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Breeding strategy and animal welfare in Sarda dairy sheep

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Alla mia famiglia...

"*….este Onanie una idda vamosa*
pro sas usanzas de s'antichitate
ospitale distinta in s'onestate
lu pote narrer viera e orgogliosa
ca bi regnat sa pache graziosa
in allegria e chin felicitate
su bonu coro de s'umanitate
de cussa zente es prodigiosa…"

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CHAPTER 1

Welfare in animals farm

1.1. What is animal welfare

Nowadays, the interest of society in the welfare of animals is similar to that in the quality of animal products., In fact, a increasing number of consumers are willing to pay higher prices for these products, if they know that they come from farms that guarantee a high level of animal welfare (Martelli, 2009). One of the reasons for this increasing interest on animal welfare was the huge change which occurred in animal farms since the middle of the last century. In particular, there was a wide spread of the intensive production systems, which have been subjected to intense criticism and have received great attention from the European Community laws.

Lately, numerous criticisms have also regarded the adoption of genetic engineering or clonation, even if these techniques are aimed to improve the quality of the productions and the welfare of animals, making them more resistant to illness (Pascalev, 2006). Indeed, these techniques are considered by a lot of people as a tool to satisfy the human interest (profit increase) more than that of the animals.

However, the public opinion about the animal welfare concept seems to be highly influenced by the country and by the direct knowledge that a person has of the surrounding breeding systems.

It has been observed that the most of people indentify the animal welfare problem with the space that the animal has in the farm (Martelli, 2009). For this reason, consumers think that the welfare of laying hens, broiler chickens and pigs, which are the most important zootechnical species for intensive breeding, needs more attention than that of sheep which are thought to be less exigent.

Unfortunately, it is very difficult for the consumer to recognize the products obtained from the farms where animal welfare is higher than those normally practiced or required by law, for which consumers accept to pay higher prices (Martelli, 2009).

At the beginning, the animal welfare concept had limitations in the scientific community as well, because researchers did not to take into account aspects which were difficult to study scientifically, such as feelings, emotions and animals conscience and concentrated on aspects which directly caused health or biological problems. The main reason for that was the fact that health indicators for welfare assessment have an immediate link with the level of animal suffering (Rushen, 2003).

Currently, it is well-known that the study of animal welfare requires the collaboration among different scientific and social disciplines which are not always easy to integrate, with consequent difficulties in interpreting the animal welfare measures collected. At the same time, it is this multidisciplinary approach that makes the animal welfare research unique, fascinating and full of contradictions among researchers, who should collaborate to properly evaluate in the welfare state of animals.

1.2. Definition and approach to study of Animal Welfare

The lack of agreement about a universal definition of animal welfare has been an unsolved scientific problem for a long time.

The term "animal welfare" is being increasingly used by farmers, corporations, consumers, veterinarians, politicians, and other agents (Hewson, 2003). However, it is often misused, as it takes different meanings according to personal interpretation.

In the past, animal welfare was associated with body state (e.g. good health and high production), rearing environment (e.g. adequate facilities and proper nutrition) and physiological parameters (e.g. plasma cortisol and heart frequency). The use of few indicators caused limitations in the assessment of welfare level, because their interpretation can be difficult. Indeed, a specific parameter of animal welfare can increase or decrease in a positive and in a negative situation as well. In other cases, different parameters that have been collected simultaneously can give contradictory information: for example, an animal can show an optimum physical condition but have a compromised mental health (Hewson, 2003)

Over time, several definitions of welfare have been proposed, which are a consequence of the approach adopted in the evaluation of animal welfare.

According to Carenzi and Verga (2009), the definition of animal welfare provided by Broom in 1986, "*the welfare of an animal is its state as regards its attempts to cope with its environment*", is based on an approach that emphasizes the biological functioning of organism, in which a hierarchical order of biological needs is established.

Other two definitions of animal welfare are those of Branbell (1965), "*welfare is a wide term that embraces both the physical and mental well-being of the animal"*, and that of Hughes (1976), "*welfare is a state of complete mental and physical health, where the animal is in harmony with its environment*". These two definitions are based on a second approach, which considers both physical and psychological conditions of the animal, such as feelings. One of the main limitations of this approach is the inability to directly measure animal feelings, since these aspects are related to subjective experiences.

A third approach is based on the concept that it is important to consider the welfare of the animal in relation to its status in natural conditions, observing the adaptability capacities and behavior expressed in nature. Even if this approach is fascinating, it is necessary to note that domestic animals differ significantly from their fellows living in nature. To properly investigate the study of animal welfare, it is now acknowledged that it is necessary to connect the emotional and biological responses of animals and to take into account the natural behavior of each species (Figure 1.1).

In general, scientists and philosophers have understood that a good understanding of animals feeling is required to deal better with welfare problems, (Rushen, 2003; Duncan, 2006; Lund et al., 2006). However, there are still strong contrasts between these two groups, who have not found a common definition of welfare and still use different concepts and terms which belong to two "distinct" cultures.

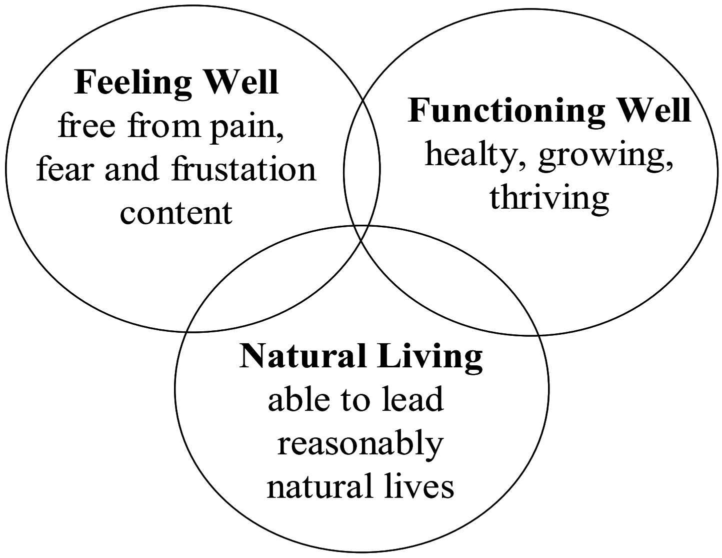

Figure 1.1 Three overlapping viewpoints for the definition of animal welfare (adapted from Widowski, 2009).

In reality, researchers who study animal welfare should not be influenced by personal feelings and moral and ethical beliefs, as this goes beyond a scientific approach of objective assessment and can thus lead to gross mistakes in evaluating animal welfare.

1.3. Animal welfare in livestock

Animal welfare as a 'formal discipline' began less than 50 years ago with the publication of the *Brambell Report* on farm animals welfare (Brambell, 1965). This report was commissioned by the British government due to the high level of public interest in the book "*Animal Machine*" by Ruth Harrison (1964), who raised the issue of the animal welfare in intensive farms, at that time of their rapid diffusion.

In 1979, the "*British Farm Animal Welfare Council*", taking into consideration the "*Brambell Report*", formulated the principle of the "*Five Freedoms*" that should be assured to the animals (FAWC, 1979):

- *Freedom from thirst, hunger and malnutrition* – by ready access to fresh and clean water and adequate diet to maintain full health and vigor;
- *Freedom from discomfort* – by providing a suitable environment, including shelter and a comfortable resting area;
- *Freedom from pain, injury and disease* – by prevention or rapid diagnosis and treatment;
- *Freedom to express normal behavior* – by providing sufficient space, proper facilities and company of other animals of the same species;
- *Freedom from fear and distress* – by ensuring conditions which avoid mental suffering.

The application of the five freedoms concept is not useful to assess whether an animal is healthy or not, but it allows to monitor the rearing conditions and to compare animal welfare among different farming conditions. For example, Webster (1999) applied the “Five freedoms” approach to identify the critical points in two different rearing systems (intensive vs. extensive) of laying hens, as presented in Table 1.1. It was showed that in intensive livestock conditions the lack of physical comfort and the occurrence of limb disorders can lead to frustration; moreover, in extensive livestock production the thermal conditions, thermal discomfort, presence of parasites and fear of living in overcrowded conditions, even if available space is adequate.

Table 1.1. Comparative analysis of the health of laying hens reared intensively or outdoors applying the concept of five freedom (adapted from Webster, 1999)

Five Freedoms	**System**	
	Intensive	**Extensive**
Thirst and Hunger	normal	normal
Discomfort:		
- *thermal comfort*	good	variable
- *physical comfort*	low	good
Ill health:		
- *disease*	low	parasitism?
- *pain*	arts	wounds
Behavior	very limited	cannibalism?
Fear and Distress	frustration	agoraphobia

Some of the set forth freedoms, such as that from hunger and thirst, are universally recognized and normally applied, whereas those concerning the behaviour and freedom from fear are aspects that are not always immediately understood (Webster, 1999).

This approach to assess welfare is not aimed to eliminate the sources of stress but to prevent suffering, which may occur because the animal fails or is unable to overcome stressful situations. In fact, stress may be too intense, complex or prolonged, or take place when the animal is prevented from taking the necessary actions that allow it to relieve stress (Webster, 2001).

The *Five Freedoms* principles do not represent a perfect model, but rather an attempt to get better results in a complex and difficult situation. Their complete observance is impossible, as to a certain extent they are mutually incompatible. In fact, total freedom of action would be improper for any animal, including humans (Webster, 1999).

Because the *Five Freedoms* are mainly based on ethics rather than on a solid scientific basis, according to Korte et al. (2007) it is necessary to introduce a new concept of animal welfare based on allostasis, i.e. on the animal capability to find stability or a new balance with a change. Indeed, this ability is crucial to a good state of health and welfare. The feeling of fear is an important stimulus in the evolutionary inheritance as it induces an organism to avoid threats. Similarly, the pain feeling has a clear importance as a means of defense and protection from potential threats or dangerous substances.

The animal organism has to set up several mechanisms to adapt to a wide range of environmental changes, which lead to various responses and attempts to achieve a good level of welfare.

Recently, welfare assessment studies take into consideration a series of reactions that are usually called "indicators" of adaptation (Table 1.2). The approach of using these indicators simultaneously gives a complete overview of the state of adaptation and welfare of the animals and can identify the problems that might have an impact on the performance of animals (production and reproduction). Therefore, the detection of a series of physiological variables and abnormal behaviors can allow an evaluation of the adaptive response (Canali, 2008).

Table 1.2. Indicators used to assess animal welfare (adapted from Canali, 2008).

Type of indicator	Examples of indicators
Behavioral	Ethogram, abnormal behavior, behavioral test
Pathological	Injury, illness
Physiological	Hormone levels, heart rate
Production	Growth, fertility

Usually any uncomfortable condition caused by multiple factors, with consequent physiological and behavioral changes in animals, as in humans, is called *"Stress"*.

This term was used for the first time by H. Selye (1936 cited in www.equilibridinamici.it), who studied the physiological responses in animals subjected to administration of harmful substances and noticed that animals subjected to stress conditions were more vulnerable to sickness. The author defined stress as *"the nonspecific response of the body to any request made to it"*, and identified for the first time two different types of stress:

- **distress,** or negative stress, when stressors cause a progressive weakness until the break of the physical and mental defenses. This implies that the activation conditions of the organism persist even in the absence of stressful events, or that the body overreacts to mild stimuli;
- **eustress,** or positive stress, when one or more stimuli, also of different nature, train the psychophysical capacity of adaptation of the individual. The eustress is a form of energy used to easily achieve a goal and each individual needs these environmental stimuli that lead to adaptation. Such

a distinction has extended the concept of stress meant as a process towards a better adaptation to the environment.

In animals, the first response to stress is an alarm reaction resulting in the stimulation of the autonomous nervous system. At this stage, it has the function to mobilize physical resources essential to the survival, through the activation of the neuroendocrine system that controls reactions to stress (e.g. adrenaline production). The second response to stress is the resistance reaction that the animal must activate to adapt to the stress and restore the physical and mental equilibrium, in case of persistent stress. Unfortunately, the organism can not maintain this state of resistance for an unlimited time, because its resources end soon or later, leading to the exhaustion phase, also called General Adaptation Syndrome. At this stage, the body is no longer able to maintain a normal functioning, since the defenses drop, and the animal becomes fatigued, weak and vulnerable to diseases (Biancifiori, 2010).

Because stress does not necessarily imply a reduction of welfare, it is possible to state that animal welfare is affected by unfavorable factors which act on the animal, but the negative consequences that may arise are closely related to its ability to adapt (Bertoni and Calamari, 2005).

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CHAPTER 2

Animal welfare assessment

2.1. How to assess animal welfare

Animals in natural or farming conditions are not in a state of complete well-being because they are normally exposed to several potential stress agents (Figure 2.1; Bertoni and Calamari, 2005), which can adversely affect their production performance, health and behavior. At the same time, if animals lived without stress, they would live in the absence of stimuli as well (Bertoni, 2001). Therefore, assessing the welfare status of animals consists of identifying and quantifying the effects of stress on them, by using indicators (or standard indexes) of the animal responses used to adapt them to the surrounding environment. In this way, it is possible to evaluate the causes of welfare reduction objectively, independently of moral considerations or biases, and to manage them properly (Bertoni, 2001; Tripaldi and Allegrini, 2000).

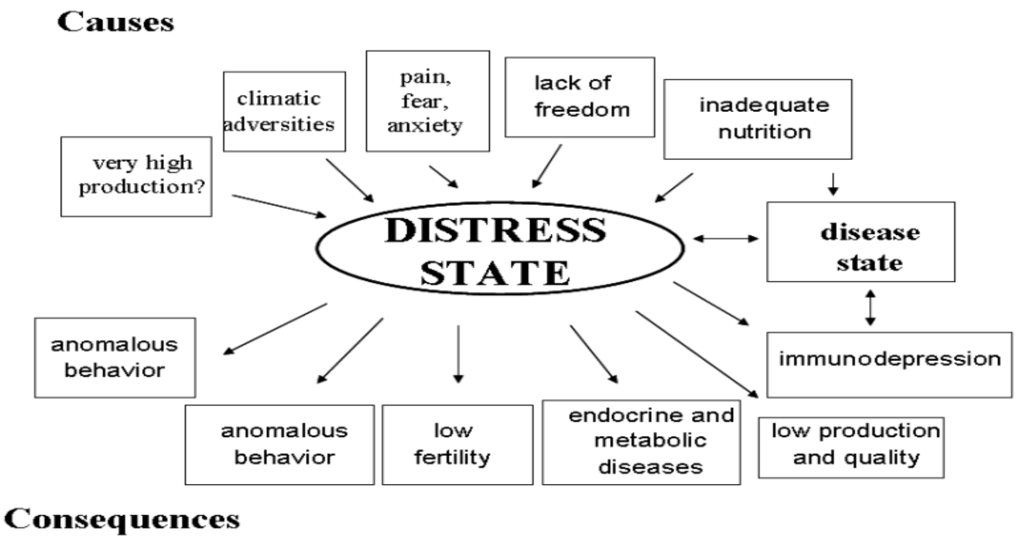

Figure 2.1. Potential causes of reduction of welfare and consequences of a lack of adaptation to stress (adapted from Bertoni and Calamari, 2005).

It is very important to take into account more than one evaluation parameter for monitoring welfare, because the animal can cope with a situation of discomfort in several ways. In particular, an animal reacts to stress through behavioral changes, first and early signs of adaptation, and physiological mechanisms (Canali, 2008). The evaluation of positive or negative feelings of the animal is one of the indicators that should be considered, even if it is difficult to assess this subjective condition. Fortunately, a good interpretation of animal feelings is now possible thank to the use of the preference tests, despite all criticism they are subjected to, and to a better understanding of the communication systems used by various species, such as vocalizations (Duncan, 2005).

When assessing welfare, the indicators used and the importance attributed to each of them as well as the way in which animal responses are evaluated are of great importance, but are difficult to put into practice. Nevertheless, a common criteria to perform this task should not be considered unrealistic (Bertoni and Calamari, 2005).

Recently, several systems for monitoring welfare in animals farms have been developed in Europe, in order to enable the breeder to identify the critical points. Even if the purposes of these systems vary, they are all based on a series of evaluation parameters that can be classified into the following two categories (Canali, 2008):

- direct parameters: based on behavioral, physiological, health and productive indicators. They have the advantage of measuring directly the animal status, but have the disadvantage of usually requiring a long detection time and being difficult to record; and

- indirect parameters: enable an assessment of the breeding environment, thus identifying critical points and risk factors. They have the advantage of being simple to detect, but have the disadvantage of not being able to sufficiently define the welfare state of animals when used alone.

The European Community promotes specific programs to develop a comprehensive system of on-farm welfare assessment. One of these projects, called Welfare Quality (www.welfarequality.net), involves 40 research centers and is aimed to evaluate the relationship between animal welfare on farm and quality of food of animal origin, through a standardization of welfare indices. However, these programs are addressed only to certain livestock species, such as cattle (dairy and beef), pigs, poultry (laying hens and broilers), and do not include other widespread species, such as sheep.

2.2. Measures to assess animal welfare

Before analyzing the main parameters used in the assessment of welfare, it is important to note that the mechanisms of adaptation to deal with various stresses differ considerably among animals, likely because of the differences among species and among individuals of the same species (Broom and Johnson, 1993).

The activation of mechanisms of adaptation of an animal to a stressful agent varies with its duration and with its possible consequences. For example, in conditions of acute stress (negative action for a short period), a rapid recovery of the original equilibrium state usually occurs, due to an adaptive response. Conversely, in conditions of chronic stress (negative action for a long period), recovery of the welfare state normally does not occur, whereas a situation of non-adaptation (distress) is triggered, with a subsequent appearance of pathological phenomena (Trevisi and Bertoni, 2009). Therefore, the evaluation parameters are not always the same for short- or long-periods of stress and require the use different measures.

In case of short-term stimuli, the behavioral, physiological and endocrine evaluation parameters consist of vocalizations, escape attempts, heart rate, respiration rate, cortisol blood level, etc. Differently, in the event of long-term stress, important behavioral, physiological and endocrine parameters can be the reproductive efficiency, growth rate, stereotype manifestation, blood hormones and metabolites concentrations, and immune responses (Broom and Johnson, 1993; Tripaldi and Allegrini, 2000; Archetti, 2007).

2.3. Short-term measures

2.3.1. Behavioral indicators

The observation of animal behavior, often overlooked, is one of the best strategies to assess the level of welfare, which is considered satisfactory when an animal expresses the natural behavior typical of the species. In fact, when an animal is in a difficult situation (environmental, physical or moral adversity), a change in behavior and communication is often the most common reaction of the animal to cope with the problem, being thus a useful indicator.

This assessment can be achieved through a sequence of analytical steps to record: what the animal does, and how, when, where, how often and for how long the animal does it. Moreover, it is necessary to establish the reasons for that behavior and if it is an expression of discomfort, pain or pleasure (Biancifiori, 2010).

Initially, behavioral changes often consist of an adaptation of orientation, with which the animal directs its sense organs towards the direction from which the stimulus is perceived. Depending on the duration and intensity of the stimulus, animals may cease the activities that they were performing previously (e.g. resting or feeding), and then adopt attitudes that allow defensive action, jumping or other sudden movements (Broom and Johnson, 1993).

Changes in normal posture or deambulation, caused by a disease, are often a diagnostic tool of that disease (Broom, 2006): for example, sheep that show lameness tend to kneel; bulls affected by traumatic reticolitis tend to walk with the characteristic stiff-legged pace, etc.

Vocalizations are another useful initial sign to indicate changes in the status of the animal. Vocalizations can describe particular moods or emotions of the animal and, even if they require good interpretative abilities, they are a non-invasive and easy-to-apply evaluation technique. The behavior and physiological status of two individuals who interact through vocalization (i.e. the sender and the receiver) can be used to reveal its meaning (i.e. its semantic content) through the phonetic analysis, which translates into numerical parameters (length, intensity, etc.) the significance of the emitted vocalization (Figure 2.2; Manteuffel et al., 2004).

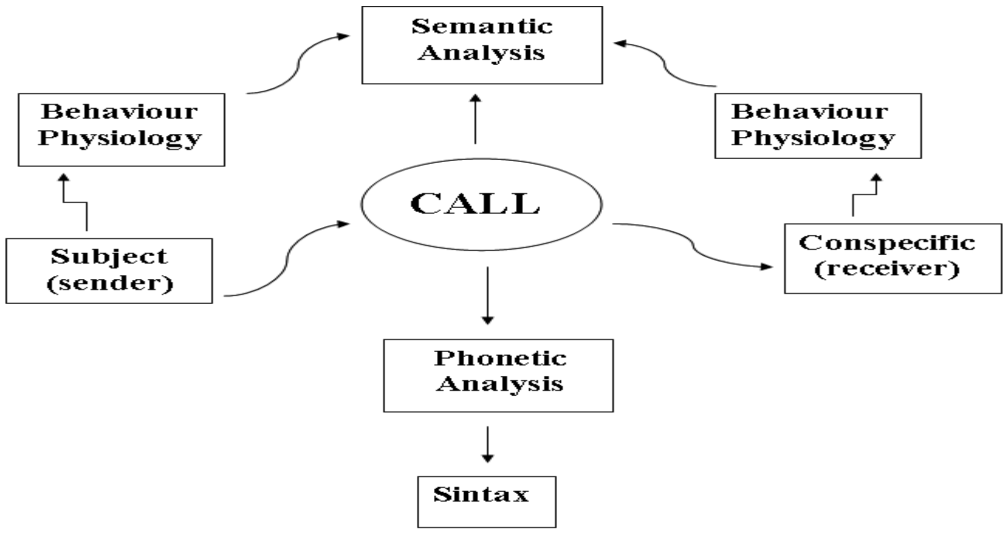

Figure 2.2. Scheme of relations and analytical procedures in acoustic animal communication (adapted from Manteuffel et al., 2004).

In most cases, vocalizations are indicators of signals of attraction or warning (e.g. to warn against a danger), but they can also be indicators of the combat ability of a competitor, the ability to attract a mate, the perceived feeding availability, or the degree of confidence on humans (Marchant et al., 2001; Manteuffel et al., 2004).

The vocal behavior is also a potentially useful tool for the physiological and psychological functioning of the animals, even if many aspects of voice communication are poorly understood and vary considerably in individual responses, thus making its interpretation difficult when assessing animal welfare (Watts and Stookey, 1999, Watts et al., 2001).

For these reasons, when behavioral measures are uncertain, they must be necessarily related to other indicators, such as physiological and endocrine parameters, to properly interpret their meaning in relation to the state of animal welfare.

