.51^{a}	0.02	0.04
C20:1cis-15	0.02	0.02	0.02	0.00	0.33
CLAtrans-9 + cis-11/C21:0	0.07^{b}	0.11^{ab}	0.13^{a}	0.01	0.01
CLAtrans-10 + cis-12	0.02	0.03	0.03	0.00	0.21
CLAtrans-12 + trans-14	0.01^{b}	0.02^{a}	0.02^{ab}	0.00	0.04
CLAtrans-11 + trans-13	0.02^{b}	0.04^{a}	0.03^{ab}	0.00	0.00
C20:2n9	0.01	0.02	0.01	0.00	0.40
CLAtrans-9 + trans-11*	0.01^{b}	0.02^{a}	0.01^{b}	0.00	0.00

Fatty acid (g/100g of FAME)	Sedentary	Semi nomadic	Transhumant	SEM	P value
C18:4n-3	0.01	0.01	0.01	0.00	0.75
C20:2n-6	0.01	0.01	0.01	0.00	0.71
C20:3n-9	0.06^{a}	0.05^{ab}	0.04^{b}	0.00	0.00
C22:0	0.14^{b}	0.27^{ab}	0.25^{a}	0.02	0.03
C20:3n-6	0.02	0.02	0.03	0.00	0.31
10,14,17 C20:3*	0.01	0.01	0.01	0.00	0.47
C22:1n-9	0.03^{a}	0.03^{a}	0.02^{b}	0.00	0.00
C20:3n-3	0.01^{b}	0.02^{a}	0.02^{b}	0.00	0.00
C20:4n-6	0.17^{a}	0.08^{b}	0.11^{b}	0.01	0.00
C23:0	0.06^{b}	0.10^{ab}	0.10^{a}	0.01	0.02
C20:4n-3	0.01	0.01	0.01	0.00	0.30
C22:2n-6	0.02	0.03	0.02	0.00	0.08
C22:5n-3 (EPA)	0.04	0.06	0.06	0.00	0.11
C24:0	0.07^{b}	0.15^{a}	0.13^{a}	0.01	0.01
C22:3n-6	0.01	0.01	0.01	0.00	0.62
C24:1cis-15	0.02^{b}	0.03^{a}	0.02^{b}	0.00	0.00
C22:4n-6	0.03^{a}	0.02^{b}	0.02^{b}	0.00	0.03
C25:0	0.02^{b}	0.03^{a}	0.03^{ab}	0.00	0.03
C26:0	0.03	0.07	0.07	0.01	0.07
C22:5n-3 (DPA)	0.09^{b}	0.15^{a}	0.13^{a}	0.01	0.01
C22:6n-3 (DHA)	0.03	0.04	0.04	0.00	0.09
SCFA	15.00^{a}	8.65^{b}	9.88^{b}	0.50	0.00
MCFA	52.30^{a}	41.95^{b}	47.93^{a}	1.02	0.00
LCFA	32.69^{c}	49.41^{a}	42.19^{b}	1.30	0.00
SFA	76.39^{a}	66.64^{b}	69.10^{b}	0.80	0.00
MUFA	19.99^{b}	28.78^{a}	26.51^{a}	0.71	0.00
PUFA	3.61^{b}	4.57^{a}	4.38^{a}	0.13	0.02
UFA	23.59^{b}	33.35^{a}	30.89^{a}	0.80	0.00
OCFA	2.71	2.50	2.72	0.05	0.30
BCFA	1.76	2.05	2.15	0.08	0.12
OBCFA	4.47	4.56	4.87	0.12	0.32
PUFA n-6	2.03	1.86	1.95	0.05	0.58
PUFA n-3	0.53^{b}	1.06^{a}	0.81^{ab}	0.06	0.01
n6:n3	4.90^{a}	1.76^{b}	3.02^{ab}	0.36	0.02
TOTAL CLA	0.51^{b}	0.66^{ab}	0.73^{a}	0.03	0.00
TFA	2.66^{b}	4.24^{a}	4.01^{a}	0.19	0.01
TFA_no_VA	1.83^{b}	3.28^{a}	3.02^{a}	0.15	0.00

1SA, VA, LA, LNA = stearic acid; vaccenic acid; linoleic acid and linolenic acid respectively; RA, EPA, DPA and DHA= rumenic acid; eicosapentaenoic acid; docosapentaenoic acid and docosahexaenoic acid respectively; SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; TFA (trans fatty acids), BCFA (branched chain fatty acids), OBCFA (odd- and branched-chain fatty acids) =sum of the individual trans fatty acids, except CLA isomers; sum of iso- and anteiso-FA; sum of odd-, iso-, and anteiso-FA respectively; SCFA (short-chain fatty acids), MCFA (medium-chain fatty acids) and LCFA (long-chain fatty acids) =sum of the individual fatty acids from C4:0 to C10:0; sum of the individual fatty acids from C11:0 to C17:0; sum of the individual fatty acids from C18:0 to DHA respectively; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids, respectively; CLA= sum of conjugated linoleic individual acids.

Table 4: Nutritional indices of Baladi goat milk based on the production system of the farm (sedentary, semi nomadic and transhumant system).

	Production systems				
Items	**Sedentary**	**Semi nomadic**	**Transhumant**	**SEM**	**P value**
AI	3.39[a]	1.83[b]	2.26[b]	0.12	0.00
TI	3.32[a]	1.79[b]	2.31[b]	0.12	0.00
h/H	0.44[c]	0.77[a]	0.61[b]	0.03	0.00
Δ 9-desaturase indices					
DI C10:1	1.48[b]	3.40[a]	1.94[b]	0.24	0.02
DI C14:1	1.40	0.92	2.28	0.30	0.18
DIC16:1	1.93	1.56	2.24	0.16	0.27
DI C18:1	61.25	57.85	62.46	1.40	0.47
DI CLA	34.82	31.88	33.93	1.28	0.77

TI, AI and h:H = thrombogenic index; atherogenic index; hypocholesterolemic to hypercholesterolemic ratio respectively. Desaturase indexes = Δ9-desaturase product / Σ Δ9-desaturaseproduct and substrate as described in the Materials and Methods. For example, the desaturase index of cis-9 14:1 is (cis-9 14:1)/ (cis-9 14:1+14:0). To improve the readability, the value was multiplied by 100.

3.4- Fatty acid profile and nutritional indices of fatty acids of Baladi goat milk based on the different altitudes categories (<1000m, 1000-1500m and >1500m above sea level)

The fatty acid profile of goat milk based on the different altitudes of the farms (<1000m, 1000-1500m and >1500m above sea level) is reported in table 5. The SCFA content was the highest at altitudes < 1000m and this is due to the higher content in C6:0, C8:0 and C10:0. The highest content of LCFA was at >1500m (P=0.013), while no difference was observed for MCFA content at the different altitudes. No significant difference was observed for the PUFAn-3 and LNA content, while EPA, DPA and DHA where the highest between 1000-1500m. These variations may be because of the minor use of supplements especially concentrates in the feed as the survey's results have evidenced, in addition, the highest content of MUFA observed at higher altitudes, could be due to the higher level of grazing on pastures and shrubs rich in MUFA and PUFA which consequently increase their levels in milk and inhibit the de novo synthesis of FA in the mammary gland (Tsiplakou *et al.,* 2010; Nudda *et al.,* 2020). No significant difference was observed for the n6: n3 ratio, vaccenic acid and rumenic acid content, while the total CLA content increased with the altitudes (P<0.05). As the altitude increase, the composition of the vegetative cover becomes richer in shrubs and lignified plants rich in tannin preferred by the Baladi goat, which can inhibit the bio-

hydrogenation process and thus increase the CLA content (Tsiplakopu *et al.,* 2010). No difference was found for the OCFA content (P=0.09), while the BCFA content increased with the altitudes due to the increased content in iso and ant-iso fatty acids. These differences could be due to the goat feeding regime, and to the biodiversity of Lebanese rangelands in plants rich in essential oils and polyphenols such as *Melissa officinalis*, *Thymus vulgaris*, *Ocimum basilicum* etc., which can improve the milk fatty acids content especially BCFA, CLA and PUFAn-3 (Kharrat, 2010; Nudda *et al.,*2020). Regarding the nutritional indices of Baladi goat milk based on the altitudes, AI and TI indexes were the lowest at altitudes above 1500 m whereas the contrary was for h/H index (P<0.05). The desaturase index of C10, C14, C16, C18 and CLA, have showed no differences (Table 6).

Table 5: Fatty acid profile of Baladi goat milk based on the different altitudes categories (<1000m, 1000-1500m and >1500m above sea level).

	Altitude categories				
Fatty acid (g/100g of FAME)	**<1000**	**1000-1500**	**>1500**	**SEM**	**P value**
C4:0	1.52	1.25	1.47	0.046	0.081
C6:0	1.68^{a}	1.39^{b}	1.30^{b}	0.047	0.001
C7:0	0.04	0.03	0.03	0.002	0.09
C8:0	2.01^{a}	1.54^{b}	1.29^{b}	0.080	0.000
C9:0	0.12	0.08	0.09	0.007	0.057
C10:0	8.01^{a}	6.14^{b}	4.75^{b}	0.360	0.000
C10:1	0.13	0.10	0.12	0.009	0.46
C11:0	0.27	0.18	0.17	0.020	0.057
C12:0	3.66^{a}	2.58^{ab}	2.42^{b}	0.181	0.006
isoC13:0	0.03	0.03	0.03	0.001	0.576
anteisoC13:0	0.04	0.03	0.04	0.004	0.321
isoC14:0	0.10	0.14	0.12	0.006	0.052
C14:0	10.02	9.42	8.80	0.251	0.105
isoC15:0	0.22	0.29	0.25	0.010	0.056
anteisoC15:0	0.35^{b}	0.47^{ab}	0.46^{a}	0.019	0.018
C14:1c9	0.14	0.11	0.24	0.032	0.205
C15:0	1.15	1.30	1.19	0.034	0.281
C15:1	0.06	0.09	0.07	0.005	0.109
isoC16:0	0.22^{b}	0.32^{a}	0.29^{a}	0.014	0.006
C16:0	29.53^{ab}	31.96^{a}	27.77^{b}	0.651	0.038
C16:1t4	0.03^{a}	0.02^{b}	0.03^{ab}	0.002	0.009
C16:1t5	0.02	0.02	0.02	0.001	0.211
C16:1t6-7	0.05	0.04	0.05	0.003	0.65
isoC17:0	0.35^{b}	0.44^{a}	0.42^{a}	0.013	0.013
C16:1t9	0.06	0.05	0.06	0.003	0.294
C16:1t10	0.02	0.02	0.02	0.001	0.264
C16:1t11-t12	0.04	0.06	0.06	0.005	0.135
C16:1c7	0.29	0.28	0.30	0.011	0.73

Fatty acid (g/100g of FAME)	<1000	1000-1500	>1500	SEM	P value
anteisoC17:0	0.37^{b}	0.47^{ab}	0.48^{a}	0.019	0.02
C16:1c9	0.56	0.56	0.70	0.059	0.496
C16:1c10	0.06	0.08	0.06	0.004	0.049
C16:1c11	0.02	0.02	0.02	0.001	0.279
C17:0	0.94^{b}	1.17^{a}	1.02^{b}	0.022	0.000
isoC18:0	0.06^{b}	0.08^{ab}	0.08^{a}	0.004	0.011
C17:1 c6-7	0.03	0.04	0.04	0.002	0.515
C17:1c8	0.01^{b}	0.02^{ab}	0.03^{a}	0.001	0.003
C17:1c9	0.25^{b}	0.37^{a}	0.33^{a}	0.013	0.000
C18:0 (SA)	12.26	10.38	13.83	0.665	0.126
C18:1t4	0.02^{ab}	0.02^{b}	0.02^{a}	0.001	0.023
C18:1t5	0.02^{ab}	0.01^{b}	0.02^{a}	0.001	0.015
C18:1t6-8	0.16^{ab}	0.12^{b}	0.21^{a}	0.011	0.001
C18:1t9	0.18^{b}	0.17^{b}	0.23^{a}	0.007	0.000
C18:1t10	0.19^{b}	0.14^{b}	0.37^{a}	0.035	0.009
C18:1t11 (VA)	0.86	0.81	1.07	0.053	0.081
C18:1t12	0.20^{b}	0.22^{ab}	0.28^{a}	0.015	0.022
C18:1t13-t14	0.55	0.53	0.63	0.050	0.712
C18:1c9	16.79^{b}	19.08^{ab}	21.42^{a}	0.582	0.001
C18:1c11	0.48^{ab}	0.46^{b}	0.57^{a}	0.019	0.019
C18:1c12	0.22	0.24	0.22	0.013	0.877
C18:1c13	0.05^{b}	0.05^{ab}	0.07^{a}	0.003	0.002
C18:1t16-c14	0.28	0.36	0.41	0.030	0.132
C19:0/C18:1c15	0.21^{b}	0.32^{a}	0.28^{ab}	0.017	0.042
C18:2t10t14	0.02	0.02	0.02	0.002	0.127
C18:2t11t15	0.02	0.02	0.03	0.003	0.358
C18:2t9t12	0.02	0.01	0.02	0.001	0.169
C18:2c9t13	0.17^{b}	0.29^{a}	0.26^{ab}	0.018	0.028
C17cyclo	0.07^{b}	0.10^{a}	0.09^{ab}	0.005	0.007
C18:2t8c13	0.09^{b}	0.15^{a}	0.13^{ab}	0.010	0.031
C18:2c9t12	0.08	0.10	0.10	0.005	0.122
C18:1c16	0.02	0.02	0.03	0.001	0.061
C18:2t11c15	0.09	0.15	0.15	0.014	0.164
C18:2n6 (LA)	1.84	1.58	1.71	0.046	0.115
C18:2t12c15	0.05	0.06	0.06	0.002	0.198
C18:2c12c15	0.02	0.02	0.03	0.002	0.19
C20:0	0.38	0.58	0.50	0.032	0.056
Δ7,9 17:2	0.02^{b}	0.03^{a}	0.03^{a}	0.002	0.019
C18:3n6	0.03^{a}	0.02^{b}	0.02^{ab}	0.001	0.012
C20:1c9	0.03^{b}	0.04^{ab}	0.05^{a}	0.004	0.01
C20:1c11	0.05^{b}	0.06^{ab}	0.07^{a}	0.003	0.01
C18:3n3 (LNA)	0.48	0.60	0.55	0.047	0.635
CLAc9t11 (RA)	0.39	0.48	0.51	0.023	0.069
C20:1c15	0.02	0.02	0.02	0.001	0.088
CLAt9c11/C21:0	0.10^{b}	0.15^{a}	0.10^{b}	0.008	0.017
CLAt10c12	0.02	0.02	0.03	0.002	0.445

Fatty acid (g/100g of FAME)	<1000	1000-1500	>1500	SEM	P value
CLAt12t14	0.01	0.02	0.02	0.001	0.054
CLAt11t13	0.03^{b}	0.04^{ab}	0.04^{a}	0.002	0.047
C20:2n9	0.01	0.01	0.02	0.001	0.044
CLAt9t11*	0.01	0.01	0.01	0.001	0.572
C18:4n3	0.01	0.01	0.01	0.001	0.277
C20:2n6	0.01^{ab}	0.01^{b}	0.02^{a}	0.001	0.023
C20:3n9	0.05	0.04	0.04	0.002	0.101
C22:0	0.18^{b}	0.31^{a}	0.23^{ab}	0.018	0.035
C20:3n6	0.02	0.02	0.03	0.002	0.055
10,14,17 C20:3*	0.01	0.01	0.01	0.001	0.604
C22:1n9	0.02	0.02	0.03	0.002	0.769
C20:3n3	0.01	0.02	0.02	0.001	0.783
C20:4n6	0.15^{a}	0.10^{ab}	0.10^{b}	0.009	0.039
C23:0	0.08	0.12	0.09	0.007	0.094
C20:4n3	0.01	0.01	0.01	0.001	0.199
C22:2n6	0.02	0.02	0.02	0.001	0.135
EPA	0.05^{ab}	0.07^{a}	0.05^{b}	0.003	0.026
C24:0	0.09	0.15	0.13	0.009	0.069
C22:3n6	0.01	0.01	0.01	0.001	0.852
C24:1c15	0.02	0.02	0.02	0.001	0.397
C22:4n6	0.03	0.01	0.02	0.002	0.108
C25:0	0.03	0.03	0.03	0.002	0.545
C26:0	0.03	0.07	0.07	0.007	0.053
DPA	0.11^{b}	0.17^{a}	0.12^{b}	0.007	0.003
DHA	0.03^{b}	0.05^{a}	0.04^{ab}	0.002	0.008
SCFA	13.51^{a}	10.53^{b}	9.06^{b}	0.503	0.000
MCFA	48.97	50.69	45.59	1.018	0.11
LCFA	37.51^{b}	38.78^{ab}	45.35^{a}	1.295	0.013
SFA	73.83^{a}	71.07^{ab}	67.45^{b}	0.799	0.001
MUFA	22.13^{b}	24.58^{b}	28.19^{a}	0.711	0.000
PUFA	4.03	4.34	4.35	0.128	0.533
UFA	26.16^{b}	28.92^{ab}	32.54^{a}	0.799	0.001
OCFA	2.62	2.90	2.61	0.054	0.091
BCFA	1.73^{b}	2.26^{a}	2.16^{a}	0.075	0.012
OBCFA	4.35^{b}	5.16^{a}	4.77^{ab}	0.117	0.038
PUFA6	2.11^{a}	1.77^{b}	1.93^{ab}	0.049	0.036
PUFA3	0.70	0.92	0.80	0.056	0.402
n6/n3	4.05	1.94	3.21	0.365	0.113
n3/n6	0.32^{b}	0.52^{a}	0.42^{ab}	0.030	0.049
CLA	0.56^{b}	0.72^{ab}	0.71^{a}	0.026	0.018
TFA	3.22^{b}	3.39^{ab}	4.29^{a}	0.189	0.024
TFA_no_VA	2.36^{b}	2.58^{ab}	3.22^{a}	0.152	0.032

1SA, VA, LA, LNA = stearic acid; vaccenic acid; linoleic acid and linolenic acid respectively; RA, EPA, DPA and DHA= rumenic acid; eicosapentaenoic acid; docosapentaenoic acid and docosahexaenoic acid respectively; SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; TFA (trans fatty acids), BCFA (branched chain fatty acids), OBCFA (odd- and branched-chain

fatty acids) =sum of the individual trans fatty acids, except CLA isomers; sum of iso- and anteiso-FA; sum of odd-, iso-, and anteiso-FA respectively; SCFA (short-chain fatty acids), MCFA (medium-chain fatty acids) and LCFA (long-chain fatty acids) =sum of the individual fatty acids from C4:0 to C10:0; sum of the individual fatty acids from C11:0 to C17:0; sum of the individual fatty acids from C18:0 to DHA respectively; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids, respectively; CLA= sum of conjugated linoleic individual acids.

