Chapter IV: Animal Welfare

Section 1. Definition

Welfare is a multidimensional concept which embraces absence of suffering, high levels of biological functioning (including absence of diseases) and the potential of animals to have “positive experience”. The multidimensionality implies that welfare is most adequately assessed through a number of measures, each linked to a specific welfare dimension (Botreau et al., 2007b). Thus welfare should be regarded as an attribute possessed by an individual and deviation of the norm can be measured in a number of ways, specifically using physical, behavioural, physiological, biochemical, pathological as well as emotional and legal indicators (Roger, 2008).

The definition of welfare has never been a static one, on the contrary, many authors have formulated their own. In 1965, F.W. Rogers Brambell issued a report suggesting that animals should be granted 5 basic freedoms: the freedom to stand up; lie down; turn around; stretch their limbs and make normal postural adjustments. Duncan and Dawkins (1983) adopt a more global approach in defining the concept of welfare, sustaining that welfare must embody various notions such as: the condition of good physical and mental health, the ability to adapt and attain a harmonious balance with one’s environment without suffering and lastly, must also consider the feelings or sensations an animal can experience. Indeed, there is an emotional response in sheep, although these animals appear very stoical to inimical stimuli. However, there is evidence that sheep are able to feel pain and react to it like other mammals; are able to learn through experience and repetition and possess memory to recognize their attendants and other sheep (Roger, 2008).

Thus the most widely accepted definition of animal welfare is that it comprises the state of the animal’s body and mind, and the extent to which its nature (genetic traits manifest in breed and temperament) is satisfied (Hewson, 2003). Nevertheless, the definition of welfare does not imply the absence of stressful factors, but simply underlines the capability of an animal to adapt to its environment. Every individual has a different tolerance towards stressful events which to a large extent depends upon the intensity of the stressful factor itself, but also upon genetic and individual aspects. Consequently, it is clear how the evaluation of welfare conditions and the selection of reliable measures of welfare aiming at identifying the level of discomfort of farm animals, is a very complex matter and a challenge for all scientists (Calamari et al., 2008).

Section 2. Sheep welfare

Sheep may face a wide range of stressful events when raised in extensive systems and our perception that in these systems welfare is good does not have a scientific basis (Sevi et al., 2009). The main compromises grazing sheep must face are those related to nutritional stress, inadequate water supply, climatic extremes, parasitical diseases and lameness.

The existence of chronic stress in extensively-managed animals is perhaps less of a central topic when studying farm animal welfare, even though free-range aspects of animal
management may not automatically guarantee overall welfare standards. Lameness and parasitism are indeed associated with physiological and behavioural responses indicating the presence of chronic forms of disease. In extensive systems, these forms of chronic stress may last fairly longer due to lack of supervision and infrequent human handling.

Grazing sheep have developed physiological and anatomical assets especially the foot structure which is profoundly adapted to dry earths and relatively warm weather. Still, animals differ greatly on the basis of their genetic traits, where some breeds are more sensitive than others. The state of health in lambs may be influenced by wind, cold and humidity. In the vast majority, the sheep’s reproductive system is season-dependent where ewes can give birth to one or more lamb. During the rest of the year, rams form separate hierarchical sub-groups. Newborns prematurely follow their mothers or other ewes. It has been established, thanks to many studies that the bond that comes between the ewe and her lamb is particularly important for the health status of the lamb. Anticipated separation can be fatal in some breeds (Sevi et al., 2009).

In most Mediterranean areas, sheep graze during daytime and are kept in barns during night-time, where integration of straw and hay can be sometimes found. It is also true that animals in extensive systems, are free to move and perform their physiological and behavioural functions. Animals should not be isolated from the flock except for emergency cases because it is known that sheep greatly suffer segregation. Members of this specific species are known for their gregarious demeanor, which is developed to the point where individual sheep can recognize each other (Roger, 2008), and therefore suffer greatly from social isolation.

Animals are also exposed to seasonal fluctuations of herbage amount and quality, and climatic variations. It is recommended that flock holders avoid allowing grazing on pastures in the early hours of the morning when grass is wet and heavily infested with parasites. Inadequate water supply can also limit animal welfare because it causes a reduction of food intake, a decrease of glycemia and a rise of urea in the blood. Therefore, water reduction can cause alteration of the metabolic profile and often reduction in live weight (Sevi et al., 2009). These factors relate directly to two of the basic five freedoms identified by the FAWC in 1994 (see below). Being hungry, thirsty, hot, fatigued, in discomfort and in pain are all aspects associated with life in nature.

Problems for lambs are even greater and range from under-nutrition, adaptability to difficult climates and high risks of predation. Lameness, endoparasites and ecto-parasites can represent significant challenges, even fatal to sheep in these production schemes, conveying the capacity of causing chronic stress and pain. Thus the provision of appropriate nutrition to pregnant ewes and care at lambing remain key human interventions, along with treatment of lameness and parasitism which are detrimental to welfare (Goddard et al., 2006).

In addition, castration, weaning, transportation, disease, social mixing, inadequate diets and dietary change can impose significant stress causing reduced performance, morbidity and even death (Carroll and Forsberg, 2007).

The Ministry of Health forbids certain mutilations unless prescribed by a veterinarian surgeon:

- Penis amputation;
- Dehorning or disbudding;
• Freeze dagging;
• Mulesing or short-tail docking;
• Teeth grinding;
• Electro-mobilisation, vasectomy and electro-ejaculation (Defra, 2002).

Welfare can indeed be assessed during a pathological condition in any animal, by studying the complex inter-relationship that binds pathology and welfare. The means for measuring welfare can be based on the following: clinical examinations, behavioural assessment, physiological assessment and immune system function (Broom, 2006).

Section 3. Why animal welfare is measured

The measurement of animal welfare can serve for various purposes: (i) for advising farmers on how to improve welfare standards; (ii) checking compliance with legislative requirements; (iii) implementing specific animal welfare certification schemes and (iv) comparing systems to refine legislation (Botreau et al., 2007a). The first goal is of great relevance seeing how recently, intensive production systems for small ruminant species have spread through the North of the Mediterranean basin which are generally managed by shepherds, who have little or no specific skills, knowledge or awareness of welfare standards relevant to this species. It is also true that scientific assessment of welfare standards in small ruminants is lacking. This can be justified by the acknowledgement that small ruminants have a higher degree of acclimatization (Caroprese et al., 2009).

Animal welfare is of increasing significance for European citizens who now expect their food to be produced with greater respect for the welfare of farm animals. Consumers’ demands are bottom-line topic, improvement of welfare must also be guaranteed with the goal of ensuring food quality and safety (Blokhuis et al., 2004). There are increasing number of studies currently in progress aimed at assessing the willingness of consumers to pay for farm animal welfare measures especially in relation to cattle, pig and poultry production, and it would appear that overall there is an increasing awareness and concern for the welfare of animals and of food quality and safety. Since human health is a more important driver of consumer choice, welfare issues must be illustrated within this multidimensional scheme. In fact, one of the priorities is that of enhancing the transparency of information provided to consumers and other stakeholders (producers, retailers and citizens) among all publicly-raised animal welfare issues (Goddard et al., 2006).