2.3.2. Physiological indicators

Although most of the physiological indicators used in the assessment of animal welfare are now considered to be valid and accurate, their use on a large scale is often limited by the time-consuming analysis and the high costs required for their determination, compared to other parameters. For this reason, these indicators are used especially to assess welfare at research level.

The main physiological parameters used to assess the effects of short-term stress on animal welfare are: hematocrit, heart rate, respiration rate, body temperature, blood enzymes and metabolites (e.g. glucose and non-esterified fatty acids, NEFA).

For a correct assessment of animal welfare, the *hematocrit*, which represents the percentage of total blood volume occupied by the corpuscular fraction (blood red and white cells, platelets), has to be compared with standard reference values established not only for each livestock species but also for each physiological stage. Indeed, hematrocrit is a sensitive parameter that increases due to normal physical activity, because the spleen releases erythrocytes when the tissues need oxygen.

In pigs, physical stress caused by prolonged transport with lack of water affects the hematocrit. For example, Becerril-Herrera et al. (2010) found that hematrocrit concentration (expressed in %) increased significantly as transport duration increased, changing from baseline values of 29.51±0.45 to values of 37.63±0.50 and 44.33±0.25 after 8 hours and 16 hours of transport, due to both spleen contraction and plasma volume reduction.

The *heart rate* has a wide range of variability of reference values for each species in rest conditions (Table 2.1; Michaëlsson and Ho, 2000). Despite that, heart rate can be proposed as an indicator of disadaptation in many situations. As explained by Broom and Johnson, (1993), heart rate increases with the level of physical activity of an animal, being associated with intensified metabolism and tachycardia (high heart rate). However, heart rate can also increase before the action occurs or because of an emotional response (Broom and Johnson), On the other hand, heart rate can decrease to such a low level, that the subject might faint (Broom and Johnson, 1993).

Table 2.1. Normal heart rate (beats/min) at rest in different species (adapted from Michaëlsson and Ho, 2000).

Species	Heart Rate at Rest (Range)
Horse	25-50
Cow	50-80
Sheep	70-120
Swine	70-120
Goat	70-120
Mouse	450-750

Normally, the heart rate tends to increase due to transport, separation from a familiar environment or isolation from the group (Foreman and Ferlazzo, 1996). In sheep, which are gregarious animals, heart rate increased because of isolation from the herd, movement from a familiar group to an unfamiliar one and during transport (Baldock and Sibly, 1990).

The heart rate also varies during different situations of daily human-livestock interaction. For example, cows trained for rectal palpation and simulated instrumental insemination during 5 weeks, with gentle attitudes of the operator, showed a lower heart rate than cows that had never undergone this training (Waibilinger et al., 2004). Similarly, the favourable behavior of the breeder towards the animal reduces its heart rate during loading and unloading of animals sent to the slaughterhouse (Table 2.2; Lensink et al., 2001).

Table 2.2. Variability heart rate during the loading and unloading in calves (adapted from Lensink et al., 2001).

Heart rate	Stockperson Behaviour		SEM	P-value
	Positive	Negative		
Heart rate during loading (Beats/min)	199.9	206.0	18.4	0.03
Heart rate during unloading (Beats/min)	185.6	193.0	22.7	0.03

As heart rate increases, the *respiratory rate* also increases. The later parameter has the advantage of being easily measured and can be taken without disturbing considerably the animal.

In heat stress conditions, the respiratory rate is the first parameter to change. In fact, an increase in respiratory rate of the animals is considered as a mechanism for thermoregulation through an increase of evaporation from lungs. Recently, it was noticed that often respiratory dysfunction in sheep and goats is due to not only adverse environmental conditions, but also physiological stress combined with viral and bacterial infections (Scott, 2011).

An abnormal respiratory rate may be caused by chest pain or severe lung disease, but can also be manifested in situations of excitement or fear (Jackson and Cockcroft, 2002). Therefore, given the numerous factors that can alter the respiratory rate, reliable data can be obtained only by using detection techniques which are easy to apply and do not disturb the animal.

Body temperature is an indicator whose values change in many stressful situations. Its increase or decrease is often related to organism dysfunctions with changes in heart rate, excessive or reduced salivation and signs of tiredness (Broom and Johnson, 1993).

Within the same flock, ewes with a rectal temperature lower than 39.8 °C during pregnancy delivered lambs with higher birth weight than ewes with a rectal temperature exceeding 39.8 °C, probably because of higher weight, and protein and DNA content of the placenta of the sheep with lower temperature (McCrabb et al., 1993; McCrabb and Bortolussi, 1996).

Changes in body temperature are related not only to physiological factors but also to animal management. Indeed, body temperature was higher in cows milked twice a day than in those milked once, likely due to a greater metabolic activity (body heat production) rather than to a physiological stress (Kendall et al., 2008).

In conclusion, the detection of body temperature in animals exposed to stress is considered a useful measure to monitor welfare. However, this parameter must be combined with others, in order to obtain reliable information.

The *enzymes and metabolites* are very useful indicators in the evaluation of short-term stress, because their basal levels can change very quickly. Indeed, various enzymes can be taken into account to assess welfare. For example, levels of rennin, a protein produced in the kidney, related to the sympathetic nervous system and involved in water balance and blood pressure, were high within 12 minutes from the stimulus perception, and returned to baseline levels within 12-20 minutes after the cessation of the stress agent (Broom and Johnson, 1993).

The increase of specific enzymes is commonly used as an indicator of muscle damage. In fact, animals subjected to transport stress had elevated plasma levels of alanine aminotransferase, aspartate aminotransferase, lactate dehydrogenase and creatine kinase. The concentrations of these enzymes were higher, the longer the waiting time before slaughter, due to stress from lack of water and food and clashes between subjects (Table 2.3; Pèrez et al., 2002b). However, because the concentrations of these enzymes also differ according to genotype and sex of the animals, the use of enzymes as a single indicator could lead to errors in the evaluation of the welfare state (Pérez et al., 2002a, b).

Table 2.3. Least square means (standard error) of the biochemical parameters in relation to lairage time, 50 animals per group (adapted from Pèrez et al., 2002b).

Parameters	Group 0 h	Group 3 h	Group 9 h	Significance
Alanine amino transferase	52.4a	59.7a	90.5b	P < 0.001
Aspartate amino transferase	103a	113a	611b	P < 0.001
Lactic dehydrogenase	2052a	2894b	7490c	P < 0.01
Creatine kinase	7692a	14918b	74691c	P < 0.05

a, b, c: Different letters indicate significant differences between groups.

The variation of some blood metabolites, such as glucose and NEFA, can also be considered a stress response. Glucose is one of the most sensitive metabolic parameters to the influence of various factors, increasing due to stimulation of adrenaline and hormones produced by the adrenal cortex and decreasing as a result of vigorous physical activity (Broom and Johnson, 1993).

Glucose levels vary due to several rearing factors, but are also influenced by age and weight of the animals (Table 2.4; Bornèz et al., 2009). Other important sources of variation of glucose level are transport, season and isolation. Indeed, sheep transported to the slaughterhouse initially increased blood glucose levels, but also showed a high degree of adaptability, as the values returned to baseline levels after 9 hours (Knowles et al., 1995; Bornèz et al., 2009). In other studies, glucose levels were higher in winter than in summer (Miranda-de la Lama et al., 2010) and after a short period of isolation (Apple et al., 1995).

Table 2.4. Glucose levels (mean±S.E.) in light and suckling Manchega Spanish breed lambs at different blood sampling times (adapted from Bornèz et al., 2009).

Biochemical Parameter	**Blood Sampling Times**					
	On Farm		After Transport		After Lairage	
	Light Lamb	Suckling Lamb	Light Lamb	Suckling Lamb	Light Lamb	Suckling Lamb
Glucose (mmol/l)	4.88±0.14x	5.27±0.16a	6.18±0.24y	6.43±0.40b	3.16±0.10z	4.75±0.09c

x, y, z Different letters indicate significant differences (P<0.05) between light lambs due to handling.
a, b, c Different letters indicate significant differences (P<0.05) between suckling lambs due to handling.

Non-esterified fatty acids can be defined as a biological effect of negative energy balance. In fact, NEFA concentrations increased when glucose did not meet energy requirements of cows (Adewuji et al., 2005) and when goats were feed restricted (Laporte-Broux et al., 2011).

2.3.3. Endocrine indicators

The indicators involved in endocrine responses to stressful stimuli are those involving the system of the sympathetic-adrenal-medullary (SAM) and the hypothalamic-pituitary-adrenocortical (HPA) axes. The adrenal glands, located near the kidneys, are constituted by two compartments (the medulla and the cortex) with endocrine function and are an important part of both SAM and HPA. The activation of the SAM nervous system in emergency situations results in the release of catecholamines (adrenaline and noradrenaline) from the adrenal medulla, within 1-2 seconds from the perception of the stimulus, and in their subsequent catabolism, with the same speed in which they were released. The mechanism involving the HPA axis, instead, is more complex than the previous one. Indeed, it starts with the production corticotropic hormone releasing factor (CRF) in the hypothalamus, which stimulates the secretion of corticotropic hormone (ACTH) by the pituitary gland, which in turn exerts its action on the adrenal cortex, where glucocorticoids, mainly cortisol, are produced. In most species, the release of glucocorticoids starts at least 2 minutes from the perception of the stimulus, and their effect lasts longer, even a few hours, than that of catecholamines, which lasts few minutes (Broom and Johnson, 1993).

Therefore, a change in the basal level of cortisol in response to short-term stress can be considered an important indicator of animal welfare. In several studies on sheep, some management activities, such as isolation, artificial milk feeding and weaning, caused stress and, consequently, an increase of blood cortisol (Napolitano et al., 1995; Orgeur et al. 1998; Napolitano et al., 2003). In a study on the effects of tail docking on lambs, levels of blood cortisol increased after stress, reached its highest concentration at approximately 1 hour from the perception of stress and decreased slowly afterwards, returning to normal values after 3-4 hours (Mellor et al., 2002).

Because therelease of cortisol is stimulated by a high number of factors and its blood level can also increase as a response to sampling stress, Broom and Johnson (1993) considered cortisol a valid indicator of welfare state only when the duration of blood sampling is shorter than 2 minutes. To overcome this limit, several studies evaluated cortisol content in other biological materials, such as saliva, milk, faeces and urine (Hay and Mormède, 1998, Morrow et al., 2002; Fukasawa et al., 2008; Yates et al., 2010), which require less invasive techniques for sampling. Unfortunately, the detection and interpretation of the values of cortisol in these materials is not easy. In fact, cortisol content is 10 times lower in saliva than in blood (Negrão et al., 2004), and cortisol might conjugate before excretion in faeces and urine or be processed by bacteria in the gut (Möstl and Palme, 2002).

2.4. Long-term measures

2.4.1. Behavioral indicators

The observation of animal behavior is a good indicator in the evaluation of the effects of long-term stress (Broom and Johnson, 1993). However, the first problem is to establish and define what is meant by "natural or normal behavior" of an animal and, consequently, what is an "abnormal behavior".

One approach to make this distinction is to compare the behavior of domesticated animals with that of their peers living in extensive natural conditions. However, a different behavior of domesticated animals is not necessarily abnormal or an index of poor welfare, as they have been selected for specific characters and also for the ability to adapt to new environmental conditions (Nowak et al., 2008).

Another approach is to associate specific behaviors with positive experiences or feelings of the animal (e.g. game, reproductive behavior and mud bath for pigs) to define the natural and pleasant situations (Boissy et al. , 2007; Opperman Moe et al., 2009). However, some researchers evidenced that the behavioral response to a positive event varies with the species, and this could mislead the assessment of the welfare state. For example, certain species increase their motor activity before the positive event of feed distribution, whereas others reduce their overall activity at the moment of feed supply (Van de Bos et al., 2003; Dudink et al., 2006; Zirmmerman et al., 2011).

Once the natural behavior of a specific species is defined, it is necessary to observe and understand the different attitudes of the single individuals and the group of animals, such as difficulty to perform normal movements, inability to escape from danger or unpleasant stimuli, time spent lying, conflicts between animals within the group, and time and methods used by an animal to reach the upright position (Fregonesi and Leaver, 2001; Plesch et al., 2010).

The inability to express a normal behavior for a long period might have a negative impact on welfare state (Spinka, 2006), because it causes a condition of suffering manifested by the appearance of transfer or stereotyped attitudes, or both (Webster, 1999).

Transfer behavior is when the animal, being unable to respond properly to a stimulus (e.g. access to food or social exclusion), produces an alternative activity (e.g. body cleaning), not pertinent to the main stimulus. It is hypothesized that animals adopt this attitude to decrease the intensity of unpleasant feelings. Stereotyped behavior occurs when the animals, especially those kept in confined spaces and without stimuli, adopt attitudes that consist of the obsessive execution and repetition of apparently senseless actions (e.g. the bear dance observed in horses) (Webster, 1999).

2.4.2. Immune indices

Immune indices are an important tool in estimating the effect of long-term stress (chronic stress), which is generally considered a "suppressor" of the immune system because it promotes the onset of diseases, metabolic disorders and infections, with a consequent reduction of welfare (Trevisi and Bertoni, 2009).

Under chronic stress conditions, the individual is forced to adopt specific adaptive responses (antibodies and lymphocytes), as it passes from one initial emotional disorder to a neuroendocrine imbalance, resulting in physiological and pathological changes and the onset of disease (Merlot, 2004). Among the parameters that best identify the immune response, there are the increase in the ratio between neutrophils and lymphocytes, the antibody production, the haemolytic complement and the serum lysozyme titration (Trevisi and Bertoni, 2009). Increased neutrophils and reduced lymphocytes in blood were observed in lambs as a consequence of isolation or separation from their mothers (Table 2.5; Cockram et al., 1994; Degabriele and Fell, 2001; Napolitano et al., 2003). Similarly, pigs in isolation or cattle subjected to 3 days of transport had a reduction of lymphocyte proliferation (Salak-Johnson and McGlone, 2007).

Table 2.5. Effect of isolation on the mean blood concentration of neutrophils and lymphocytes (n. of cells x 10^9/l) (adapted from Cockram et al., 1994).

Time since start of isolation (h)	**Total number**					
	Neutrophils			Lymphocytes		
	C	I	s.e.d.	C	I	s.e.d
0	3.24	2.92	0.539	7.56	6.54	0.876
3	3.24	5.96	0.304	6.47	5.60	0.399
24	3.88	4.43	0.438	6.89	5.36	0.517

C: adjusted mean of control group; I: adjusted mean of isolated group; s.e.d.: standard error of the difference between means

Therefore, considering that the ratio between neutrophils and lymphocytes tends to increase under chronic stress conditions, this parameter might be suitable to assess welfare. Nemi (1993) reported that this ratio was higher than 1 in adult cattle exposed to prolonged stress or after a prolonged inflammatory condition, whereas it was lower in non-stressed animals. However, the use of this index is rather complex and requires a lot of attention, because any disease can affect the number and type of these immune cells in blood.

The production of antibodies, i.e. immunoglobulins (IgA, IgE, IgG and IgM) produced by B lymphocytes, subsequent to inoculation of antigens also represents a good index of evaluation in long-term stress. Normally, the antibody response is induced by injecting an antigen into muscle or under the skin of the animal (ovalbumin or limpet hemocyanin keyole), and the subsequent production trend of antibodies is then monitored in the following weeks. However, the use antibody responses to assess the welfare state is not so easy, because they can vary (i.e. increase or decrease) depending on the type of stress suffered and, especially, on the subclass of antibodies considered (Pollok et al., 1992; Mackenzie et al., 1997; Napolitano et al., 1995; Grasso et al., 1999; Caroprese et al., 2005).

Amadori (2007) suggested that the haemolytic complement, which is an protein-related immunological mechanism responsible for the body's defense against viruses or bacteria, can be a good indicator for assessing the state of welfare. This alternative immunological pathway recognizes the repeating sugar structures in the cell wall of viruses and bacteria. Indeed, in sick animals, the haemolytic complement system consumes fractions of viral or bacterial walls, thus decreasing its concentration. Therefore, if the amount of haemolytic complement in blood decreases, this means that this mechanism has been activated and the animal is stressed.

A recently proposed indicator of welfare is the lisozyme, which is an enzyme with antibacterial action present in various biological liquids (blood, milk, saliva and lachrymal secretion). This enzyme, which acts synergically with the humor immune system and with complement factors, is able to attack and destroy nitrogenous polysaccharides (e.g. peptidoglycan) present in the cell walls of several microorganims. Because the bacteriolytic action of lysozyme consists of hydrolysing the glycosidic bond of the bacteria cell wall, determining the lysozyme concentration in biological liquids can be a useful indicator of animal welfare (Sotirov, 2006; Moscati et al., 2008). However, in order to properly use this indicator, it would be important to determine the normal reference values for different species. In swines, Moscati et al. (2003) determined reference values for lisozymes and complement (Table 2.6; Moscati et al., 2003), by collecting blood samples from 30 pigs reared in a closed system, managed according to the multi-site system and respecting hygiene and sanitary regulations, during the various phases of the production cycle (weaning, growth, finishing) for two years. According to the Authors, the reference values for lisozyme should be within the range of 1-3 μg/ml and those for complement should be higher than 80 CH50/150 μl.

Table 2.6. Normal values of lisozyme and complement in blood of pigs at various phases of the production cycle (mean±s.e.) (adapted from Moscati et al., 2003).

Parameters	Weaning	Growth	Fattening
Lisozyme in serum (μg/ml)	2.6±0.22	2.1±0.22	2.9±0.22
Complement (CH50/150 μl)	89±0.13	97±0.13	99±0.13

In conclusion, monitoring the natural immune system properly can give be useful to indicate critical phases of adaptation of the animal to the environment and to the rearing system.

2.4.3. Endocrine indicators

Normally, in animals under short-term stress a temporary increase of glucocorticoids and catecholamines in plasma occurs, whereas in those under long-term stress (chronic stress) the SAM and HPA axes seem to adapt to the situation (Broom and Johnson, 1993).

Some studies evidenced that during long-term stress, such as social isolation, confinement in metabolic cages, lameness and prolonged effort, there is initially an increase of the cyrchadian rythm of cortisol secretion, even if as time goes by cortisol concentration usually goes back to normal values (Apple et al., 1993; Janssens et al., 1995; Ruis et al., 2001).

As a consequence, glucocorticoids cannot be used as a reliable single indicator of the welfare state in the long term, because, if not associated with other parameters, their low or stable concentration could erroneously suggest that the stress is overcome when, in reality, it still persists.

2.4.4. Reproduction indicators

Hormones secreted by hypothalamus and pituitary gland are strictly associated with responses to stress factors. Because these secretions regulate and control the reproductive process, long term stress influences the reproductive state of animals.

In males and females, re production is controlled by the secretion of gonadotropins (GnRH) by hypothalamus, which act in the anterior lobe of the pituitary gland, stimulating the secretion of the follicle-stimulating hormone (FSH) and the luteinizing hormone (LH), which in turn act at the ovary and testicle level, influencing gametogenesis and sexual behavior (Dwyer and Lawrence, 2008).

Therefore, during chronic stress, hormonal disorders might occur during estrous or ovulatory phases, and can reduce fertility and reproductive efficiency, depending on the degree of the symptoms (Dobson and Smith, 2000; Turner et al., 2005).

Long-term under nutrition can influence the reproductive capacity of animals, causing a delay of puberty in male and female lambs (Da Silva et al., 2001), modification of the cellular structure of testicles (Bielli et al., 2002) and reduction of ovulation rate (Rae et al., 2001). Similarly, long-term heat stress can influence fertility, by causing endocrine changes which can reduce the number of oocytes and embryos of prolificous species (De Rensis and Scaramuzzi, 2003), the duration of estrus, due to a lower production of estradiol, and the production of progesterone (Wolfenson et al., 2000).

Management of animals can also affect their reproduction. For example, when animals are divided into groups, the presence of social dominance can reduce the number of animals in estrus and the duration of estrus (Orihuela, 2000). In addition, a favorable human-animal interaction is able to reduce the negative effects of long-term stress, for example, by reducing animal restlessness during instrumental insemination and thus increasing the probability of pregnancy (Waiblinger et al., 2004).

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CHAPTER 3

Animal welfare of sheep

3.1. Sheep farming

Sheep, together with cattle, are the most widespread livestock species worldwide, with a world population of 1,077,762,456 heads (Faostat, 2012). The large diffusion of sheep is given by: i) their ability to adapt to difficult environmental conditions, which characterize many areas of the world (e.g. poor vegetation, limited rainfall, poor water supply), and ii) their importance as a source of goods of primary importance (wool, meat and milk). Sheep farming is quite common in less-favored areas where it is often the only possibility of economic exploitation (Africa, Latin America, Middle East, China, etc.). Even in regions with more favorable environmental, structural and economic conditions, such as the Mediterranean area, sheep farming is almost always performed in marginal areas, where it is difficult to find equally-profitable alternatives (Idda et al., 2010). However, despite the high adaptive capacity and resilience of sheep, exposing this species excessively to difficult farming conditions could endanger animal welfare.

An important characteristic of sheep is that this species is reared in various systems which differ for breed, purpose (milk, meat and wool), and production level.

Intensive farming system is adopted when breed are highly specialized and have high production level. In this system, the animals are kept indoors during all or most of the year, because the main purpose is to maximize production, by reducing the time and cost of management. The animal has the advantage of receiving more care by humans, being totally dependent on them for food and water supply, thermal comfort and health care, and the disadvantage of loosing its freedom and, to a great extent, its natural behavior (Dwyer, 2009).

Since sheep are gregarious animals and live mainly outdoors, they may be subjected to greater anxiety, fear or frustration due to managerial errors (inadequate environmental conditions or an inappropriate attitude of the operator), or may show low adaptability when forced to live in confined areas (Fitzpatrick et al., 2006 cited by Caroprese, 2008).

In most dairy sheep farms in the Mediterranean, the most common farming system is *semi-extensive or semi-intensive* ones, which consists of a more or less extensive grazing associated with farming techniques typical of traditional intensive systems (e.g. mechanical milking and feed supplementation during almost the entire lactation). Animals reared in this system have much more freedom than those reared in confined systems, but, on the other hand, they face many more problems which can influence their welfare, such as adverse weather conditions (high or low temperatures), greater risk of contracting parasitic diseases, fear of predators, and nutritional deficiencies due to lack of pasture in critical seasons (Dwyer, 2009). For example, in some cases ewes reared extensively had to increase the time of grazing from the normal 8 hours to a maximum of 13 hours, to make up for the shortage pasture which would otherwise cause weight loss (Lynch et al., 1992 cited by Dwyer, 2009). In these extensive conditions, nutritional deficiencies and adverse weather conditions can affect the survival of newborn lambs, especially those born from multiple births (Waterhouse, 1996). This is due to the fact that sheep are normally pregnant during the cold season (autumn-winter) and give birth at the final part of this unfavourable period, which worsens their body condition and their ability to properly raise their young. In order to counteract these detrimental effects, farmers often supply feed supplements, based concentrates and hay, and animal shelter at least during the night.