Table 6: Nutritional indices of Baladi goat milk based on the different altitudes categories (<1000m, 1000-1500m and >1500m above sea level).

	Altitude categories				
Fatty acid (g/100g of FAME)	**<1000**	**1000-1500**	**>1500**	**SEM**	**P value**
AI	2.95^{b}	2.53^{ab}	2.03^{b}	0.123	0.002
TI	2.89^{a}	2.54^{ab}	2.08^{b}	0.121	0.009
h/H	0.53^{b}	0.54^{ab}	0.68^{a}	0.028	0.018
DI C10:1	1.67	1.61	2.64	0.243	0.125
DI C14:1	1.29	1.21	2.49	0.300	0.121
DIC16:1	1.79	1.74	2.35	0.160	0.191
DI C18:1	59.29	64.73	61.10	1.395	0.385
DI CLA	33.48	36.97	32.40	1.284	0.399

TI, AI and h:H = thrombogenic index; atherogenic index; hypocholesterolemic to hypercholesterolemic ratio respectively. Desaturase indexes = Δ9-desaturase product / Σ Δ9-desaturaseproduct and substrate as described in the Materials and Methods. For example, the desaturase index of cis-9 14:1 is (cis-9 14:1)/ (cis-9 14:1+14:0). To improve the readability, the value was multiplied by 100.

3.5- Fatty acid profile and nutritional indices of fatty acids of Awassi sheep milk based on the production system of the farm (semi nomadic and transhumant system)

The fatty acid profile of sheep milk based on the production systems of the farms (semi nomadic and transhumant) is reported in table 7. Samples of Awassi sheep milk in the sedentary system were not available for analysis. The SCFA and MCFA content was the highest in the transhumant compared to the semi nomadic system ($P \leq 0.01$), due to the higher content of C6:0, C8:0 and C10:0, C14:0 and C16:0; whereas the LCFA content was the highest in the semi nomadic system ($P < 0.01$). These differences could be described by the higher content of MUFA and PUFA in the animal diet in the transhumant system, which in addition to the pasture and shrubs, is constituted of crop residues and this high content may inhibit the de novo synthesis of FA in the mammary gland and decrease the content of SCFA and MCFA (Tsiplakou *et al.,* 2010; Nudda *et al.,* 2020). The high LCFA content in the semi nomadic system could be explained by the seasonal changes and differences of the feed ratio between silage, fresh herbage and grass, especially during the summer where the quality of the herbage decreases (Djordjevic *et al.,* 2019). PUFAn-3 content was also the highest in the semi nomadic system due to the higher content in LNA. No differences were observed for

LA, EPA, DPA, and DHA content. This could be explained by the sheep preference to fresh pasture and grass rich in PUFAn-3 and containing about 60% of LNA of the total fatty acid content (Tsiplakou *et al.,* 2010; Nudda *et al.,* 2003), which consequently increase the content in milk of the FA originated from elongation and desaturation of alpha-linolenic acid (Tsiplakou *et al.,* 2010; Nudda *et al.,* 2020). The n6: n3 ratio was the highest in the transhumant system ($P<0.01$). Vaccenic, rumenic acids and total CLA were the highest in the semi nomadic system ($P<0.01$), and no significant difference was showed for the OCFA, BCFA and OBCFA content in the different systems. The similar results of the content in OCFA and BCFA obtained by our study could be due to the lactation stage, since the collection of milk samples was at the final stage of lactation (Nudda *et al.,* 2021). The nutritional indexes AI and TI were the highest in the transhumant system while h/H was the highest in the semi nomadic (Table 8). No significant difference was showed for the desaturase index of C10 and C16 between the two systems ($P>0.05$), while the desaturase index of C14, C18 and CLA were the highest in the transhumant system.

Table 7: Fatty acid profile of Awassi sheep milk based on the production system of the farm (semi nomadic and transhumant system).

	Production system			
Fatty acid (g/100g of FAME)	**Semi nomadic**	**Transhumant**	**SEM**	**P-value**
C4:0	1.74	1.91	0.052	0,111
C6:0	0.99	1.26	0.052	0,006
C8:0	0.83	1.15	0.059	0,004
C9:0	0.07	0.07	0.004	0,665
C10:0	2.91	4.18	0.249	0,008
C10:1	0.11	0.12	0.006	0,453
C11:0	0.15	0.27	0.022	0,002
C12:0	2.24	2.98	0.160	0,016
isoC13:0	0.04	0.03	0.002	0,306
anteisoC13:0	0.04	0.06	0.003	0,009
isoC14:0	0.18	0.16	0.010	0,215
C14:0	10.02	11.14	0.259	0,028
isoC15:0	0.44	0.36	0.025	0,120
anteisoC15:0	0.65	0.55	0.030	0,098
C14:1c9	0.23	0.34	0.022	0,013
C15:0	1.24	1.23	0.036	0,832
C15:1	0.10	0.09	0.007	0,960
isoC16:0	0.43	0.39	0.017	0,368
C16:0	27.49	32.36	0.692	0,000
C16:1t4	0.03	0.03	0.002	0,874
C16:1t5	0.03	0.03	0.002	0,771
C16:1t6-7	0.05	0.05	0.002	0,211
isoC17:0	0.52	0.44	0.024	0,084
C16:1t9	0.07	0.06	0.005	0,235

Fatty acid (g/100g of FAME)	Semi nomadic	Transhumant	SEM	P-value
C16:1t10	0.03	0.02	0.002	0.005
C16:1t11-t12	0.09	0.06	0.006	0,008
C16:1c7	0.33	0.29	0.010	0,059
anteisoC17:0	0.60	0.53	0.025	0,169
C16:1c9	1.12	1.51	0.084	0,017
C16:1c10	0.11	0.08	0.009	0,090
C16:1c11	0.02	0.02	0.001	0,506
C17:0	0.93	0.90	0.024	0,502
isoC18:0	0.15	0.10	0.008	0,000
C17:1 c6-7	0.04	0.05	0.002	0,104
C17:1c8	0.03	0.02	0.002	0,001
C17:1c9	0.36	0.36	0.014	0,935
C18:0 (SA)	11.22	7.75	0.461	0,000
C18:1t4	0.03	0.02	0.001	0,139
C18:1t5	0.02	0.02	0.002	0,196
C18:1t6-8	0.21	0.14	0.011	0,002
C18:1t9	0.25	0.20	0.008	0,001
C18:1t10	0.24	0.26	0.017	0,459
C18:1t11 (VA)	1.35	0.75	0.075	0,000
C18:1t12	0.32	0.23	0.017	0,006
C18:1t13-t14	0.69	0.55	0.036	0,067
C18:1c9	21.20	18.35	0.490	0,002
C18:1c11	0.58	0.53	0.014	0,068
C18:1c12	0.20	0.26	0.012	0,007
C18:1c13	0.07	0.07	0.004	0,808
C18:1t16-c14	0.46	0.31	0.025	0,002
C19:0/C18:1c15	0.36	0.28	0.018	0,026
C18:2t10t14	0.04	0.03	0.002	0,098
C18:2t11t15	0.05	0.02	0.004	0,000
C18:2t9t12	0.02	0.02	0.002	0,632
C18:2c9t13	0.40	0.33	0.019	0,055
C18:2t8c13	0.20	0.16	0.009	0,030
C18:2c9t12	0.15	0.11	0.006	0,002
C18:1c16	0.04	0.03	0.003	0,031
C18:2t9c12	0.00	0.00	0.002	0,326
C18:2t11c15	0.41	0.15	0.031	0,000
C18:2n6 (LA)	1.80	2.08	0.081	0,084
C18:2t12c15	0.07	0.06	0.003	0,014
C18:2c12c15	0.03	0.02	0.002	0,004
C20:0	0.71	0.56	0.031	0,016
C18:3n6	0.04	0.05	0.005	0,141
C20:1c9	0.04	0.03	0.002	0,008
C20:1c11	0.08	0.08	0.003	0,219
C18:3n3 (LNA)	1.06	0.61	0.061	0.000
CLAc9t11 (RA)	0.83	0.61	0.033	0,000
C20:1c15	0.02	0.03	0.001	0,177
CLAt9c11/C21:0	0.14	0.14	0.008	0,885
CLAt10c12	0.05	0.02	0.003	0,000

Fatty acid (g/100g of FAME)	Semi nomadic	Transhumant	SEM	P-value
CLAt12t14	0.02	0.02	0.001	0,057
CLAt11t13	0.05	0.03	0.002	0,000
C20:2n9	0.02	0.02	0.001	0,054
CLAt9t11*	0.04	0.03	0.002	0,054
C18:4n3	0.01	0.02	0.001	0,149
C20:2n6	0.02	0.02	0.001	0,733
C20:3n9	0.08	0.06	0.003	0,000
C22:0	0.37	0.32	0.020	0,226
C20:3n6	0.03	0.03	0.001	0,056
C22:1n9	0.07	0.04	0.006	0,001
C20:3n3	0.03	0.02	0.001	0,000
C20:4n6	0.14	0.16	0.008	0,150
C23:0	0.14	0.14	0.009	0,786
C20:4n3	0.02	0.01	0.001	0,133
C22:2n6	0.06	0.04	0.003	0,001
EPA	0.11	0.09	0.003	0,005
C24:0	0.19	0.14	0.010	0,012
C22:3n6	0.02	0.01	0.001	0,040
C24:1c15	0.05	0.04	0.003	0,001
C22:4n6	0.02	0.02	0.001	0,601
C25:0	0.04	0.03	0.002	0,291
C26:0	0.09	0.08	0.007	0,396
DPA	0.21	0.19	0.008	0,083
DHA	0.09	0.08	0.005	0,177
SCFA	6.68	8.72	0.387	0,006
MCFA	47.83	54.57	0.975	0,000
LCFA	45.49	36.71	1.246	0,000
SFA	64.64	69.32	0.766	0,001
MUFA	29.02	25.36	0.605	0,001
PUFA	6.33	5.31	0.184	0,004
UFA	35.35	30.67	0.765	0,001
OCFA	2.60	2.68	0.053	0,501
BCFA	3.05	2.62	0.128	0,095
OBCFA	5.65	5.30	0.173	0,314
PUFA6	2.12	2.41	0.088	0,089
PUFA3	1.53	1.01	0.070	0,000
n6/n3	1.39	2.75	0.225	0,001
n3/n6	0.72	0.44	0.034	0,000
CLA	1.12	0.85	0.042	0,000
TFA	5.20	3.61	0.236	0,000
TFA_no_VA	3.85	2.86	0.169	0,002

1SA, VA, LA, LNA = stearic acid; vaccenic acid; linoleic acid and linolenic acid respectively; RA, EPA, DPA and DHA= rumenic acid; eicosapentaenoic acid; docosapentaenoic acid and docosahexaenoic acid respectively; SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; TFA (trans fatty acids), BCFA (branched chain fatty acids), OBCFA (odd- and branched-chain fatty acids) =sum of the individual trans fatty acids, except CLA isomers; sum of iso- and anteiso-FA; sum of odd-, iso-, and anteiso-FA respectively; SCFA (short-chain fatty acids), MCFA (medium-chain fatty acids) and LCFA (long-chain fatty acids) =sum of the individual fatty acids from C4:0 to C10:0; sum of the individual fatty

acids from C11:0 to C17:0; sum of the individual fatty acids from C18:0 to DHA respectively; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids, respectively; CLA= sum of conjugated linoleic individual acids.

Table 8: Nutritional indices of Awassi sheep milk based on the production system of the farm (semi nomadic and transhumant system).

	Production system			
Fatty acid (g/100g of FAME)	**Semi nomadic**	**Transhumant**	**SEM**	**P-value**
AI	2.01	2.68	0.111	0,001
TI	1.83	2.58	0.113	0,000
h/H	0.68	0.52	0.025	0,000
DI C10:1	3.66	3.15	0.315	0,429
DI C14:1	2.22	2.87	0.145	0,021
DIC16:1	3.86	4.39	0.179	0,143
DI C18:1	65.57	70.49	0.798	0,001
DI CLA	38.68	45.66	1.051	0,000

TI, AI and h:H = thrombogenic index; atherogenic index; hypocholesterolemic to hypercholesterolemic ratio respectively. Desaturase indexes = Δ9-desaturase product / Σ Δ9-desaturaseproduct and substrate as described in the Materials and Methods. For example, the desaturase index of cis-9 14:1 is (cis-9 14:1)/ (cis-9 14:1+14:0). To improve the readability, the value was multiplied by 100.

3.6- Fatty acid profile and nutritional indices of fatty acids of Awassi sheep milk based on the different altitude's categories (<1000m, 1000-1500m and >1500m above sea level)

The fatty acid profile of sheep milk based on the different altitudes of the farms (<500m, 500-1000m, 1000-1500m and >1500m above sea level) is reported in table 9. All FA groups were significantly affected by altitude. The SCFA content was higher at <1000m and at 1000-1500 m of altitude (P=0.02), due to the higher content in C6:0 and C8:0. Similar pattern has been observed for MCFA, due to the higher content in C12:0 and C14:0. The opposite trend is evidenced for LCFA being the lowest at <1000m and at 1000-1500 m of altitude mainly related to lower content of both mono- and poly-unsaturated FA (P<0.01 and P=0.017 respectively). The lower MUFA content is due to lower C18:1 cis9 (P<0.01), whereas the lower PUFA is due to lower PUFAn-3 of which ALA represent the most abundant FA. The content of ALA derivatives EPA, DHA and DPA were the lowest at <1000 m compared to the others altitudes. On the other hand, n6: n3 ratio content was the highest between 1000-1500 m, CLA at altitudes lower than 1000m (P<0.01), while similar pattern was observed for VA and RA content were the lowest content was observed between 1000-1500m (P<0.01). No significant difference was showed for the OCFA and OBCFA content among the different altitudes, while the content of BCFA was the lowest between 1000-1500m (P=0.035). The

large differences observed in the Awassi sheep milk FA composition at different altitudes could be attributed to the sheep diet which prefer grazing on pasture characterized by wide variation and distribution of the vegetative cover (Kharrat, 2010) especially plants rich in essential oils, polyphenols or vegetable oils which could have a marked effect on the milk FA composition (Nudda *et al.,* 2020). In addition, these variations, could also be attributed to the summer season where plant cover become scarce and the content in NDF and ADF increase whereas the protein content decrease, with effects on milk composition (Nudda *et al.,* 2021) (Table 9). The nutritional indices of Awassi milk at different altitudes are reported in table 10. AI and TI indexes were the highest at 1000-1500m altitudes, and the h/H at above 1500m ($P<0.05$). The CLA/C18:1 trans-11 Δ9-desaturase was significantly higher in 1000-1500 m compared to the other altitudes. The correlation coefficient between rumenic acid and vaccenic acid was high ($R2 = 0.82$) because of the precursor-product relationship via the Δ9-desaturase enzyme (Figure 5). Desaturase index of C10 was the highest at <1000m, whereas the desaturase index of C14 and CLA were the highest between 1000-1500m, and no differences were observed for the desaturase index of C16 and C18 ($P>0.05$).

Table 9: Fatty acid profile of Awassi sheep milk based on the different altitudes categories (<1000m, 1000-1500m and >1500m above sea level).