Consumers’ concern and the apparent demand for information on animal welfare is the starting point of an EU funded project-Welfare Quality®. This project dates from 2004 to 2009 and is the largest piece of integrated research work yet carried out in animal welfare in Europe. The main aims are:

• To develop practical strategies to improve animal welfare;
• To develop a protocol for the assessment of animal welfare on-farm and at slaughter;
• To develop a protocol to translate assessment data into product information;

• To integrate and interrelate the most appreciated specialist expertise in the multidisciplinary field of animal welfare in Europe (Blokhuis, 2008).

Welfare Quality® is funded by the European Commission under the sixth Framework Programme. The project aims at integrating animal welfare in the food quality chain which means that future EU policy will include consideration of animal welfare. Another objective of the project is to provide consumers with information on the products they buy and for this purpose, we need a system that can be used routinely throughout Europe; that is sensitive to fluctuation in the animal welfare status, that reflects welfare status of the herd as a whole, that remains transparent to retailers and stakeholders and that corresponds to the current state of the art in animal welfare science (Botreau et al., 2007).

The reform of the Common Agricultural Policies (CAP) follows the trend of more market-oriented measures decoupling subsidies from production (Blokhuis et al., 2004). Indeed the new European rural policy (2007-2013) announced that direct payments to farmers within the EU will depend on whether they follow ‘Good farming practices’ that inevitably must incorporate animal welfare legislation. In addition, farmers may be paid for delivering extra public services in the field of environmental protection and animal welfare. To achieve this, farms will need to demonstrate levels of animal welfare through farming practice which move beyond the basic level that is defined by law. Thus, the implementation of welfare improvement strategies and reliable monitoring systems will support the development of genotypes and of husbandry systems and practices that offer different facets of animal welfare, thus contributing to the diversification and societal sustainability of farm animal production in Europe (Blokhuis et al., 2004) Information on the activities of the EU to protect animals can be found at http://europa.eu.int. (Veissier et al., 2008).

The private sector is currently developing new production schemes mainly quality assurance schemes, but some also aim to design specific animal welfare schemes which largely surpass national regulations (Veissier et al., 2008). In Italy, “Qualità Sicura Coop” is a practical example of a general quality assurance scheme. The contents of the product specification underlying these private labels can be highly variable.

The use of private labels offers a quality guarantee which may cover environmental-friendly ways of production, respect of animal welfare, provide guarantees about the use of chemicals and veterinary medicines which go beyond the legal minimum or they may focus on the complete traceability of the production process. Naturally, these labels are certified by quality organizations such as the International Organization of Standardization (ISO). A quality organization responds to customers’ needs today and in the future. Quality organizations would have an internal audit to assure the process is of high quality and they would have an independent audit to assure their customers that they are doing exactly what they say they are doing in the area of animal welfare. Therefore, the internal audit provides feedback to management about what is happening on the farm. It provides factual data which gives space for improvement in the process. And finally, the internal audit provides documentation that can be reviewed by an independent auditor. Animal welfare audits are in some cases a market requirement. They will meet custom preferences (McGlone, 2004).

Furthermore, in certain European countries, an important initiative has taken place in favour of welfare product labeling with an assurance scheme to signify the animal welfare
provenance of meat and other animal products (Mayfield et al., 2007). The OIE (World organization for animal health) Conference in 2004, first extended the concept of welfare onto farm animals, creating a strong correlation between animal health and food quality and safety. Welfare succeeds in being recognized as a fundamental issue in certifying hygiene of animal products destined to consumers. The OIE International Conference of the 30th of March 2006 entitled “Animal Welfare... a part of the EU Food Chain Policy”, launched a request directly from citizens who demonstrated striking interest towards animal welfare and willingness to invest economically in order to witness the creation of welfare-friendly products on European markets. Thus the duo animal welfare and food quality could be a resource for food industries and food producers (Fossati and Ruffo, 2006).

Section 4. EU and national regulation

Current animal welfare regulations can be directly linked to the “Strasbourg Convention”. Through internal legislation introduced with law number 623/85, Italy has officially adopted all aspects decided during the Strasbourg Convention regarding the protection of farm animals and animals for slaughter.

Later in time, The Treaty of Amsterdam in 1997, established a protocol on the protection and welfare of animals; “desiring to ensure improved protection and respect for the welfare of animals as sentient beings”, and impelling the Community and the Member States to pay full regard to the welfare requirements of animals (Treaty of Amsterdam, Official Journal C340, 10 November 1997). The Convention for the Protection of Animals kept for Farming Purposes was translated into an EU directive (Directive 98/58/EC) (Veissier et al., 2008), which – in Italy - was implemented at a national level through legislative and administrative practice by the D.lvo 26th March 2001 n.146. Nevertheless, there are several recommendations regarding the protection of sheep and goats that has been produced the 16 November 1992 (Veissier et al., 2008).

The Italian legislation (D.lvo 146/01) establishes minimal standards to focus upon for the protection of farm animals, and it grants to each region the freedom to identify novel and more specific welfare parameters. One of the main obligations stated in the D.lvo cited is intended for animal owners and/or caretakers, who must adopt adequate measures in order to guarantee the welfare of their animals and avoid causing them unnecessary pain, suffering or bodily harm. Stockholders, intended as any person who manages the animals or is legally responsible for them, must ensure that they are tended to by a sufficient number of competent and capable people (D.lo 146/01). In fact, the stockholder is often considered the second most important factor to be evaluated when assessing animal welfare (Aerts et al., 2006).

Another fundamental point underlined by the cited law is that of ensuring the freedom of movement, relative to the species regarded. Sheep are generally little accustomed to handling, especially if reared in extensive systems where there is less opportunity for this to happen, therefore the quality of human-animal interactions play a principle role in sustaining welfare (Goddard et al., 2006; Caroprese et al., 2009). Goddard (2006) points out that lambing time is a crucial moment because human interference will cause a response in the sheep that can be of a great relevance. The law recommends that animals reared under extensive management be ensured a suitable shelter or at the least, be protected from predators or other possible health risks. This statement must be stressed when managing animals which are
largely predated such as sheep are. Naturally, animals must be guaranteed an acceptable quantity and quality of water.

In 1993, the UK Farm Animal Welfare Council published more precise standards concerning freedoms directly linked with animal welfare: freedom from hunger and thirst; freedom from discomfort; freedom from pain, injury and disease; freedom to express normal behaviour and freedom from fear and distress (McGlone, 2004).

According to Broom (Broom, 2006), the definition of health refers to the state of the body and brain in relation to the effects of pathogens, parasites, tissue damage and physiological disorders. Since all of these aspects involve pathology, the health of an animal is its state as regards its attempts to cope with pathology. Any kind of pathology involves some degree of poor welfare. The individual coping with pathology, will inevitably have more difficulty in coping with its environment and this can have some harmful effects on its functioning, leading the animal to a state of worst welfare.. Animals with a high degree of pathology can have very poor welfare, which can be evaluated scientifically. However, sometimes it is tricky establishing whether the pathology caused the state of poor welfare or vice versa.