3.2. Factors which influence sheep welfare

Studies on factors which can influence sheep welfare are very limited, compared with those carried out on other livestock species reared intensively. Among these factors, the most important ones are human, environmental and technical or managerial factors.

3.2.1 Human factor

Studies on human behavior towards animals started in the seventies with Seabrook (1972), who highlighted the importance of this interaction as a factor able to influence the animal well-being. This interaction is a dynamic process that influences relationships based on mutual past experiences and highlights the degree of confidence that the animal have on man. The human-animal relationship, which implies different perceptions (olfactory, tactile, visual, auditory), can be classified into the following five different types, according to the action performed by man: visual presence, movement of animals without physical contact (even if vocal interaction can occur); physical contact, feed administation (rewarding behavior) and invasive attitude, i.e. bad handling of animals (Waiblinger et al., 2006).

The human-animal relationship can vary from positive to negative, with corresponding pleasant and unpleasant (fear, pain, frustration) emotions, due to rewarding (e.g. feeding and grooming) or punishing (e.g. social isolation, veterinary treatment and rough handling) events. Any type of communication between animal and man can influence the way in which they interact. For example, humans can unconsciously deliver signals of tranquility or danger, to which the animal responds with signs of tranquility, fear or aggression (Waiblinger et al., 2006).

Unfortunately, even if domestication has undoubtedly increased the docility in different livestock species, many animals still fear man strongly. To ensure a good human-animal relationship, first of all it is necessary to develop farming techniques that improve quickly and effectively how animals perceive humans. Secondly, it is important to assess the effect of animal genetics on this interaction, so that appropriate selection criteria can be established. Sheep are subjected to various farming practices (e.g. milking, shearing, nail clipping, and tail shortening) which can cause more or less negative or unpleasant feelings, according to the way in which they are carried out. Indeed, animals respond to a negative stimulus by adopting reluctant attitudes or keeping a greater distance from it (Waiblinger, 2010).

At the farm level, humans and animals can vary in the way they interact with each other. For example, if animals stay calm and accept being managed by unfamiliar people, it is likely that the farmer will treat them kindly and friendly; differently, if sheep run away as a stranger comes closer, it is likely that the farmer will adopt an abrupt or aggressive attitude (e.g. screaming and hitting the animals) (Waiblinger, 2010).

In young animals, the administration of feed is a very important stimulus to increase animal confidence on man. Boivin et al. (2000) found that lambs fed milk manually (hand-feeding) spent more time near the handler, had less physical activity and bleated less than lambs that either suckled milk without being helped by a person or had limited contact with man. This behavior was still evident even after 7 weeks from weaning. Tallet et al. (2005) observed that the presence of man is not sufficient to guarantee an adequate confidence level of animals on man, because it is fundamental to establish a good human-animal relationship in terms of both physical contact (handling of animals) and voice interaction. In fact, in a study on 6-month-old lambs from two different genotypes and rearing systems, lambs treated gently had earlier (i.e. shorter latency period) and more frequent number of contacts (e.g. sniffing humans) with man than lambs which had undergone forced handling or which had not been in contact with man before (Mateo et al., 1991). However, the same authors observed that many behaviors varied depending on the breed and previous experience the lambs had in the herd of origin.

The behavior of the lambs also changes if environmental conditions are modified. In fact, in a familiar environment and in the presence of fellows, lambs fed milk manually (i.e. hand-bottle fed) had a higher number of contacts with the caregiver than lambs that either suckled from a container without man's help (i.e. wall-bottle fed) or were only handled (but not fed) by man. Differently, when lambs were isolated in an unfamiliar environment, even if in the presence of a known person, their attitude towards man was not influenced by the above mentioned treatments (i.e. hand-bottle fed, wall-bottle fed and handled) (Tallet et al., 2009). Boivin et al. (1997) observed that lambs kept in continuous contact of with the same person during the first 3 weeks of life were able to recognize that person in an unfamiliar environment even after 3 weeks of no contact. In particular, those lambs showed a shorter latency period to interact, a longer duration of the interaction, a smaller number of vocalizations and less physical activity than lambs which had been milk-fed by different people during the same period. Nevertheless, these behavioral differences disappeared with age, mostly because of changes in livestock management, such as the establishment of new groups which consequent changes in social hierarchies.

Animal contact with humans from a young age facilitates the formation of a positive interaction between them, but this is not true when this contact occurs in the presence of the mother such interaction, even when feed is supplied by humans (Boivin et al., 2002). However, this has not been confirmed by Goddard et al. (2000), when comparing two different genotypes (Scottish Balckface and Texel * (Blue-faced Leicester * Scottish Blackface)) and rearing conditions (extensive management, with limited contact with humans vs. Intensive management, with more contacts with humans). In particular, increased heart rate in the presence of man in lambs reared extensively was the only difference observed from lambs reared intensively in an arena test, whereas all other parameters studied did not show clear differences, with the effect of race being confused with that of management.

Caroprese et al. (2005) found that gentle handling of animals, i.e. gently handling each lamb for 5 minutes, once a day throughout the trial, influenced positively immune responses in lambs reared artificially, but not in lambs suckled by their mothers. It was concluded that a correct approach by operators can limit the negative effects of the normal farming practices to which confined animals are more subjected.

The human-animal interaction is also strongly influenced by animal temperament (calm or nervous) assessed The temperament of ewes in the milking parlour can be assessed on the basis of the following behavioral characteristics: position occupied in the milking parlour, reaction to feed, activity towards neighbours, reaction towards forage offered by hand by unfamiliar person; reaction towards positioning of teatcups, persistent occupation of the same position in the milking parlour (Dimitrov et al., 2005). Dimitrov (2008) observed that, in response to the same human behavior, the nervous ewes had higher values of cortisol and lower levels of lysozyme during the milking operations (23.7±6.1 vs. 8.1±1.4 nmol/l for cortisol, 0.173±0.013 vs. 0.300±0.032 µg/ml for lysozyme; $P<0.05$) and due to shearing (19.9±5.2 vs. 38.5±6.1 nmol/l for cortisol; 0.102±0.0014 vs. 0.065±0.005 µg/ml for lysozime; $P<0.05$) and worse immune responses than calm ewes.

Hart et al.(2009) observed that temperament also influenced the quality of colostrum, as calm ewes produced colostrum with a higher content of IgG than nervous ewes (Table 3.1; Hart et al., 2009). This is important because offspring from calm ewes have a higher chance of survival in the neonatal period than those from nervous ewes, due to improved passive immunity acquired by lambs.

Table 3.1. Body weight, lamb birth weight and the IgG concentration of colostrum available at birth from calm and nervous ewes (mean±S.E.) (adapted from Hart et al., 2009).

Parameter		**Temperament of ewes**	
		Calm	Nervous
Ewe body weight	kg	57±1.3	56±1.3
Lamb Birth Weight	kg	5.0±0.20	5.3±0.24
IgG	mg/ml	35.7±2.5a	30.3±1.5b

a, b: Different letters indicate significant differences (P<0.05) between groups.

In Australia, since 1990 two genetic lines have been selected for temperament (calm and nervous), starting the evaluation at weaning (14-16 week-old lambs), with a value of heritability of expressed characters ranging between 0.2 and 0.4 (Blache and Bickell, 2010). These selected genetic lines selected have differed also for reproduction, with calm sheep showing higher ovulation rate and proportion of twin gestations than nervous ones, probably because of a better use of the available energy and a higher rate of survival of their lamb from birth to weaning, associated with a a better maternal attitude and a closer presence to their youngs.

An important problem that still has to be solved is the definition of reliable parameters to assess the human-animal interaction at the farm level.

Currently, among the several methods available to evaluate the degree of confidence on (or fear to) humans that an animal has, the most diffused are based on the following (de Passillè and Rushen, 2005):

- *distance measures:* these measures are usually taken by placing the animal together with a person in a limited space and assessing, for example, how close the animal gets voluntarily to a stationary person ("approach distance") or how close the animal will allow a person to approach it ("flight distance"). In either case, the greater the distance, the more fearful the animal is;
- *handling measures*: these measures are based on the observation of the responses of the animal during routine operations on the farm, such as milking, feeding, moving, and shearing. In this case, because there is no agreement on a specific measure, different parameters are used (e.g. time required to perform a certain practice, escaping actions and kicking).
- *evaluation score*: it is based on the attribution of a subjective value to the behavior of the animal by an estimator.

Unfortunately, the use of non standardized parameters to evaluate the degree of confidence on humans poses great difficulties when comparing the results obtained in different experimental situations by different authors.

3.2.2. Environmental factors

Environmental factors considered are those related especially to high temperatures, ventilation in confined environments and physical comfort.

Temperature. Thermal comfort is an essential condition for the maintenance of adequate welfare of animals, including sheep, which may experience stress when temperature conditions are unsuitable.

Even though sheep are considered resistant to extreme weather conditions, their well-being is reduced when temperatures are outside their optimal range of survival (5 to 25 ºC). In fact, an animal exposed to non-optimal temperatures (< 5 ºC and > 25 ºC) has a series of adaptive physiological responses, which can influence negatively milk yield and quality, reproductive performance and immune system and can lead to diseases (Peana, 2005).

The animal adopts various methods to defend itself effectively against hyperthermia. One of these methods is the synthesis of new heat shock proteins, which contribute to an increase of the resistance at the cellular level and possibly at the systemic level (Heimbach et al., 2001). It is also possible to limit the damage caused by hyperthermia on ewes through simple management techniques, such as providing shade or shelter during the hottest hours of the day, and changing feeding time to late afternoon, so that the production of metabolic heat is higher when environmental temperature are lower (i.e. during the night) (Sevi et al., 2007).

Sevi et al. (2001a, 2002b) noticed that shading and evening feed-administration caused, above all, an increase in protein and fat content (Table 3.2; Sevi et al., 2001a), with an increase of unsaturated and long chain fatty acids (e.g. oleic, linoleic and linolenic acids) and a decrease of short-chain saturated acids (e.g. caproic, capric, lauric, myristic and staeric acids. The same authors observed that good management of the animals in hot weather conditions ensured an environmental temperature 4 °C lower than that of the unprotected animals, with consequent lower rectal temperature (39.5 vs 41.1 °C) and lower frequency respiratory (103 vs 120 beats/min) in ewes protected from solar radiation than in unprotected ones.

Table 3.2. Milk yield and composition in ewes when protected or exposed to solar radiation and fed in the morning (PROM, EXPM) or in the afternoon (PROA, EXPA) (adapted from Sevi et al., 2001a).

						P-value		
Parameter	**PROM**	**EXPM**	**PROA**	**EXPA**	**SE**	**SR**	**FD**	**TM**
Milk yield, g/d	784	706	767	766	31.3	NS	NS	***
Protein yield, g/d	51.0	46.7	50.0	51.2	1.9	NS	NS	***
Casein Yield, g/d	38.4a	34.8b	38.9a	39.2a	1.1	NS	NS	***
Fat yield, g/d	51.8a	46.4b	52.3a	52.1a	1.9	NS	NS	***
Lactose yield, g/d	37.2	32.8	35.8	36.3	1.6	NS	NS	***
Protein content, %	6.50	6.62	6.51	6.69	0.09	NS	NS	***
Casein content, %	4.90	4.93	5.07	5.12	0.07	NS	NS	***
Fat content, %	6.61	6.58	6.82	6.80	0.11	NS	NS	***
Lactose content, %	4.75	4.64	4.67	4.74	0.04	NS	NS	***

a,b Means within row with different superscripts differ ($P < 0.05$). SR–Solar radiation, FD–time of feeding, TM–time of trial. NS, Not significant. ***P < 0.001.

Although sheep have a small body size, a feature that would be expected to facilitate heat loss, this species seems to be more susceptible to heat stress than to cold stress. Low temperatures are not a major disturbing event in ewes reared in a Mediterranean environment, where cold periods are normally short and effects are limited to a drop in milk yield and a variation milk quality (Pazzona et al., 2005).

Peana et al. (2007) observed that milk yield decreased by 15% (c.a. 0.30 kg/d per head) when maximum and mean temperature ranges were higher than 21-24°C and 15-21°C, respectively, whereas it decreased by 20% (c.a. 0.39 kg/d per head), as minimum temperatures changed from 9-12 °C to 18-21 °C.

Sevi (2007) observed that high temperature changes also the plasma NEFA concentration and the body condition score (BCS), evidencing an increased energy demand for thermoregulation, with increased heart rate, respiratory rate and water intake, and decreased feed intake; thus hampering the reconstitution of body reserves and probably the content of body fat available for milk synthesis.

Ventilation. Ventilation plays a main role in maintaining the welfare and performance of housed sheep, by affecting thermal exchanges between the animal's body surface and the environment, by avoiding an excessive increase in relative humidity, and by keeping levels of noxious gases and airborne particles under control

Th efficiency of ventilation in a sheepfold and its effects on environment and animals depend on several parameters, such as daily flow, air speed and length of ventilation cycles. For example, turbulent air currents caused by very high ventilation rates may result in more dust entering the animal house and in a longer period in which dust particles remain suspended in the air. On the other hand, if ventilation rate is low, dangerous gases (mostly ammonia) can accumulate. (Sevi et al., 2003a,b).

Another issue is that indoor ventilation must be applied differently depending on the season, because animals needs differ considerably between winter and summer.

Several studies in dairy sheep showed that summer ventilation rate lower than 40 m^3/h per head altered behaviour, physiological parameters (higher rectal temperature and respiratory rate; Sevi et al., 2003a) and production performance (10% lower milk yield; Sevi et al., 2003b; 2003c; Albenzio et al., 2005; Table 3.3) of the animals.

Table 3.3. Least square means ± SEM of air dust and gaseous pollutants concentrations as affected by low, moderate, and programmed ventilation regimen (adapted from Sevi et al., 2003c).

Parameter		**Low** (40 min/cycle- 1m/sec 23 $m^3/h/ewe$)	**Moderate** (40 min/cycle- 2m/sec 47 $m^3/h/ewe$)	**Programmed** (70% RH-1m/sec 73 $m^3/h/ewe$)
Total dust	mg/m^3	0.50b	0.50b	0.66a
Respirable dust	mg/m^3	0.16b	0.19b	0.26a
NH3	ppm	11.5a	7.5b	6.5b
CO2	ppm	858a	669b	642b
6.5%FCM yield	g/d	807b	831ab	891a
Fat	%	5.7	5.3	5.2
Protein	%	6.2	6.1	6.1
Casein	%	4.6b	4.8a	4.8a
SCC	Log10 cells/ml	5.05	5.10	5.16

a, b: Different letters indicate significant differences (P<0.05) between groups.

The role of air exchange in the sheepfold during winter is often overlooked, but can have a negative impact on animal welfare and milk quality. In fact, in Mediterranean environment, a good ventilation rate for lactating sheep in winter season should be equal to $47m^3/h$ per head, with an air speed of 2 m/sec and 10 daily cycles, each cycle characterized by 40 minutes of ventilation (Sevi et al., 2003c)

Physical comfort. The issues of the space available for the animals and the quality of the environment in which they live have regarded mainly farm animals reared intensively, which correspond to the minority of sheep reared. These aspects have often been overlooked when dealing with semi-extensive or semi-intensive rearing systems, which characterize most dairy sheep farms, probably because those sheep make large use of grazing. However, in these systems, farmers usually confine the animals for limited periods of time (e.g. in the period of parturition, during the night and during rainy and cold days), in facilities which often are not large enough for that purpose, with the risk of influencing negatively their well-being.

For sheep kept permanently indoors, Chiumenti (2004) reccomends a minimum area of 0.9-1.2 m^2/head when animals are kept on straw litter, and of 0.8-1.0 m^2/head when sheep are on slatted floor. However, the surface area per head varies with the age and weight of the animals and with the farming system adopted as well. In extensive farming, in which the animals spend most or all day grazing and are confined in the pen for a short time, space can be reduced by 15-20% compared to that needed for animals reared intensively. For animals permanently confined, it is recommended to use a paddock area (open paddock) of 2.0-2.5 m^2/head, where the animal can perform a minimum amount of functional exercise (Chiumenti, 2004).

Sevi et al. (1999) found a significant decrease in air concentrations of total microorganisms, especially coliforms, when the where sheep were kept had an area of 2 m^2/head compared to rooms which had 1.5 or 1 m^2/head. In addition, the ewes housed in the least crowded room showed an increase in milk yield, casein and fat content and a decrease of somatic cell count, which determined an overall improvement of milk cheese making properties.

The space available for an animal regards not only surface but also volume. Sevi et al. (2001b) observed a better environmental quality for ewe rearing, due to lower relative humidity and lower concentrations of microorganisms in the air (*Mesophilic, Coliforms* and *Staphylococci*), when space available was greater than 7.0 m^3/head compared to 5.6 m^3/animal or 4.0 m^3/head. In addition, the reduction of the volume available per head caused a reduction of milk yield and quality, i.e. lower milk protein, casein and fat contents and higher milk somatic cell count and presence of harmful microorganisms (Coliforms), accompanied by a lower aptitude for cheese making.

In intensive farming, characterized by a high density of animals per unit of area or volume, the management of the sheepfold is important to reduce the incidence of mammary infections. In Comisana sheep reared intensively, the incidence of mastitis tended to increase as lactation progressed, with the predominant pathogen in the milk of infected sheep being *Esclerichia coli* (Albenzio et al., 2002). In addition, sheep with mastitis had a reduction in milk yield (-21%), quality and cheese making aptitude, evidencing that the hygiene in the farm is important not only for animal well-being, but also for its economic implications, (Albenzio et al., 2002).

A critical point of the environmental pollution of the stables is associated with the management of litter. In fact, proper litter management, based on its removal at regular intervals (4 weeks) or on the addition of granular bentonite (0.5 kg/m2) to it, restricts the proliferation of bacteria and slows down the processes of nitrogen degradation, clearly improving air quality (Sevi et al, 2001c, 2003a).

In addition to its effects on animal health and production, high animal density also alters the behavior of animals. Under these conditions, the sheep are likely to show different abnormal behavior (e.g. detrimental action and stereotyped or repetitive actions), which can be used as indicators of poor welfare (Dwyer and Lawrence, 2008)

The overcrowding of animals in confined environments leads to increased competition for water and food, with some animals, especially the weakest, not being able to get them (Nowak et al., 2008). In fact, when the space of the manger was reduced from 24 to 4 cm/sheep, the percentage of animals that did not eat passed from 0% to 31% (Lynch et al., 1992 cited by Nowak et al., 2008).

In the study of Bøe et al. (2006), reducing the area available for ewes from 0.75 or 1.0 m^2/head to 0.5 m^2/head reduced the overall lying time, increased the rest time performed near other sheep and increased the walking time (Table 3.4; Bøe et al., 2006). Regardless the space available, most sheep prefered to rest in the vicinity of the perimeter of the box rather than in its central part.

Animals behavior is also influenced by the characteristics of the floor in the lying area. Færevik et al. (2006) found that sheared sheep prefered a floor with low thermal conductivity (straw or wood), whereas non-sheared sheep had no preference for floor type.

Caroprese (2008) highlighted the beneficial effects of free-access to an open space on immune response, behavioral activities and production efficiency of sheep, probably due to a better translation of the information received from photoperiod.

Table 3.4. Lying behaviour and displacements (mean ± standard error) of ewes in pens (adapted from Bøe et al., 2006).

Parameter	Size of lying area (m^2/ewe)		
	Small 0.50	**Medium 0.75**	**Large 1.00**
Lying time (% of total observations)	63.0 ± 1.1 a	67.4 ± 1.3 b	66.9 ± 1.1 b
Lying close to one or more ewes (% of lying in lying area)	81.8 ± 2.5 c	69.3 ± 3.8 d	59.4 ± 2.6 e
Standing (% of total observations)	7.7 ± 0.5 a	7.7 ± 0.5 a	10.6 ± 1.0 b
Walking (% of total observations)	8.5 ± 0.7 a	3.7 ± 05 b	1.3 ± 0.2 b

a, b: Different letters indicate significant differences (P<0.05) between groups;
c,d,e,: Different letters indicate significant differences (P<0.001) between groups.

3.2.3. Management factors

In sheep, management aspects are certainly one of the main factors that can affect animal welfare. State of discomfort caused by stress from poor management can change animal behaviour and, above all, can negatively affect production performance, with consequent economic losses.

The main operational factors that can influence sheep welfare and milk production are milking and lactation/weaning.

Milking. In dairy sheep, lactation is the most important physiological phase of the entire production cycle. Therefore, the way in which milking is performed (manual or mechanical) can influence the emotional state of the animal and, consequently, its welfare.

Villagrà et al. (2007) found a close relationship between the order of entry into the milking parlour in the first two weeks after weaning and the aptitude of sheep for machine milking. Order of entry did not influence the total amount of milk milked, but affected the different fractions of milk extract. In particular, the first sheep entering the milking parlour had a higher percentage of milk milked mechanically, lower percentage of milk obtained with machine and manual stripping, and lower percentage of residual milk than the third and last sheep entering the parlour (Table 3.5; Villagrà et al., 2007). The fact that no differences were found in the total milk production is attributed to the fact that this parameter is the sum of all fractions of milk removed.

Table 3.5. Milk yield and milk fractioning during milking according to the order of sheep entry into the milking parlour (Ls means ± S.E.M.) (adapted from Villagrà et al., 2007).

Order	Variable				
	Yield (ml)	MMP (%)	MSP (%)	HSP (%)	RP (%)
1	965±27	81a±0.7	11a±0.5	8a±0.4	16a±0.9
2	1011±56	80a±0.6	11a±0.4	9b±0.3	19b±0.7
3	964±34	76b±0.9	14b±0.2	11c±0.5	24c±1.1

MMP = machine milking percentage; MSP = machine stripping percentage; HSP = hand stripping percentage; RP = residual milk percentage;
a, b: Different letters indicate significant differences (P<0.05) between groups.

Dimitrov and Djorbineva (2003) showed that animal temperament (calm and nervous) affects the ability of the animal to release the milk when milked mechanically. Calm animals had higher milk production (592 vs. 477 g/head/d), higher milk flow rate (15.6 vs. 13.6 ml/s) and lower milk ejection latency (1.9 vs. 5.3 s) than nervous animals.