	Altitude categories				
Fatty acid (g/100g of FAME)	**<1000**	**1000-1500**	**>1500**	**SEM**	**P value**
C4:0	2.10	1.84	1.77	0.050	0.071
C6:0	1.23^{ab}	1.32^{a}	1.03^{b}	0.050	0.029
C8:0	1.06^{ab}	1.25^{a}	0.87^{b}	0.057	0.008
C9:0	0.07	0.08	0.07	0.004	0.431
C10:0	3.69^{ab}	4.71^{a}	3.04^{b}	0.238	0.006
C10:1	0.15^{a}	0.11^{b}	0.11^{b}	0.006	0.01
C11:0	0.19^{b}	0.33^{a}	0.16^{b}	0.020	0.000
C12:0	2.49^{ab}	3.36^{a}	2.31^{b}	0.150	0.006
isoC13:0	0.03	0.03	0.04	0.002	0.399
anteisoC13:0	0.04^{b}	0.06^{a}	0.04^{b}	0.003	0.000
isoC14:0	0.15	0.15	0.18	0.010	0.287
C14:0	10.08^{ab}	11.63^{a}	10.27^{b}	0.244	0.028
isoC15:0	0.36	0.33	0.44	0.024	0.131
anteisoC15:0	0.63^{ab}	0.47^{b}	0.66^{a}	0.028	0.011
C14:1c9	0.24^{b}	0.39^{a}	0.24^{b}	0.021	0.005
C15:0	1.29	1.18	1.26	0.034	0.504
C15:1	0.10	0.07	0.10	0.007	0.074
isoC16:0	0.36	0.39	0.43	0.016	0.295
C16:0	32.21^{a}	32.99^{a}	28.17^{b}	0.667	0.001
C16:1t4	0.03	0.03	0.03	0.002	0.884
C16:1t5	0.03	0.03	0.03	0.002	0.797
C16:1t6-7	0.05	0.04	0.05	0.002	0.056

Fatty acid (g/100g of FAME)	<1000	1000-1500	>1500	SEM	P value
isoC17:0	0.55^{ab}	0.39^{b}	0.52^{a}	0.023	0.016
C16:1t9	0.08	0.05	0.07	0.005	0.085
C16:1t10	0.02^{ab}	0.02^{b}	0.03^{a}	0.002	0.003
C16:1t11-t12	0.06^{ab}	0.05^{b}	0.09^{a}	0.006	0.004
C16:1c7	0.30^{ab}	0.25^{b}	0.33^{a}	0.010	0.001
anteisoC17:0	0.59^{ab}	0.44^{b}	0.61^{a}	0.024	0.006
C16:1c9	1.29^{ab}	1.67^{a}	1.16^{b}	0.079	0.015
C16:1c10	0.08	0.08	0.10	0.008	0.323
C16:1c11	0.02	0.02	0.02	0.001	0.657
C17:0	0.89	0.85	0.94	0.024	0.294
isoC18:0	0.09^{b}	0.09^{b}	0.15^{a}	0.008	0.000
C17:1 c6-7	0.05	0.04	0.04	0.002	0.821
C17:1c8	0.02^{b}	0.02^{ab}	0.03^{a}	0.002	0.011
C17:1c9	0.36	0.33	0.37	0.013	0.441
C18:0 (SA)	8.68^{ab}	7.41^{b}	10.62^{a}	0.435	0.002
C18:1t4	0.03	0.02	0.02	0.001	0.399
C18:1t5	0.03	0.02	0.02	0.001	0.208
C18:1t6-8	0.21^{a}	0.14^{a}	0.19^{a}	0.010	0.033
C18:1t9	0.25^{ab}	0.19^{b}	0.24^{a}	0.008	0.022
C18:1t10	0.50^{a}	0.27^{b}	0.23^{b}	0.026	0.000
C18:1t11 (VA)	1.31^{a}	0.65^{b}	1.28^{a}	0.079	0.000
C18:1t12	0.28^{ab}	0.21^{b}	0.30^{a}	0.016	0.03
C18:1t13-t14	0.58	0.50	0.67	0.035	0.116
C18:1c9	18.26^{ab}	17.55^{b}	20.85^{a}	0.494	0.005
C18:1c11	0.57	0.51	0.57	0.013	0.082
C18:1c12	0.28^{a}	0.25^{ab}	0.20^{b}	0.011	0.009
C18:1c13	0.07	0.07	0.07	0.003	0.823
C18:1t16-c14	0.31^{ab}	0.27^{b}	0.44^{a}	0.024	0.003
C19:0/C18:1c15	0.32^{ab}	0.24^{b}	0.35^{a}	0.017	0.021
C18:2t10t14	0.05	0.03	0.04	0.003	0.281
C18:2t11t15	0.02^{b}	0.02^{b}	0.05^{a}	0.004	0.000
C18:2t9t12	0.03	0.02	0.02	0.002	0.678
C18:2c9t13	0.33^{ab}	0.29^{b}	0.40^{a}	0.018	0.02
C18:2t8c13	0.15^{ab}	0.14^{b}	0.20^{a}	0.009	0.006
C18:2c9t12	0.10^{b}	0.11^{b}	0.14^{a}	0.006	0.007
C18:1c16	0.03	0.03	0.04	0.003	0.08
C18:2t9c12	0.02	0.01	0.00	0.003	0.166
C18:2t11c15	0.14^{b}	0.10^{b}	0.38^{a}	0.031	0.000
C18:2n6 (LA)	2.21^{a}	2.11^{a}	1.74^{a}	0.077	0.025
C18:2t12c15	0.06^{ab}	0.05^{b}	0.07^{a}	0.003	0.013
C18:2c12c15	0.02^{ab}	0.02^{b}	0.03^{a}	0.002	0.001
C20:0	0.51^{ab}	0.51^{b}	0.70^{a}	0.032	0.007
C18:3n6	0.04	0.06	0.04	0.004	0.051
C20:1c9	0.02^{b}	0.03^{b}	0.04^{a}	0.002	0.001
C20:1c11	0.08^{ab}	0.09^{a}	0.07^{b}	0.003	0.002
C18:3n3 (LNA)	0.69^{ab}	0.47^{b}	0.99^{a}	0.062	0.000
CLAc9t11 (RA)	0.86^{a}	0.56^{b}	0.82^{a}	0.037	0.002
C20:1c15	0.03^{a}	0.03^{ab}	0.02^{b}	0.001	0.011

Fatty acid (g/100g of FAME)	<1000	1000-1500	>1500	SEM	P value
CLAt9c11/C21:0	0.14	0.12	0.14	0.008	0.584
CLAt10c12	0.02[b]	0.02[b]	0.04[a]	0.003	0.000
CLAt12t14	0.02[a]	0.01[b]	0.02[a]	0.001	0.001
CLAt11t13	0.04[a]	0.03[b]	0.05[a]	0.002	0.000
C20:2n9	0.02	0.01	0.02	0.001	0.066
CLAt9t11*	0.03	0.03	0.03	0.002	0.492
C18:4n3	0.02	0.01	0.02	0.001	0.182
C20:2n6	0.01	0.02	0.02	0.001	0.239
C20:3n9	0.06[b]	0.05[b]	0.08[a]	0.003	0.000
C22:0	0.28	0.28	0.37	0.020	0.082
C20:3n6	0.03	0.03	0.03	0.001	0.41
C22:1n9	0.05	0.04	0.07	0.006	0.176
C20:3n3	0.02[b]	0.02[b]	0.03[a]	0.001	0.000
C20:4n6	0.18	0.15	0.14	0.007	0.168
C23:0	0.15	0.12	0.14	0.009	0.448
C20:4n3	0.01	0.01	0.01	0.001	0.23
C22:2n6	0.03[b]	0.04[b]	0.05[a]	0.003	0.002
EPA	0.08[b]	0.08[b]	0.10[a]	0.004	0.01
C24:0	0.12[ab]	0.12[b]	0.18[a]	0.010	0.008
C22:3n6	0.01	0.01	0.02	0.001	0.14
C24:1c15	0.03[b]	0.04[b]	0.05[a]	0.003	0.004
C22:4n6	0.02	0.02	0.02	0.001	0.769
C25:0	0.03	0.03	0.04	0.002	0.345
C26:0	0.05[a]	0.07[a]	0.10[a]	0.007	0.025
DPA	0.16	0.18	0.21	0.009	0.102
DHA	0.06	0.08	0.09	0.005	0.056
SCFA	8.32[ab]	9.34[a]	6.91[b]	0.372	0.01
MCFA	52.72[ab]	55.84[a]	48.94[b]	0.932	0.002
LCFA	38.95[ab]	34.81[b]	44.15[a]	1.198	0.001
SFA	68.12[ab]	70.65[a]	65.32[b]	0.743	0.004
MUFA	26.12[ab]	24.36[b]	28.54[a]	0.585	0.003
PUFA	5.75[ab]	4.99[b]	6.13[a]	0.180	0.017
UFA	31.86[ab]	29.34[b]	34.67[a]	0.742	0.004
OCFA	2.65	2.62	2.63	0.051	0.973
BCFA	2.79[ab]	2.36[b]	3.06[a]	0.120	0.035
OBCFA	5.45	4.97	5.69	0.163	0.166
PUFA6	2.54[a]	2.45[a]	2.06[a]	0.083	0.033
PUFA3	1.04[b]	0.86[b]	1.46[a]	0.073	0.000
n6/n3	2.81[a]	3.34[a]	1.44[b]	0.225	0.000
n3/n6	0.39[b]	0.37[b]	0.70[a]	0.035	0.000
CLA	1.12[a]	0.77[b]	1.10[a]	0.043	0.001
TFA	4.64[ab]	3.25[b]	4.99[a]	0.223	0.001
TFA_no_VA	3.33[ab]	2.60[b]	3.72[a]	0.159	0.006

[1]SA, VA, LA, LNA = stearic acid; vaccenic acid; linoleic acid and linolenic acid respectively; RA, EPA, DPA and DHA= rumenic acid; eicosapentaenoic acid; docosapentaenoic acid and docosahexaenoic acid respectively; SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; TFA (trans fatty acids), BCFA (branched chain fatty acids), OBCFA (odd- and branched-chain fatty acids) =sum of the individual trans fatty acids, except CLA isomers; sum of iso- and anteiso-FA; sum of

odd-, iso-, and anteiso-FA respectively; SCFA (short-chain fatty acids), MCFA (medium-chain fatty acids) and LCFA (long-chain fatty acids) =sum of the individual fatty acids from C4:0 to C10:0; sum of the individual fatty acids from C11:0 to C17:0; sum of the individual fatty acids from C18:0 to DHA respectively; PUFA n-3 and PUFA n-6 = sum of individual n-3 and n-6 fatty acids, respectively; CLA= sum of conjugated linoleic individual acids.

Table 10: Nutritional indices of Awassi sheep milk based on the different altitudes categories (<1000m, 1000-1500m and >1500m above sea level).

	Altitude categories				
Fatty acid (g/100g of FAME)	**<1000**	**1000-1500**	**>1500**	**SEM**	**P value**
AI	2.40^{ab}	2.89^{a}	2.10^{b}	0.107	0.003
TI	2.45^{ab}	2.80^{a}	1.93^{b}	0.112	0.001
h/H	0.53^{ab}	0.48^{b}	0.66^{a}	0.025	0.003
DI C10:1	4.78^{a}	2.42^{b}	3.48^{ab}	0.296	0.033
DI C14:1	2.32^{ab}	3.16^{a}	2.29^{b}	0.136	0.012
DIC16:1	3.84	4.73	3.92	0.168	0.079
DI C18:1	67.38	70.59	66.60	0.759	0.068
DI CLA	40.04^{b}	47.49^{a}	39.69^{b}	0.998	0.001

TI, AI and h:H = thrombogenic index; atherogenic index; hypocholesterolemic to hypercholesterolemic ratio respectively. Desaturase indexes = Δ9-desaturase product / Σ Δ9-desaturaseproduct and substrate as described in the Materials and Methods. For example, the desaturase index of cis-9 14:1 is (cis-9 14:1)/ (cis-9 14:1+14:0). To improve the readability, the value was multiplied by 100.

3-7- Multivariate Statistical Analysis:

The FA profiles were analyzed by a multivariate approach to identify the FA able to discriminate between goat and sheep milk. In particular, the Stepwise Discriminant Analysis (SDA), which was applied using the stepclass function of the klaR R package (Weihs *et al.,* 2005), was used to select the most discriminant fatty acid able to separate between the two species. The selected stopping criterion was set to an improvement less than 0.50%. This technique selected four FA from the 101 original pool: CLAt9t11*, C16:1c9, C14:1c9, C10:0. These four variables were used in the Linear Discriminant Analysis (LDA), applied using the lda function of the MASS package of R (Venables and Ripley, 2002). Results of the LDA procedure are reported in Table 11. Since only two species were analyzed, a single new Linear Discriminant Function (LDF) was estimated (Figure 7). This function is a linear combination of the four selected FA, each one with its scaling factor. In particular, the following equation was computed for each animal:

$$LDF = C10:0 * -0.28 + C14:1c9 * -9.39 + C16:1c9 * 4.85 + CLAt9t11 * 81.70$$

The two species showed a Mahalanobis distance of 3.29, which was highly significant at the Hotelling test ($P<0.0001$). The analysis of the scaling coefficients (Table 11) can be used to

understand how the new LDF was computed. The most important FA to determine the LDF was the CLAt9t11; the most negative coefficient was showed by the C14:1c9, which was the second most important FA.

Table 11: Results of the Linear Discriminant Analysis

	Average		
Variable	**Goat**	**Sheep**	**Scaling**
C10:0	6.07	3.61	-0.28
C14:1c9	0.18	0.28	-9.39
C16:1c9	0.63	1.32	4.85
CLAt9t11	0.01	0.03	81.70

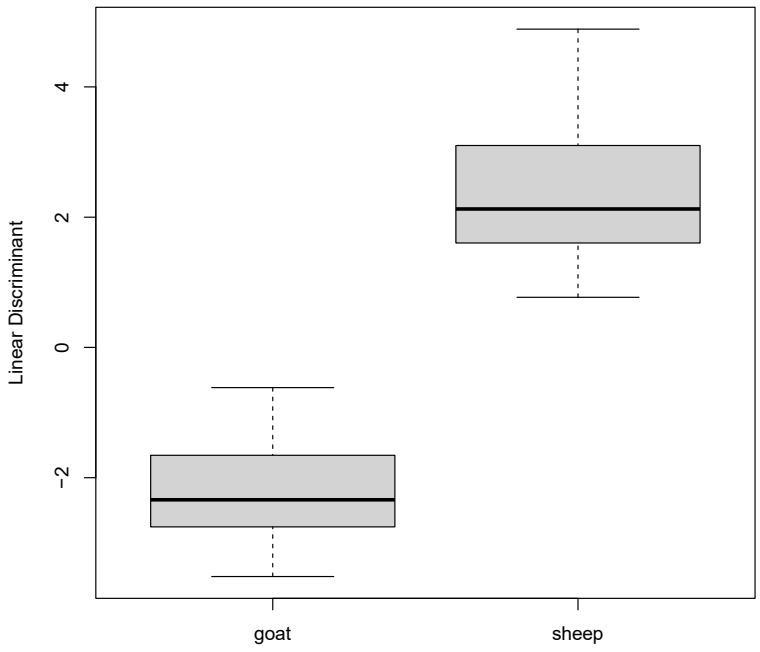

Figure 7: Coefficients for the new Linear Discriminant Function computed for goat and sheep animals.

In order to test the potentiality of the 4 selected FA to discriminate between goat and sheep milk, the dataset was randomly splitted in two subsets: 90% of observations (58 animals) were used as training, whereas 10% (6 animals) as validation. The training dataset was used to create the LDA model and to assign the scaling coefficients to the 4 involved FA. The validation dataset was used to assign the 10% of the observations to one of the two species.

This procedure was repeated 1,000 times and the assignment ability was evaluated through the number of animals assigned to the wrong species. In a total of 892 rounds all validation animals were correctly assigned to their species, whereas in 108 rounds only 1 animal (17% of error) was assigned to the wrong species. Thus, the four selected FA can be used to discriminate between goat and sheep milk, instead of using the whole FA profile.

4- Conclusion

This is the first study that report the FA composition of milk from sheep and goats bred in Lebanon. The sheep and goats maintained in the same environmental and feeding conditions evidenced marked differences in FA profile of their milk, with particular attention to FA of nutraceutical interest as rumenic acid and PUFA of the omega3 family. Substantial differences within specie have been observed for the FA composition of milk obtained in different production system. In particular goats and sheep in semi-nomadic and transhumant condition showed a more favorable FA profile of milk, for higher content in LNA, LA, and their long derivative EPA, DPA, and DHA, then sedentary farming system. Differences in the fatty acid profile of goat and sheep milk based on the different altitudes of the farms has been also observed, always related to the feed's availability and to the biodiversity of Lebanese rangelands at different altitudes in shrubs and woody plants rich in essential oils and polyphenols.

5- References

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CHAPTER 4

Cardoon (*Cynarara Cardunculus*) by-products: potential use in dairy sheep nutrition

This part of the thesis regard two experimental trials conducted as part of the research project "Bio products for feeding lactating sheep", funded by COMETA PON 2017 project (Indigenous Mediterranean Crops and their Enhancement with Advanced Green Chemistry Technologies).

The experiment 1 was carried out to study the effect of Cardoon (*Cynara cardunculus*) extracted flour (CEF) byproduct on the production, composition and fatty acid profile of milk in dairy Sarda sheep.

The experiment 2 was carried out to study the effects of Cardoon (*Cynara cardunculus*) polyphenolic extracts (CPE) on the feeding behavior, and milk production, composition and coagulation properties in Sarda dairy ewes.

Introduction

Cardoon (*Cynara cardunculus L.),* is a perennial herbaceous plant from the *Asteraceae* family and grows naturally in Mediterranean countries. It is characterized by a wide range of application such as: biomass for energy production, pulp paper manufacture due its richness in fiber (hemicellulose, cellulose and lignin), oil extraction from seeds (Fernandez *et al.,* 1996), and inulin production from the roots: cardoon roots are rich in sugar content (about 367 g/Kg of dry matter) with inulin having the highest content: 85% of total sugar (Raccuia and Melilli, 2004). Inulin has a positive effect on the gut microflora composition: it can increase the mineral absorption, have a good effect on blood lipid composition and also prevent colon cancer (Fernandez *et al.,* 2006). Cardoon plant is also used as a green forage due to its high biomass yield (6.5 ton of dry matter/ ha), (Fernandez *et al.,* 1996; Cajarville et al, 2000). In addition, cardoon is considered as source of several active compounds (Fernández *et al.,* 2006; Raccuia and Melilli, 2004, 2007) with potential health properties (Kukić *et al.,* 2008). Studies on the utilization of cardoon byproducts in small ruminant feed are increasing due to their nutritional potential.