**Section 5. Evaluation of welfare**

A few single indicators have been suggested to provide an evaluation of animal welfare including, for example behavioural parameters (Trentini et al., 2008); corticosteroids (Carroll et al., 2007); acute phase proteins (Deni, 2009); haematological (Giuliotti et al., 2004), immunological parameters (Moscati and Beghelli, 2008); mortality rate and productive traits. However, none of these assets evaluated singularly can cover all the dimensions of welfare. For instance, an animal can be diseased with no impairment of the corticotropic axis activity and vice versa (Botreau et al., 2007a).

Research on aspects of animal welfare has also focused on the body, using physiological measures, such as endorphins, plasma cortisol, and heart rate, to examine how the animal is coping with its environment. However, there are limitations to seeing animal welfare only in terms of the body. One limitation is that genetics and the environment can produce desirable physical outcomes, even though the animal's mental state is compromised (Hewson, 2003). Another limitation is that some physical parameters (heart rate, plasma cortisol) are difficult to interpret, because they can be increased by both positive and negative experiences, such as the presence of a mate and the presence of a predator. Moreover, blood is not always easily obtained due to cost and difficult interpretation of results (Aerts et al., 2006).

The above would suggest that animal welfare includes not only the state of the animal’s body, but also its feelings. Most would agree that animals have feelings (fear, frustration), and it has been proposed that animal welfare consists entirely in sensations and that these have evolved to protect the animal’s primary needs (Hewson, 2003).

Stress has been defined by Broom (2006) as an environmental effect on an individual which over-taxes its control system, inevitably reducing its fitness or activity and leading to pathology. The endocrine system is known to have a leading role in the organism’s response
under stress. Indeed there is an activation of physiological coping mechanisms during stress which is majorly modulated by glucocorticoids and other hormones. In fact, they are capable of immunomodulation and increasing the susceptibility of an individual to other pathogens (Broom, 2006). Glucocorticoids have many biological effects:

1. Effects on the protein, carbohydrate and lipid metabolism
2. Anti-inflammatory, anti-toxic and anti-allergic effects
3. Enhances synthesis and secretion of catecholamines
4. Increases blood glucose concentration
5. Suppression of the immune system
6. Increase in gastric hydrochloric acid and pepsin secretion
7. Alterations of growth and reproductive axes
8. Regulation of the stress response (Preziuso and Preziuso, 1999; Carroll and Forsberg, 2007).

Glucocorticoids stimulate gluconeogenesis from other organic molecules such as lactate, pyruvate and amino acids during the “flight or fight” response. Contemporarily, catecholamines are enhanced which control heart rate, pupil dilation, vasoconstriction in the skin and gut, increase glucose production by the liver and so forth. Suppression of the immune system is the function that is one of the central topics in this specific research, of fundamental relevance in order to fully comprehend and interpret data collected. In more specific terms, glucocorticoid effects on the immune system may include the following:

1. Lymphopaenia
2. Neutrophilia
3. Leucopenia
4. Suppression of B cells and T-cytotoxic CD8+ cells
5. Decrease the synthesis of IL-1 by macrophages
6. Decrease the synthesis of IL-2 by T-helper CD4+ cells
7. Regulation of CD4+/CD8+ ratio (Broom, 2006; Carroll and Forsberg, 2007; Moscati and Beghelli., 2008).

These functions are carried out thanks to the presence of specific receptors for glucocorticoid hormones which are found on the following cells: lymphocytes, macrophages and granulocytes which undergo proliferation, cytokine secretion, antibody production and cytolitic activity.

Other hormones are synthesized during stress response for instance beta-endorphins, vasopressin and oxytocin. It is known that beta-endorphins promote T-helper cells in explicating their functions especially that of stimulating phagocytes. Furthermore, catecholamines which regulate the acute phases of stress, have been found to suppress cell-mediated immune responses whilst enhancing humoral immunity (Broom, 2006). Chronic exposure to high levels of glucocorticoids can cause severe physiological and psychological problems such as excessive protein catabolism, hyperglicaemia, immunosuppression and
depression. In domestic livestock, conditions such as reduced rates of reproduction, suboptimal growth, suppressed milk production and suppression of the immune system are all possible consequences that in turn can predispose the individual to disease (Carroll and Forsberg, 2007).

Scientists have come to the conclusion that during stress, there is a fine line that separates the various systems responsible for its regulation, such as the neuroendocrine system, the endocrine system and the immune system. Better still, there are many cross-communications between these pathways, which lead to the acknowledgement that the stress response is no longer considered an all-or-nothing activity associated with the “flight or fight” response, nor is it strictly immunsuppressive. Infact, it has been determined that acute stress can be immuno-enhancing, whilst chronic stress can be immunosuppressive. Generally, acute stress is associated with the initiation of the innate immunity. During chronic stress instead, there is a shifting of events which tend towards suppression first at a cellular level then across the entire immune system spectrum. Infact, under chronic stress, effector cells are stimulated constantly to be prepared to a large-scale immune response and continue to secrete proinflammatory cytokines, which stimulate further secretion of glucocorticoids thus establishing a vicious circle.

However, prolonged exposure to glucocorticoids can cause severe thymus atrophy and an overall decrease in the lymphocyte proliferative response of calves injected with dexamethasone. In the long term, catecholamines have been reported to decrease phagocytosis and inhibit lymphocyte proliferation, antibody secretion and production of proinflammatory cytokines as well (Carroll and Forsberg, 2007).

5. a) **Hematological parameters**

Hematological parameters are indeed influenced not only by the status of health, by the instauration of disease and thus welfare, but also by the physiological stage animals are in, the productive level, nutrition and management systems. In verifying animal welfare in ovine farms, across the various managerial aspects (nutrition, weaning, milking, pregnancy, lactation, transport and slaughter), innumerable parameters have been identified as valid for this specific goal. Among these, the ones hereby explained include the complete blood panel, the metabolic profile, the liver enzymes and certain immunological parameters (Cavallina, 2004).

- **Red Blood Count (RBC):**

Red blood cells are corpuscular elements without a nucleus characterized by having the fundamental role of transporting oxygen and carbon dioxide within the organism. They are measured from blood samples withdrawn using a test tube coated with an anticoagulant. Haemolysis can create false lower red blood counts, PCV counts and false increases of AST. Fluctuations may be due to numerous factors: a decrease in RBC can be due to an anemic state, hemorrhage, parasitism, lysis of erythrocytes, malnutrition, vitamin deficiencies, systemic diseases, renal insufficiency and intoxication from derivatives of coumarin; an increase (polycythemia) can be due to dehydration, chronic respiratory insufficiency (Archetti and Ravarotto, 2002).
The three main classes of anemia include excessive blood loss (acutely, such as a hemorrhage or chronically, through low-volume loss), excessive blood cell destruction (hemolysis) or deficient red blood cell production (ineffective hematopoiesis).