In Lacaune sheep, ewes subjected to a mixed management system (suckling three times a day and machine milking twice a day) were more prone to release milk to the lamb rather than to the milking machine. In fact, even though the baseline values of oxytocin were similar in suckling and milking, the peak of secretion of this hormone was significantly higher at the beginning of suckling than at the start of machine milking (91.7±26.1 vs. 13.1±1.8 pg/mL). On the other hand, the concentration of prolactin and cortisol in blood increased in both conditions of milk extraction, without significant differences between them (Marnet and Negrão, 2000).

Machine milking can have such a considerable negative impact on primiparous ewes, that it can affect production performance during their entire life. If the animal memorizes their first machine milking as a negative event, to which it has never been able to adapt, this management practice is likely to become a chronic stressor and that animal will probably always enter last in the milking parlour.

In primiparous ewes, levels of cortisol, adrenalin and noradrenalin were higher at the first machine milking made the day after delivery than in the following days of machine milking (Negrão and Marnet, 2003). However, because lambing is also a stressor, its effects may have interacted with those caused by milking in the first 4 days. In fact, lower values of these parameters were observed on the 15th day of milking when 75% of the animals showed signs of adaptation to this procedure. Another interesting finding was that, as days from first milking progressed, sheep considered adapted to machine milking showed an almost exponential increase of oxytocin, whereas the non-adapted ewes showed a limited increase of this hormone.

Rassu et al. (2006) found that a week of training of primiparous in the the milking parlour (ambient and noise) before weaning is not sufficient to reduce the stress of the animals, thus not influencing milk production and quality, except for the significantly lower SSC values in the trained than in the non-trained ewes, at the first 4 days of milking.

A malfunction of the machine milking system, especially non-suitable values of vacuum level, frequency and ratio of milking, can cause stress, even in multiparous sheep already trained to this practice.

The vacuum level recommended for sheep is between 36 and 42 kPa, which is greater than that exercised by the lamb during feeding (26 kPa). This recommended range appears to be adequate, because if vacuum level is too low, teat cups can fall and milking time increases (Salaris et al., 2005). Peris et al. (2003a) milking In machine-milked Manchega ewes, vacuum levels of 36 and 42 kPa did not differ for udder health, for milk SCC, and for the variation in teat thickness after milking.

Sinapis et al. (2006) showed that high vacuum levels of machine milking (> 40kPa) can compromise the udder health, because vacuum of 44 or 50 kPa not only increased the rate of milk removal, but also reduced the percentage of machine milking in relation to the percentage of machine stripping. In addition, high vacuum level (50 kPa) increased SSC in milk (Table 3.6; Sinapis et al., 2006), which is the main indicator of udder health.

Table 3.6. Effects of vacuum level on the fractions of milk milked and milk quality (adapted from Sinapis et al., 2006).

Parameter		**Vacuum level (kPa)**		
		38	**44**	**50**
Milk yield	ml/d	921±27	887±25	896±26
Machine milk	ml	771±24	719±23	737±24
	%	83±0.5a	80±0.7b	81±0.6b
Machine stripped milk	ml	150±5	168±6	159±5
	%	17±0.5b	20±0.7a	19±0.6a
Fat	%	6.23±0.06	6.30±0.07	6.35±0.09
Protein	%	5.50±0.10	5.50±0.10	5.40±0.10
SCC	n.x10^3	218±140	254±220	277±102
Log SCC	n	5.34±0.3a	5.38±0.5ab	5.44±0.4b

a, b: Different letters indicate significant differences ($P<0.05$) between groups.

In mechanical milking, in addition to the vacuum level, the rate and the ratio of pulsation are also important. A correct suction action and teat massage prevents the formation of edema and congestion, thereby reducing the risk of contracting infections and feeling pain (Pazzona et al., 2005; Salaris et al., 2005).

Milking machine for sheep are normally used with a pulsation rate of 120-180 cycles/min, depending on the breed and production level. A high rate seems to increase the amount of milk from machine stripping, whereas a low rate can increase teat cups drop off. The incidence of mastitis and the udder health are influenced, above all, by pulsation ratio and duration of teat massage. Mammary diseases are positively associated with high pulsation ratio (i.e. when aspiration phase is longer than the massage phase). Currently, a pulsation ratio of 50% is normally adopted (Salaris et al., 2005) In fact, when pulsation ratio was kept at 50%, ewes subjected to pulsation rates of 180 and 120 cycles/min during milking did not differ significantly for milk SCC, variations in teat thickness after milking and incidence of mastitis (Peris et al., 2003b).

In addition to the proper functioning of the milking plant, it is important to favour animal well-being by assuring proper hygiene of the plant and the operator, because this is a critical point for the microbial contamination of the udder and milk, which almost always results in clinical and sub-clinical mastitis. (Pazzona et al., 2005).

Suckling and weaning. The suckling period is a critical stage during which the link between ewe and lamb is estabilished. This relationship is particularly important in the first days postpartum, when the conditions for mutual recognition are laid down.

Maiorano et al. (2009) compared the following systems of suckling techniques and lamb management: i) suckling twice a day until slaughter (C); ii) only maternal milk until 15th day of age, with sheep being machine milked in the afternoon, and then, till slaughter, maternal milk, concentrate and hay ad libitum (T1); iii) only maternal milk until 15th day of age, with sheep being machine milked in the afternoon, and then, from 16th to 30th day of age, maternal milk, concentrate and hay ad libitum, and, from 31st day of age till slaughter, only concentrate and hay ad libitum (T2). Lamb weight and growth did not vary among systems during the trial, except for the period from 15th to 30th day of age, in which these parameters were significantly higher in animals milk-fed twice a day (C) (Table 3.7; Maiorano et al., 2009).

The temporary separation of lambs from their mothers during suckling for 1h/d for a week from the 13th to the 20th day (S1), or from the 20th to the 27th day (S2) of age did not affect neither their growth nor their behavior after weaning (evaluated at 45th day of age; Table 3.8; Simitzis et al., 2012) when subjected to an isolation test (Simitzis et al., 2012).

Table 3.7. Effect of management on lamb growth and on lamb slaughter performance (adapted from Maiorano et al., 2009).

Group	C	T1	T2	SEM
Live weight (kg)				
Day 0	4.9	4.8	4.9	0.2
Day 15	8.6	8.5	8.7	0.3
Day 30	12.3	11.1	11.2	0.3
Slaughter	19.6	19.3	16.8	0.5
ADG (g/d)				
Day 0-15	250	250	252	11
Day 16-30	243a	174b	166b	13
Day 31-slaughter	211	256	172	14
Empty body weight (kg)	18.2	18.5	15.5	0.7
Cold carcass weight (kg)	12.3	12.1	10.0	0.5
Cold dressing (%)	67.7a	65.1b	64.5b	0.8

a, b: Different letters indicate significant differences (P<0.05) between groups.

Table 3.8. Effect of treatment on average heart rate (beats per min, L.S. mean ± S.E.) and on number of vocalizations and jumps during the isolation test (adapted from Simitzis et al., 2012).

Age	Parameters	Treatment		
		C	S1	S2
2 months	Jump	0 (0.0)	0 (0.1)	1 (0.3)
	Vocalizations	15 (12.16)	14 (12.15)	17 (13.18)
	Heart rate	172.8±9.7	178.7±9.7	175.3±9.7
3months	Jump	0 (0.1)	0 (0.6)	1 (0.3)
	Vocalizations	11 (9.16)	10 (9.13)	12 (6.16)
	Heart rate	167.9±8.42	166.6±8.42	167.1±8.42

C=control, S1=lamb isolation for 1 h between the 13th and 20th day of age, S2=lamb isolation for 1 h between the 20th and 27th day of age.

Sheep seem to adapt within three days of temporary separation repeated during suckling. In fact, Cockram et al. (1993) found no significant differences in cortisol, leucocytes, prolactin and beta-endorphin contents between sheep separated temporarily from their lambs and those kept always with their lambs.

Management of the ewe-lamb interaction also influences milk yield and quality. McKusick et al. (2001) observed that ewes separated from their lambs for 15 h during the evening, machine milked once daily in the morning, and then kept with their suckling lambs for 9 h, produced less milk, with a lower fat content, than ewes that had been separated from their lambs after birth, but produced more milk than ewes suckled by their lambs (not machine milked) until weaning. Even after weaning, sheep that had been separated from their lambs after birth continued to produce more milk with a higher fat content than ewes reared in the other two systems. On the other hand, lambs fed artificially from birth had lower growth performance than those that suckled for 9 h/d or those fed exclusively by their mothers.

In a similar study, Dikmen et al. (2007) observed that milk production, even after weaning, was higher in the machine milked (once a day) and suckling ewes (MIX) than in the suckling and not machine-milked ewes (DY 60)., whereas milk fat content did not differ between treatments (Table 3.9; Dikmen et al., 2007). In MIX ewes, milk fat content during the suckling period was about half that found after weaning, due to the stress caused by the daily separation from the lambs. The weight and growth of lambs were not statistically different before and after weaning, although they tended to be higher in lambs kept constantly with their mothers.

Table 3.9. Least-squares means (±SEM) of ewe lactation traits for MIX and DY60 group (adapted from Dikmen et al., 2007).

Parameter	**Weaning system**	
	MIX	**DY60**
Lactation length (day)	171±5.4	168±4.9
Milking period (day)	168±2.7a	110±3.7b
Commercial milk yield	103±7.9a	64±5.4b
60-day milk fat (%)	3.8±0.2	-
Average milk yield (kg/day)	0.634±0.04a	0.568±0.04b
Milk fat (%)	5.7±0.1	5.7±0.1

a, b: Different letters indicate significant differences (P<0.001) between groups.

Weaning has more negative effects when lambs are naturally fed by their mothers than when they are artificially fed. In natural conditions or in sheep meat farms, weaning occurs gradually over a few months, because as the lamb grows up, maternal milk is not sufficient to satisfy its requirements. In this way, the lamb gradually ingests increasing amounts of solid feed until 3-5 months of age, when it will become the only nutrition source for the animal.

Weaning is even a greater problem in dairy sheep farms where precocious weaning and separation of lambs from the ewes are adopted. Nowadays, two weaning techniques are used: progressive or sudden weaning. The first technique consists of training the lambs to ingest solid feeds in association with milk. This is done by increasing gradually the hours of separation of lamb from the mother and by providing appropriate amounts of solid feeds (hay and concentrates) in farms using natural milk feeding, or by reducing gradually the amount of milk provided and providing solid feeds in farms using artificial feeding. The second technique, i.e. the sudden weaning, consists of separating the ewe from the lamb when the latter is old enough to be able to ingest and utilize a sufficient amount of solid feeds, without any type of training.

Regardless of the technique used, weaning is definitely stressful for lambs and suckling ewes. Orgeur et al. (1998) compared the following two techniques for weaning lambs at 3 months of age: i) progressive weaning, by gradually increasing the hours of daily separation of lambs from the mother, starting when lambs were 25 days of age and ending with definitive weaning at 3 months; and ii) sudden weaning at 3 months. The authors found that sheep from sudden weaning only had a higher content in the leukocytes day after the separation than sheep from progressive weaning, with no difference in cortisol levels being observed. In addition, lamb growth during the period of differentiated weaning and afterwards was not influenced by weaning system, probably because feed supplementation during the milking phase favoured the adaptation of lambs from both groups to solid feed.

The stress caused by separation from the mother is certainly related to the age of the lamb, because the ewe-lamb bond decreases with age. In fact, Galeana et al. (2007), monitoring the ewe-lamb interaction on pasture between 35th and 63th day of age of the lambs, found that the distance between the mother and the lamb increased progressively with age. In the first three days after weaning (64 days of age), when lamb and ewe were separated physically but not visually, the sheep reduced rapidly their presence in proximity (<1 meter) of the fence, whereas lambs continued to stand in that location more frequently than the mothers.

Lambs separated early (2 days of age) from the mother, even if fed with artificial feeding, had a lower growth, a higher cortisol level and a worse immune response than lambs separated at a greater age (15 and 28 days of age) (Napolitano et al., 1995).

The technique of weaning also seems to affect the behavior of lambs. Orgeur et al. (1998) observed that the number of vocalizations of lambs and ewes was significantly higher after sudden weaning than after progressive weaning, although 48 h from weaning the frequency of bleats of lambs and, above all, of ewes decreased significantly, in both systems. The visual and auditory contact with their mothers after weaning can be stressful for lambs, with their state of discomfort being expressed by a greater number of bleats (Orgeur et al., 1999).

For better animal welfare, it is important to establish a positive interaction between man and lambs within the time of weaning, because after this moment lambs are in stricter contant with the farmer.

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OBJECTIVE

The production cycle of dairy sheep currently adopted in Sardinia has a century of history, even if some modifications have occurred over time. This system can be traced back to the early 900's, when the industrial processing of milk into cheese, especially *Pecorino Romano* cheese, began to spread in the island.

As milk demand increased, farmers desynchronized the production cycle of sheep from that of pasture. In order to maximize sheep milk yield for commercial purposes, lambing of multiparous ewes became concentrated in autumn, with a major production of the so-called "Christmas lamb", slaughtered at the age of 30 days with a slaughter weight of about 7 kg. In this way, the sheep could be milked immediately after weaning, without constraints due to lamb rearing.

In current rearing systems, the milk produced during early lactation by primiparous and multiparous ewes is normally suckled by newborn lambs (for approximately 30 d). Thereafter, i.e. from weaning to dry-off (by mid-summer), sheep are milked twice a day.

Nevertheless, the farming sheep sector is currently experiencing a worst crisis. For that reason the overall objective of the present thesis is to evaluate the effects of new weaning techniques aimed at increasing profitability of farms keeping the traditional practices consolidated over time and without compromising animal welfare and quality of productions. The study was carried out in two physiological stages: early and late lactation, where margins for improvement profitability are expected.

CHAPTER 4

Effect of partial weaning technique on milk and meat production in Sarda sheep

4.1. Introduction

In Sarda dairy sheep, like in other dairy sheep breeds, the first month of lactation is very important. During this period, milk yield increases until the lactation peak is reached, i.e. within 3-4 weeks from lambing (Cappio-Borlino et al., 1997). During the first 30-40 days of lactation, however, in traditional rearing systems the farmer can make profit exclusively from meat production of slaughtered lambs, because all milk is suckled by the lambs.

In modern systems, the most widely adopted technique is the artificial weaning, which involves both the separation of lambs from the mother immediately after birth (within 24 hours) and the artificial feeding of the lamb with a milk replacer. Therefore, sheep can be milked for commercial purposes from the beginning of lactation. However, a disadvantage of this system is that the amount and quality of meat from artificially fed lambs seems to be lower than that of naturally fed lambs.

Several studies demonstrated that, at the same age or slaughter live weight, artificially fed lambs had a lower growth performance, with a lower carcass weight, and lower fat, n-6 polinsatured fatty acid and CLA contents in meat than naturally fed lambs (Barone et al., 2007; Lanza et al., 2006; Rodríguez et al., 2008).

In addition, even though ewes could be milked immediately after lambing in these systems, artificial milk feeding is not currently adopted because its management costs are considerably higher than those of the natural suckling system. In fact, a feeding regime based on artificial milk is rare in Sardinia, because of the low market price of milk (0.65-0.75 €/l) and the high cost of milk powder (about 0.50 €/l). The high equipment costs, the low growth performance of lambs and the high risks associated with digestive diseases also discourage this practice.

For the above reasons, to increase commercial milk yield during lactation, new mixed management systems, based on both suckling and machine milking, have been recently studied. For example, McKusick et al. (2001) compared the following three weaning systems during the first 30 days post-partum of East Friesian crossbred dairy sheep: i) lambs were weaned at 24 h of age and raised artificially, ewes were machine milked twice a day; ii) from 24 h after birth, lambs were separated from their mothers for 15 h (from evening to morning) and allowed to suckle for the remaining 9 h, ewes were machine milked once a day in the morning until weaning and twice a day after weaning; iii) lambs suckled the ewes, which were not machine milked until weaning and were machine milked twice a day after weaning. Milk yield until weaning from ewes milked once a day was equal to 60% of that from ewes milked twice a day. Differences in lamb growth were observed only after weaning, with lower growth increase in artificially fed lambs than in lambs from the other two rearing systems. Although financial returns of the entire lactation (milk plus meat sales minus feeding plus management costs) were not significantly different among treatments, the mixed weaning system had a numerically higher return (+7.4%) than the natural and the artificial suckling systems.

In a similar experiment on Awassi dairy sheep, Dikmen et al. (2007) evaluated the effects of the following two weaning systems on lamb growth and commercial milk production: i) mixed weaning system, ewes were milked once a day and suckled by their lambs for 9 h during the day until weaning (i.e. 60 days of age), ewes milked twice a day afterwards; ii) natural weaning system, ewes were not milked and were suckled by their lambs until weaning (60 days of age), ewes milked twice a day afterwards. It was concluded that growth performance of lambs was similar in both treatments until weaning, probably due to dietary supplementation offered to mixed-weaning lambs from 15 days of age. Milk production was higher in sheep with mixed system not only until weaning but also afterwards (634 vs. 568 g/d, for mixed weaning and natural weaning, respectively), suggesting that this technique was able to increase by 60% the milk yield in 170 days of lactation.

In crossbred Friesian sheep, Jaeggi et al. (2008) found that ewes from the mixed weaning system, i.e. partial suckling by the lambs (15 hours/day), produced milk with lower fat and solids, with resulting lower cheese yield, than ewes weaned from their lambs at 24 hours postpartum.

The objective of this study is to evaluate the effects of a new weaning technique, aimed at increasing milk yield of Sarda dairy sheep, on lamb growth performance and milk yield after weaning.

4.2. Materials and Methods

4.2.1. Animals and treatments

The trial was carried out in a dairy sheep farm located in Tula (40°44’0’’60 N lat, 08°59’5’’64 E long), northern Sardinia, Italy. Twenty-two multiparous Sarda ewes (2-4 years old) were used. Ewes were homogeneous for age and lambing type, i.e. within a lambing period of 2 days and giving birth to a single male lamb. Animals were handled in the same way during the first 5 days postpartum, in which lambs were kept with their dams to facilitate the establishment of the mother-young relationship, staying on pasture during the day and indoors during the night. From the 5th day postpartum until weaning, ewes were divided into two groups (11 ewes/group) and submitted to one of the following rearing systems:

- Total suckling group (**TSG**), handled according to the traditional farming system adopted for sheep in Sardinia. Lambs were allowed to suckle their mother until slaughtering. Both ewes and lambs were kept on pasture during the day and stalled at night, after the residual daily milk had been milked;
- Partial suckling group (**PSG**), lambs were stalled for 24 hours and separated from their mothers in the morning. Ewes were kept on pasture during the day and stalled with their lambs during the night, after the milk produced during the grazing time had been milked.

The feeding regime was the same for all ewes: herbage grazing from 8.00 to 16.00, concentrate (600 g/head per d) in two daily meals, i.e. before going to pasture and at the evening milking, and hay ad libitum during the night.

TSG lambs suckled maternal milk for 24 hours a day, with the exception of the residual milk removed with milking in the afternoon.

PSG lambs suckled maternal milk only during the night (from 16.00 to 8.00). Milk accumulated during the separation from the mothers (from 8.00 to 16.00) was milked in the evening. From the 5th day of age, **PSG** lambs were supplemented with a concentrate (Table 4.1) to compensate for the lower milk availability. This concentrate was balanced for suckling lambs and available at a market price of 0.40 €/kg.

In both groups, lambs was slaughtered at 10 kg of live weight, corresponding to the typical weight of traditional suckling lamb.

Table 4.1. Chemical composition (%) of the feed offered to lambs from the partial suckling group until weaning.

Parameters	% DM
Dry matter	88.8
Crude protein	17.9
Ether extract	4.2
NDF	33.5
ADF	14.3
ADL	2.7
Ash	8.0

4.2.2. Measurements

Animals

The milk yield and composition were determined weekly during the suckling period and until the 28th day after weaning and also every 48 hours during the first week post-weaning.

The lambs were weighed at birth, at 5 days of age and once a week thereafter until slaughtering. Average daily gain, concentrate intake, slaughtering live weight, carcass weight (hot and cold after 24 h of refrigeration at 4° C) and dressing percentage were also determined.

Milk

Individual milk samples were analyzed for milk fat, protein and casein contents using infrared spectrophotometric method (IRMA) by Milkoscan-605 (Foss-Eletric S.A., Hillerød, Denmark). Urea content was also determined with differential pH method and the somatic cells count (SCC) with Fossomatic 5000 (Foss-Eletric S.A., Hillerød, Denmark).

Meat

The five heaviest lambs of each group were slaughtered, with *Longissimus dorsi* (LD) muscle being excided and analyzed for protein (Kjeldal method) and fat (modified by Folch method) content. Fatty acid profile of LD was determined from the intramuscular lipid fraction extracted according to the Folch method (Clark et al., 1982). Fatty acids were quantified as fatty acid methyl esters (FAME, as suggested by Nudda et al., 2011), which were separated on a gas chromatograph Varian 3400 (Varian Instrument, Palo Alto, California, U.S.A.) equipped with a capillary column (100m x 0.32mm i.d.; 0,25 μm film thickness).

4.2.3. Statistical analysis

Data were analysed using MINITAB® software (Version 12.1, Minitab, State College, PA, USA). A general linear model was used for milk yield, milk fat, protein, casein, urea and SCC contents, and lamb live weight and growth with the following model:

$$y_{ijk} = \mu + group_i + period_j + (group\ x\ period)_{ij} + \varepsilon_{ijk}$$

where y = dependent variable; μ = overall mean; $group_i$ = fixed effect of the treatment (i = TSG, PSG); $period_j$ = fixed effect of the sampling period (j = sampling period); (group x period)ij = fixed effect of the interaction between treatment and sampling; and ε_{ijk} = residual error.

A covariance analyses was conducted for lamb live weight, slaughter weight and dressing percentage, to take into account possible differences in initial body weight of lambs.

Meat fat, protein and fatty acid profile were analyzed according to a one-way analysis of variance using the following model:

$$y_{ij} = \mu + group_i + \varepsilon_{ij}$$

where y_{ij} = dependent variable; μ = overall mean; $group_i$ = fixed effect of the treatment; and ε_{ij}= residual error.

4.3. Results and Discussion

4.3.1. Suckling period

Ewes and lambs of **PSG** group showed an immediate positive attitude to the temporary separation (8.00 to 16.00 h). Separating the ewes from their young before going to pasture in the morning was performed easily. In addition, a calm behaviour and absence of bleat in ewes during the evening milking was observed. These positive elements of welfare evaluation were not verified in ewes of **TSG**, which showed higher bleating activity and a nervous state while milking the residual milk in the presence of their lambs.

Milk production of **PSG** group was significantly higher than that of the **TSG** (645±38 vs. 83±38 g/head/day; P=0.0001) (Table 4.2), because of the daily temporary separation of **PSG** ewes from their lambs. Actually, the milking of **TSG** ewes cannot be considered a regular practice, considering that in the traditional farming systems only ewes with a conspicuous quantity of residual milk are milked.