Each year in Sardinia, about 61.500 tons of soya, 42.000 tons of peas, 14.000 tons of field bean, 11.000 tons of sunflower and 3.000 tons of lupine are imported and are used as a protein supplement (Falce, 2013). The imported soybean into Europe are from the United States (69%) and Brazil (25%) increasing the costs in animal nutrition. Cardoon byproducts and more specifically cardoon extracted flour, being rich in protein, can replace the soybean use in the feeding of ruminants, and are convenient from an economical point of view (Falce, 2013). In fact, the biomass yield of cardoon varies between 10-20 tons of dry matter/hectar (Gominho *et al.,* 2018), and is composed of 40% stalk, 35% capitula and 25% leaves, and the seeds can be used as a source of renewable energy (Cavarjille *et al.,* 1999; Gominho *et al.,* 2011). The hull of the cardoon seeds represents about 45% of the seed, which is higher than in other oil seeds where the hull represents 15-20% of the rapeseed, and 30% in the sunflower seed, being highly rich in fiber and lignin. The seed represent high soluble fraction formed by cellular contents and a lower value of fiber bound nitrogen, and represents also an un-degradable fraction composed of residual hulls containing high content of fiber and lignin which indicates that the kernel is highly degradable while the hull is poorly degradable (Cajarville *et al.,* 2000). The byproducts remaining after the harvest of cardoon, when ensiled have a good smell and can be used as a good silage that can replace the conventional feeds

such as hay, and have a crude protein content of about 88 g/Kg of DM, and a fiber content of 509 g/Kg of DM (Meneses *et al.,*2007). Cajarville *et al.*, (2000) have evidenced that the green leaves of cardoon have revealed high quality characteristics for forage due to their high digestibility coefficients (86.1% for OM, 78.3% for DM, and 77.6% for CP), while cardoon residues and dry leaves, due to their low CP content (78.4 g/kg and 72.0 g/kg of DM respectively), need a supplementation with urea in order to increase their digestibility.

Studies on cardoon seeds in animal nutrition have showed a composition of about 225 g/kg of CP, 250 g/kg of EE (ether extract) and 338 g/kg of NDF. Cardoon seeds contain about 29% of fats and 19% of highly fermentable proteins at the rumen level (19%) (fractions A and B1), they are rich in PUFA, mainly linoleic and oleic acids (Curt *et al.,* 2002), amino acid, and α-tocopherol, (Maccarone *et al.,*1999). Cardoon seeds showed the highest oil content between the *Cynara* species (about 25%) (Curt *et al.,* 2002; Raccuia *et al.,* 2007) and regarding the fatty acid profile, the predominant fatty acids are linoleic acid (C18:2; 59.7%), followed by oleic acid (C18:1; (25%), palmitic acid (C16:0; 10.6%) and stearic acid (C18:0; 3.7%) (Raccuia *et al.,* 2011, 2004; Curt *et al.,* 2002). Studies of seeds inclusion in ruminant diet, have evidenced the possibility to include 10% of cardoon seeds in the diet in order to benefit of its digestive effects in rumen degradability (56.8%) and CP degradability (82.9%). The addition of whole cardoon seeds in wethers diet have showed that the addition of around 10% of cardoon seeds is the most beneficial and permits to exploit its digestive effects (Cajarville *et al*. 2000). Higher concentration, can limit the feed intake due to their high fiber content.

Curt *et al.* (2002) suggested the addition of the seed press-cake in ruminant fodder, obtained after the oil extraction from the seeds, due to its high nitrogen content similar to that of the sunflower press-cake. After seed processing and fats extraction, two types of byproducts can be obtained: the cardoon cake which is a dark brown flour where almost all fat is extracted by mechanical extraction with a residue of 5%; and the cardoon extracted flour which is produced after the hot extraction of lipids from the seeds by using solvents. The latter is completely fat free and contain about 30% of protein, with a good amino acid profile, rich in glutammic and aspartic aminoacids (Cajarville *et al.,* 2000). Both by-products, have a soluble protein concentration lower than the seeds due to the heat treatment in fat extraction process, thus improving the efficiency of protein use by the rumen microorganisms (Fernadez *et al.,* 2006). The un-degradable and un-digestible portion (fraction C) does not change through the process from seeds to cake, which preserve the qualitative characteristics of the feed. The cardoon press-cake after oil extraction has a high content of fiber fraction: NDF, ADF and

ADL, with a moderate content of CP and EE (Genovese *et al.,* 2016). Compared to the sunflower press cake, cardoon press-cake showed a higher content of NDF (+24%), ADF (+36%) and ADL (+30%), and a lower content of CP (-37%). Starch in cardoon press cake is only 1.2% of dry matter (Cabiddu *et al.,* 2019). Also, cardoon press cake presented higher concentrations of linoleic acid (+40%), palmitic acid (+220%) and lower oleic acid (−46%) compared to sunflower cake. The seed press-cake is rich in nitrogen, these characteristics make it possible to be used as a feedstock (Genovese *et al.,* 2016), and according to Cajarville *et al.,* (2000), and Cannas *et al.* (2006), the addition of 250 g of cardoon press cake can substitute 100 g of soybean or 150 g of sunflower cakes, in dairy sheep grazing ryegrass, supporting a milk production of 2.5 kg/day/ewe, thus reducing the feed costs without affecting negatively the performances of the animal. In addition, studies have showed that the addition of cardoon seed to the diet up to 25% did not affect the rumen degradation of NDF and DM of lucerne hay. The 0.1 addition of cardoon seeds to the basal diet have increased the degradation of NDF (41%) and for DM (26%), and did not influenced the ruminal fermentation parameters (pH, ammonia, volatile fatty acid VFA), but cardoon seed inclusion at 0.25 substitution rate, decreased total VFA concentrations, and increased ammonia concentrations (Cajarville *et al.,*2000).

Moreover, several phenolic compounds have been characterized in cardoon leaves and seeds: caffeoylquinic acids and glycosides of luteolin and apigenin characterized by a great antioxidant activity (Pinelli *et al.,* 2007; Valentao *et al.,* 2002; Kukic *et al.,* 2008). Polyphenols in cardoon byproducts, could act as antioxidant substances (Fernandez *et al.,* 2006), have anticarcinogenic (Moon *et al.,* 2006), and hepato-protective properties, due to its capacity to increase the metabolic activity of the liver cells by accelerating the regeneration process of the liver (Fernandez *et al.,* 2006). Polyphenols transferred into animal products, such as dairy products and meat, can enhance their shelf-life and their nutritional quality (O'Connell and Fox, 2001). The most important polyphenolic compounds in cardoon leaves are: caffeoylquinic derivatives with chlorogenic acid, and glycosides of luteolin and epigenin, representing about 19 mg/g extract and 5.7 mg/g extract respectively (Valentao *et al.,* 2002; Pinelli *et al.,* 2007; Kukic *et al.,* 2008). These polyphenolic compounds are characterized by different pharmacological activities: for example, luteolin have an essential function in inhibiting de novo cholesterol biosynthesis (Ramos *et al.,* 2014). Also, succinylcaffeoylquinic acid compounds are present in high concentration in wild cardoon compared to cultivated cardoon (between 26.1-35.2% and 3.9-17.6% respectively) (Pinelli *et al.,* 2007). Additional compounds were found in the involucre bracts of *Cynara*, including: b-sitosterol, sitosteryl-3b-glucoside, apigenin, apigenin 7-glucoside, apigenin 7-methylglucuronide, scopolin,

cynarine and chlorogenic acid (Pandino *et al.,* 2011ab; Ramos *et al.,* 2014; Fernandez *et al.,* 2006). Chlorogenic acid is among the most representative phenolic acids being the most active antioxidant constituent and having anti-carcinogenic properties (Kollia *et al.,* 2016). The antioxidant activity of 3-O-caffeoylquinic acid and 4-O-caffeoylquinic acid have showed the same activity of chlorogenic acid due to their effect on the inhibition of the methyl linoleate oxidation and their effect on scavenging the superoxide anion radical. The 3,4-Di-O-caffeoylquinic acid, 3,5-di-O-caffeoylquinic acid, and chlorogenic acid, can inhibit the peroxidation of lipid (Pandino *et al.,* 2010; Pinelli *et al.,* 2007). Also b-sitosterol, have a stronger antioxidant activity than α-tocopherols, because it exerts a preventive action by inhibiting the excess production of active oxygen by various cells, whereas caffeic acid derivatives and polyphenols are radical scavenger because they capture hydroxyl and superoxide anion radicals, which concludes that b-sitosterol works on the stabilization of the cell membrane in order to have an antioxidant activity (Kollia *et al.,* 2016; Couveria *et al.,* 2012; Fratianni *et al.,* 2007).

Moreover, a study done by Kukic *et al.,* 2008, have showed that all compounds from the cardoon involucre bracts, are characterized by an antimicrobial activity against all tested strains of fungi and bacteria (the minimum inhibitory concentrations (MICs), the minimum bacterial concentration (MBCs) and the minimum fungicidal concentration (MFCs) are in a range of 0.03–0.10 mg/ml). Among these compounds, luteolin exert the best antimicrobial activity. Also, apigenin, apigenin 7-glucoside, luteolin and other flavones have showed an interesting antimicrobial activity (Fernandez *et al.,* 2006; Grammelis *et al.,* 2008; Ramos *et al.,* 2014).

In summary, cardoon plant and byproducts and their use in animal diets is of great interest because of their potential positive effect on animal health and production, and on the quality of dairy products. To deep the knowledge of the inclusion of cardoon byproducts in small ruminant diets, two experiments have been carried out with the aim to study their effects on the milk production traits, milk composition, coagulation properties and fatty acid profile in lactating dairy ewes.

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Experiment 1

Effect of byproduct of Cardoon (*Cynara cardunculus*) on the production, composition, and fatty acid profile of sheep milk

1- Objectives

The purpose of this study was to investigate the inclusion in lactating Sarda dairy ewes of two different doses of cardoon flour on the production, composition and fatty acid profile of milk.

2- Material and Methods

2.1- Experimental design

This work was a part of the research project "Bio-Products for feeding lactating sheep", funded by the "COMETA PON 2017" project (Indigenous Mediterranean Crops and their Enhancement with Advanced Green Chemistry Technologies). The experiment was conducted at Ledda's farm located near Sassari. During the pre-experimental period 100 multiparous Sardinian sheep at the beginning of lactation (100 days after calving) were identified and monitored for individual milk yield, milk composition, BW and body score conditions (BCS). Blood samples were collected to check the health status of the animals. Based on the pre-experimental measurements, 36 animals were selected. The ration was composed of a total mixed ration (TMR) in addition to dehydrated ryegrass hay and soybean extracted flour (Table 1). Diet was prepared with the Small Ruminant Nutrition System (Cannas *et al.,* 2004; Tedeschi, 2010). After 21 days of the adaptation phase, animals were divided into three groups of 12 animals each homogenous for their weight, BCS and milk production (Table 2). The experimental phase lasted 28 days. The group control (CON group) received the diet of the adaptation period described above, while for the other two groups, soybean meal and ryegrass hay were replaced with 250 g/head (low dose; LD group) and 550 g/head (high dose; HD group) of cardoon extracted flour (CEF; Table 3). The diets were isoproteic (17% CP, DM basis) and similar in their fibrous content and energy (Table 4). During the experiment, each 3 animals were allocated in one pen and the feed ration was offered four time a day (5:00, 10:00, 15:00 and 20:00). Every day, the residues of the TMR of each pen were weighted and were collected for analysis once a week. Clean water was always available. The cardoon floor was provided by an Italian feed industry.

Table 1: Chemical composition of the feed ration during the adaptation phase.

Item	TMR	Hay	Soybean
Dry matter DM	87.32	88.46	90.00
Crude protein, % DM	14.33	12.66	52.80
NDF, %DM	37.28	55.50	7.79
ADF, %DM	24.79	47.31	5.20
ADL, %DM	4.02	4.88	2.50
Fat, % DM	4.49	2.20	1.10
Ash, % DM	8.45	10.00	6.70

DM, NDF, ADF and ADL: dry matter, neutral detergent fiber; acid detergent fiber: acid detergent lignin respectively.

Table 2: Characteristics of the animals in the three experimental groups (mean ± sd).

Group	Milk Yield	Body weight	BCS
	Kg/d	Kg	
CON	1.859±0.28	43.79±5.98	2.72±0.09
LD	1.819±0.34	44.43±4.70	2.67±0.12
HD	1.807±0.37	43.97±5.11	2.65±0.07

Stdev: Standard deviation; CON: control; LD: low dose; HD: high dose

Table 3: Chemical composition of cardoon extracted flour.

Item	Cardoon extracted flour
Dry matter DM	90.21
Crude protein %DM	21.91
NDF, %DM	72.35
ADF, %DM	52.24
ADL, %DM	19.10
Fat, %DM	1.02
Ash, %DM	6.39

NDF, ADF and ADL: neutral detergent fiber; acid detergent fiber: acid detergent lignin respectively.

Table 4: Feed ration used in the 3 experimental groups, quantity of feed/animal/day in gram and their chemical composition

Feed g/day	CON	LD	HD
TMR	1426	1426	1426
Soybean	263	200	115
Dehydrated ryegrass hay	577	390	175
Cardoon extracted flour	-	250	550
Total g/d	2266	2266	2266
NDF, %DM	35.6	37.3	39.5
Crude protein, %DM	17.5	17.3	17.0
NEmilk Mcal/Kg DM	1.59	1.50	1.40

NDF: neutral detergent fiber; NEmilk: net energy milk

2-2- Sampling and measurements

Feed samples

Feed samples of the three experimental groups were collected weekly and were analyzed for their physical and chemical characteristics. Diet was weighted daily and was administered 4 times a day. The next day ration was calculated based on the residues of each day in order to administer a higher percentage (30%) compared to the ingestion of the previous day. The dry matter content, NDF, ADF, ADL and crude protein content were analyzed as detailed in chapter 7 paragraph 1.

Body weight, body score conditions and ingestion

BW and BCS of the animals were measured at the beginning, middle and the end of the experiment. BCS was measured during the animals weighing, and was done by three operators who assigned individually a score from 0 to 5 for each animal. Ingestion of each pen was monitored on a daily basis, and the individual ingestion was calculated at the end of the experiment by the administration of a known feed quantity and by weighing the residues: 4 times a day 1,000 ±5 g of feed mix was presented for each animal in a plastic basin (30×30 cm) with metal side panels in order to avoid contact with other animals; feed was left available for 90 minutes and then the residues were weighted with an approximation of ±0.1g. This test was carried out for two consecutive days, and the residues were collected for

each of the 4 administrations during the second day to analyze their chemical composition and the particle size of residuals.

Milk samples

Individual milk yield was measured and individual milk samples were collected each week and divided in two aliquots for the analysis of fatty acids (10 ml) as detailed in Chapter 7 paragraph 2.2, and of the proximate milk composition (50 ml). The fatty acid methyl esters (FAME) were analyzed as detailed in Chapter 7 paragraph 2.3. Milk samples were delivered to the laboratories of the regional LAORE agency, to determine the fat, lactose, casein, protein and urea content using infrared method (Milkoscan 6000), and somatic cells content (SCC) using the flow cytometry method (Bactoscan 6000). The fat corrected milk was calculated using the formula reported by Pulina and Nudda (2001):

$$FCM = M\ (0.37 + 0.097F)\ (\text{reference value kcal/kg} = 1.020)$$

Where:

FCM = fat corrected milk

M = Milk yield (kg);

F = Milk fat content (%).

2-3- Statistical analysis

The data of weight, BCS, milk yield and composition and fatty acids profile were analyzed according to the Proc Mixed procedure of the SAS software (SAS, 2001), applying a mixed linear model, using diet and time as fixed effects (date of sampling) and their interaction, and animals as a random effect.

3- Results and Discussion

3.1- Body weight

The BW and BCS did not showed a significant difference between the 3 experimental groups (Table 5). The BW increase numerically over time in both groups fed CEF compared to the CON, even if did not reach the statistical significance (Figure 1).

Table 5: Effect of diet, time and their interaction (diet × time), on the weight (Kg) and body score condition (BCS) of the animals.

	Diet				Significative P		
Variables	Con	LD	HD	SEM	Diet	Time	Diet×Time
Weight (Kg)	43.44	45.49	44.21	1.382	0.57	0.14	0.17
BCS	2.715	2.61	2.666	0.026	0.42	0.22	0.47

SEM: standard error; Con: control; LD: low dose; HD: high dose

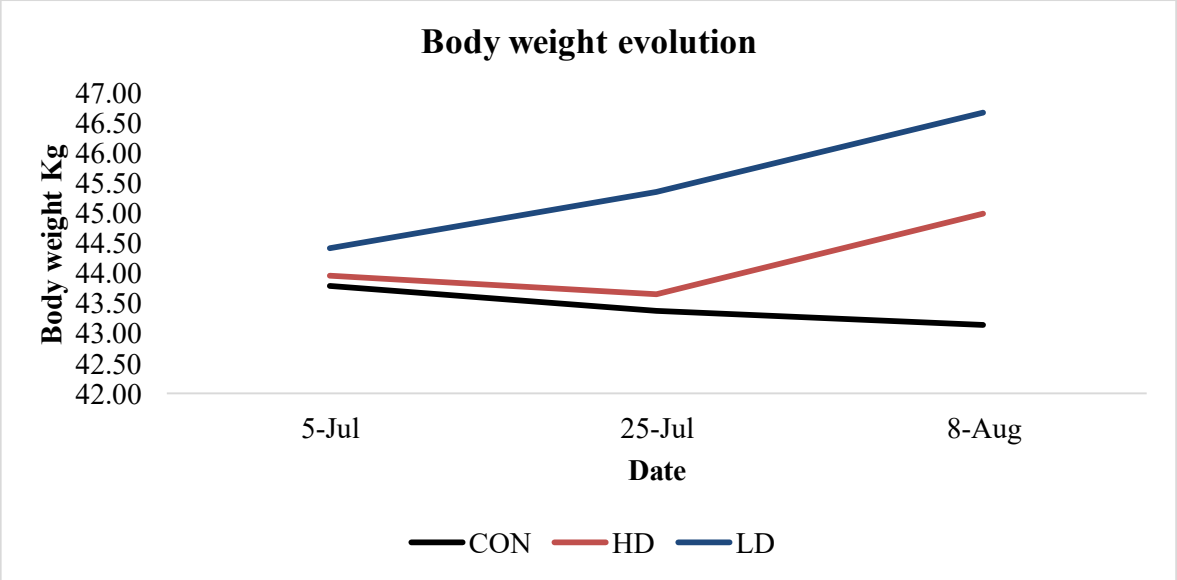

Figure 1: Evolution of animal weight from the pre-experimental phase till the end of the experiment.