Classification of anemia is achieved thanks to the erythrocyte parameters:

<table>
<thead>
<tr>
<th>Erythrocyte indexes</th>
<th>High MCHC</th>
<th>Normal MCHC</th>
<th>Low MCHC</th>
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<tbody>
<tr>
<td>Normal MCV</td>
<td>Normocytic Hyperchromic</td>
<td>Normocytic Normochromic</td>
<td>Normocytic Hypochromic</td>
</tr>
<tr>
<td>High MCV</td>
<td>Macrocytic Normochromic</td>
<td>Macrocytic Hypochromic</td>
<td></td>
</tr>
<tr>
<td>Low MCV</td>
<td>Microcytic Hyperchromic</td>
<td>Microcytic Normochromic</td>
<td>Microcytic Hypochromic</td>
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</table>

Table 4. Classification of anemia (Lubas, 2005).

Intestinal parasitism of blood-sucking nematodes can cause loss of plasma and/or blood through intestinal damage. This leads to iron deficiency and in the long term would cause a microcytic hypochromic anemia, sometimes characterized by the presence of anisocytosis and reticulocytosis (the presence of nucleated immature red blood cells). These last two laboratory results would indicate that the anemia is regenerative (Lubas, 2005). In a regenerative anemia, the bone marrow responds appropriately to the decreased red cell mass by increasing RBC production and releasing reticulocyte.

- **Haemoglobin (HgB):**

  Haemoglobin is synthesized in immature red blood cells in the bone marrow and consists of an assembly of four globular protein subunits. Each subunit is composed of a protein chain tightly associated with a non-protein heme group. A heme group consists of an iron (Fe) ion (charged atom) held in a heterocyclic ring, known as a porphyrin. A porphyrin ring consists of four pyrrole molecules cyclically linked together with the iron ion bound in the centre which is the site of oxygen binding. In this way oxygen is transported in red blood cells of vertebrates.

  An increase in number can indicate dehydration, stress and fear, shock in which there is splenic contraction, polycythemia and muscle fatigue. A decrease indicates anemia or late pregnancy.

  Normal range: 8,50-13 g/dL

- **Packed Cell Volume (PCV):**

  This measure represents the volume in percentage occupied by red blood cells in venous blood and not leucocytes nor platelets (Lubas, 2004).
An increase of PCV can be justified by the presence of burning on skin, polycythemia, loss of plasma, diarrhoea, vomiting, undernutrition, interstitial nephritis and malnutrition. Instead, a diminishment is caused by loss of hydration, circulatory congestion, congestive cardiomyopathies and cortisone intoxication (Archetti and Ravarotto, 2002). Lubas (2004) states more causes of increase and decrease of PCV which are identical to those mentioned for haemoglobin.

Lastly, during acute stages of inflammation, the oxidative burst performed by neutrophils leads to the release of large quantities of peroxidases, enzymes able to rupture the erythrocyte membrane. This would definitely cause a lowering of the PCV values (Bertoni, 1999).

Normal range: 20-45%

- **Mean Corpuscular Volume (MCV):**
  Along with MCH it is used to classify the state of anemia.
  MCV is influenced by the number of cell divisions carried out by the red blood cell lineage during erythrocyte differentiation.
  Normal range: 23-48 fL
  MCV >48 fL => macrocytic anemia (i.e. augmented bone marrow activity and lack of erythropoiesis precursors)
  23<MCV<48=> normocytic anemia (i.e. acute stage of hemorrhage, hemolysis and reduced erythropoiesis)
  MCV <23 fL => microcytic anemia (i.e. iron and copper deficiency) (Lubas, 2004).

- **Mean Corpuscular Haemoglobin Concentration (MCHC):**
  It indicates the average cellular concentration of haemoglobin. It is an estimate of the concentration of haemoglobin contained in the mass of erythrocytes. False fluctuations may be due to false alterations of Hgb and PCV. A decrease can be caused by iron deficiency anemia, hemoglobinopathy and instead will remain normal during non sideroblastic anemia.
  Normal range: 31-34 g/dL
  MCHC>34 => hyperchromic anemia (i.e. there are no existing conditions where MCHC can exceed the normal range because it would signify a supersaturation of Hgb in red blood cells. It is most likely due to technical errors).
  31<MCHC<34 => normochromic anemia (i.e. even if MCV and Hgb increase or decrease, MCHC can remain within range)
  MCH<31 => hypochromic anemia (i.e. Hgb will be relatively more decreased) (Lubas G., 2004).
Mean Corpuscular Haemoglobin [MCH=(Hgb*10)/RBC]:

This measure identifies the average quantity of haemoglobin contained in red blood cells.
Normal range: 8,0-12,0 pg

Red blood cells distribution width (RDW):

This is the most important index of anisocytosis, a condition where red blood cells are of unequal size. This index must be interpreted together with MCV for the classification of the type of anemia because alterations of RDW indicates early signs of anemia even if MCV is within normal range.
Normal range: 18-24.6 %

Wide blood cell count (WBC):

White blood cells are nucleated cells capable of defending the organism from non-self elements. The WBC count includes neutrophils, basophils, eosinophils (granulocytes) and monocytes and lymphocytes (agranulocytes) (Archetti and Ravarotto, 2002).

In sheep, total count should be around 3,0 × 10⁹/L at birth and then gradually decrease up to 3 months of age when they stabilize around 5,5-9,5 × 10³/μL (Lubas, 2005).

Leukocytosis (increase in WBC counts) can be due to physiological factors such as adrenaline production which causes mobilization of the neutrophil pool from small capillaries to larger veins.

Pathological leukocytosis can be caused by numerous factors:

- Local and general infections
- Metabolic and chemic intoxication or poisoning
- Tissue necrosis
- Hemorrhage or hemolysis
- Tumours
- Internal or external corticosteroids

As already mentioned, administration of corticosteroids causes neutrophilia, eosinopenia, lymphopenia and slight monocytosis. Lymphopenia is the most reliable factor in evaluating stress signs (Lubas, 2005).

Leukopenia (or leukocytopenia) is caused by:

- Viral infections
- Bacterial infections (only during initial stage of infection, subsequently it can dramatically increase)
- Septic shock, endotoxemia and anaphylaxis
- Bone marrow hypoplasia
Table 5. Causes of fluctuation in the leukocyte population. (Archetti and Ravarotto, 2002).