During the suckling period, milk protein, casein, urea and SSC were not influenced by management system, whereas milk fat content was higher in **TSG** than in **PSG** ewes (6.3%±0.3 vs. 4.6%±0.2; P=0.0001) (Table 4.2). This may be partially due to the concentration effect (Pulina et al., 2006), associated with the low milk yield of **TSG** ewes, and due to the separation stress. Indeed, McKusick et al. (2001, 2002) and Jaeggi et al. (2008) compared the milk quality of ewes weaned from their lambs after birth with that of ewes machine milked once a day, with their lambs allowed to suckle for 9 hours/d, and found that mother-young separation inhibits the total ejection of milk and also that of fat, particularly abundant in the last fractions of ejected milk.

Table 4.2. Milk production (g/head/d; mean ± s.d.) of Sarda ewes from total suckling (TSG) and partial suckling (PSG) groups during the suckling period.

Parameters		TSG	PSG	Significance (P)		
				M	S	MxS
Milk	g/d	83±38**b**	645±38**a**	<0.001	0.503	0.198
Fat	%	6.3±0.3**a**	4.6±0.2**b**	<0.001	0.615	0.297
Protein	%	5.0±0.1	5.1±0.1	0.430	0.134	0.338
Casein	%	4.0±0.1	4.0±0.1	0.526	0.283	0.433
Urea	mg/dl	49±2	48±2	0.520	0.002	0.110
Somatic cells count	n.x10^3	618±286	131±265	0.220	0.448	0.409

M=management; S=sampling period
a,b: Different letters indicate significant differences (P<0.05) between treatments.

The optimal slaughtering weight of the lambs was obtained at 25 days of age, when lambs of both groups reached an average live weight just above 11 kg (Table 4.3). Overall, it can be stated that the slaughtering age of lambs from both groups was lower than the 30 days normally required by Sarda suckling lambs.

This result can be attributed to several factors as follows:

- in the **TSG** group, the favorable environmental conditions, i.e. good temperatures and pasture abundance during the trial, very likely favored milk production and, consequently, lamb growth. Moreover, it is likely that under those favorable temperatures less energy was spent by lambs for thermoregulation to limit the negative effects of adverse weather conditions, as previously found by Peana et al. (2007);
- in the **PSG** group, the management conditions adopted allowed lambs to achieve the same slaughtering weight of **TSG** group, because the lower

amount of available milk was likely compensated by the supplementation with concentrate and a lower energy consumption for moving and thermoregulation. This result is in agreement with that observed by Dikmen et al. (2007) in lambs separated from their mothers and fed concentrate from the 15th day of age.

The effect of management on lamb growth varied according to the period considered as follows:

- similar average daily growth (ADG) between **TSG** and **PSG** lambs in the first 5 days of life (about 300 g/head/d) (Table 4.3.) when both groups were managed in the same way (lambs and mother kept together on pasture during the day and housed during the night);
- higher daily growth in **TSG** lambs than **PSG** (277±17 vs. 190±17 g/head/d; P<0.05), between the first and second weeks of age, which coincided with the first week of temporary daily separation of **PSG** lambs from their mothers;
- similar growth rates between **TSG** and **PSG** lambs (about 250 g/head/d) during the third week of life;
- higher growth rates in **PSG** than **TSG** lambs (318±17 vs 210±18 g/head/d) in the last rearing week before slaughtering (Table 4.3).

Over time, the daily growth of **TSG** lambs decreased progressively, whereas that of **PSG** lambs was significantly higher on the 25th day than on the 12th day of age, with the ADG on the 5th and 19th having an intermediate value.

The different pattern between the two groups may be due to the following reasons:

- during the initial experimental period, the low concentrate feed intake of **PSG** lambs (23 g/head/d) was likely caused, as reported by Napolitano et al. (2008), by both the stress of mother-young separation and the inability of lambs to use high amounts of solid feed at such an early age. Thus, the decreased milk availability to **PSG** lambs, due to the temporary separation from the mother, probably was only partially compensated by concentrate intake.
- during the last experimental period, the amount of available milk was likely inadequate to satisfy the increased requirements of **TSG** lambs. Conversely, **PSG** lambs probably met their needs by increasing concentrate intake (from 23 g/head/d on 12 th day to 117 g/head/d on 25th day), thus showing higher ADG than **TSG** lambs (Table 4.3).

Table 4.3. Weight and daily growth rate of lambs from total suckling (TSG) and partial suckling (PSG) groups during the suckling period.

Sampling period	TSG		PSG		
	Live weight Kg	**ADG g/d**	**Live weight Kg**	**ADG g/d**	**Concentrated food intake g/head/d**
Birth	5.0±0.8	-	5.0±0.8	-	-
5th day	6.1±0.2	320±17**a**	6.5±0.2	304±17**ab**	-
12 th day	8.3±0.2	277±17**ab***	8.0±0.2	190±17**b***	23
19 th day	10.0±0.2	243±17**b**	10.0±0.2	257±17**ab**	54
25 th day	11.1±0.2	210±18**b***	11.4±0.2	318±17**a***	117
Average		***263±9***		***267±9***	***65***

a,b: Different letters indicate significant differences (P<0.05) between sampling period within treatment.
*: significant differences between treatment within sampling period (P<0.05)

At slaughtering (Table 4.4), both groups of lambs showed similar values for all parameters evaluated, with the exception of the cold dressing out, which was significantly higher, in **TSG** lambs than in **PSG** lambs (65.5%±1.8 vs. 60.0%±1.8; P<0.05). This may be due to the numerically, even if not statistically significant, lower slaughter weight and greater drip loss observed in the lambs of the **PSG** group.

Table 4.4. Weights at slaughter, dripping losses and dressing percentage of lambs from total suckling (TSG) and partial suckling (PSG) groups.

Parameters		**TSG**	**PSG**
Slaughter weight	Kg	11.0±0.3	11.4±0.3
Hot carcass weight	Kg	7.4±0.3	7.0±0.3
Cold carcass weight	Kg	7.2±0.3	6.8±0.3
Drip loss	%	2.3±0.2	2.9±0.2
Cold dressing out	%	65.5±1.8**a**	60.0±1.8**b**

a,b: Different letters indicate significant differences (P<0.05) between treatments.

The chemical composition (meat fat and protein content) and fatty acid profile of *Longissimus dorsi* muscle were not influenced (P>0.05) by management (Table 4.5). Even if not statistically significant, meat from **TSG** lambs had higher values of unsatured fatty acids content (UFA) than meat from **PSG** (57.28%±2.08 vs. 55.53%±1.83; P>0.05), probably because of the numerically higher content in PUFA of the omega 6 and omega 3 series in TSG lambs. Meat from TSG also had slightly lower, but not significant, omega 6/omega 3 ratio than lambs allowed to suckle maternal milk 24 hours/day. Because of the the nutraceutical properties of polyunsaturated fatty acids, in particular those of the omega 3 series (Parodi, 1997; Belury, 2002), it is important to monitor their content in meat in animal welfare studies. In our experiment, partial weaning did not affect significantly lamb meat quality.

Table 4.5. Meat chemical composition and fatty acid profile of lambs from total suckling (TSG) and partial suckling (PSG) groups.

Parameters		**TSG**	**PSG**
Fat	%	2.31±0.43	1.98±0.20
Protein	%	20.42±0.25	20.31±0.41
Fatty acid (g/100g FAME):			
PUFA n-6	%	9.98±1.71	8.79±1.40
PUFA n-3	%	3.65±1.00	2.82±0.93
MUFA	%	43.66±2.38	43.92±1.69
UFA	%	57.28±2.08	55.53±1.83
SFA	%	42.72±2.08	44.47±1.83
CLA	%	1.18±0.20	1.23±0.11
n6/n3	%	2.79±0.30	3.27±0.66
SFA/UFA	%	0.75±0.06	0.80±0.06

PUFA=polyunsaturated fatty acids; MUFA=monounsaturated fatty acids; UFA=unsaturated fatty acids; SFA=saturated fatty acids

4.3.2. Milking period

The different management system of lactating sheep during suckling affected their milk yield even after the slaughtering of lambs. In fact, in the first month of lactation after slaughtering, **PSG** ewes had a significantly higher milk production than **TSG** ones (1534±36 vs 1366±38 g/d; P<0.05) (Table 4.6).

In both groups, the milk production showed a decreasing trend after weaning, mainly due to the end of the lactation peak (Cappio-Borlino et al., 1997) and partially due to the occasional adverse climatic conditions (i.e. lower temperature, windy conditions, frost). Milk yield at the various sampling dates had higher numerical values in **PSG** ewes than in **TSG**, although such differences were not significant (Figure 4.1). In particular, **PSG** ewes showed some reduction in the milk yield during the first 3 days post-weaning and a more stable pattern in the subsequent period in respect to **TSG** ewes.

Table 4.6. Milk yield and composition of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

Parameters		TSG	PSG	Significance (P)		
				M	S	MxS
Milk	g/d	1366±38**b**	1534±36**a**	0.002	0.032	0.950
Fat	%	5.6±0.1	5.4±0.1	0.126	0.000	0.968
Protein	%	5.0±0.1	5.1±0.1	0.245	0.000	0.976
Casein	%	4.0±0.0	4.0±0.0	0.301	0.000	0.978
Urea	mg/dl	46±1**a**	44±1**b**	0.027	0.000	0.979
Somatic cells count	n.x10^3	200±79	376±75	0.107	0.085	0.900

M=management; S=sampling period
a,b: Different letters indicate significant differences (P < 0.05) between treatments.

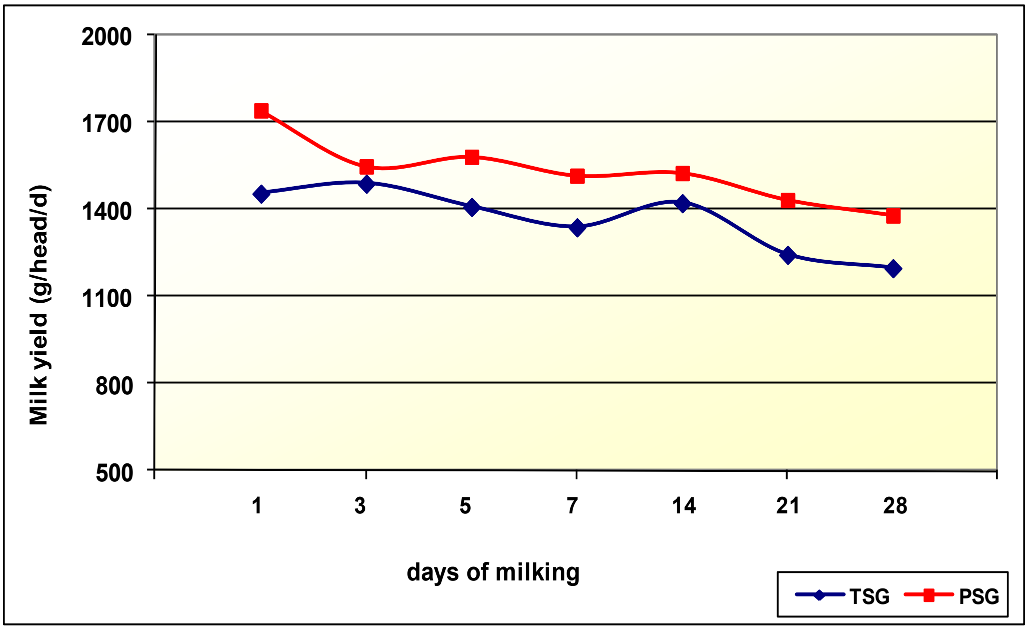

Figure 4.1. Evolution of milk yield of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

The different rearing techniques adopted during the suckling period did not influence milk composition, except for the milk urea content, which was lower in **PSG** than in **TSG** ewes (44±1 vs. 46±1 mg/dl; P<0.05) (Table 4.6). Regardless of the rearing system, on average, milk content of fat, protein, and casein of the ewes were 5.5%, 5.0% and 4.0%, respectively. Somatic cell count tended to be higher in **PSG** ewes than in **TSG** ones (376±75 vs. 200±79 n.x10^3/ml; P=0.107). Milk composition varied significantly (P<0.05) over time, except for SSC (Table 4.6; Figures 4.2-4.5)In particular, milk fat content was very low (4.1% in **TSG** and 3.7% in **PSG**) on the first day of milking after weaning, increased significantly until the 3rd day of milking in **PSG** and **TSG** ewes, and did no change significantly afterwards (Figure 4.2). The low content of fat in milk of all ewes on the first day after weaning could be attributed in part to the stress caused by the separation of ewes from their lamb, which was shown to inhibit milk fat content (McKusick et al., 2002), and, in a small part, by the increased production of milk during the first days after weaning, with its consequent dilution effect (Pulina et al., 2006).

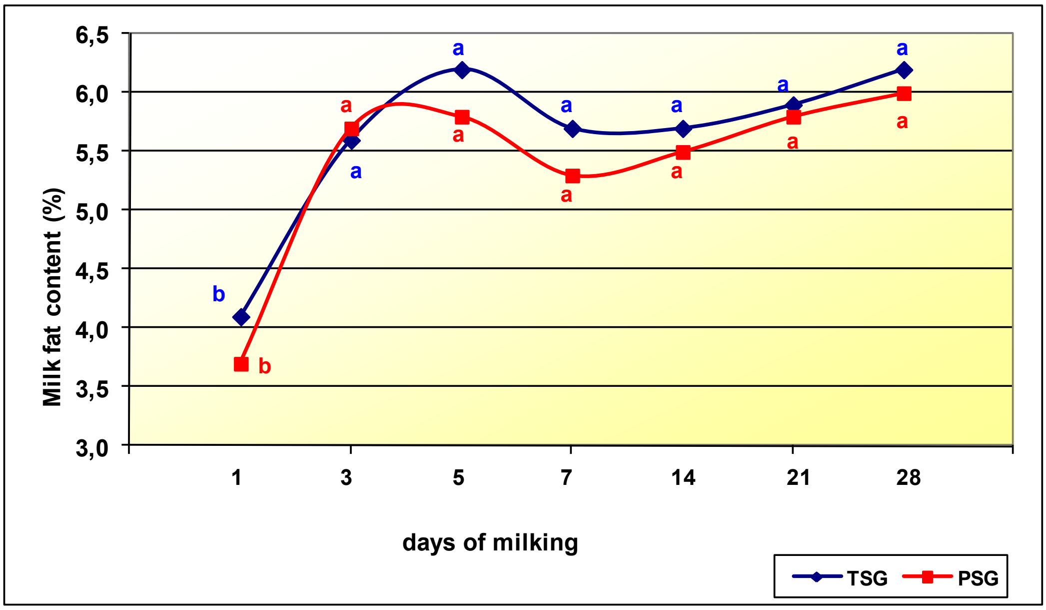

Figure 4.2. Evolution of milk fat content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

Milk content of protein and nitrogen fractions, during the 4 weeks after weaning, varied as follows:

- milk protein content increased in both groups, although this growth was significant only in the **TSG** group (Figure 4.3). This is probably due to the greater reduction in milk yield observed in this group than in **PSG** during the first month of milking;

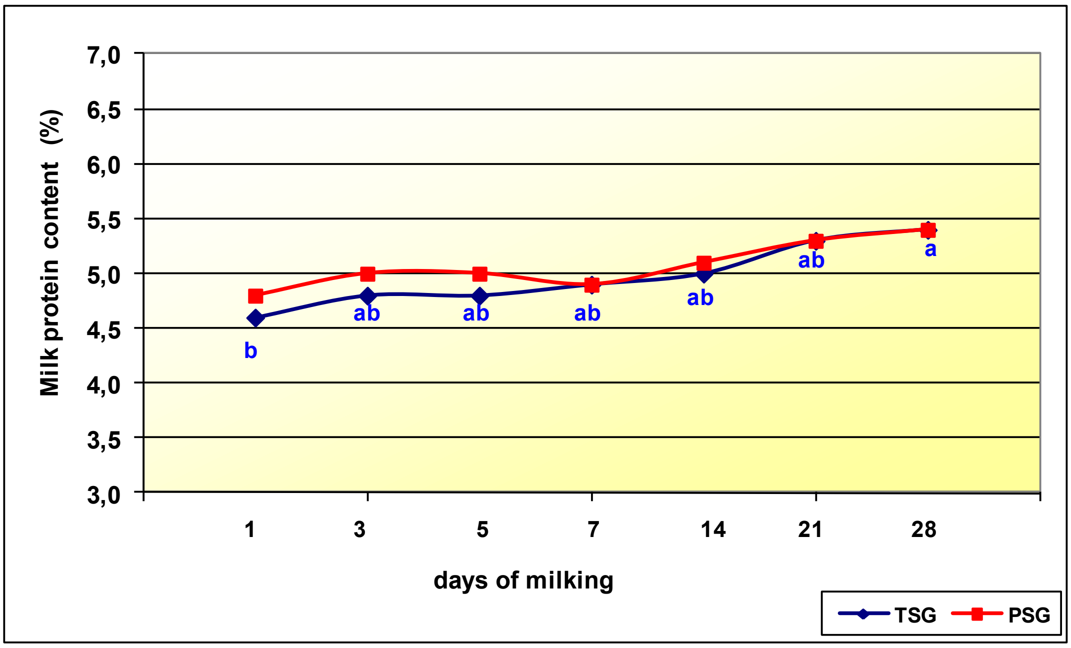

Figure 4.3. Evolution of milk protein content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

- milk content of casein and urea (Figures 4.4 and 4.5, respectively) showed significant and more marked variations than those observed for total protein. In particular, casein increased, whereas urea decreased over time. This fact may be attributed to variations in the diet, especially herbage quality considering that the quality of concentrate and hay did not change.

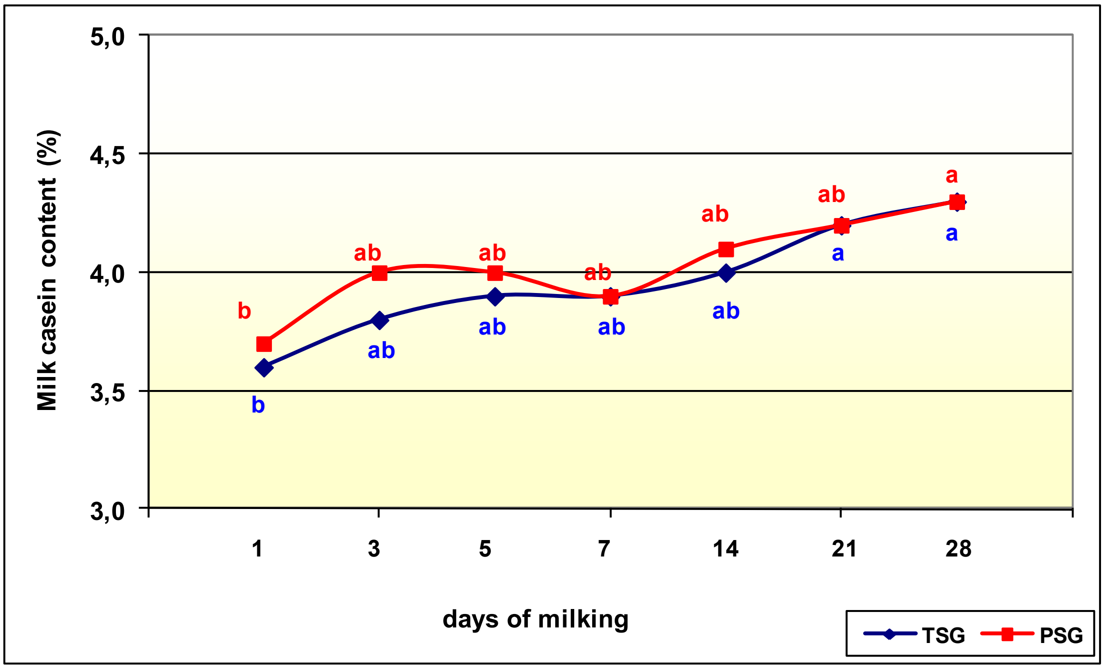

Figure 4.4. Evolution of milk casein content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

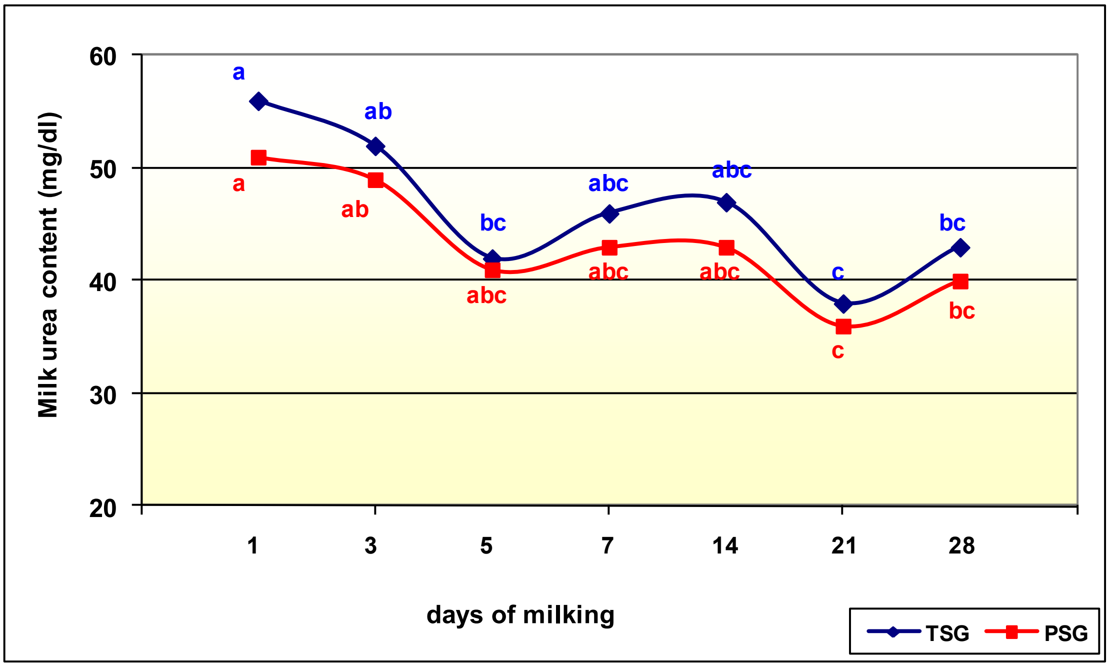

Figure 4.5. Evolution of milk urea content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

The milk SCC content tended to be influenced by sampling (P=0.085). It was similar in the first 14 days of milking and tended to increase during the last two weeks of the experiment, especially in **PSG** ewes, although such changes were not significant. These results are in agreement with those observed by McKusik et al. (2001) who found that management system of lambs and ewes after birth had no effect on the SCC content after weaning. The SCC values detected in our experiment were lower than average values found in Sardinian dairy farms, which are usually more than one million.