3.2- Ingestion

Group ingestion was not significantly influenced by the tested diets (Table 6). However, a numerical increase in the DMI in the both CEF groups compared to the CON group (Figure 2) was evident, even if did not reach the significant level. However, the net energy intake calculated for each experimental group (Table 6) shows a numerically higher NE ingestion in the CON group compared to the other two groups.

Table 6: Effect of diet and time on the group ingestion of dry matter.

	Diet			
Variables	**CON**	**LD**	**HD**	**SEM**
Ingestion Kg of DM	1.909	1.983	1.963	0.121
NEIngestion Mcal/Kg DM	3.035	2.975	2.748	

SEM: standard error; Con: control; LD: low dose; HD: high dose

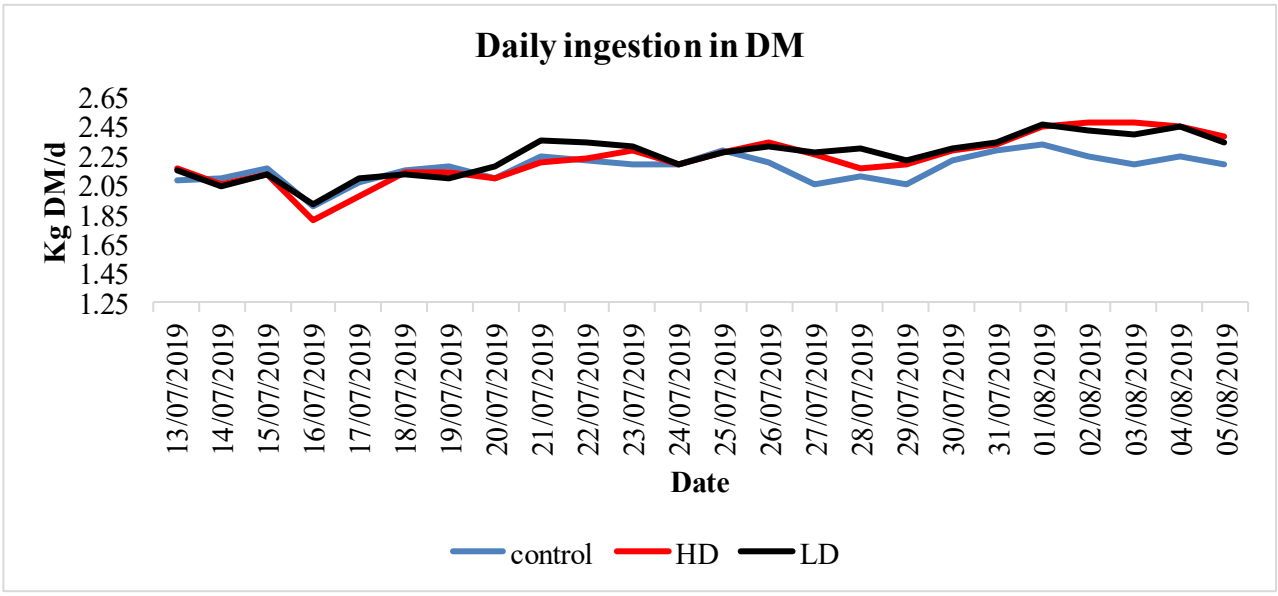

Figure 2: Evolution of the daily ingestion in DM of the three experimental groups.

3.3- Effect on milk production

Diets effect on the production and composition of milk are shown in table 7. The CEF did not influence milk production and milk components, but time affect significantly all the variables studied (P<0.001). Some studies showed that polyphenols in ruminant diet, can be effective in increasing the milk production (Harris *et al.,* 1998; Hymes-Fecht *et al.,* 2013), whereas other studies showed a decrease in milk production often linked to reduced ingestion (Griffiths *et al.,* 2013). Moreover, the conflicting results in the literature (Dung, 2010), are due to the fact not only the quantity of polyphenols but also by the source, the chemical characteristics and also by the other ingredients which constitute the diet can exert different effects on animal performance. Time was always significant for all production traits. All the tested groups have showed a decrease in the milk yield during the experimental period (Figure 3) consistent with the natural lactation curve evolution of Sarda sheep (Macciotta *et al.,* 1999).

Table 7: Effect of diet, time and their interaction on milk production and milk composition

Variables	Diet			SEM	Significative P		
	CON	LD	HD		Diet	Time	Diet×Time
Milk production Kg	1.622	1.625	1.649	0.069	0.95	<0.001	0.91
Normalized milk Kg	1.476	1.508	1.489	0.059	0.93	0.011	0.89
Fat %	5.84	5.802	5.608	0.145	0.48	<0.001	0.81
Protein %	5.14	5.52	5.22	0.141	0.14	0.003	0.89
Lactose %	4.89	4.93	4.93	0.064	0.90	<0.001	0.56

SEM: standard error; Con: control; LD: low dose; HD: high dose

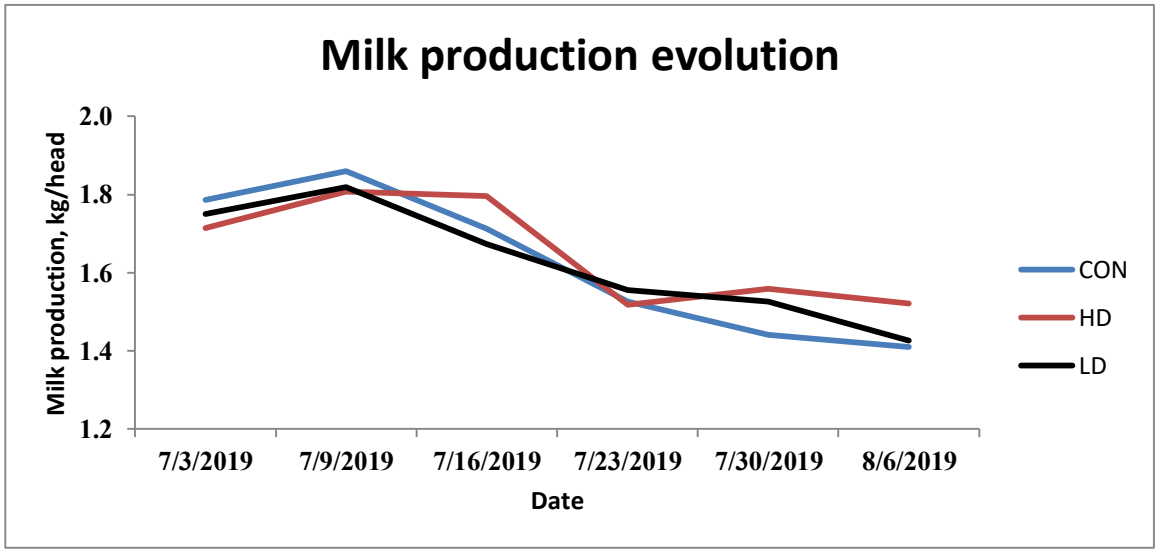

Figure 3 : Evolution of the milk production in the groups during the experiment.

3.4- Milk fatty acid profile

The effect of the cardoon floor on milk fatty acids profile is reported in Table 8. The average content of SFA, MUFA and PUFA were in agreement with other studies on the same breed (Correddu *et al.,* 2019). The cardoon floor did not modify the milk fatty acid profile ($P>0.05$). This was an unexpected results because polyphenols in ruminant feed, can affect the FA profile of milk due to their effects on rumen bio-hydrogenation process, by the inhibition of rumen micro-organisms growth, responsible of the final step of bio-hydrogenation: the reduction of vaccenic acid into stearic acid. The lack of effect in this study could be related to the chemical structure or to the polyphenols content in cardoon floor; also to the diet composition and the ingestion (Correddu, 2015). The fatty acid profile was significantly influenced by the sampling date. The evolution of fatty acid during the experiment evidenced the increase of SFA towards the 4 sampling, whereas the opposite trend was observed for the MUFA (Figure 4-5), PUFA, CLA and TFA concentrations (Figure 6-7-8). A decrease of OBCFA content is reported with sampling (Figure 9). The variation of the fatty acid profile could be due to physiological factors related to the different phases of the lactation (Kay *et al.,* 2005). The experiment was conducted on animals at their final stage of lactation that were kept indoor. The observed variation of fatty acid among time may be due to the advancement of lactation. Significant interaction diet×time has been observed for C10:0, C12:0, C14:0 and C18:1cis-9 due to similar concentration of the fatty acids in CON and LD groups from the beginning till the end of the experiment, whereas in the HD group they decreased during the 2nd and 3rd sampling and then increased again in the 4th sampling.

The evolution of C18:1cis-9 is opposite to the evolution of the SCFA and MCFA (C10:0; C12:0; C14:0), probably because in milk the proportion between the content of short and medium chain fatty acids synthesized de novo in the mammary gland and the concentration of MUFA, in particular C18:c1cis-9, is maintained constant to maintain constant the fluidity of the milk fat in the different phases of lactation (Timmen and Patton, 1988). This result has also been observed on other species such as buffaloes and cattle (Conte *et al.,* 2016; Correddu *et al.,* 2017).

Table 8: Effect of the diet, sampling time and their interaction on the fatty acid composition 9g/100g).

	Diet			SEM[2]	Significative P		
Variables[1]	**CON**	**HD**	**LD**		**Diet**	**Time**	**Diet*Time**
C4:0	2.87	2.80	2.81	0.04	0.866	<.0001	0.001
C6:0	1.91	1.85	1.82	0.02	0.559	0.100	0.003
C8:0	1.73	1.66	1.64	0.02	0.448	<.0001	0.000
C10:0	6.01	5.61	5.66	0.10	0.367	<.0001	<.0001
C12:0	3.55	3.41	3.43	0.07	0.681	<.0001	<.0001
C14:0	9.99	9.63	10.32	0.10	0.125	<.0001	0.024
C16:0	28.62	28.91	29.06	0.20	0.762	<.0001	0.070
C18:0	8.76	9.20	8.07	0.18	0.072	<.0001	0.262
C18:1trans-11	1.18	1.07	0.99	0.05	0.253	<.0001	0.961
C18:1cis-9	19.11	19.50	19.84	0.25	0.511	<.0001	0.027
C18:2n-6	2.65	2.83	2.71	0.04	0.398	<.0001	0.557
C18:3n-3	0.32	0.29	0.30	0.01	0.225	<.0001	0.663
CLAcis-9,trans-11	0.79	0.71	0.76	0.03	0.407	<.0001	0.995
EPA	0.04	0.04	0.04	0.00	0.062	<.0001	0.062
DPA	0.08	0.09	0.08	0.00	0.066	<.0001	0.014
DHA	0.03	0.04	0.03	0.00	0.294	<.0001	0.031
SFA	68.10	67.74	67.45	0.24	0.702	0.000	0.045
UFA	31.78	32.14	32.44	0.24	0.696	0.000	0.044
MUFA	26.46	26.70	27.12	0.20	0.615	0.085	0.015
PUFA	5.28	5.37	5.25	0.07	0.789	<.0001	0.923
SCFA	12.64	12.03	12.05	0.14	0.330	<.0001	<.0001
MCFA	48.58	48.32	49.33	0.28	0.559	<.0001	0.155
LCFA	38.79	39.65	38.62	0.33	0.581	<.0001	0.030
CLA	0.93	0.84	0.89	0.03	0.362	<.0001	0.994
TFA	5.70	5.36	5.43	0.21	0.622	<.0001	0.978
TFA (-CLA e VA)	3.59	3.45	3.55	0.17	0.879	<.0001	0.953

OBCFA	4.21	4.19	4.18	0.05	0.969	<.0001	0.413

[1] EPA, DPA and DHA= eicosapentaenoic acid; docosapentaenoic acid and docosahexaenoic acid respectively; SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; SCFA (short-chain fatty acids), MCFA (medium-chain fatty acids) and LCFA (long-chain fatty acids) =sum of the individual fatty acids from C4:0 to C10:0; sum of the individual fatty acids from C11:0 to C17:0; sum of the individual fatty acids from C18:0 to DHA respectively; CLA= sum of conjugated linoleic individual acids; TFA (trans fatty acids) and TFA (-CLA e VA): sum of the individual trans fatty acids, except CLA isomers; trans fatty acids without CLA and C18:1 trans-11 (vaccenic acid, VA) respectively; OBCFA (odd- and branched-chain fatty acids) = sum of iso- and anteiso-FA. [2]SEM: standard error of the mean.

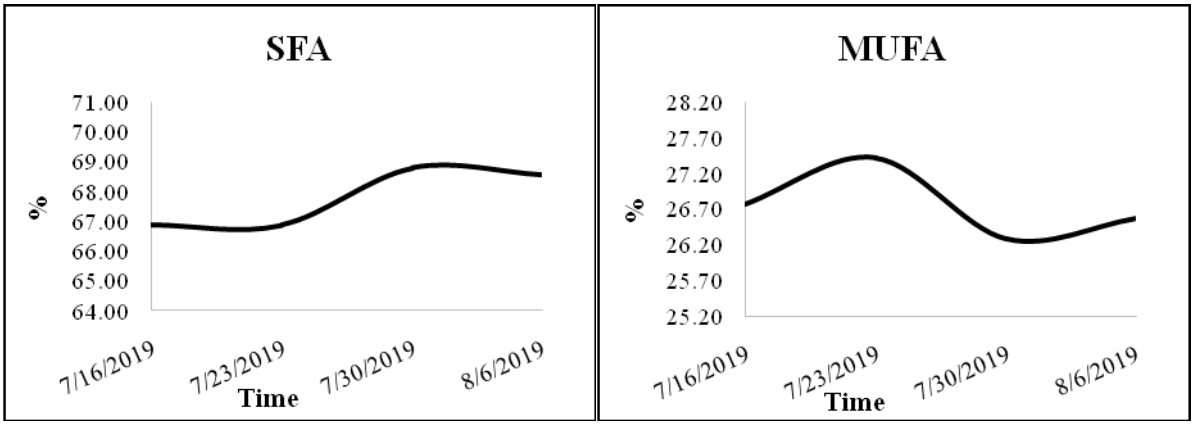

Figure 4-5: Evolution of the concentration of SFA and MUFA fatty acids during the experiment

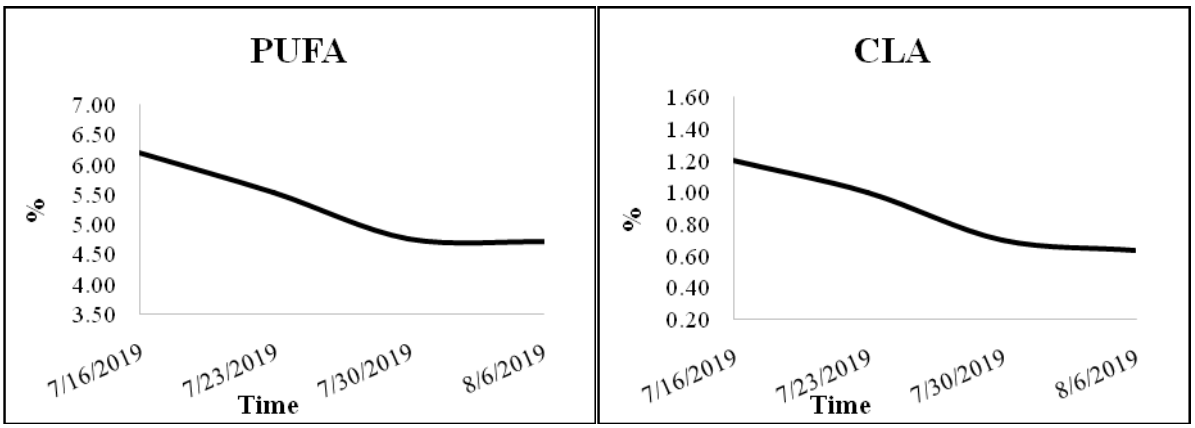

Figure 6-7: Evolution of the concentration of PUFA and CLA fatty acids during the experiment.

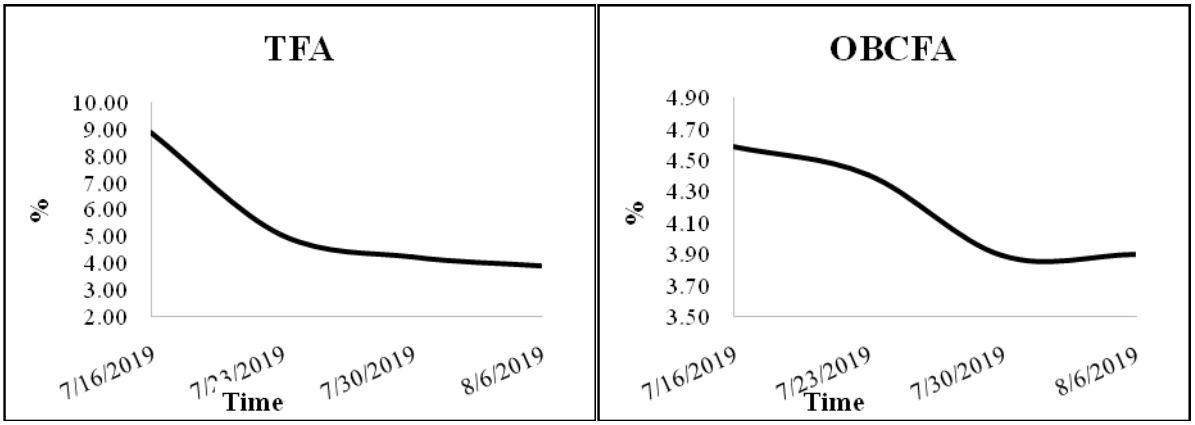

Figure 8-9: Evolution of the concentration of TFA and OBCFA fatty acids during the experiment.