<table>
<thead>
<tr>
<th>Leukocyte population</th>
<th>Reasons for increase</th>
<th>Reasons for decrease</th>
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<tbody>
<tr>
<td>NEU</td>
<td>o Corticosteroids</td>
<td>o Hypoplasia</td>
</tr>
<tr>
<td></td>
<td>o Hemolysis</td>
<td>o Necrosis of bone marrow</td>
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<td></td>
<td>o Acute inflammation</td>
<td>o Viral infections</td>
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<tr>
<td></td>
<td>o Tissue necrosis</td>
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<tr>
<td></td>
<td>o Myositis</td>
<td></td>
</tr>
<tr>
<td>LYM</td>
<td>o Brucellosis</td>
<td>o Acute bacterial inflammations</td>
</tr>
<tr>
<td></td>
<td>o Actinomycosis</td>
<td>o Stress</td>
</tr>
<tr>
<td></td>
<td>o Babesiosis</td>
<td>o Corticosteroid administration</td>
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<tr>
<td></td>
<td></td>
<td>o Immunodeficiency syndrome</td>
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<tr>
<td>MONOC</td>
<td>o Inflammatory damages</td>
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<tr>
<td></td>
<td>o Septic myocarditis</td>
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</tr>
<tr>
<td>EOS</td>
<td>o Antibiotic administration (especially tetracyclines)</td>
<td>o Administration of corticosteroids</td>
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<tr>
<td></td>
<td>o Hypersensitivity reactions</td>
<td>o Acute inflammation</td>
</tr>
<tr>
<td></td>
<td>o Parasitic diseases</td>
<td>o Stress</td>
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<td></td>
<td>o Fungal diseases</td>
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<td></td>
<td>o Tumours</td>
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</table>

On the basis of leukocyte counts, it is possible to distinguish between acute and chronic phases of infections. During acute stages of infection, WBC counts are considerably increased due to higher production and release of neutrophils into blood circulation from the bone marrow. However, once the organism’s demands exceed this compensatory mechanism and large amounts of neutrophils migrate from the blood to tissue compartments, the bone marrow will start to release immature neutrophils named band neutrophils (or band cells) as opposed to normal segmented neutrophils. Lymphopenia and monocytosis are indicators normally associated to this phenomenon. On the other hand, during chronic phases of infection, the leukocyte will be slightly different where MBC counts are more or less within range and there is no associated lymphopenia (Lubas, 2005).

The ratio calculated between neutrophils and lymphocytes (N/L) is a proportional measure of the global white blood cell response (Lubas, 2005). The physiologic immune
response of circulating white blood cells to various stressful events as tissue injury, severe trauma, major surgery, burns and sepsis syndrome, is characterized by elevated neutrophils and decline in lymphocyte counts. The development of neutrophilia and lymphocytopenia is caused and maintained by many factors. Lymphocytopenia is induced by factors such as hormones, cytokines and chemokines. Lymphocytopenia reflects the strength and intensity of the stressful event, as well as the resistance and adaptability of the immune system.

The inflammatory response is mainly generated by the innate components whilst the immune response, by the adaptive or acquired immunity. Thus, the inflammatory/immune response to stress can be effectively characterized by the ratio of relative neutrophil counts (%) to relative lymphocyte counts (%). This index is referred to as neutrophil/lymphocyte stress factor (NLSF).

Under physiological conditions, the NLSF is less than five e.g. normal values of neutrophils are less than 75% and relative counts of lymphocytes are higher than 15%, thus the NLSF ratio is five. Under pathological conditions, the NLSF is increased to higher values than 6. In conclusion, NLSF is a reliable parameter for the monitoring and evaluation of the systemic inflammatory response (Zahorec, 2001).

5. b) Metabolic profile

- **AST/GOT (aspartate transaminase) and ALT/GPT (alanine transaminase) transaminase enzymes:**

  These liver enzymes are often analyzed when studying metabolic profiles. They are part of the cellular metabolism of certain organs and do not have a plasmatic functionality but are located within cells and sometimes within cellular organs (i.e. mitochondrion). Factors which can influence their presence in the blood circulation are for instance:

  ✓ Tissue hypoxia
  ✓ Alteration of cell membrane permeability
  ✓ Cytolysis
  ✓ Cellular senescence

  One or more of the above events indicates cellular damage (caused by infection or toxins) which invariably leads to the liberation of certain enzymes in the extracellular liquid compartments from which they diffuse into the plasma and can thus be measured. ALT is located in the cytoplasm of hepatocytes and high plasmatic levels are considered to be directly correlated with cytolysis of liver cells. AST on the contrary, is located 50% in cytoplasm and 50% in mitochondrions of hepatocytes, cardiac and skeletal muscle cells. Variations of this enzyme are correlated with the three mentioned organs; therefore it can be used as an indicator of cytolysis of liver cells but also of cardiac and muscle fibers. Infact, it is important to consider the alterations of this enzyme together with other enzymes such as the three isoenzymes (MM, MB and BB) of CPK (creatine phosphokinase) which can be directly correlated to muscle, cardiac and brain functionality, and the five isoenzymes of LDH (lactate dehydrogenase) which are found in the muscle, brain, heart, liver and other organs (Bizzeti, 2007).

  Normal range of ALT: <18 U/L
Normal range of AST: 40-123 U/L

• **Blood proteins:**

  Also called serum proteins, they have many essential functions:

  ✓ circulatory transport molecules for lipids, hormones, vitamins and metals
  ✓ enzymes, complement components, protease inhibitors, and kinin precursors
  ✓ regulation of acellular activity and functioning, as well as regulation of the immune system

  Blood proteins can be divided into two groups, depending on the way in which they are collected from the animal. Briefly, by using an anticoagulant-coated test tube (with EDTA or Heparin), we will avoid coagulation of the blood thus usage of fibrinogen and other factors of the coagulation cascade. By doing so, it is possible to measure their specific quantity in the plasma obtained. On the other hand, if we wish to obtain serum thus blood from which cellular elements and coagulation factors have been “extracted” thanks to coagulation then a normal test-tube will be used without anticoagulant.

  Serum=albumin, α, β and γ-globulins

  Plasma= albumin, α, β, γ-globulins, fibrinogen, factor V and VIII (Lubas, 2004).

  Albumin represents 35-50% of total serum proteins. It is synthesized in the liver and is majorly responsible for the oncotic pressure of plasma. It is also one of the principle molecules used for transporting substances. Globulins can be divided into α, β and γ where the first two are synthesized by hepatocytes and the third represent antibodies (IgM, IgG and IgE) thus secreted by B lymphocytes and plasma cells. Hypogammaglobulinemia is usually correlated to immunodepression or stress. Hypergammaglobulinemia on the contrary, can be consequential to vaccination (Bertolin et al., 2002). α and β globulins include many lipoproteins and acute phase proteins. Separation of blood proteins by electrophoresis allows the clinician to analyze the different fractions and interpret various disorders characterized by increase of the blood protein amounts.

  Normal range: 6-7.9 g/dL

  Hyperproteinemia can be due to:

  ✓ dehydration
  ✓ vomiting
  ✓ plasmocytoma

  Hypoproteinemia can be due to:

  ✓ liver failure
  ✓ renal failure
  ✓ enteropathies
  ✓ malabsorption
Malnutrition (Bertolin et al., 2002).