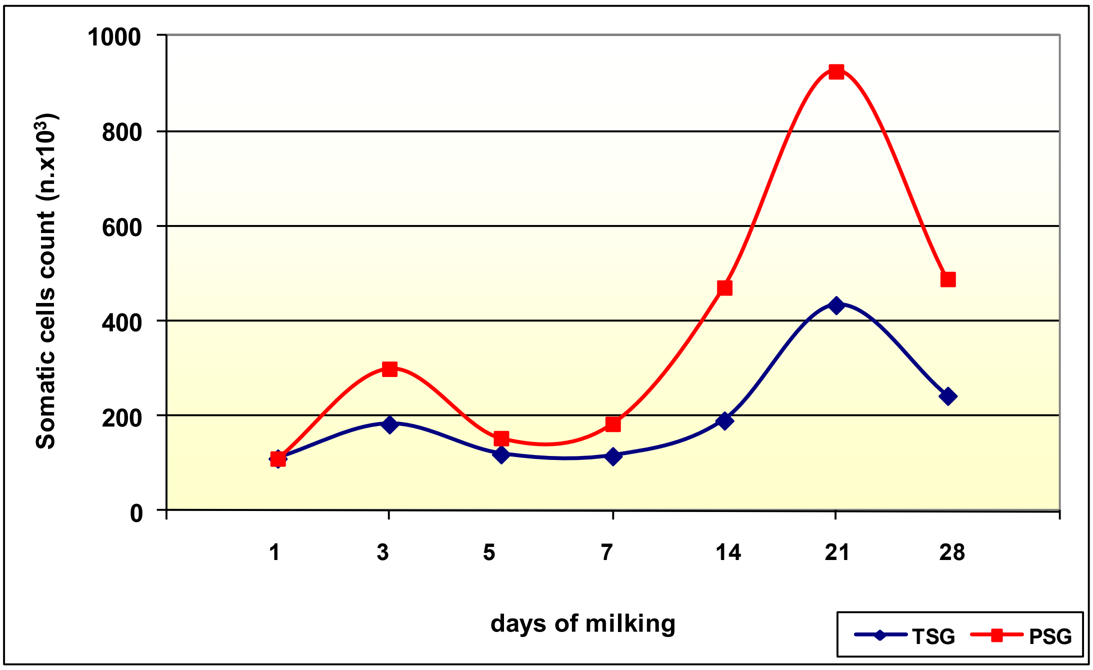

Figure 4.6. Evolution of milk somatic cells count of ewes from total suckling (TSG) and partial suckling (PSG) groups during the first month of lactation after weaning.

Overall, the partial suckling management positively affected milk yield in the first month after weaning without compromising its quality. This could be attributed to the fact that sheep kept on pasture without lamb spent more time grazing and, consequently, were able to maintain a better body condition even after weaning, with a consequent better milk production efficiency.

4.4. Conclusions

The adoption of the partial suckling technique, by milking ewes once a day from the 5th day after parturition until weaning, allowed to recover 11-14 l/ewe of milk more than the traditional farming system, in which the lamb was kept together with the mother during the entire suckling period. This new rearing system did not affect the meat production and quality. In addition, it favored milk production even during the first month after weaning.

Since most of the births of lamb are normally concentrated in late autumn-early winter, it is expected that, in adverse weather conditions, the advantages of the new rearing system in terms of milk and meat production efficiency would be greater than those obtained in our experiment, which was conducted in optimal climatic conditions.

It must be added that the technique under study is characterized by simplicity of application. In fact, it could be easily implemented in farms equipped with stall, by building only mobile fences that can separate the ewes from their lambs, as well as in those without stall, by building economic and mobile huts (similar to those for pig raised outdoors) in which lambs can stay and be supplemented during the time of separation from their dams.

4.5. References

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CHAPTER 5

Effects of partial suckling technique on milk production, growth and behaviour of Sarda replacement ewe lambs

5.1. Introduction

In Sardinian traditional farming systems, replacement ewe lambs are chosen among those born in autumn, naturally suckled until 40-50 days of age and then weaned. The weaning can be done suddenly or progressively, by separating female lambs from their mothers at approximately 30 days of age. As other farm animal species, farm lambs are normally weaned at much younger ages than in natural conditions, and sometimes face different kinds of problems (physiological, behavioral, healthy etc.; Weary et al., 2008).

Napolitano et al. (2008) observed that the pair ewe/lamb is in very close contact until 60 days of age. When lambs grow, there is a gradual reduction in the frequency (from 36 suckles per day at 1–2 weeks of age to 14 at 6–7 weeks) and duration (from 41 s per bout at 1–2 weeks of age to 12 s at 6–7 weeks) of suckling.

After weaning, the ewes are milked twice a day for almost the entire of lactation, which usually ends in mid-summer with the dry-off period. Weaned lambs are kept in stall equipped with paddock for at least one month and fed hay and concentrate, in order to ensure an adequate feeding regimen, to limit the negative effects of separation from the mother and to train the animals to human presence.

In this traditional rearing system, the milk produced during the first 40 days of lactation is given to lambs and, therefore, cannot be used for commercial purposes.

The most common alternative, used until recently, was the artificial weaning with a milk replacer. From one hand, this technique allows the farmers to milk the ewes immediately after lambing, but on the other hand, it increases management costs and causes different problems in lambs, such as immunosuppression, lower growth and abnormal behavior (e.g. sucking objects or anatomical parts of other lambs). Other negative effects attributed to the absence of the mother during the suckling period are the reduced level of vocalizations and the propensity to decrease the ambulatory behavior (Napolitano et al., 2008).

Several techniques have been studied to reduce the stress associated with artificial rearing, such as the administration of a mixture composed by 50% of artificial milk and 50% of natural milk in the first week after the separation of the lambs from their dams (Sevi et al., 1999). Lambs fed a milk-mixture showed higher growth, better immune responses, lower blood levels of cortisol and increased ambulatory activity than artificially fed ones.

In the last decade, new rearing techniques have been proposed aimed at improving the production of commercial milk and reducing the negative stress effects associated to the early separation of the lambs from their mothers and to the use of milk replacer.

McKusick et al. (2001), evaluated the effects of natural suckling, i.e. lambs always kept with the mother or suckling only 9 hours/d and being separated from the mother for the rest of the day, and artificial suckling. It was observed that the rearing system adopted did not affect the live weight and growth at weaning (about 30 days of age) of the lambs. On the contrary, at 120 days of age, the naturally fed lambs showed higher live weight than artificially fed ones (kg 47.3±1.6 vs. kg 43.7±1.2), with an intermediate live weight being observed in partially suckled lambs (kg 45.9±1.8). The duration of lactation was similar between groups even if the milking period was lower in ewes that permanently suckled their lambs. This led to a significantly higher commercial milk yield in ewes that did not suckle, compared to partially and naturally suckled ewes (kg 260±10, 236±9 and 172±10, respectively), associated to a lower milk fat content. The more profitable rearing system was the partial suckling compared to the artificial one because of the higher costs connected to the second system.

In a similar trial, conducted by Dickmen et al. (2007), lambs were allowed to suckle permanently or partially (in the morning for 9 hours/day). Similar weight at weaning (60 day of age) and daily growth were found, but these parameters tended to be higher in permanently suckled lambs at older age than in the others. Milk production was significantly higher in partially suckled ewes than in the counterpart (kg 103±8 vs. 64±5). whereas milk fat content did not differ.

All the rearing systems previously described adopted the partial suckling with both ewes and lambs being confined in stall and separated from evening until the moment of milking in the morning

Since these systems do not fit the Sardinian sheep farming system, this experiment aimed to evaluate the effect of partial suckling with grazing sheep on dairy sheep milk production and composition, and lamb growth and behavior after weaning. The partial suckling system tested consisted of keeping the ewes alone on pasture during the day and in stall during the night with the lambs, which were confined from the 5th day of age.

5.2. Materials and Methods

5.2.1. Animals and treatments

The trial was carried out in a dairy sheep farm located in Tula (40°44’0’’60 N lat., 08°59’5’’64 E long.), northern Sardinia, Italy. Twenty-six multiparous Sarda ewes (2-4 years old) were used. Ewes were homogeneous for age and lambing type, i.e. within a lambing period of 2 days and giving birth to a single female lamb. Animals were handled in the same way during the first 5 days postpartum, in which lambs were kept with their dams to facilitate the establishment of the mother-young relationship, staying on pasture during the day and indoors during the night. From the 5th day postpartum until weaning, ewes were divided into two groups (13 ewes/group) and submitted to one of the following rearing systems:

- Total suckling group (**TSG**), handled according to the traditional farming system adopted for sheep in Sardinia. Lambs were allowed to suckle their mother until two weeks before weaning. Both ewes and lambs were kept on pasture during the day and stalled at night, after the residual daily milk had been milked;
- Partial suckling group (**PSG**), lambs were stalled for 24 hours and separated from their mothers in the morning. Ewes were kept on pasture during the day and stalled with their lambs during the night, after the milk produced during the grazing time had been milked.

During the two weeks before weaning, that is before the total separation of lambs from the mothers, all the animals were managed in the same way using the **PSG** rearing technique, in order to stimulate the intake of solid food in all lambs and to limit the stress of separation in **TSG** lambs.

The feeding regime was the same for all ewes: herbage grazing from 8.00 to 16.00, concentrate (600 g/head per d) in two daily meals, i.e. before going to pasture and at the evening milking, and hay ad libitum during the night.

PSG lambs suckled maternal milk only during the night (from 16.00 to 8.00). Milk accumulated during the separation from the mothers (from 8.00 to 16.00) was milked in the evening. From the 5^{th} day of age, **PSG** lambs were supplemented with a concentrate to compensate for the lower milk availability (Table 5.1). This concentrate was balanced for suckling lambs and available at a market price of 0.40 €/kg.

TSG lambs suckled maternal milk for 24 hours a day, with the exception of the residual milk removed with milking in the afternoon, until two weeks before weaning and subsequently were offered the same feed used for **PSG** lambs, so that the two groups were handled in the same way.

Table 5.1. Chemical composition (%) of the feed offered to lambs from the partial suckling group until weaning and to those of the total suckling group during the two weeks before weaning.

Parameters	**% DM**
Dry matter	88.8
Crude protein	17.9
Ether extract	4.2
NDF	33.5
ADF	14.3
ADL	2.7
Ash	8.0

In order to train the lambs to ingest solid food and to facilitate their daily separation from their mothers a small fence was built inside the box where all of them were confined. This fence was accessible only to lambs and there, both feed and water were present.

5.2.2. Measurements and sample collection

Animals

The lambs were weighed at birth, at 5 days of age and once a week thereafter until weaning. Average daily gain and the feed consumption were also determined.

Milk

Milk yield and composition were determined weekly from the suckling period until the 20th day after weaning and also every 48 hours during the first week post-weaning.

Individual milk samples were analyzed for milk fat, protein and casein contents with infrared spectrophotometric method (IRMA) by Milkoscan-605 (Foss-Eletric S.A., Hillerød, Denmark), for urea content with differential pH method and for somatic cells count (SCC) with Fossomatic 5000 (Foss-Eletric S.A., Hillerød, Denmark).

Behavior

An Arena test was performed to evaluate the effects of the breeding method on the behavior of female lambs during the suckling period, especially the level of fear of or confidence on humans.

The Arena test was performed one week after weaning in the box where the animals were confined during the entire period of pre-weaning. The test adopted was modification of that proposed by Beausoleil et al. (2008), creating a pen or Arena (Figure 5.1.), 3.0 meters wide, 7.0 meters long and with the side walls covered with a cloth up to 1.5 meters in height. The box was divided into 5 zones with colorful stripes, 1.4 meters apart from each other.

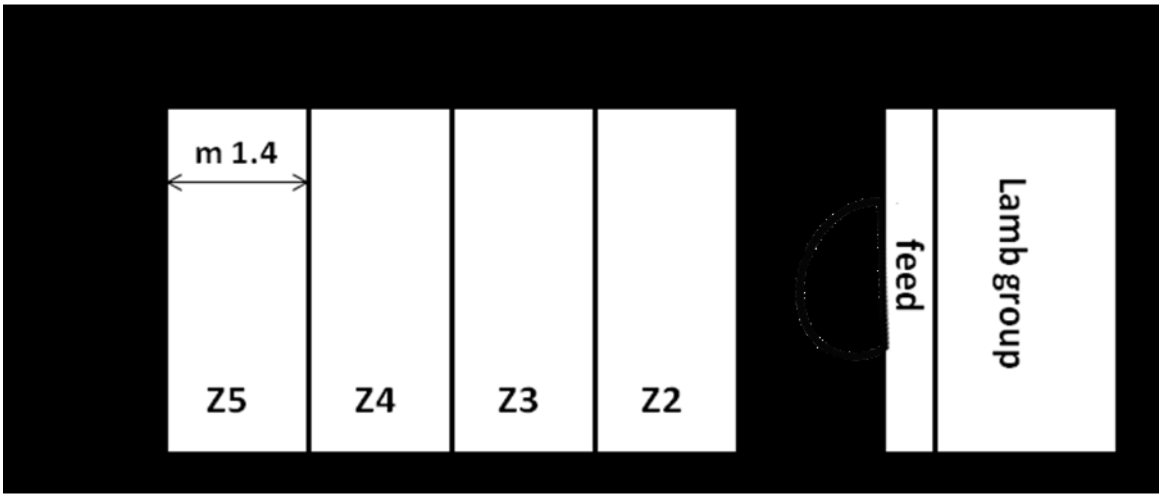

Figure 5.1. Dimensions of test Arena. Zone lines were marked on the floor of the Arena in paint. Z0 marks the position of human. The test lamb could move anywhere in Zones 1–5 and was physically (but not visually) separated from the group sheep by wooden fence. The sides of the Arena were 1.5 m high and covered in shade cloth to create a visual barrier

In **Zone 0** of Arena, localized at the end of the pen, inside a traced semicircle of a diameter of 0.80 meters, there was a not familiar man for the lambs. Behind him, there was a feeding trough where the female lambs could find their feed, and a group of lambs (separated by a fence to prevent physical contact) which served to attracted the lambs tested.

The female lamb to be evaluated was introduced into the box in the opposite side to Zone 0, where it remained for 3 minutes, during which its behavior was filmed by a video camera located outside the box.

At the end of the video shooting, the video of all tests were analyzed, taken into account the following parameters of behavioral assessment as proposed by Beausoleil et al. (2008): **Mean distance to group sheep**, considering at 15-second intervals in which of the five areas of the arena the lamb was; **Time in Zone 0**, which indicates the confidence on man; **Zones crossed**, which indicates the motor activity of the animals; **Latency to sniff human**, which indicates the time that the animal takes to get in touch with the man; **Bleats,** whose number detected during the test is an indicator of the state of psychic activity of the animal (Table 5.2; Beausoleil et al., 2008).

Table 5.2. Parameters of individual sheep behaviour measured in an Arena test with stationary human and a group of flockmates present (adapted from Beausoleil et al., 2008).

Behaviour	Definition of behaviour
Mean distance to group of lambs	Mean distance (m) test the lamb maintained from the group of lambs during the test. Calculated by multiplying the distance from the group sheep (estimated by recording the zone occupied by test sheep's right front foot every 15 s) by the proportion of time spent there.
Time in Zone 0	The amount of time (min) test sheep spent in the zone closest to the human (within 0.8 m).
Zones Crossed	Number of times the sheep crossed from one zone of the arena into another, defined as moving the right front foot across a zone boundary
Latency to sniff human	Time taken (min) for test sheep to first sniff the human
Bleats	Vocalizations

5.2.3. Statistical analysis

Data were analysed using MINITAB® software (Version 12.1, Minitab, State College, PA, USA). A general linear model was used for milk yield, milk fat, protein, casein, urea and SCC contents, and lamb live weight and growth with the following model:

$$\mathbf{y_{ijk} = \mu + group_i + period_j + (group\ x\ period)_{ij} + \varepsilon_{ijk}}$$

where y = dependent variable; μ = overall mean; $group_i$ = fixed effect of the treatment (i = TSG, PSG); $period_j$ = fixed effect of the sampling period (j =

sampling period); (group x period)ij = fixed effect of the interaction between treatment and sampling; and ε_{ijk} = residual error.

A covariance analyses was conducted only for lamb live weight, to take into account possible differences in initial body weight of lambs.

Since the data related to behavioral parameters failed to meet the assumptions of homogeneity of variance and normal distribution, they were analyzed by a non-parametric analysis of variance (ANOVA). The five behavioral measures. i.e. Average Distance, Time in Zone 0, Zone crossed, Number of bleats, Latency time to sniff human, were analyzed by Mann-Whitney U test. The group was entered as a main effect in the model.

In order to analyse the potential reason on the basis of the behaviour pattern of the Arena test, a multivariate principal component analysis of factors was performed. The five types of behaviour were included in the analysis. Factors with eigenvalues greater than 1.0 were retained. In the same way, the amount of variance explained by them, and the interpretability of the factor structure were used to determine the number of factors to be retained.

These factors were rotated using Varimax rotation to facilitate interpretation. Only factor loadings of 0.700 or higher were considered in the interpretation of each factor.

5.3. Results and Discussion

5.3.1. Suckling period

In this trial we observed a the successful and rapid adaptive response of ewes and lambs of the **PSG** group to the new technique. A similar behavior was noticed in our previous experiment described in chapter 4.

The breeder was able to separate easily the lambs from the ewes each morning and to perform calmly the afternoon milking of the sheep. **PSG** ewes showed a calm behavior (not urinating, not kicking and not bleating), contrary to the **TSG** ewes, which showed higher bleating activity and a nervous state during the milking of the residual milk in the presence of their lambs.

Milk production of **PSG** ewes was significantly higher than that of the **TSG** (641±26 vs. 103±26 g/head/d; $P<0.001$) (Table 5.3), because of the daily temporary separation of **PSG** ewes from their lambs. This can be explained by the fact that these ewes performed a complete milking each day, due to the separation of lambs for 8 hours daily, contrary to the **TSG** ewes, which were milked only the residual milk because .the lambs were always with them.

For the milk quality, significant differences were observed only in fat content, which was higher for **TSG** than **PSG** ewes (6.5%±0.2 vs. 5.3%±0.1; $P<0.001$) (Table 5.3). The reasons for this difference, as reported in a previous experimental test (chapter 4), were likely the concentration effect and the stress effect of the separation from the lambs. As observed by McKusick et al. (2001 and 2002) and by Jaeggi et al. (2008), this can restrict the passage of milk and, therefore, of fat, particularly abundant in the last fractions of milk from the udder.

Table 5.3. Milk yield and composition (g/head/d; mean ± s.d.) of Sarda ewes from total suckling (TSG) and partial suckling (PSG) groups during the suckling period.

Parameters		TSG	PSG	Significance (P)		
				M	S	MxS
Milk	g/d	103±26**b**	641±26**a**	<0.001	0.422	0.779
Fat	%	6.5±0.2**a**	5.3±0.1**b**	<0.001	0.274	0.712
Protein	%	5.0±0.8	5.1±0.6	0.645	0.285	0.331
Casein	%	4.0±0.1	4.0±0.1	0.730	0.339	0.300
Urea	mg/dl	46±2	45±1	0.590	0.450	0.159
Somatic cells count	$n.x10^3$	223±380	737±247	0.261	0.862	0.898

M=management; S=sampling period; MxS=management and sampling period interaction

a,b: Different letters indicate significant differences (P<0.05) between treatments.

This was confirmed by the values of fat content on the week before weaning, when the two groups were managed in the same way, i.e. daily lambs/ewes separation and total milking of ewes before the union with their daughters in the evening. In particular, the fat content was significantly lower in **TSG** ewes than in **PSG** ewes (3.9%±0.9 vs. 5.0%±0.9; P<0.001), mainly due to the stress caused by temporary separation, because the **TSG** ewes were not trained as those of the other group. No significant differences (P>0.05) were observed for the other parameters between the two groups, with milk yield of 625±152 and 673±127 g/d (P=0.388), protein 5.2%±0.5 and 5.2%±0.3 (P=0.879), casein 4.1%±0.4 and 4.1%±0.2 (P=0.848), urea 52±7 and 47±10 mg/dl (P=0.127) and SCC 246±531 and 334±552 $n.x10^3$ (P=0.684) for **TSG** and **PSG** groups, respectively.

The weight of lambs increased linearly from birth to weaning, being statistically different between sampling within group, but not between groups, even if growth rates were sometimes numerically different between the later (Figure 5.2).

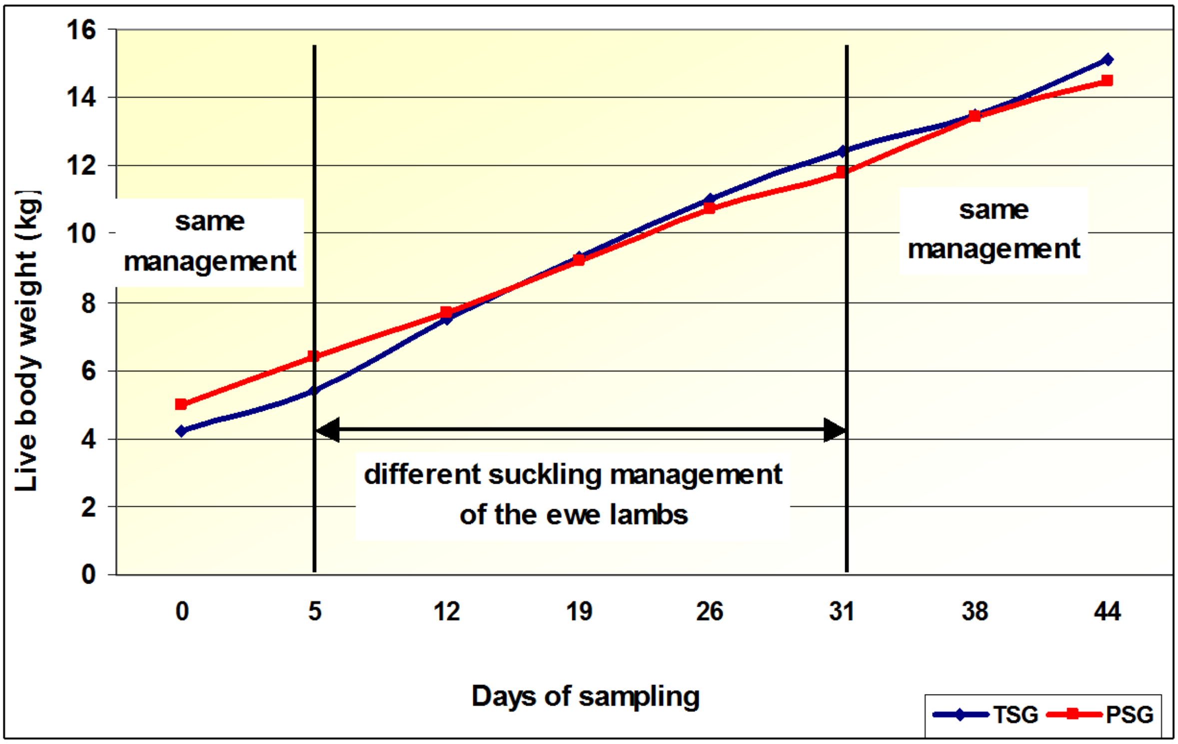

Figure 5.2. Evolution in body weight of lambs from total suckling (TSG) and partial suckling (PSG) groups during the suckling period and at weaning.