4- Conclusion

This study suggests, that the supplementation of cardoon flour in two different doses 250 and 550 g/head in replacement to soybean meal and ryegrass hay, did not affected negatively the ingestion, milk yield and milk main components: protein, fat and lactose. Furthermore, the analysis of the effect this by-product on the milk fatty acid profile, did not showed significant differences in the quality of milk fat. In conclusion, this byproduct can be used at the tested doses without negative effect on milk quality and production. However, longer supplementation period is necessary to evaluate the role of this type of byproduct during others phases of lactation.

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Experiment 2

Effect of polyphenolic extracts of cardoon (*Cynara cardunculus*) on the production, composition and coagulation properties of sheep milk

1- Objectives

The aim of this study was to investigate the effects of cardoon polyphenolic extracts (CPE) administration on the ingestion and feeding behavior of lactating Sardinian sheep, and on milk production, milk composition and milk coagulation properties.

2- Material and Methods

2.1- Experimental design

The experiment was conducted at the "Mauro Deidda" experimental didactic farm of the University of Sassari, located in Ottava. During the pre-experimental phase, 50 multiparous Sarda ewes were identified at 90 days after calving. The health status of the animals and BCS were evaluated and the individual production level and milk quality, were measured. Twenty ewes were selected and adapted to the experimental diet for a period of 15 days. The experimental diet consisted of a TMR (total mixed ration) (Table 1) formulated with software of the Small Ruminant Nutrition System (Cannas *et al.,* 2004; Tedeschi, 2010). The experiment lasted for 42 days (6 weeks) from May to June 2021, divided into 3 cycles of 14 days each. After the adaptation period to TMR, the animals were divided into 2 groups of 10 animals each homogenous for milk production, BW and BCS (Table 2): a control group fed TMR (CON group) and the treated group (TR group) was supplemented with increasing dose of cardoon polyphenolic extracts CPE starting from 5 g/head for the first cycle, 10 g/head for the second cycle and 20 g/head for the 3rd cycle. Animals were milked two times a day (8:00 and 17:00) and at the end of each milking, post dipping was carried out using a foaming solution. The CPE was offered in the evening milking in a single dose, orally using a spoon with a little water without use of appetizers. The 2 experimental groups were fed individually with individual self-feeders system. Feed was administered daily, and were weighted, in addition to the residues, automatically by the software of the automatic feeders.

Table 1: Chemical composition of the TMR ration.

Items[1]	TMR
Dry matter DM, %	86.45
Crude Protein, % DM	17.53
NDF, % DM	32.92
ADF, % DM	23.92
ADL, % DM	7.85
Ash, % DM	7.99

[1]NDF, ADF and ADL: neutral detergent fiber; acid detergent fiber: acid detergent lignin respectively.

Table 2: Characteristics of the two groups of ewes during the pre-experimental phase.

Group	Milk (kg) [1]	Body weight (kg)	BCS	Ingestion (kg/DM)
Control	2.016±0.65	54.33±6.95	2.85±0.32	2.3±0.30
Treatment	2.056±0.65	52.21±6.41	2.88±0.28	2.1±0.20

[1]data are reported as mean ±standard deviation

2-2-Sampling

Animals

At the beginning and the end of each cycle, animal weight and body conditions score (BCS) were measured. BCS was assessed by 3 operators who individually assigned each animal a score from 0 to 5 (including quarters). The individual intake was monitored daily through the automatic self-feeders (Bio-Control feeding system), which managed automatically the individual access inside the feeders, and calculate the amount of feed intake of each animal.

Feed samples

Samples of feeds were collected weekly. Residues were collected daily, 24 hours after their administration, and twice a week after 12 hours of their administration to evaluate the residual chemical composition and physical characteristics. The DM, ADF, ADL, NDF and CP were determined as reported in Chapter 7, paragraph 1.

Individual ingestion measurement and feeding behavior of ewes by Bio-Control feeding system

The individual ingestion, number of visits during the 24 hours, duration and time of each visit, to the self-feeders from the company Biocontrol AS (Rakkestad, Norway) were registered by the software. During the trial 3 feeders per groups were used. Each feeder, through an input panel, detected the presence of the animal through a special ear tag (HDX frequency), thus authorizing the animal to feed. The individual amount ingested was automatically measured and recorded by the software.

Figure 1: Automatic feeders linked to the Bio-control software.

Milk samples

Individual milk production was measured for two consecutive days at the end of each experimental week, both at morning and evening milking, for a total of 8 measurements per cycle. Milk samples were collected in 40 mL tube for chemical and physical analysis and were delivered to the laboratory of the regional LAORE Agency. The following parameters were determined: fat, lactose, protein, casein and urea content using the infrared method (Milkoscan 6000; Foss Electric, Hillerod, Denmark); and the SCC by flow cytometry (Fossomatic 360; Foss Electric, Hillerod, Denmark). The normalized milk was estimated using the formula reported in Pulina and Nudda (2001). The concentration of saturated, monounsaturated and polyunsaturated FA and the concentration of individual FA as vacceninc acid (C18:1trans11), alpha-linolenic acid (C18:3n3) and cis9trans11 CLA were determined by MIR spectroscopy (Milkoscan 6000). An aliquot of the milk samples was used to determine the coagulation properties of the milk using a Formagraph (FossElectric A/S,

Hillerod, Denmark) according to the method proposed by Zannoni and Annibaldi (1981) and detailed in Chapter 7 paragraph 2.5.

2-3- Statistical analysis

The data of feed ingestion were analyzed separately for the three cycles of the experiment, using the PROC MIXED procedure of the SAS for repeated measurements, according to the following linear model:

$$Yijkl = \mu + Di + Sj + (D \times S)ij + eijkl$$

Were:
Y= variable: ingestion of feed for each cycle
μ = general mean
D = fixed effect of the diet (i= control or treatment)
S = fixed effect of the sampling day (j = 14 days)
(D × S) = interaction between the diet and the sampling day
e = residual error

The data related to the body weight and BCS were analyzed separately for the three cycles of the test, using the PROC MIXED procedure of the SAS for repeated measurements, according to the following mixed linear model:

$$Yijk = \mu + Di + Rj + (D \times R)ij + eijk$$

Where:

Y= variable, body weight and BCS
μ = general mean
D = fixed effect of the diet (i=2, control, treatment)
S = fixed effect of the sampling day (j=2)
(D × S) = interaction between diet and sampling day
e = residual error

3- Results and Discussion

3.1- Body weight

The polyphenolic extract supplementation did not affect significantly the BW and BCS score, but there was a significant effect of time (Table 3; Figure 2).

Table 3: Evolution of body weight and BCS for the two groups during the three cycles of the experiment.

Variables	Group		SEM[1]	Significative (P)		
	Con	Treat		Group	Time	Group*Time
Body weight, kg						
First cycle, (dose 5g/head)	57.61	54.30	2.34	0.331	0.067	0.596
Second cycle, (dose 10g/head)	58.49	55.05	2.36	0.316	<0.001	0.662
Third cycle (dose 20g/head)	57.24	53.80	2.42	0.329	0.431	0.236
BCS						
First cycle, (dose 5g/head)	2.973	2.918	0.715	0.595	0.762	0.293
Second cycle, (dose 10g/head)	3.003	2.918	0.759	0.442	0.516	0.365
Third cycle (dose 20g/head)	2.956	2.887	0.550	0.388	1.000	1.000

[1]SEM = standard error; Con = control; Treat = treated group with the three doses of the polyphenolic cardoon extract.

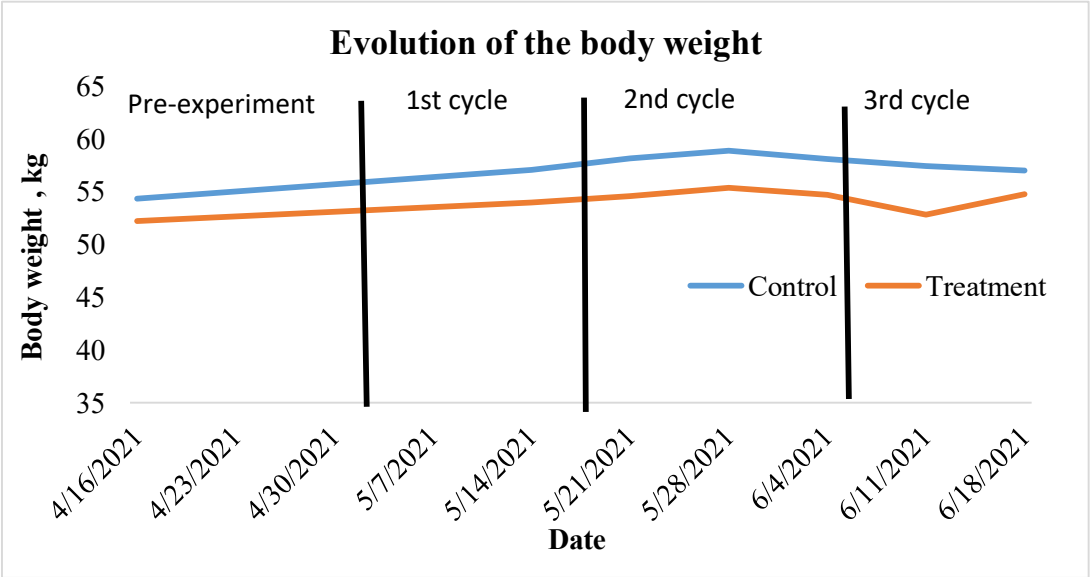

Figure 2: Evolution of the body weight from the pre-experimental phase till the end of the experiment in control and treated group.

3.2- Intake

The ingestion was not significantly affected by CPE supplementation (Table 4). The average group ingestion was significantly influenced by time (P<0.001; Table 4). The intake in both groups shows a similar trend during the first and second cycles, but decrease significantly during the third cycle when ewes received the highest dose of polyphenols (P=0.006) (Figure 3).

Table 4: Effect of the diet, time and their interaction on the feed intake.

Variable	Group		SEM[1]	Significative (P)		
	Con	Treat		Group	Time	Group*Time
Ingestion, g di TQ						
First cycle, (dose 5g/head)	2561	2530	81.88	0.749	<0.001	0.577
Second cycle, (dose 10g/head)	2432	2415	102.73	0.903	0.001	0.993
Third cycle (dose 20g/head)	2272	1822	101.65	0.006	<0.001	0.436

[1]SEM = standard error; Con = control; Treat = treated group with the three doses of the polyphenolic cardoon extract.

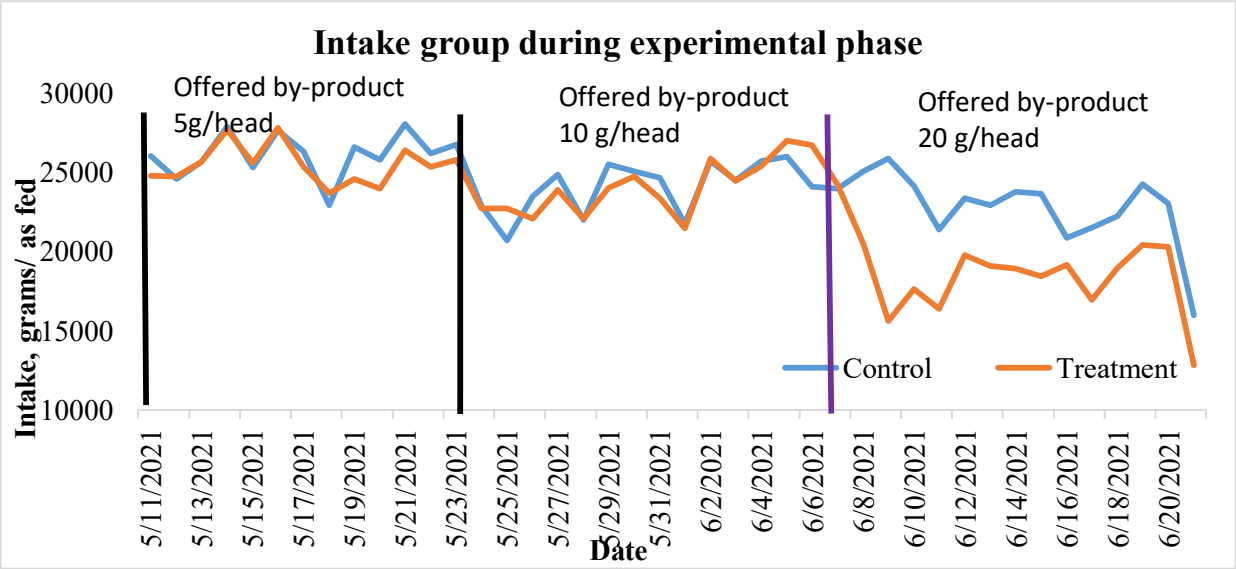

Figure 3: Evolution of the daily ingestion during the experimental test.

3.3- Eating behavior of the ewes

The ingestion pattern during the 24 hours showed an increase in the ingestion in both groups in correspondence of the offer of the ration at morning at 8:00 and at night 18:00 (Figure 4-5-6). Before the night feeding, the treated group received the CPE in the dose established at each cycle (5, 10 and 20 g/head). The daily intake peaked in the hours immediately after the new feed administration, both for the first and second cycle (Figure 4-5), while for the third cycle, the group with the high dose of CPE, did not showed the usual peak of the ingestion after the evening meal, which was observed instead for the CON group (Figures 6). The observed differences in intake with the higher dose of polyphenolic extracts could be due to alteration in rumen fermentation and digestive process, thus reducing the digestibility of the rations, feed ingestion and production. They may cause also a reduction in the palatability of the rations and therefore of their ingestion (Makkar *et al.,* 2003). The effect of palatability could be easily overcome by dividing the dose into several meals or by masking the taste and smell of the polyphenolic compounds with appetizers rich in sugar such as molasses or carob.

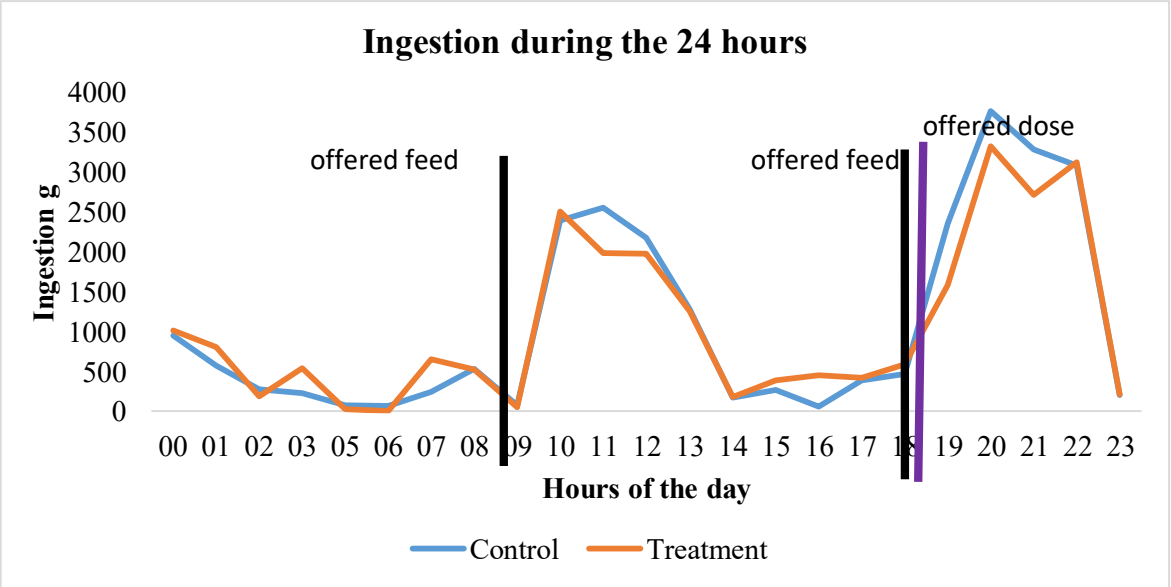

Figure 4: Evolution of group intake during the 24 hours of the 6th day in the first cycle.

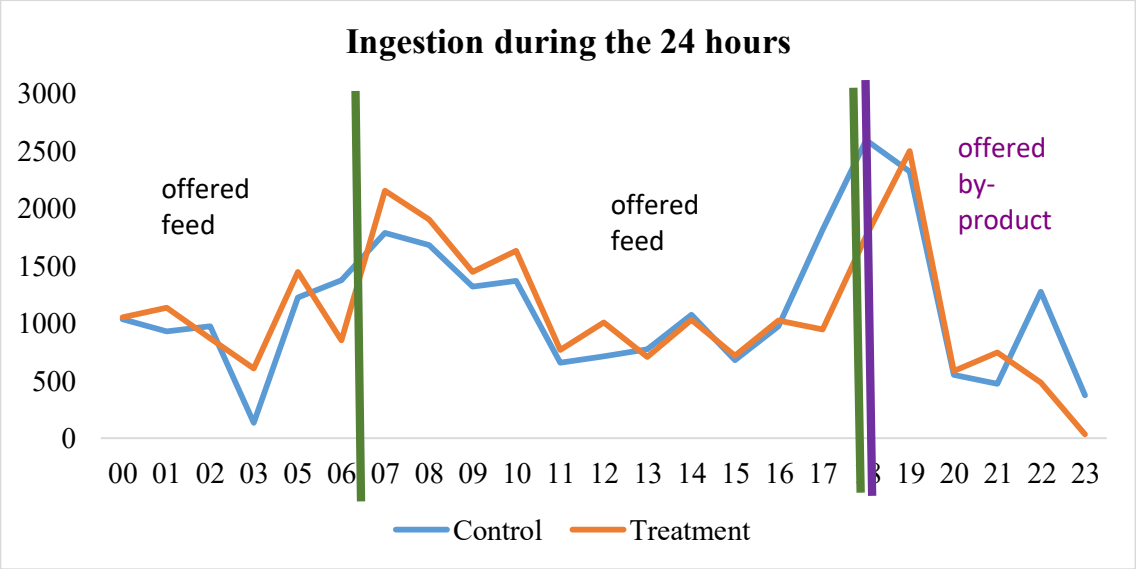

Figure 5: Evolution of group ingestion during the 24 hours of the 6th day in the second cycle.