- **Glucose:**
  Glucose, along with NEFA, lipoproteins and ketone bodies, are the main indicators studied when evaluating the status of the energetic metabolism. In ruminants, glucose derives to a good extent from digestion in the rumen, specifically from proprionic acid. Starch digestion generates a good amount of glucose as well, not to mention amino acid transformation (Bertoni, 1999). The amount of energy deriving from a ruminant's diet is particularly important in avoiding syndromes caused by glucose deficiency such as ketosis, metabolic acidosis and tympanites (Bertolin et al., 2002). However, it must be emphasized that glucose is not the essential molecule used by the metabolism as a source of energy as volatile fatty acids are (Bertolin et al., 2002). Regardless, increase of glycaemia can be due to cortisol and/or catecholamine activity which both induce hyperglycemia and lipolysis (therefore more circulating NEFA) (Bertoni, 1999).

**Causes of hyperglycemia are:**
- Diabetes mellitus
- Cushing syndrome
- Hyperthyroidism
- Shock
- Trauma
- Pancreatic disease

**Causes of hypoglycemia are:**
- malabsorption
- hepatorenal syndrome

Normal range: 50-80 mg/dL

- **Cholesterol:**
  It is an essential component of cell membranes in mammals and is synthesized mainly by the liver. The total amount of lipids in blood is given by cholesterol (over 1/3), phospholipids, triglycerides, beta-lipoproteins and fatty acids. Its primary function is that of acting as a precursor molecule for the production of bile, vitamin D and adrenal gland steroids such as cortisol, aldosterone, progesterone, estrogens and testosterone hormones (Bertolin et al., 2002). Blood cholesterol can be found in two states; free or esterificated. The ratio between esterificated cholesterol and total quantity is an index of hepatocyte activity and levels inferior to 2/3 would insinuate hepatic failure, seeing as this organ is responsible for the enzymatic conversion. Thus, esterificated cholesterol is a direct parameter of liver parenchyma functionality whereas total cholesterol, on the other hand, is correlated to cholestasis - the condition where bile cannot flow from the gall bladder to the duodenum via the duct system.
Cholestasis may be due to gall stones and can be diagnosed by analyzing, together with total cholesterol levels, ALP (alkaline phosphatase), γGT (gamma-glutamyl transferase), bilirubin levels, phospholipids, triglycerides and acid bile amounts. These substances all increase during cholestasis (Bizzeti, 2007).

During inflammation, the liver’s functionalities are temporarily deviated and the assets of proteins and lipoproteins normally produced, result in being modified. In particular, IL-1 cytokine, mainly responsible for the onset of sickness behaviour, activates the synthesis of acute phase proteins which occurs in hepatocytes. Contemporarily, hepatocytes synthesize and secrete reduced amounts of “normal” liver proteins such as albumins and lipoproteins (cholesterol) (Bertoni, 1999).

Cholesterol is mainly evaluated in farm animals in order to verify the extent of the mobilization of lipid deposits in order to relate this to an energetic deficiency which can be observed in lactating animals. In addition, blood cholesterol is used as an indicator of the productivity of lipoproteins from the liver which have the duty to transport triglycerides synthesized using NEFA. Clearly, glucose and lipoproteins are influenced by the type of diet supplied to these animals (Bertoni, 1999).

In cases where the animal is in energetic deficiency, due to the critical physiological period which too often is not sustained by a rich diet, glucose, triglycerides and NEFA mean values would all be superior to the normal range. However, as the period of lactation proceeds, cholesterol levels increase in parallel to the energetic deficiency the animal can encounter. Cholesterol levels seem to be correlated to the quantity of milk produced. When sheep have finished lactating, cholesterol levels tend to drop since the lipids supplied with the diet are quite low at this time and anabolic processes are predominant (Bertoni, 1999).

Moreover, cholesterol, being associated with lipoproteins, can be used as an index, if well interpreted, of an imbalanced energetic metabolic state present up to three months prior to blood analysis, which is useful especially around parturition time. Infact, lipoproteins are known to decrease after giving birth and gradually increase during the first 3-4 months of lactation in cattle. This increase is directly proportional to the energetic disparity which predominates in this critical phase (Bertoni, 1999).

Normal range: 60-150 mg/dL

High cholesterol values can imply:
- Diabetes mellitus
- Jaundice
- Hepatocyte damage

Low cholesterol values can imply:
- Hyperthyroidism
- Anemia
- Serious liver disease
- Cachexia
- Serious infections
• Non-essential fatty acids (NEFA):

Non-essential fatty acids originate from lipo-mobilization that occurs when fatty deposits are needed for energy utilization. They are destined to be transformed into ketone bodies in the liver which will subsequently be used in order to obtain ATP. Normally, they are found in low concentrations in the blood (Bertolin et al., 2002). Similar to glucose concentration, NEFA are influenced by cortisol and catecholamine levels in the blood thus increasing their concentration thanks to the enhancement of lipolysis in adipose tissue (Bertoni, 1999).

Normal range: 102-450 μmol/L

• Triglycerides:

Triglycerides or lipids derive from the chemical bonding called esterification between three molecules of fatty acids and one molecule of glycerol which occurs in the liver. Triglycerides accumulate in adipose tissue acting as the main fatty deposit in the organism. When their blood concentration increases in an animal on an empty stomach then they receive more clinical attention and need to be interpreted in relation to a possible case of pancreatitis or malabsorption.

Normal range: 13-23 mg/dL

• Blood Urea Nitrogen (BUN):

The sole fundamental parameter to consider when studying the protein metabolism is urea. This is clarified when knowing that in ruminants urea is particularly influenced by the existing relationship between dietary protein and energy, where an excess in proteins or proteins of scarce biological value (BV) can both induce higher BUN values, which are risky for this species (Bertolin et al., 2002).

BUN is influenced by the internal and external amounts of ammonia (NH3) where the first pool derives from gluconeogenesis and amino-acidic catabolism, whilst the second from rumen absorption. The inner quantity of NH3 can be increased essentially by 4 physiological events:

1. parturition and lactation represent a particular moment in which the organism deals with an energetic deficiency. This deficiency in turn stimulates all mechanisms from which energy is produced; therefore gluconeogenesis and amino-acidic catabolism are activated. This is particularly evident in sheep;
2. stress is known to increase the breakdown of proteins thus produce NH3;
3. cytokines produced during endotoxemia are able to increase oxidation of amino-acids thus augmenting blood urea nitrogen;
4. the consumption of a meal will inevitably increase NH₃ levels to higher values, which in this case are also associated with the amount of NH₃ absorbed in the rumen (Bertoni, 1999).

The outer quantity is thus obtained thanks to rumen degradation of protein but is balanced out by the usage of amino-acids destined to the synthesis of microbial products.

In sheep, it has been estimated that during pregnancy and even more so during lactation, the organism develops a necessity to form a supply of amino acids calculated of about 17-27% of all bodily proteins. It is still not crystal clear whether this newly created reserve of amino acids is destined to be utilized for gluconeogenesis or for the synthesis of milk proteins. Nevertheless, these amino acids allow better usage of lipid deposits (Bertoni, 1999).