The weight of **TSG** and **PSG** lambs, recorded at different ages, were, respectively, the folllowing:

- at birth: 4.2±0.4 and 5.0±0.4 kg;
- day 5: 5.4±0.4 and 6.4±0.4 kg;
- day 31: 12.4±0.4 and 11.8±0.4 kg;
- weaning day: 15.1±0.4 and 14.5±0.4 kg.

The average daily growth (ADG) during the suckling period was significantly higher in **TSG** lambs than in **PSG** lambs (254±6 vs. 220±6 g/d respectively; P<0.05) (Table 5.4), due to the different growth rate during the first week of the experiment because:

- in the first 5 days post-partum — when the lambs in both groups were handled in the same way, and they were always with the mother, to ensure as much as possible the bond sheep/ewe lamb — the growth was similar between both groups, although slightly higher in **PSG** than in **TSG** lambs (278±15 vs. 230±15 g/d; P>0.05) (Table 5.4);
- between 5th and 31th days of age, significant effects of treatment were observed only in the first week when the lambs of the **PSG** group showed statistically lower growth rates than **TSG** lambs (164 ±15 vs. 274±15 g/head/d; P<0.05) (Table 5.4).

This can be attributed to the stress caused at the beginning of the temporary separation and also to the inability of lambs at this age to ingest a sufficient quantity of concentrated feed specific for lambs during the suckling period, such as to compensate for the reduced milk availability compared to lambs of **TSG** group. This is in accordance with the study of Napolitano et al. (1995), in which lambs separated precociously from the ewe had a reduced growth due to a nutritional stress cause by low ability to feed properly in the first days of separation.

In fact, although in the first week the ewe lambs of **PSG** group showed a daily concentrate intake of 23 g/head/d, in the next weeks this ingestion increased noticeably and arrived to 160 g/head/d in the 4th week of differentiated treatment. This allowed the **PSG** lambs to grow at a rate similar to that of the **TSG** lambs.

In the last two weeks before the weaning (from 31st to 44th days of age) — when the ewe lambs of both groups were handled and fed in the same way, all following the group management of the **PSG** — the overall growth of lambs was similar (214 vs. 205 g/d, for **TSG** and **PSG,** respectively) (Table 5.4.), probably because of the ingestion of a similar amount of concentrate. However, in the first of these two weeks, **TSG** lambs showed a significantly lower growth, likely due to the fact that they were not used to the separation from the mother, contrary to that was observed in the **PSG** group.

However, it is difficult to explain why the growth of **PSG** lambs slowed down in the last week, despite the fact that the consumption of concentrate was similar in both groups (Table 5.4).

Overall, despite the differences observed in different stages of the suckling period, the ADG from birth to 44th day of age was similar between the two groups of ewe lambs (242±6 vs. 216±6 g/d for **TSG and PSG,** respectively), demonstrating that the early but partial separation of the lambs from the ewe did not influence negatively their growth.

Table 5.4. Weight and daily growth rate of lambs from total suckling (TSG) and partial suckling (PSG) groups during the suckling period.

Sampling	TSG		PSG	
	ADG g/d	**Concentrated food intake g/head/d**	**ADG g/d**	**Concentrated food intake g/head/d**
0-5th day	230±15**ab**	-	278±15**a**	-
5-12th day	274±15**aA**	-	164±15**bB**	23
12-19th day	246±15**a**	-	211±15**ab**	54
19-26th day	253±15**a**	-	218±15**ab**	117
26-31th day	265±15**a**	-	229±15**ab**	160
Average 0-31th day	**254±6A**	**-**	**220±6B**	**88±62**
31-38th day	167±15**b**	300	228±15**ab**	291
38-44 th day	262±15**aA**	346	182±15**bB**	321
Average 31-44th day	**214±11**	**323±33**	**205±11**	**306±21**
Average 0-44th day	**242±6**	**-**	**216±6**	**161±122**

a,b: Different letters indicate significant differences between sampling period within treatment($P<0.05$).
A,B: Different letters indicate significant differences between treatment within sampling period ($P<0.05$)

5.3.2. Behavior of lambs.

The behavioral observations of lambs were carried out in an arena test after weaning. The lambs of the **PSG** group expressed more loud vocalizations ($P<0.05$) and crossed more zones than did lambs of the **TSG** group. Latency to sniff human was higher in **TSG** than in **PSG** but no significance was found. The significance ($P<0.05$) in this behavioural response was found when all animals that had not sniffed at all (withing the 180 seconds of monitoring) were eliminated. The groups did not differ significantly in their expression of the behavioural responses “Time in Zone 0” and “Distance to human” (Table 5.5).

Table 5.5. Behavioral parameters during the Arena test of lambs from total suckling (TSG) and partial suckling (PSG) groups after weaning.

Parameters	Number Animal/Group	TSG	PSG	Significance (P)
Mean Distance (m)	12	2.85±0.3	2.59±0.2	0.510
Bleats (n)	12	35.5±3.7**a**	51.91±2.1**b**	0.000
Zone Crossed (n)	12	20.83±2.9	22.67±1.8	0.597
Time Zone 0 (s)	12	9.58±5.4	10.08±3.8	0.942
Latency to Human (s)	12	146±13.9	119±19.5	0.263
Latency to Human* (s)	6	113.3±20.2**a**	58.16±13.9**b**	0.048

a,b: Different letters indicate significant differences (P<0.05) between treatments.
* with elimination of the animals that had not sniffed the human within 180 seconds.

Following the non-parametric analysis, not related factors (P>0.700) were extracted to explain the answers to common changes in behavior.

Two component with eingenvalues of 2.11 and 1.26 were extracted with principal components analysis. The first and second Factors explained, respectively, 42.14% and 25.24% of the total variance. The cumulative effect of the two factors explained 67.38% of the total variance (Table 5.6).

Table 5.6. Extraction principal component.

Component	Eingevalue	% Total Variance	CumulativeEingevalue	Cumulative %
1	2,11	42,14	2,10	42,14
2	1,26	25,23	3,37	67,38

The first Factor reflected the amount of time spent in close proximity with the human (Time in Zone 0), distance to human and latency to sniff human (Table 5.7). There was no significant difference between the groups for Factor 1 scores (LS means: First group= 0.22; Second group= -0.46; Group effect F= 1.14; P=0.296). The second Factor reflected the locomotion and vocal activity (zones crossed and loud bleating). The lambs of **PSG** had significantly higher scores (P<0.05) for this Factor than did the **TSG** lambs (LS means: First group= -0.21; Second group= 0.46; Group effect F= 6.24; P=0.021) (Table 5.7).

The data showed that the Factor 1 explains the difference in behaviour and that the **PSG** group had more confidence on humans compared with **TSG**. The lambs of the **TSG** group expressed less confidence on man, due to an increased fear and insecurity inside the Arena test, keeping always at a greater distance (Mean distance 2.85 vs. 2.59 m for **TSG** and **PSG,** respectively) and avoiding or delaying contact with humans (Time 0: 9.58 vs. 10.08 s for **TSG** and **PSG,** respectively; Latency to sniff human: 146 vs. 119 s for **TSG** and **PSG,** respectively). The parameter "Latency to sniff human" was also calculated and analysed only in lambs (6 per group) who have had a direct contact with the man before 180 seconds. In this case there was a greater and significant difference between groups (113 vs. 58 s for **TSG** and **PSG,** respectively, P<0.05) in comparison with the case when all lambs were included in the analysis.

Table 5.7. Varimax rotated factor loadings of behaviours performed by individual lamb in 3-min arena test with a stationary human and a group of flockmates present.

Variables	**Factor 1** *"Confidence on Human"*	**Factor 2** *"Exploration of new ambience"*
Distance	**0.879**	0.253
Bleats	-0.091	**0.811**
Zone Crossed	0.256	**0.769**
Time 0	**-0.789**	0.118
Latency to Human	**0.753**	-0.029

Significance P>0.700

This study showed how the “Confidence on human” (Factor 1) and “Exploration of new ambience” (Factor 2) was expressed in the two groups. In the literature, the state of fear, provoked by human presence is normally demonstrated by keeping distance (Erhard, 2003; Beausoleil et al., 2005; 2008). In the arena test, fear of the stimulus may be expressed as avoidance, reluctance to investigate the stimulus, and suppression of active behaviours such as locomotion, vocalisation and exploration. There are significant individual differences in behavioural responses to social isolation and presence of a human (Romeyer and Bouissou, 1992; Vandenheede at al., 1998; Vierin and Bouissou, 2003).

In our study, it was observed that the habit of human presence reduced the time that the animal used to make contact with and to stay in the vicinity of man (Factor 1 - latency to sniff human; time zone 0). Similarly Beausoleil et al. (2008) and Erhard et al. (2006) observed that when the animal was aware of the environment and of the person, the distance between the two was lower and the state of fear became a state of curiosity to the new environment.

Considering the Factor 2, which reflected the locomotion and vocal activity, the **PSG** group showed a significant effect on the number of bleating and movement inside the Arena between one area and the other (Zone Crossed) compared to **TSG** group. The analyzes showed that the presence of a human and of the new environment (Arena test) generated on lambs **PSG** only reactions of curiosity and not reactions of fear (greater movement between one area and another and greater number of bleating to draw the attention of the group of origin).

Some authors revealed that the high locomotion activity may be associated with low levels of fear, because it can be caused by situations of isolation and not necessarily by the presence of a man (Erhard, 2003). Probably, these reactions are due to the fact that **PSG** lambs have been separated partially since the 5^{th} day of age. In this way, they were more used to humans, and the man was not seen as a source of danger, but as part of routine management.

5.3.3. Period of milking

In the first twenty days after weaning, the different suckling treatments did not affect the milk production (1501±38 vs 1523±38 g/d, **TSG** and **PSG,** respectively, P>0.05) (Table 5.8).

Milk production decreased over time (Figure 5.3) after the production peak, which usually occurs in 3-4 weeks of lactation (Cappio-Borlino et al., 1997). With regard to milk quality, significant differences were observed between the two groups only for fat and SCC contents which were lower in **TSG** ewes than in **PSG** (fat 5.6%±0.1 vs. 5.9%±0.1, P=0.018; SCC n.x10^3 933±416 vs. 2198±416, P=0.034) (Table 5.8).

Table 5.8. Milk yield and composition (g/head/d; mean ± s.d.) of Sarda ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

Parameters		TSG	PSG	Significance (P)		
				M	S	MxS
Milk	g/d	1501±38	1523±38	0.674	0.000	0.965
Fat	%	5.6±0.1**b**	5.9±0.1**a**	0.018	0.000	0.276
Protein	%	5.3±0.0	5.4±0.0	0.648	0.034	0.982
Casein	%	4.3±0.0	4.3±0.0	0.630	0.007	0.995
Urea	mg/dl	43±1	40±1	0.079	0.000	0.896
Somatic Cells Count	$n.x10^3$	933±416**b**	2198±416**a**	0.034	0.091	0.683

M=management; S=sampling;
a,b: Different letters indicate significant differences (P<0.05) between groups.

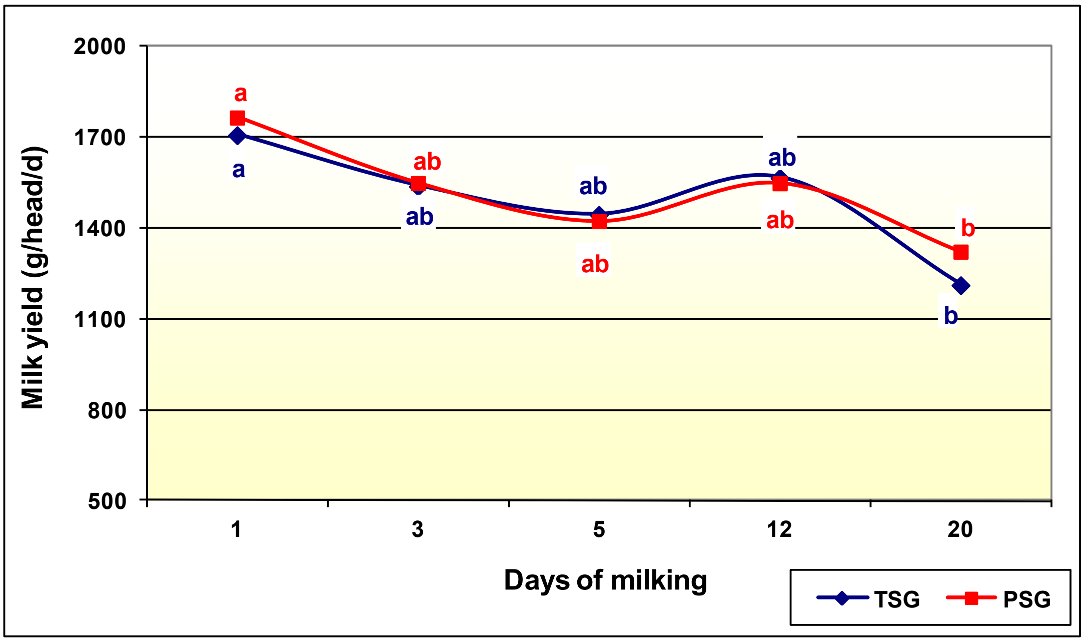

Figure 5.3. Evolution of milk yield of ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

In particular, an interesting patern was observed for the milk fat content (Figure 5.4), which showed low values in both groups at the first day of milking (4.1 vs.

4.8 % **TSG** and **PSG,** respectively), soon after the total separation of the lambs from the mothers. These values stabilized from the 5th day of milking, when the typical values for the Sarda breed (slightly above 6%) were reached and the variability between samplings was reduced. Also in this case, the low fat content of the first sample can be attributed in part to the dilution effect (Pulina et al., 2006), because milk production was significantly higher in the first day of milking, and in part to the stress effect of the separation, which may have inhibited the passage of the last fraction of milk, which is more rich in fat (McKusick et al., 2001, 2002; Jaeggi et al., 2008).

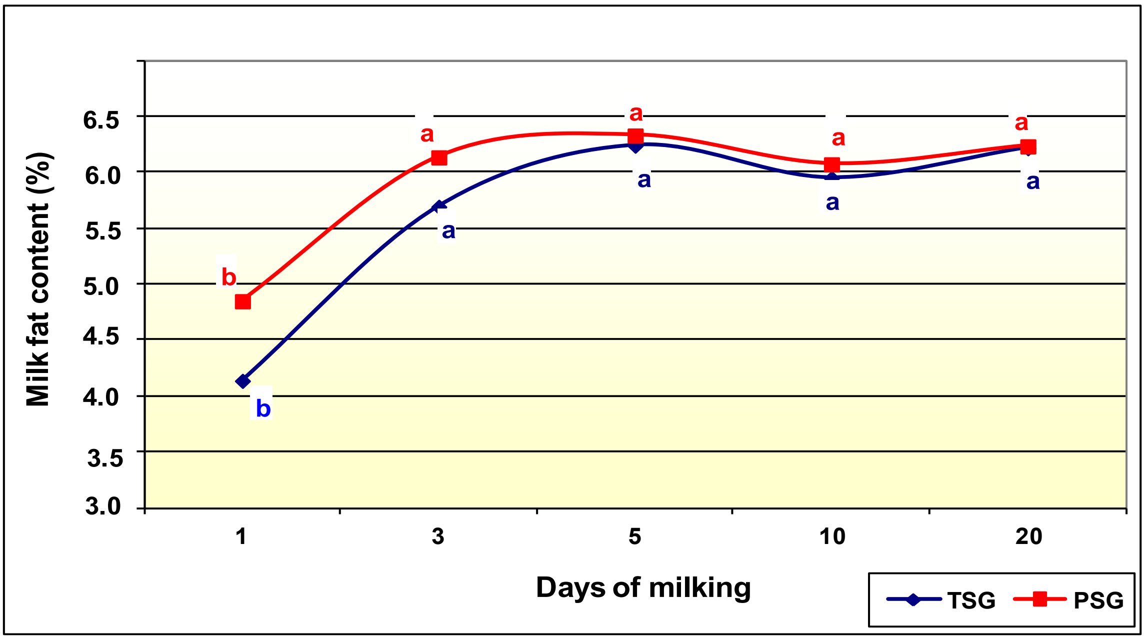

Figure 5.4. Evolution of milk fat content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

The somatic cell content, although being significantly different between two groups, showed a variable trend over time (Figures 5.5), with high variability within each group and an unexpected peak on the 5th day of milking, especially in the **PSG** group.

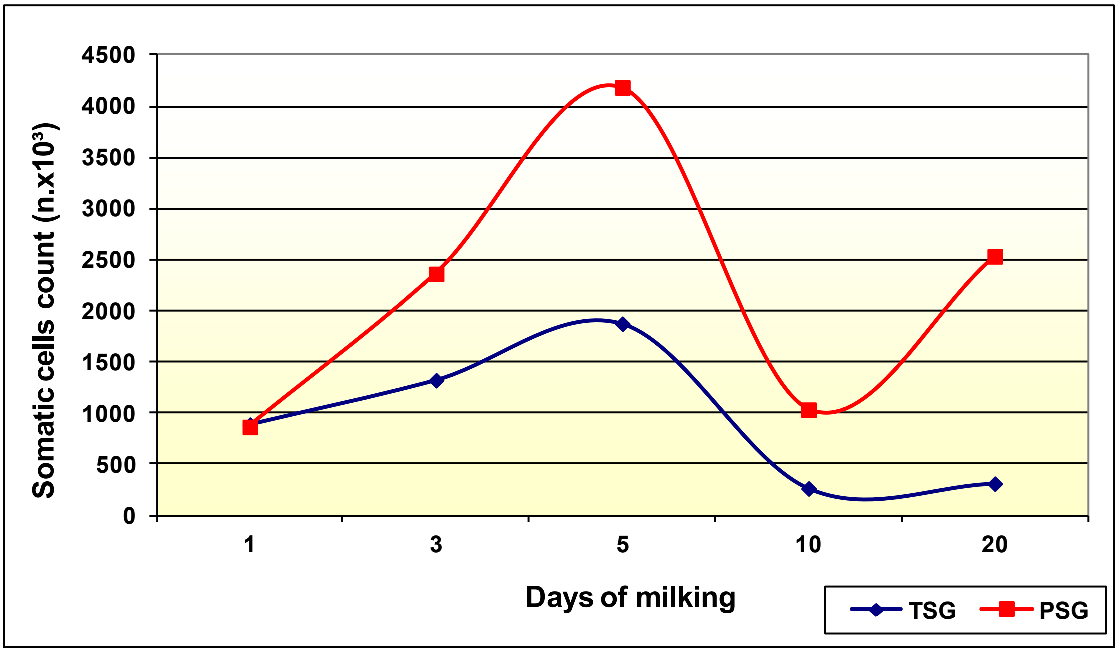

Figure 5.5. Evolution of milk somatic cells count of ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

Milk protein, casein and urea content did not significantly differ between groups (P>0.05). During the first 20 days of milking, the protein and casein contents remained quite constant (Figures 5.6 and 5.7), whereas the urea content was more variable and differed between samplings (Figure 5.8).