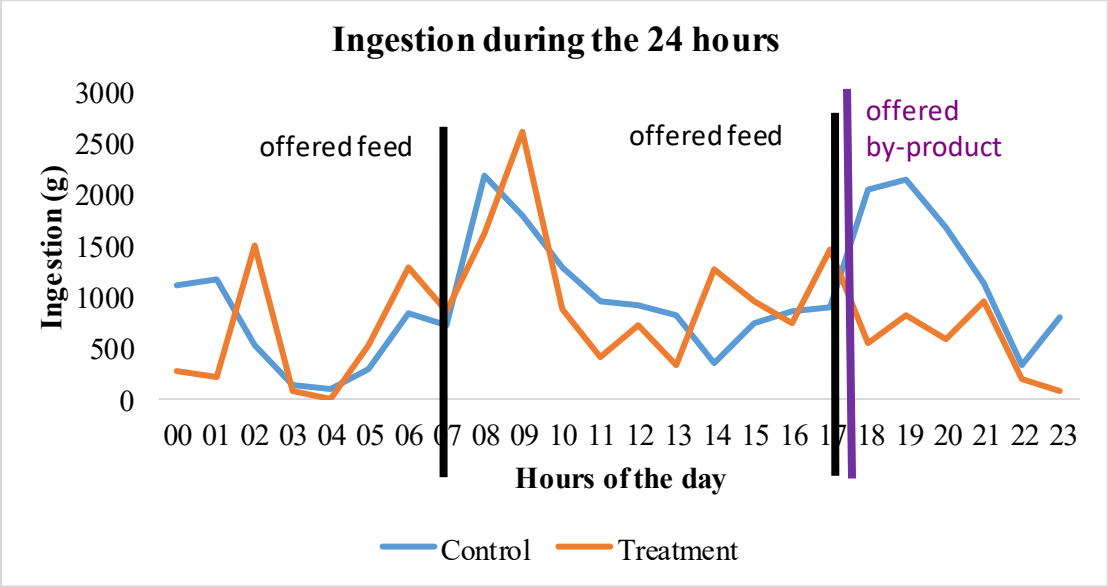

Figure 6: Evolution of group ingestion during the 24 hours of the 6th day in the third cycle.

3.4- Milk production and milk composition

The addition of 5 g/head of the cardoon polyphenolic extract (CPE) did not influenced the milk yield and normalized milk yield (Table 5). The CPE at the dose of 5 g/d did not affected milk composition but decreased the pH of the milk even if remain within the optimal range values of the milk (Table 5).

The addition of 10g/head of CPE during the second cycle (Table 6), did not affect milk yield. The lipid content increased significantly ($P<0.05$) in the TRT group, likely due to concentration effect related to the numerical reduction of milk production. The SCC was reduced by the dose of 10g/d of CPE in comparison to the CON group ($P=0.058$) (Table 6).

The dose of 20 g/d decreased significantly milk yield ($P<0.05$) compared to the CON group (-24%). The reduction in milk production was accompanied by an increase in lipid content (+12%; $P<0.01$) and a tendential increase in protein content (+3.6%; $P<0.10$). The lactose content ($P = 0.03$) and the urea content (-11%; $P=0.021$) decreased significantly compared to the CON ($P=0.03$) (Table 7). The decrease in urea was previously observed in other studies in which the ruminant diet was directly integrated with polyphenols or feeds containing polyphenols (Dschaak *et al.,* 2011; Santos *et al.,* 2014). Some studies have shown that also the administration of feeds rich in polyphenols decreases the NH3 production in the rumen and consequently nitrogen and methane excretions (Theodoridou *et al.,* 2010).

Table 5: Production and composition of the milk in the control and the treatment group after the administration of 5 g/head of polyphenolic extract during the first cycle.

	CON	TRT	SEM[1]	Group	Time	Group*Time
Milk, kg/d	1616.8	1617.9	121.9	0.995	0.643	0.742
Norm. milk., kg/d	957.37	890.70	71.20	0.510	0.081	0.767
Fat, %	6.33	5.99	0.21	0.285	0.337	0.830
Protein, %	5.15	5.18	0.09	0.783	0.116	0.700
Lactose, %	5.04	5.05	0.04	0.893	0.040	0.406
SCC, log10/ml	363.85	107.79	68.54	0.150	0.422	0.695
Casein, %	3.91	3.96	0.07	0.653	0.176	0.646
Urea, mg/dl	43.83	42.71	1.30	0.466	0.000	0.102
pH	6.68	6.63	0.01	0.021	0.336	0.919
NaCl, mg/100ml	96.78	96.63	4.00	0.981	0.493	0.721

[1]SEM=Standard error

Table 6: Production and composition of the milk in the control and the treatment group after the administration of 10 g/head of polyphenolic extract during the second cycle.

	CON	TRT	SEM[1]	Group	Time	Group*Time
Milk, kg/d	1532.5	1391.6	88.20	0.279	0.879	0.750
Norm. milk., kg/d	908.48	824.99	51.85	0.273	0.997	0.539
Fat, %	6.29	6.69	0.13	0.047	0.789	0.824
Protein, %	5.52	5.69	0.07	0.128	0.434	0.793
Lactose, %	4.95	4.94	0.04	0.831	0.184	0.688
SCC, log10/ml	554.48	131.14	141.46	0.058	0.823	0.836
Casein, %	4.23	4.40	0.06	0.067	0.517	0.793
Urea, mg/dl	43.19	42.73	1.23	0.754	<.0001	0.831
pH	6.62	6.57	0.02	0.059	0.557	0.983
NaCl, mg/100ml	107.0	99.2	4.58	0.247	0.673	0.877

[1]SEM=Standard error

Table 7: Production and composition of the milk in the control and the treatment group after the administration of 20 g/head of polyphenolic extract during the third cycle.

	CON	TRT	SEM[1]	Group	Time	Group*Time
Milk, kg/d	1285.9	981.2	81.24	0.013	0.316	0.852
Norm. milk., kg/d	786.27	608.90	52.02	0.024	0.466	0.975
Fat, %	6.59	7.39	0.14	0.001	0.573	0.925
Protein, %	5.62	5.82	0.08	0.092	0.538	0.594
Lactose, %	4.97	4.84	0.04	0.030	0.394	0.676
SCC, log10/ml	234.92	118.95	39.06	0.178	0.896	0.630
Casein, %	4.33	4.51	0.07	0.077	0.510	0.611
Urea, mg/dl	43.97	38.94	1.51	0.021	0.036	0.901
pH	6.63	6.62	0.02	0.655	0.625	0.848
NaCl, mg/100ml	98.12	97.25	4.20	0.886	0.438	0.979

[1]SEM=Standard error

The non-univocal results of polyphenols in the small ruminant diets on milk production (Dung, 2010; Griffiths *et al.,* 2013; Hymes-Fecht *et al.,* 2013), is probably due to the different amount of polyphenols used, the source of polyphenols, their chemical characteristics and the potential interactions with other ingredients of the ration. The depressive effect of polyphenols on the milk production could also be related to a reduction in total feed intake (Figure 7). The time affected significantly almost all variables. Milk

production in the two experimental groups during the three cycles, highlights the reduction in milk production in both groups similar to the natural lactation curve evolution of Sarda dairy sheep (Macciotta *et al.,* 1999). The evolution of the lipid and protein content showed an opposite trend due to the expected concentration effect following the reduction in the milk production (Figure 8-9). The casein content showed a trend similar to that of the protein, which can be explained by the fact that the casein fraction constitutes about 80% of the total milk protein content (Figure 10). The lactose content showed a progressive decrease with the progress of the lactation like that of the milk production (Figure 11). Interesting the results observed for the SCC during the three experimental cycles of the two groups, due to the stable evolution trend in the TRT group regardless the amount of the dose used, while in the CON group showed a higher variability among time (Figure 12).

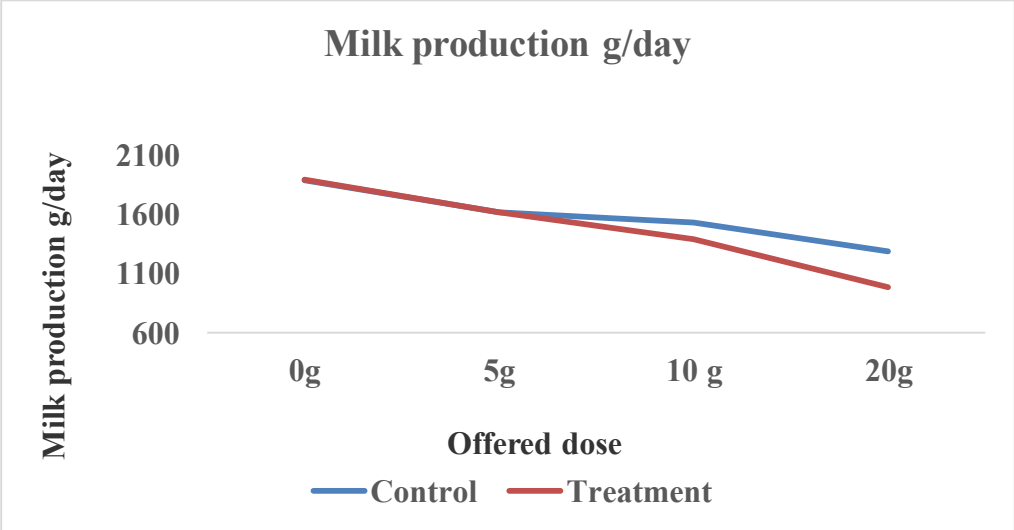

Figure 7: Temporal evolution of the milk production following the progressive increase in the administration dose of the polyphenolic extract (Pre-experimental phase: 0g/d; cycle 1: 5g/d; cycle 2: 10g/d; cycle 3: 20 g/d).

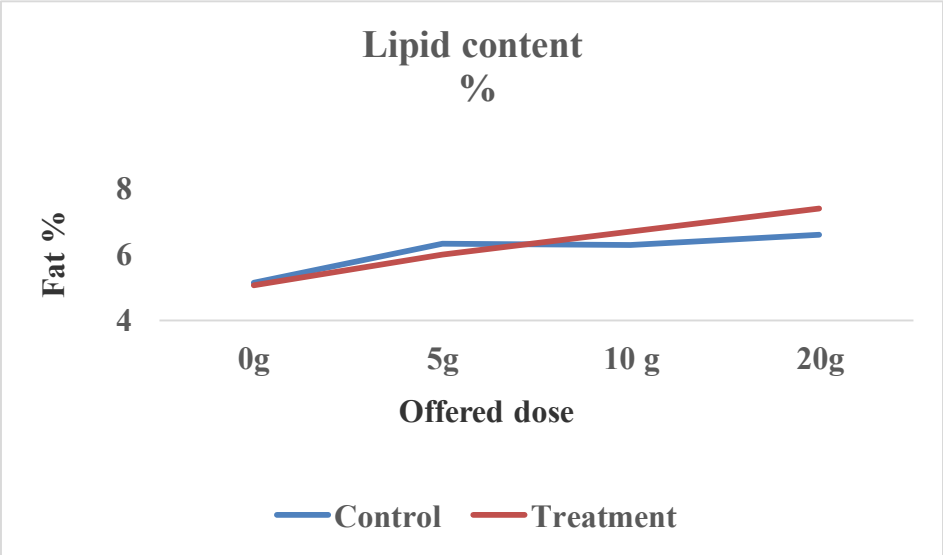

Figure 8: Temporal evolution of the lipid content following the progressive increase in the administration dose of the polyphenolic extract (Pre-experimental phase: 0g/d; cycle 1: 5g/d; cycle 2: 10g/d; cycle 3: 20 g/d).

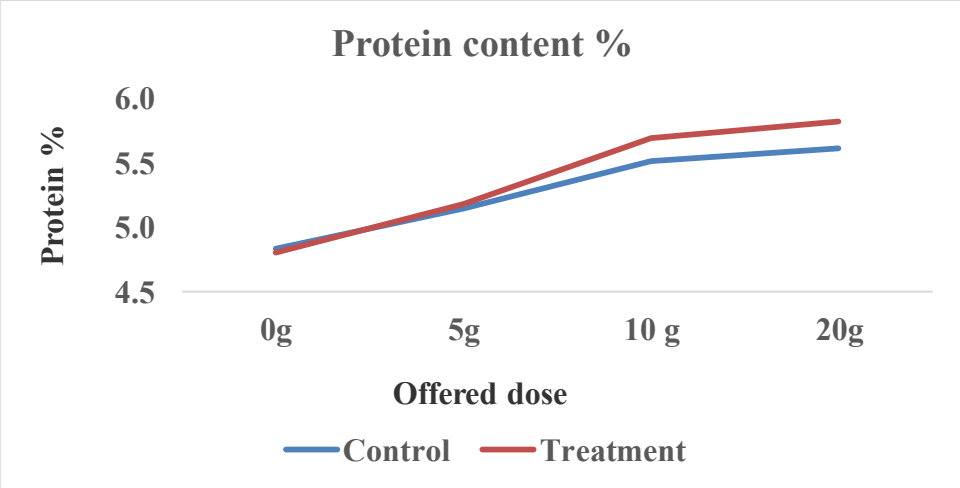

Figure 9: Temporal evolution of the protein content following the progressive increase in the administration dose of the polyphenolic extract (Pre-experimental phase: 0g/d; cycle 1: 5g/d; cycle 2: 10g/d; cycle 3: 20 g/d).

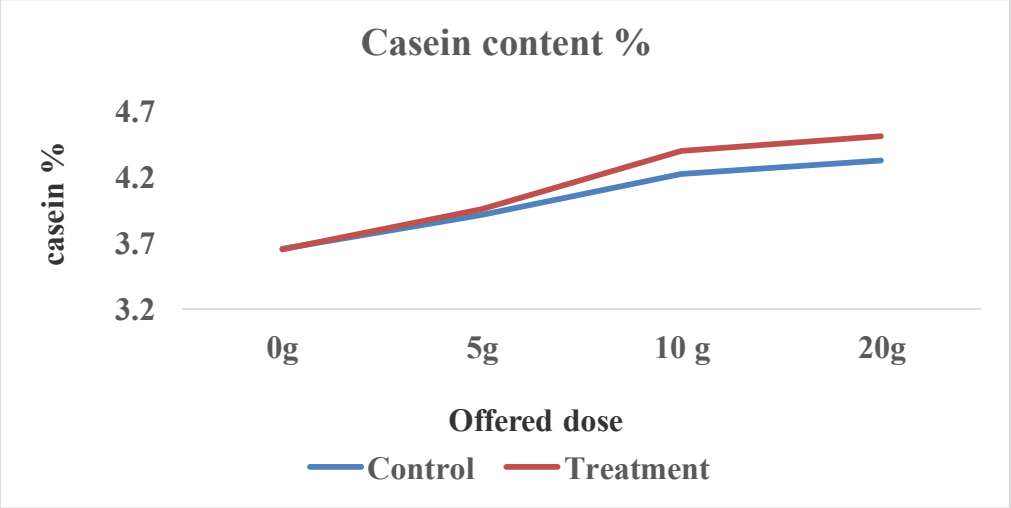

Figure 10: Temporal evolution of the casein content following the progressive increase in the administration dose of the polyphenolic extract (Pre-experimental phase: 0g/d; cycle 1: 5g/d; cycle 2: 10g/d; cycle 3: 20 g/d).

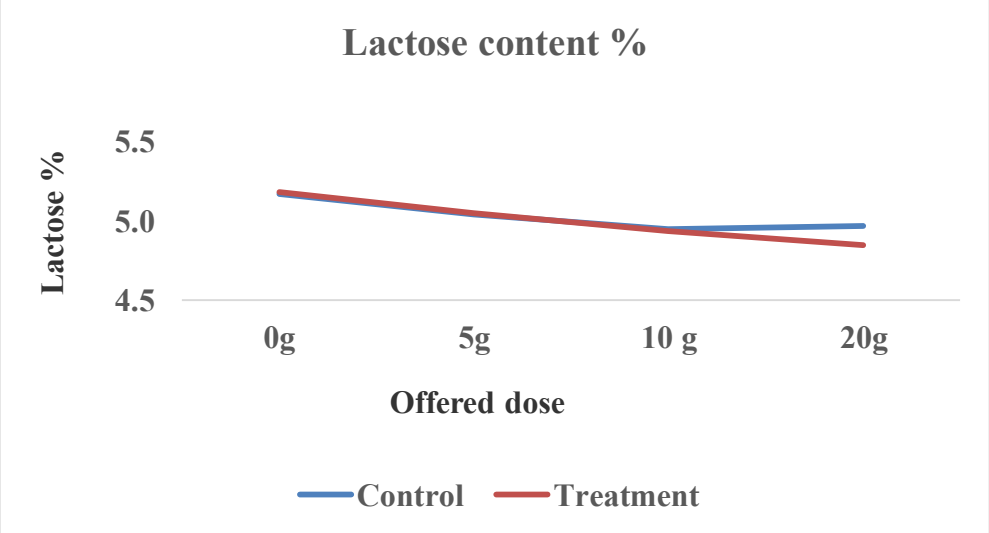

Figure 11: Temporal evolution of the lactose content following the progressive increase in the administration dose of the polyphenolic extract (Pre-experimental phase: 0g/d; cycle 1: 5g/d; cycle 2: 10g/d; cycle 3: 20 g/d).