Urea is the most important end product of the protein metabolism, produced in the liver and excreted from the kidneys with urine (Bizzeti, 2007). Urea is indeed correlated with gluconeogenesis and is influenced by many factors of regulation, of which dietary protein input (Bertoni, 1999). Alterations of serum urea levels must be considered in relation to the state of hydration of animals, potential heart failure, renal obstruction and the glomerular filtration rate.

It is important to state that among several effects of cortisol on metabolism, it stimulates liver gluconeogenesis and simultaneously reduces insulin levels, which enhances glucagon activity. Cortisol levels can be high during stress or at the initial stages of parturition but then decrease with the start of lactation, where, instead, glucagon levels increase. Catecholamine levels are known to activate gluconeogenesis as well which is interesting seeing as they are present in early phases of stress. This explains the frequently observed outcome of imbalanced nitrogen equilibrium caused by stress (Bertoni, 1999).

Moreover, in ruminants, it is common knowledge that the protein asset derives not only from dietary input but also from rumen activity which primarily assigns non-amino acid nitrogen to microbial syntheses. However, when non-amino acid nitrogen is in excess, proteins and amino acids can be fermented to produce energy, yielding ammonia (NH₃). Ammonia is absorbed across the reticulorumen wall, into the blood stream and converted into urea in the liver. This is why blood urea levels must be considered when analyzing protein metabolism alongside blood protein counts (Bertoni, 1999).

Main causes of increased BUN levels are:

- Reduced glomerular filtration rate
- Inadequate renal perfusion which can occur during shock, congestive heart failure, dehydration and hypovolemia
- Increase of nitrogen waste products due to fever, burns, stress
- Dehydration due to vomiting, diarrhea, profuse sweating
- Increased protein catabolism in neoplasia, thyroid toxicosis and lymphoma
- Urinary tract obstruction due to urolithiasis or stenosis

Main causes of decreased BUN levels are:

- Under-nutrition
✓ Excessive fluid administration
✓ Hypoprotein diet
✓ Cirrhosis (Bizzeti M., 2007).

Normal range: 8-20 mg/dL

• Creatinine:

Creatinine is an end product of creatine phosphate in the muscle and is eliminated via the kidneys. It is the second most important nitrogen-based substance found in urine after urea. Its filtration through the glomerular is basically total and it is not submitted to filtration nor to re-absorption across renal tubules therefore, it directly measures glomerular filtration rate. So if the glomerular clearance is deficient for whatever reason, creatinine levels in the blood will rise, reflecting renal function (Bizzeti, 2007). It is not influenced by nutrition or by the state of hydration, but only by physical exercise, hyperthyroidism, glomerular failure, acute or chronic nephritis and uremic syndrome (Bertolin et al., 2002). Moreover, its blood levels may vary also due to alteration in the mass of muscle protein, especially when this is being rapidly mobilized from tissue to blood. If on the other hand, mobilization occurs at a slow rate and is prolonged in time, for example in early stages of lactation, then creatinine blood levels will be reduced (Berton, 1999).

Normal range: 0.7-3 mg/dL

• Cortisol:

Cortisol levels have been the subject of many studies aimed at evaluating animal welfare (Fagiolo et al., 2002; Fagiolo et al., 2004). Cortisol plasma concentration has been shown to increase during transportation of small ruminants along with body temperature and heart frequency, especially when they are being loaded on-board (Trentini et al., 2008). Cortisol is present as a constituent in saliva, urine and faeces and can be collected in an easier and less stressful manner. In fact, it is known that the collection of blood can be the immediate cause of increase of cortisol in the blood therefore it is a poorly accurate indicator.

The hypothalamic Corticotropin-releasing hormone (CRH) stimulates the synthesis and secretion of Adrenocorticotropic hormone (ACTH) which in turn stimulates the synthesis and release of cortisol among other hormones from the adrenal cortex, along the Hypothalamic-pituitary-adrenal axis (HPA). This phenomenon prevails during a stress response and is therefore often quantified when studying an organism’s physiological coping mechanisms used to endure stress. Cortisol enhances the organism’s catabolic pathways over the anabolic ones which are fundamental in preserving vital operations (especially the circulatory system) (Preziuso and Preziuso, 1999). Unfortunately, cortisol explicates suppression of the immune system which has been explained in depth.

Moreover, cortisol has a certain role during the initial phases of lactation where it stimulates the production of caseins (predominant phosphoprotein in milk). Nevertheless, stress is known to gradually decrease the synthesis of milk due to a negative effect on the oxytocin levels which are fundamental for stimulating the milk ejection. In fact, during lactation, glucocorticoid and catecholamine levels should be maintained low in order to avoid interference with milk productivity (Preziuso and Preziuso, 1999).
5. c) Immunological parameters

Clinical immunology has acquired importance among other parameters in evaluating the effort an animal must undergo in order to adapt to its own environment. The laboratory tests carried out supply interesting data regarding the discomfort or malaise an animal could be experiencing when coping with reduced welfare and greater susceptibility towards pathogens (Moscati and Beghelli, 2008).

- **Bactericidal/permeability-increasing protein (BPI):**

  This is an antibacterial protein that has been associated with the activity of innate immunity because it is a major constituent of the primary granules of neutrophils. Neutrophils and other phagocytes (such as complement components, Natural killer cells and T-cytotoxic cells) on encountering bacteria, coat them with positively charged molecules and consequently promote phagocytosis. This is called opsonization. The subsequent destruction occurs thanks to the respiratory burst, where superoxide anion is released from neutrophils (Tizard, 2004). This chemical burst literally produces holes in the cellular membrane (Carroll and Forsberg, 2007). At this point, primary granules migrate through the cytoplasm, fuse with the phagosome and release their lytic enzymes and antimicrobial peptides.

  BPI has the capacity of binding to the lipopolysaccharides (LPS) which are the main chemical substances that make up the cell walls of gram-negative bacteria (Tizard, 2004). Laboratory analysis of this parameter allows to determine the capacity of serum in inhibiting bacterial growth and is relevant in assessing welfare because for instance in swine it is one of the first non-specific defense mechanisms to be reduced when coping with stressful events (Moscati and Beghelli, 2008).

  Normal range: >90

- **Lysozyme:**

  The enzyme lysozyme, also known as muramidase or N-acetylmuramide glycanhydrolase, is contained in secondary granules of neutrophils and destroys bacterial peptidoglycans, which are abundant constituents of the cell wall of gram-positive bacteria. Lysozyme is also found in high concentrations in tears, urine, milk, saliva and other fluids of mammals. It has the ability of acting as an opsonin, binding to bacterial surfaces and facilitating phagocytosis during activation of the innate immune response (Tizard, 2004). By measuring lysozyme, information regarding the activity of granulocytes and monocytes is gathered which are possible indicators of inflammation (Moscati and Beghelli, 2008).