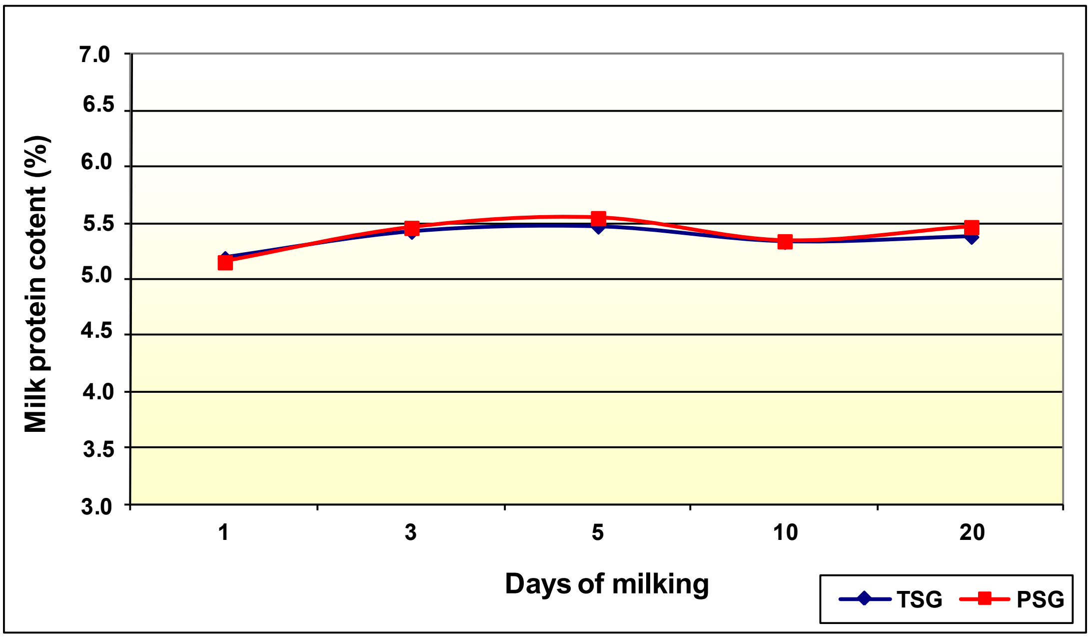

Figure 5.6. Evolution of milk protein content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

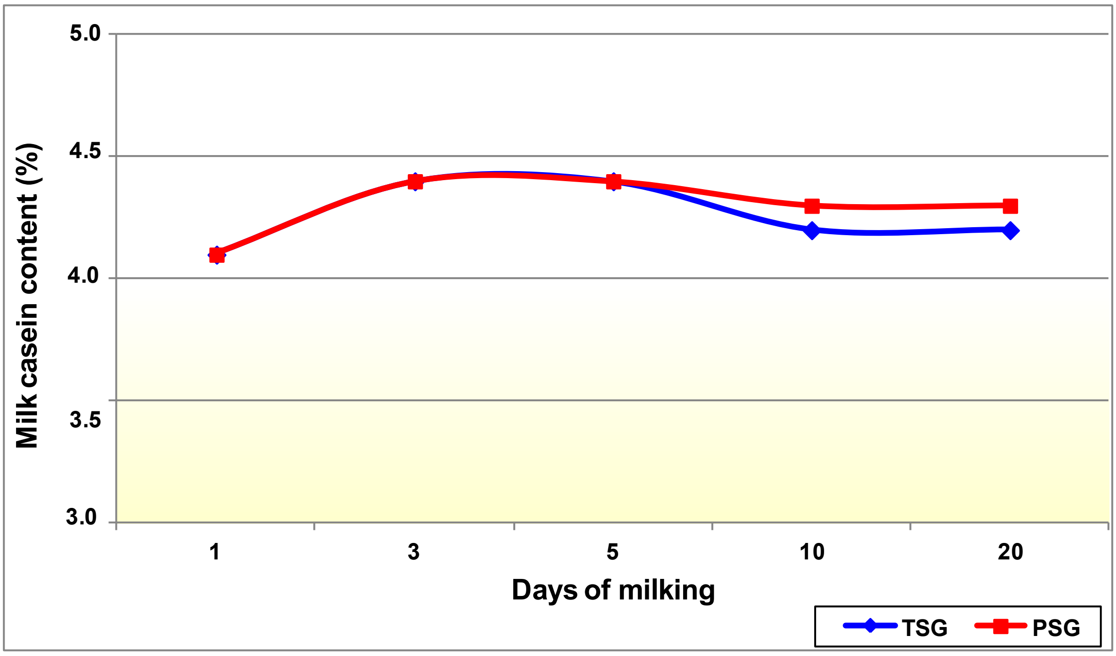

Figure 5.7. Evolution of milk casein content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

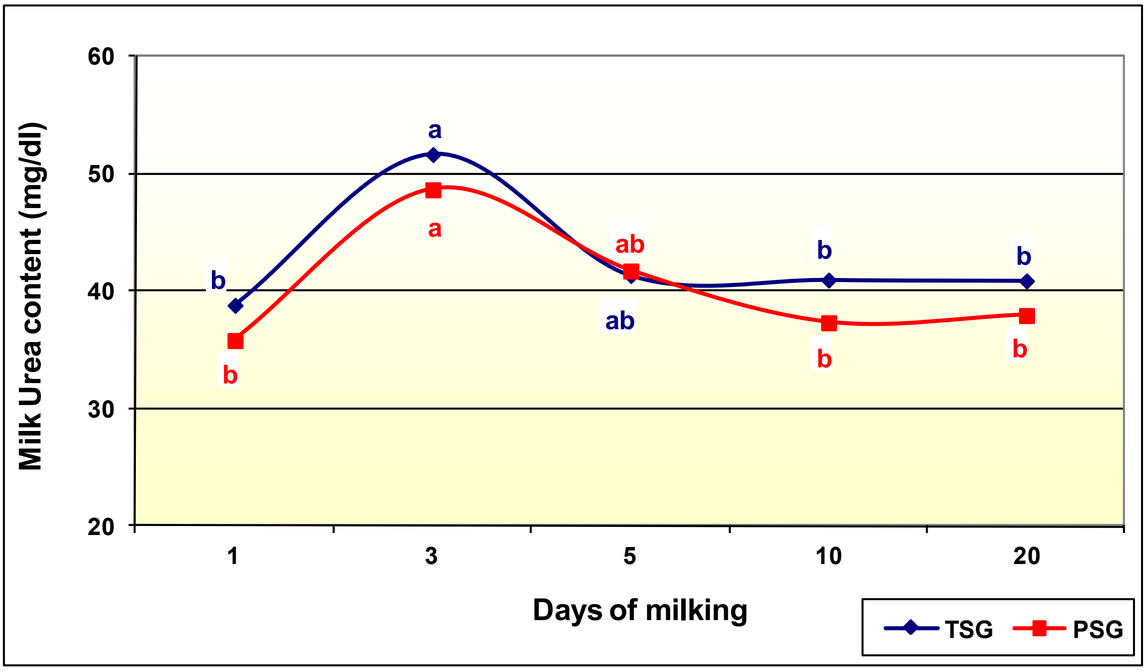

Figure 5.8. Evolution of milk urea content of ewes from total suckling (TSG) and partial suckling (PSG) groups during the 20 days of lactation after weaning.

5.4. Conclusions

The adoption of the partial suckling technique in lambs destined for the replacement more than double the production of commercial milk (26 vs. 11 liters/sheep **PSG** and **TSG**) with respect to the traditional sheep farming system in which ewes were always (except for the last two weeks pre-weaning) with the lamb. The **PSG** system did not influence substantially milk yield and composition during the 20 days post-weaning period of milking. At the same time, the lambs were not affected by the daily temporary separation, showing the same body weight at weaning of those reared traditionally. Indeed, early contact with the man, who administered their daily dietary supplementation, caused less fear and a higher degree of curiosity about the new environment in **PSG** lambs.

It should be added that the technique of partial suckling is easy to apply also in farm that do not have enough buildings, because it is possible to use mobile structures at low cost.

Additional studies would be necessary to deepen the knowledge of the effects of partial separation on the most frequently used blood and physiological parameters in the assessment of the state of animal welfare. It would also be necessary to check if the connection observed between human and animal at weaning remains unchanged over time.

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CHAPTER 6

Economic impacts of partial weaning technique adoption on female/male lambs

6.1. Economic analysis

The introduction of new rearing techniques in the context of livestock, as in any other economic field, is not always seen as a beneficial factor. In particular in ovine breed, although the use of partial weaning technique leads to evident positive effects (in economic terms, better job conditions and animal welfare), to change practices traditionally adopted by farmers represents the main problem to overcome.

In our experiments have been reported the better productive results of the partial weaning technique than the traditional technique, in terms of higher milk yield during suckling period, similar meat production, quality and also growth in replacement ewe lambs. It remains to evaluate the financial returns that the new technique may produce.

As regard the experiment with slaughtered lambs, about 650 g/head/day of milk have been milked by partially suckled ewes (**PSG**) during the suckling period instead of totally suckled (**TSG**) which recorded 85 g/head/day on average.

Considering that in Sardinia the mean duration of suckling period for slaughtering lambs is 30 days and during the first 5 days post-partum the young is kept with its mother, the productive differences of the new weaning technique could last 25 days. As a consequence in a dairy sheep farm adopting the partial weaning technique it is possible to produce 14 kg milk/head more than a traditional farm (Table 6.1). Since the market price of milk is about 0.70 €/kg, the above cited differences of milk produced with partial weaning system results in 10 €/head more in financial returns than the traditional one.

The major financial returns obtained has to be reduced by the cost of concentrate offered to the partially suckled lambs, that is 100 g/head/d (2.5 kg/head during the suckling period). Since the market price of concentrate is 0.40 €/kg the daily costs of supplementation is 0.04 €/head/d and 1.0 €/head considering the entire suckling period (Table 6.1).

In conclusion, the effective gain obtained adopting the partial weaning system is 10.3 €/head versus 1.5 €/head typical of the traditional rearing system with a difference between them of about 8.0 €/head.

Similar values can be also obtained with replacement ewe lambs. Indeed, as reported in chapter 5 of this thesis, partially and traditionally suckled lambs were submitted to different weaning technique until the thirty day of age which followed the same management from 31 to 45 day.

Table 6.1. Economic analysis of partial weaning technique adoption.

Parameters		**Partial Suckling**	**Traditional Suckling**
Milk production	g/head/d	645	83
Days of different suckling	n.	25	25
Milked milk	kg	16.1	2.1
Price of milk	€/kg	0.70	0.70
Return during 25 suckling days	€/head	11.3	1.5
Concentrated feed intake by lambs	g/head/d	100	-
Cost of lamb food	€/head/d	0.04	-
Cost of lamb feeding in 25 suckling days	€/head	1.0	-
Gain (milk returns – cost of feeding lambs)	€/head	**10.3** (11.3-1.0)	**1.5** (1.5-0.0)

Considering that the mean size of a Sardinian flock is about 300 sheep/farm, and assuming a fertility of 85% and a prolificacy of 120%, economic and productive results would be that reported in Table 6.2 and Figure 6.1.

A farm with 255 ewes managed with partial suckling system could produce over 4000 liters of milk during the suckling period versus 500 liters obtained with traditional system thus resulting in a return of approximately 2.500 € more in 30 days.

Assuming a total consumption of 765 kg of concentrate/lamb, the feeding costs to supplement the potential 306 lambs amount at 306 €. Deducing the feeding cost by the returns, a gain of about 2000 € could be obtained during the suckling period.

The mentioned results can be evaluated under another point of view. Assuming that in a traditional farm can be produced 160 kg/head of milk, the total amount of milked milk will be 160 l x 255 ewes = 40800 liters. The same amount of milk can be obtained in a farm adopting the partial weaning technique with 20 ewes less, considering the 14 liters produced during the suckling period (174 l x 235 ewes = 40890 l).

In conclusion, despite the advantages of the partial weaning technique remains to evaluate how the farmer are willing to change their traditional culture of work.

Table 6.2. Productive and economic impact of partial weaning technique in a tipical flock.

Parameters		Partial Suckling	Traditional Suckling
Flock	n.	300	300
Fertility	%	85	85
Prolificacy	%	120	120
Ewes milked	n.	255	255
Lambs bred	n.	306	306
Milked milk during suckling	kg	4.106	536
Milk returns during suckling	€	2.627	383
Consumption of concentrated for lambs	kg	765	-
Cost of the concentrated for lambs	€	306	-
Gain (milk returns – cost of feeding lambs)	€	2.321	383

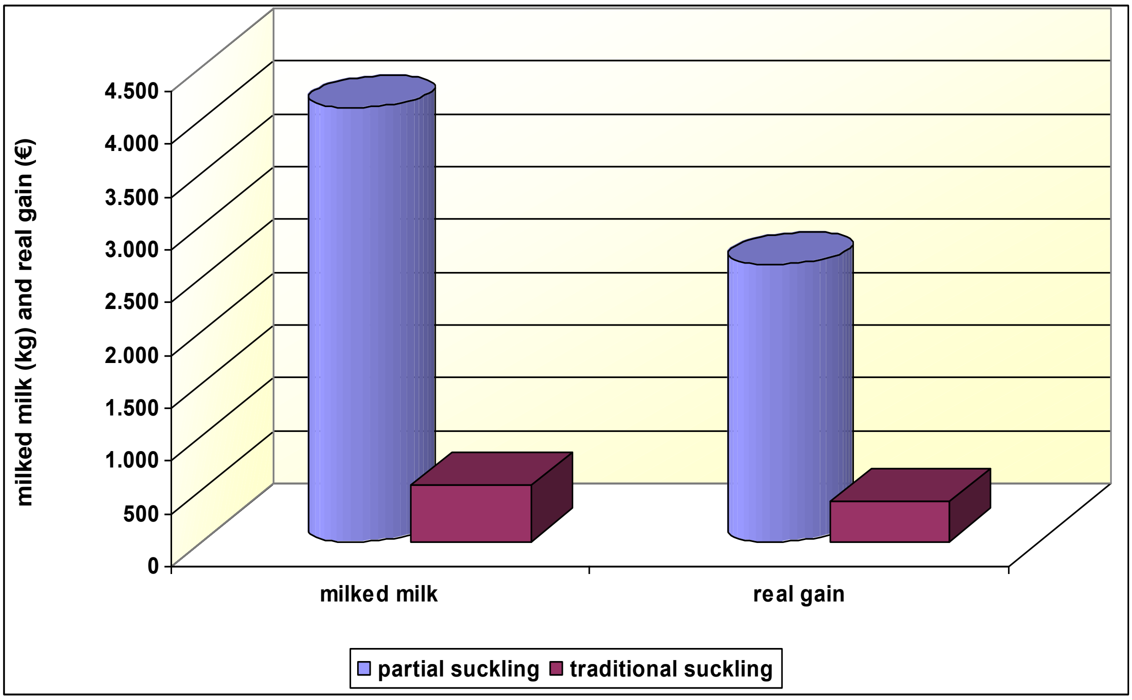

Figure 6.1. Milk production and real gain during suckling period under two weaning systems.

CHAPTER 7

Influence of dry period length on immune response in primiparous Sarda dairy sheep and their offspring

7.1. Introduction

In Sardinia the milk production of sheep is wholly obtained with Sarda breed sheep, which is generally raised in semi-extensive farming system.

In this area, adult sheep breeding is based on a mating season in late spring and a lambing season in late autumn/early winter. The lactating period lasts around 245-275 days (suckling + milking) while the dry period lasts 120-90 days on average. The dry period is a critical stage of the lactation cycle and it is essential for achieving optimal milk yield in the subsequent lactation (Capuco et al., 1997; Bachman and Schairer, 2003) as well as for ensuring damaged mammary epithelial cells renewal (Bernier-Dodier et al. 2011).

However, due to ovine milk price contraction in recent years, milk yield increase through dry period shortening is considered to be a valuable option for improving dairy sheep farmers economic benefits.

In this work we aimed to evaluate if different dry period length could influence immune response of dairy ewes and consequently of their lambs. At this purpose blood leukocytes subpopulation and immunoglobulin during the first month post partum were detected.

7.2. Materials and Methods

After estrus synchronization and pregnancy diagnosis two groups of eight lactating primiparous ewes were dried off 60 days (short dry off – SDO) and 90 days (long dry off – LDO) before lambing. After birth, six lambs per group were included in the same treatment group of their mothers.

Ewes and lambs blood samples were collected in EDTA vials at 1, 2, 7 and 30 days post partum and analysed for leukocytes subsets. White blood cells (WBC) differential count was performed using an automatic cell counter (ADVIA 2120, Siemens).

A three colour *lyse and wash* immunofluorescence staining procedure was developed to identify lymphocytes subsets in flow cytometry. For this purpose, ovine specific monoclonal antibodies against T helper lymphocytes (CD4+), T cytotoxic lymphocytes (CD8+) and a subset of γδ T lymphocytes (WC1) were used.

Colostrum and milk samples were collected at the same frequency of blood sampling in order to analyze immunoglobulin also. These were analyzed for total IgG enzyme immunoassay with a commercial ELISA kit (Alpha Diagnostic).

Data were analyzed by a GLM model, using dry off length, sampling time and their interaction as fixed factors.

7.3. Results and discussion

Compared to **LDO** group, **SDO** ewes showed lower percentages of total WBC (9.9 vs. 13.1 cells x 10^3/µL; P=0.001) and eosinophils (2.3 vs. 4.1 %; P=0.002) as well as an increased number of lymphocytes (51.1 vs. 47.7 %; P=0.009) (Table 7.1). No significative differences were found between lambs, except for monocytes which were higher in **SDO** respect to **LDO** (3.4 vs. 2.1 %; P=0.006) (Table 7.1).

In particular, during the first month post partum, for both groups has been observed that: the white blood cell content is almost constant, the percentage of LYM tends to be increasing and that of EOS increases significantly, especially for the group **LDO** (Figure 7.1).

Table 7.1. WBC differential count in SDO and LDO ewes and lambs (LSM ± s.d.).

			Groups		**Significance (P)**		
			SDO	**LDO**	**Group (G)**	**Sampling (S)**	**G x S**
ewes	WBC	(n.x103/µl)	9.9±0.6	13.1±0.6	0.001	0.777	0.994
	NEUT	%	39.5±2.1	41.2±2.1	0.555	0.001	0.997
	LYM	%	51.1±1.9	47.7±1.9	0.009	0.021	0.920
	MONO	%	4.8±0.8	5.4±0.8	0.554	0.067	0.667
	EOS	%	2.4±0.4	4.1±0.4	0.002	0.059	0.635
	BASO	%	0.45±0.04	0.42±0.04	0.587	0.007	0.646
lambs	WBC	(n.x103/µl)	8.4±0.6	9.6±0.6	0.204	0.004	0.075
	NEUT	%	47.7±3.5	43.0±3.5	0.350	0.053	0.079
	LYM	%	45.1±3.2	52.3±3.2	0.116	0.159	0.022
	MONO	%	3.3±0.3	2.1±0.3	0.006	0.000	0.002
	EOS	%	0.7±0.1	0.6±0.1	0.297	0.011	0.980
	BASO	%	0.50±0.08	0.62±0.08	0.313	0.011	0.300

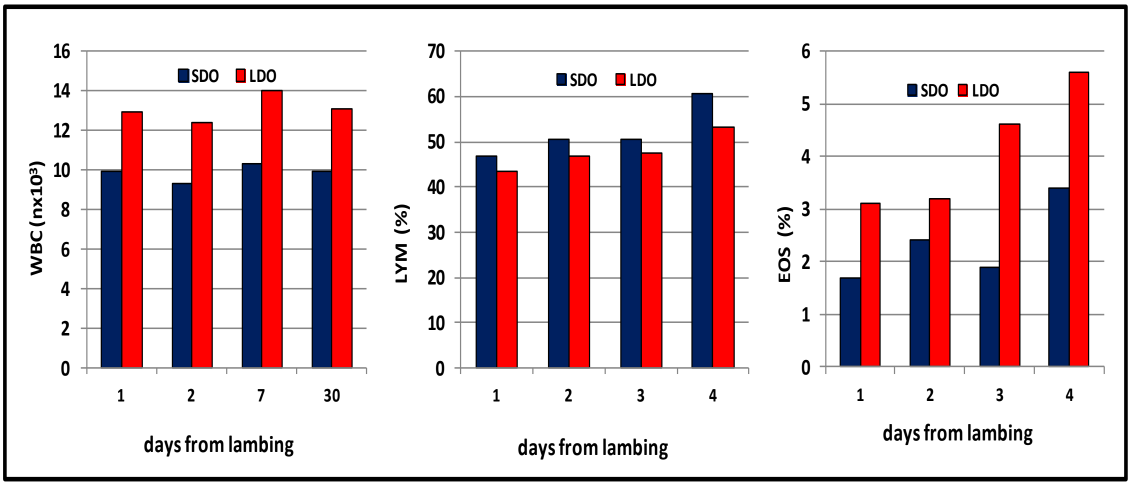

Figure 7.1. White blood cells, lymphocyte and eosinophils content on first month after lambing.

Lymphocytes subsets of ewes belonging to the two treatment groups were similar, while in **SDO** lambs lower CD4+ (44.56 vs. 50.07 %; P=0.042) and WC1+ (7.07 vs. 9.65 %; P=0.039) percentages were found in comparison to **LDO** lambs (Table 6.2).

Table 7.2. Lymphocytes subsets (on %) in SDO and LDO ewes and lambs (LSM ± s.d).

Lymphocytes subsets	Groups		Significance (P)		
	SDO	LDO	Group (G)	Sampling (T)	G x S
	ewes				
CD4+	30,13±0,96	32,09±0,96	0,154	0,059	0,399
CD8+	24,58±0,84	23,23±0,84	0,262	0,004	0,059
WC1+	4,09±0,36	4,33±0,36	0,649	0,307	0,280
	lambs				
CD4+	44,56±1,86	50,07±1,86	0,042	0,104	0,638
CD8+	15,12±0,97	14,89±0,97	0,868	0,118	0,189
WC1+	7,07±0,85	9,65±0,85	0,039	0,861	0,067

With respect to the immunoglobulins content in milk, **LDO** ewes showed overall higher values (15.1±2.8 vs. 7.6±2.9 mg/ml; P=0.070), even if not statistically significant, respect ewes of **SDO** group. This was mainly due to higher immunoglobulins content in the first two days post partum, because after one week and one month from lambing values were similar (Figure 7.2).

As the sheep have lambing at different times (15 days apart), the differences observed could be attributed to different climatic conditions, rather than the effect of the treatment.

In all cases, for both groups the values of the parameters considered fall always within the normal reference interval of ovine species.

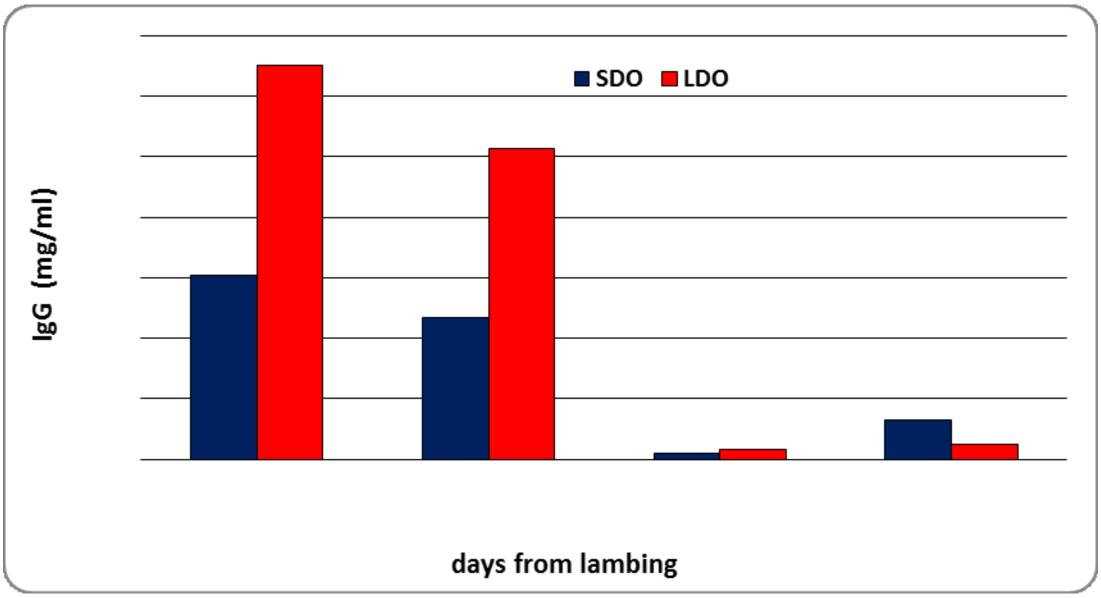

Figure 7.2. Trend of immunoglobulins white blood cells, lymphocyte and eosinophils content on first month after lambing.

7.4. Conclusions

Dry period shortening does not appear to affect significantly white blood cells and leukocytes subpopulation of ewes and lambs, as well as IgG in milk during the first month post partum. This implies the feasibility to extend the lactation up to two months before lambing with consistent improvements in dairy farm profitability.

7.5. References

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