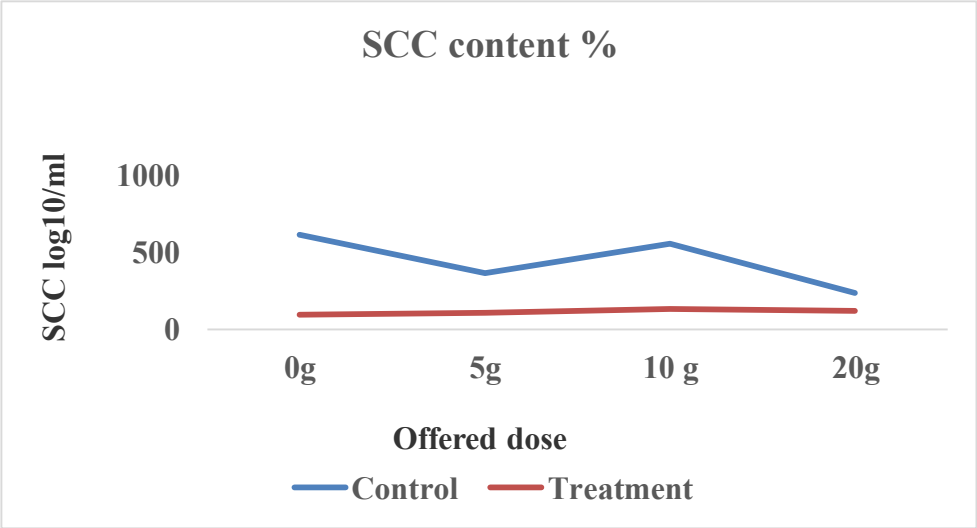

Figure 12: Temporal evolution of the somatic cells count content following the progressive increase in the administration dose of the polyphenolic extract (Pre-experimental phase: 0g/d; cycle 1: 5g/d; cycle 2: 10g/d; cycle 3: 20 g/d).

The observed positive effect of polyphenols on the SCC has been previously reported in experiments in which byproducts containing polyphenols have been tested in the same breed (Nudda *et al.,* 2019; Carta *et al*., 2020). Other study on Sarda sheep where the diet consisted of grazing on pasture rich in legumes (76% *Medicago polymorpha L.*, 20% *Lolium multiflorum L.*) in addition to chestnut tannin extracts, evidenced a significant reduction in the SCC in comparison to the control group (Pulina and Nudda, 2010). Castanares *et al.,* (2011), have found and confirmed these results in an experiment on Sarda sheep where 40g/day of chestnut and quebracho tannins were added. Also, the positive effect of tannins on the SCC was found on goats, where the diet consisted of grazing on *Lespedeza cuneata* forage known by its richness in tannins (Min *et al.,* 2005). Tannin effect could be due to the transfer of secondary metabolites characterized by a bacterial action to the mammary gland. In fact, the proliferation of mammary gland pathogens such as *E. coli, Klebsiella pneumonia* and *S. aureus*, was inhibited by different tannin extracts (Min *et al.,* 2008). Similar results were observed after the addition of pomegranate extract to dairy cow's diet (Shabtay *et al.,* 2012) and to calves before weaning (Oliveira *et al.,* 2010), which has consequently increased the lymphocyte function and reduced the SCC of milk.

3.5- Effect of cardoon polyphenol on milk fatty acid profile

Milk fatty acid profile of CON and TRT groups is shown in table 8. The TRT group highlighted an increase in MUFA with the increase of the CPE dose. The SFA and PUFAs showed a discontinuous variation as function of the dose probably linked to a gradual adaptation of the ruminal microflora to the polyphenolic extracts. Particularly interesting the variation of the vaccenic acid (VA) and CLA which are both intermediates from the linoleic acid in the rumen bio-hydrogenation process. The low concentration of both fatty acids shows a fast bio-hydrogenation activity into stearic acid (C18:0) with a consequent limited escape of CLA and VA acid from rumen.

Table 8: Concentration (g/100g of fat) of the fatty acids groups and of the individual fatty acids VA, CLA and LA during the 3 cycles.

Group	**CON**			**TRT**		
Dose	**Cycle1**	**Cycle2**	**Cycle3**	**cycle1**	**Cycle2**	**Cycle3**
SFA	71.0	65.5	68.2	64.8	66.2	70.2
UFA	21.9	18.8	20.6	19.5	19.8	21.5
MUFA	17.5	15.2	16.5	15.8	16.2	17.5
PUFA	4.37	3.69	4.22	3.80	3.68	4.01
VA	0.89	0.81	1.10	0.84	0.58	0.44
C18:3 n3	0.58	0.47	0.44	0.60	0.41	0.42
CLA c9t11	0.57	0.52	0.57	0.52	0.41	0.28

[1]SFA, UFA, MUFA, PUFA= sum of the individual saturated fatty acids; sum of the individual unsaturated fatty acids; sum of the individual monounsaturated fatty acids; and sum of the individual polyunsaturated fatty acids respectively; VA, CLA= vaccenic acid; CLA= sum of conjugated linoleic individual acids.

3.6- Milk coagulation parameters

The milk coagulation parameters (MCP) measured in the second and third cycles are reported in table 9. The dose of 10 g/head did not affect the MCP whereas the dose 20 g/head reduced significantly the RCT (P=0.05), and have increased the curd firmness A30 (P<0.01) mainly due to the increase in protein and fat content of milk

Table 9: Milk coagulation properties in the control and treated groups.

Dose		CON	TRT	SEM[1]	*P*
10 g	RCT (min)	17.20	16.42	49.70	0.648
	A30	54.10	52.92	1.32	0.669
	K20 (min)	1.93	2.08	10.60	0.683
	Amax	57.13	59.39	1.32	0.408
20 g	RCT (min)	18.58	14.13	69.41	0.051
	A30	41.75	57.09	3.08	0.009
	K20 (min)	1.97	1.62	7.88	0.198
	Amax	45.28	63.31	3.43	0.005

[1]SEM=Standard error

4- Conclusion

In conclusion, the results of our study have showed that the supplementation of cardoon polyphenolic extracts did not have any negative effects on the BW and BCS of the animals. However, the highest dose of polyphenols from cardoon affected negatively the feed intake and milk yield. The poor palatability of the ration in the hours following the administration could be the more probable explanation. The increase of fat, proteins and caseins concentration and the positive effect on SCC affected positively the milk coagulation properties. The supplementation of polyphenolic extracts resulted also in an acceleration in the bio-hydrogenation processes of the linoleic acid decreasing the escape of unsaturated intermediates such as CLA and VA into the mammary gland. These results suggest that cardoon polyphenolic extracts can be introduced in the diet of sheep in doses not exceeding 5 g/head, to avoid depressive effect on the animal production. However, further tests are needed to confirm the effect of the bioactive components of the cardoon polyphenolic extracts with longer administration times and intermediate doses. Finally, the effect observed on the milk SCC suggests to investigate on the bactericidal effect of the cardoon polyphenolic extracts on micro-organisms pathogens for the mammary gland.

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Chapter 5

Laboratory analysis

1- Feed chemical composition analysis

The content of dry matter was determined by placing the grinded feed samples at 105 ºC for 24 hours until reaching a constant weight. NDF content was determined according to Mertens (2002), using a heat stable enzyme called amylase and then expressing its residual ash. ADF were analyzed according to AOAC method 973.18 ("AOAC Official Method of Analysis", 1990, method 973.18), while ADL was analyzed based on the Robertson and Van Soest (1981) method and by using sulfuric acid. The crude protein (CP) was determined using the Kjeldahl method (AOAC, 2000; method 988.05). The ash was determined using a muffle at 550 ºC (AOAC, 2000; method 942.05).

2- Milk analysis

2.1- Milk fat extraction and protein content

Milk fat content was determined using the Gerber method (ISO, 1975) and the total protein (N × 6.38) by the Kjeldahl method. The milk fat extraction was made based on the Rose-Gottlieb method (AOAC, 1990) with the modifications detailed by Nudda *et al.,* (2013). Briefly, 0.4 ml of ammonia (25%), 2 ml of ethyl alcohol (95%), and 4 ml of hexane were added to 2 g of milk sample. Then the prepared samples were centrifuged for 15 minutes at 3-4ºC at a velocity of 3000 rpm. After the first centrifugation, the upper layer obtained was collected. A second extraction was made using 2 ml of ethyl alcohol (95%) and 5 ml of hexane only. Samples were centrifuged for a second time for 15 minutes, 3-4 ºC and at 3000 rpm. The upper layer was collected again. The extraction was repeated for the third time using only 5 ml of hexane and then the samples were centrifuged at 3000 rpm at 3-4 ºC for 15 minutes and the upper layer was collected. After the three extractions, the hexanic phase which contains the lipid was evaporated in a rotary evaporator using vacuum.

2.2- Milk fat rapid lipid separation

Milk separation was done based to the described method by Fang *et al.,* 2014, with some modified steps. The samples of milk were centrifuged for 15 minutes at 12,000 rpm at a temperature of 7 ºC, then the upper aliquot of fat in the tube (11-14 mg) was transferred into vials. The fat methylation was done using 500 μl of sodium methoxide (0.5 N), and then the obtained solution was stirred for 2 minutes. After stirring, 1000 μl of C5:0 and C13:0 internal standard were added to the vials. The vials were stirred again for 1 minute. The hexane phase was recovered and transferred into tubes of 1.5 ml capacity, and then were centrifuged for 5 minutes at 14000 rpm at a temperature of 8 ºC, to eliminate any residual sodium methoxide

collected during the recovery phase. The hexane phase was recovered again and transferred again to a new vial to be used in the gas chromatograph.

2.3- Milk fatty acid profile (FAME)

A base-catalyzed transesterification was used in order to determine the fatty acid methyl esters (FAME) using the standard procedure of FIL-IDF (1999), with the details cited by Nudda et al. (2013). Fatty acids were determined using a 7890A Gas Chromatography System (Agilent Technologies, Santa Clara, CA, USA), equipped with 7693 auto sampler (Agilent Technologies Santa Clara, CA, USA) and a flame ionization detector (FID). For the FAME separation, a CP-SIL 88 capillary column (100m×0.250 μm i.d., 0.25 μm film thickness, Agilent Technologies, Santa Clara, CA, USA) was used. The carrier gas used was Helium (1 ml/min flow rate) and its pressure was equal to 28 psi and 1 ml is injected per sample. The starting temperature of the gas chromatography was 45 ºC. This temperature was maintained for 4 minutes and was then increased each minute 13 ºC, until reaching 175 ºC. The temperature 175 ºC remained for 26 minutes and then was increased by 4 ºC each minute until reaching a temperature of 215 ºC. This temperature was maintained equal for 35 minutes. 250 ºC was the temperature fixed for the injector and the detector. Split ratio was 1:80. OpenLAB CDS GC ChemStation Upgrade software data system (Revision C.01.04, Agilent Technologies Inc., Santa Clara, CA, USA) was used to calculate the retention time and the area of each individual FA. The FA peaks were identified through the comparison of their retention time with others already published as mentioned by Nudda *et al.* (2005). The individual FA were expressed as g/100g of total FAME and the groups of FA were calculated as follow: short chain fatty acid (SCFA) were calculated using the sum of single fatty acids ranging from C4:0 to C10:0; medium chain fatty acids (MCFA) by calculating the sum of single fatty acid from C11:0 till C17:0; whereas the long chain fatty acids (LCFA) were calculated by adding the single fatty acids from C18:0 to C22:6 (DHA). The saturated fatty acid (SFA) represented the sum of the individual saturated FA; the unsaturated fatty acid (UFA) represented the addition of the individual unsaturated FA; the monounsaturated fatty acids (MUFA) were calculated using the sum of the individual monounsaturated FA; the polyunsaturated fatty acids (PUFA) represented the addition of the individual polyunsaturated fatty acids. On the other hand, the trans fatty acids (TFA) were calculated through the addition of the individual trans FA, while the branched-chain fatty acids (BCFA), odd-and branched-chain fatty acids (OBCFA), PUFA n-3, PUFA n-6 and total conjugated linoleic acids (CLA) were calculated using the sum of individual branched-FA, the sum of individual

odd-and branched-chain FA, the addition of individual n-3 FA, the addition of individual n-6 FA, and by the sum of individual conjugated linoleic acids respectively.

2.4- Nutritional indexes calculation

The fatty acids nutritional properties were calculated as the ratio between n-6 and n-3 and three indices AI, TI and h/H. The atherogenic index (AI) was calculated using the formula: AI= [12:0 + (4×14:0) +16:0] / [(PUFA) + (MUFA)]; while the trombogenic index (TI) was estimated using the formula TI= (14:0 + 16:0) / [(0.5 × MUFA) + (0.5 × n-6) + (3 × n-3) + (n-3: n-6)]; and the hypocholesterolemic to hypercholesterolemic ratio (h/H) was computed using the formula [(sum of 18:1cis-9, 18:1cis-11, 18:2 n-6, 18:3 n-6, 18:3 n-3, 20:3 n-6, 20:4 n-6, 20:5 n-3, 22:4 n-6, 22:5 n-3 and 22:6 n-3) / (14:0 + 16:0)]. On the other hand, in order to calculate the capacity of the diet/species to desaturase the saturated fatty acids into Δ9-UFA, the C10, C14, C16 and CLA index were calculated using the different formulas: [C10:1/ (C10:0 + C10:1)] ×100; [C14:1 cis-9/ (C14:0 + C14:1 cis-9)] ×100; [C16:1 cis-9/ (C16:0 + C16:1 cis-9)] ×100; [C18:1 cis-9/ (C18:0 + C18:1 cis-9)] ×100; and [CLA cis-9, trans-11/ (C18:1 trans-11 + CLA cis-9, trans-11)] ×100 respectively (Carta *et al.,* 2020).

2.5- Milk coagulation properties

The milk coagulation properties were determined using a formagraph (FossElectric A/S, Hillerod, Denmark) according to the method proposed by Zannoni and Annibaldi (1981). Briefly, 10 ml of individual milk were heated until reaching a temperature of 35 ºC and then 200 µL of rennet was added (Hansen Naturen 215, Pacovis Amrein AG, Bern, Switzerland; with 80±5% chymosin and 20±5% pepsin) diluted in distilled water (0.8:100 vol/vol) obtaining a final concentration of 0.034 IMCU/ml. After adding the rennet, the changes in the consistency of the milk as the coagulation proceeds was detected by oscillating pendulums. After 30 minutes, two types of outputs were generated from the program: the first is the graphic one that represents the typical coagulation bell, and the second is the tabular form where the values corresponding to each sample for the 3 lacto-dynamo-graphic parameters are shown:

- RCT= rennet clotting time or coagulation time (expressed in minutes) is the time that elapses between the addition of the rennet (t0) and the time where the milk viscosity starts to change into aggregation micelles and therefore the start of the coagulation process.

- K20: clot formation time (minutes) represent the time required for the clot to reach a mechanical strength such as to determine a path width of 20 mm
- A30: consistency of the clot measured at 30 minutes (mm), corresponds to the width of the path reached 30 minutes after adding the rennet.

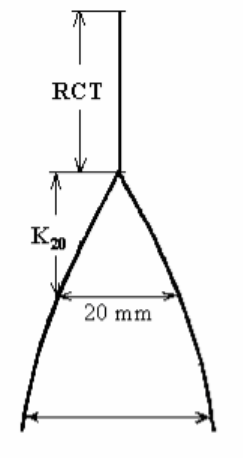

Figure 1: Milk coagulation bell and the lacto-dynamo-graphic parameters (RCT, K20 and A30)

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General conclusion

The prevalence of the extensive production systems for small ruminants in the Mediterranean region has been expressed in Lebanon by the dominant widespread of the semi nomadic and transhumant systems relying on grazing pasture, rangelands and crop residues. In these systems, Awassi sheep and the rustic Baladi goat are the dominant breeds, due to their high adaptation capacities to the environmental conditions. The size and composition of the flock, the reproductive performance of animals, in addition to the managerial aspect of the farms including the type of feeds, livestock diseases, income and constraints varied with the production system and the farm altitude. These differences are mainly due to the availability and the type of rangelands (pasture, crop residues, shrubs and lignified plants), and to the economic status of the farmer. Nevertheless, these systems need to be improved, in particular the low production and the scarcity of feeds are the most important constraints facing this sector.

The milk fatty acid composition of the Awassi sheep and Baladi goats, evidenced differences between the two species even if reared in the same environment and feeding conditions. The fatty acid profile varies also depending on the production system of the farm and on the altitude: this variation is mainly related to the feed type and feed availability.

The production of small ruminants in Lebanon can be significantly improved through a better feeding, and proper management and breeding programs, by monitoring infectious diseases, and by creating cooperative structures for farmers, which can work togheter to the improvement of the production and the quality.

On the other hand, the increasing demand for animal feed in the world, and the competitiveness between cropped land for human food and animal feed, suggest the use of agro industrial byproducts rich in bioactive compounds in the animal nutrition. Cardoon is a plant present in Lebanon and their by-product can be included in the ruminant diets. The experimental trial carried out in Sardinia, evidenced that the supplementation of cardoon byproducts (cardoon flour and polyphenolic extracts) in dairy sheep diets, did not exert negative effects on the production traits and livestock health if used in the tested doses. However, at high levels, cardoon byproducts supplementation in dairy sheep feed could exert some detrimental effects, due to the high variation of bioactive compounds composition (polyphenol in the case of cardoon byproducts). However, further studies are needed to

clarify the effect of cardoon byproducts supplementation for a longer period in ruminant diets.

The supplementation of byproducts into animal diet is gaining much interest nowadays, and is considered one of the important pilars for meeting the European Green Deal. Moreover, it might be also, an effective solution for the improvement of the Lebanese small ruminant sector, by providing the possibility to reduce the feed costs and the production costs, to decrease the environmental impact more specifically the overgrazing of the Lebanese rangelands and to improve the milk yield and quality.