  Normal range: 1-3 μg/ml

- **CD4+/CD8+**

  Infection with parasitic nematodes is known to stimulate antigen-specific T-cell proliferation during the acute stage. However, there is profound lack of information on the
activity of CD4-positive (CD4+) T-cell subsets during the chronic phase of nematode infections (when T-cell reactivity is suppressed) and how exactly these T-cell subsets contribute to host-protective beneficial immune responses (Rausch et al., 2008). Regulatory T-helper cells represent a subset of CD4+ T cells that are critically involved in balancing the reactivity of the immune system and preventing autoimmunity. T cells have been shown to control excessive inflammatory responses against pathogens. Rausch (et al., 2008) aimed at investigating the role of the various CD4+ T-cell subsets during chronic phase of H. polygyrus in mice with regard to worm expulsion.

In another field trial, Moro (et al., 2008) selected CD4+ and CD8+ as relevant criteria in studying the immune profile during an assessment of animal welfare based on the knowledge that stress can reduce CD8+ T-cell subset as a consequence of cortisol activity. In normal conditions, endogenous glucocorticoids regulate the pool of T cells and the CD4+/CD8+ ratio (Carroll and Forsberg, 2007).

In domestic livestock, these are valid parameters used to measure acquired immunity. Together with parameters belonging to the innate immunity, assessing the relationship between the two systems becomes possible. They do not function independently and sometimes rely on similar communication molecules. It is known that up-regulation of innate immunity provides an important feed-forward for antibody production when, for instance, activated neutrophils stimulate the acquired system by releasing certain cytokines. In fact, the overall success of the immune system lies in maintaining an intricate balance between pro-inflammatory and anti-inflammatory cytokine profiles, and cellular versus humoral immunity (Carroll and Forsberg, 2007).

The following table illustrates the two main categories of the immune system and their principle components. In each division, the role of cytokines is of essential importance. The immune system, thanks to a fine and sophisticated balance between the two categories, is capable of defending the organism against almost any pathogen without attacking its own components (Moscati and Beghelli, 2008).

<table>
<thead>
<tr>
<th>IMMUNE SYSTEM</th>
<th>Humoral components of immunity</th>
<th>Cellular components of immunity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Innate immunity</td>
<td>Complement</td>
<td>Macrophages, neutrophils and NK cells</td>
</tr>
<tr>
<td>Acquired Immunity</td>
<td>Antibodies</td>
<td>Lymphocytes</td>
</tr>
</tbody>
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Table 6. Simplified classification of the immune system (Moscati and Beghelli, 2008).

It has been established that depression of innate cellular immunity (macrophages etc) is characterized by the persistent decline of CD4+ T-helper cells and by the elevation of CD8+ T-helper cells. CD4/CD8 ratio should never be inferior to 1 because this would predict immunosuppression (Zahorec, 2001).

What obstacles can be found?
Clearly the evaluation of welfare is an extremely complex matter and is fraught with specific constraints of which a few are the following: the validity of measures still needs appraisal; the interpretation is often difficult; parameters do not all have the same importance and data is sometimes missing. Generally speaking, scientists have classified measures used to assess animal welfare under two large groups: those measuring aspects of the animal’s environment and those directly measuring aspects of the animals themselves. Naturally, animal-based measures such as health parameters are generally considered more valid. Nevertheless, when no reference method exists, predictive validity will be looked at and many postulations may be formulated. Indeed, measures do not all have the same contribution to overall welfare and assigning relative weighting is always a critical task (Botreau et al., 2007b). Furthermore, measurements of health, productivity, stress physiology, immunology, normal and abnormal behaviour represent aspects of difficult interpretation, because they may show large differences between individual animals and yield conflicting evidence about a given set of circumstances (Edwards, 2008).

Section 6. Homeopathy, Immunity & Welfare

It has been suggested that homeopathic treatments essentially stimulate an organism’s natural responses aiming at balancing out the equilibrium with which an animal interacts with its proper environment. Thus, homeopathic remedies, when correctly applied, have the capacity to enhance the body’s self-curate mechanisms. However, scientific research supporting this fundamental concept is lacking, which calls for further studies that can demonstrate precisely how this effect is elaborated.

Currently, data which is in favour of the beneficial homeopathic effects towards immunity is scarcely consistent and insufficiently reliable, according to the scientific community. Nevertheless, promising results, although limited, should not be under-estimated nor underrated since in homeopathy, a remedy is validated when able to re-establish the animal-environment equilibrium once it has been shifted.

Homeopathic medication is not intended to have an antagonizing effect directed against the pathogen itself but enhances the immune response in a certain number of ways. Zacharias (et al., 2008) employed homeopathic medicines on the control of H. contortus in sheep reared in a semi-intensive system and the evaluation of the effectiveness was achieved through the following parameters: fecal parasitological examinations, immunoglobulin concentrations, haematological and serum biochemical analyses, weight gain and cost benefit. Two experimental groups were created: the homeopathic one and the control group. Parasite counts demonstrated no significant difference between the homeopathic group and the control group in considering FEC levels, however reduction of larvae per gram of faeces in the homeopathic group was highly significant compared to the control group. Haematological and biochemical parameters showed no significant differences among the two study groups; however, there was a significant correlation between parasitological and haematological results in homeopathy group and control group. The most interesting data which highlights the relation homeopathic medicine has with immunity is that IgG concentrations were higher in the homeopathic than the other two groups, where the rise of specific immunoglobulins was
higher. IgA and IgG levels were found to be negatively correlated with FEC counts therefore indicating that these are specific antibodies which hinder larvae establishment and participate in eliminating the worms (Zacharias et al., 2008).

It can be deduced that homeopathic medicine acts as a stimulator for the organism while conventional medicine directly eliminates the nematodes. Infact, in the homeopathic group there was a better recovery of erythropoiesis. Results also outline that the homeopathically treated animals have a higher count of circulating eosinophils which are fundamental in combating parasitism (Zacharias et al., 2008).

In another study, it was observed that the homeopathic remedy applied was able to keep FEC under the threshold level in terms of health and zootechnical damage (=300 UPG) in the long term which once again denotes that its effects may be more subtle but more enduring than conventional medicine (Benvenuti et al., 2008).

In naturally infested ewes, a monitoring program was carried out which resulted in total elimination of EGP in 37% of individuals, whereas only 7% exceeded 600 EPG level. This is again probably thanks to a certain level of resistance deriving from homeopathy (Pisseri et al., 2008).

The precise mechanism with which homeopathic medicine cures certain pathological conditions has not been entirely elucidated. Infact, no homeopathic remedy has received a registered brand name in Italy and there is not a sole model of assessment of the effects of homeopathy on immunity which has been certified as accurate. Nevertheless, numerous clinical studies are published, unfortunately these often involve humans as the objects of research and are deficient in the validity of results due to scarce funds.

Obviously, in order to specify and clarify the true instruments with which homeopathic remedies explicate their effects, further clinical research is needed to be achieved for this purpose.

The main objective of this study was to assess several welfare parameters and verify their reliability as useful tools in analyzing the responses manifested by an animal when treated with homeopathic medication, in the aim of controlling parasitological diseases in sheep raised extensively.