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IDENTIFICAZIONE ELETTRONICA DEGLI OVINI IN SARDEGNA: ANALISI RETROSPETTIVA DI UN QUINDICENNIO

ELECTRONIC IDENTIFICATION OF SHEEP IN SARDINIA: A RETROSPECTIVE ANALYSIS OF THE PAST FIFTEEN YEARS

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ABSTRACT

This doctorate thesis provides a general background and new research findings on the electronic identification of ruminants. The first chapter is a literature review on electronic devices used for livestock identification. The other chapters describe experimental work on electronic identification devices, mostly in dairy sheep but also in goats. Chapter 2 deals with the effect of the presence and permanence time (from few days to 9 years) of four types of ceramic boluses (differing in capsule size, weight and material) used for electronic identification of Sarda sheep in six farms on the reticulum and chemical-physical characteristics of the interface surface. The boluses showed the presence of calcium, manganese, and zinc salts. The long-term permanence of the bolus *in situ* caused little changes in the mucosa which did not seem to impair the organ function. Chapter 3 comparing traditional and electronic animal identification in dairy sheep farms showed that the electronic identification device was more realiable, effective and efficient than the ear tag and ear tattoo. Chapter 4 studied possible correlations between the presence of rumen bolus and calcium content in blood, during pregnancy and lactation, and in milk in early, mid and late lactation in Sarda sheep. Calcium content in blood and milk did not differ between ewes with or without ceramic bolus. Chapter 5 concerns animal reading and data collection efficiency of automated milk recording in dairy sheep and goats.

CHAPTER 1

The livestock identification

1.1 Electronic Identification and Legislation

Livestock production represents a driving force for the economy of Sardinia, a region with a clear agropastoral vocation. Sardinia boasts traditional local products with a range of local varieties. The added value of the island's products reflects the peculiarities of a production in which quality prevails predominantly over quantity with the exception of the sheep and goat dairy sector. However, at present, local productions in Sardinia are facing a dire situation, especially in the meat sector, with imported product such as lamb and suckling piglet sold as local production. This severely damages local farmers dismissing regional brand and misleading consumers.

An efficient system of traceability is not possible without a solid animal identification system, especially in relation to the requirements of EC Reg. 178/2002. A tool that would allow the traceability of animal products is the electronic identification based on RFID technology that provides for the use of transponders. Instructions governed by the European Union by Council Regulation (EC) No. 1760/2000 ask for a system of identification and registration of bovines and regarding the labeling of beef and beef-based products and Reg. (EC) No 21/2004 establishing a system of identification and registration of sheep and goats, D.M. May 5, 2006 for equine registry replaced by D.M. 29 December 2009 and No. 272 November 2011. These provisions will allow for the use of an efficient and difficult to counterfeit animal identification system, and will join the EU Reg. No. 1169/2011 of the European Parliament and the Council on the labelling of meat that require the mandatory declaration of origin for products that come into force in all Member States in 2015. This shows how traceability is, currently and probably will be even more in the future, an important theme for the industry of animal production. Therefore, considering production levels, the sheep and goat sectors deserve particular interest according to the territorial districts, followed by the bovine and equine sectors with specific differences of each sector. It is also worth mentioning that for the swine industry there is not any individual regulated anagraphical data and the industry, referred to in the Presidential Decree 317/96, is still somewhat behind.

Numerous contributions have been produced internationally over the years on a trial basis (Caja G. 2003 Caja et al., 1999, 2014; Cappai et al, 2013ab; Ghirardi et al., 2006; IDEA Project; Pinna et al., 2012, 2010ab, 2005, 2004) regarding the use of RFID

technology for animal identification in Europe with the devices used in agreement with the ISO standards 11784 and 11785.

Therefore the electronic identification arises as a robust instrument that can support different levels of traceability of livestock in the production chain. Last but not least by virtue of promising practical-application puratical, this technology could also facilitate considerable improvement of the management aspects for the farmer himself (i.e. the farm register), and the sector's operators (LP and NHS veterinary doctors, specialists) allowing a higher degree of transparency of the information available to the final consumer.

The European Regulation 21/2004 of 17 December 2003, published in the Official Gazette of January 9, 2004, established a system of identification and registration of small ruminants, sheep and goats, which amended the EC Regulation No.1782/2003 and Directives 92/102/EEC and 64/432/EEC. The requirement for the creation of this Regulation arose when the Council of the European Union considered outdated and unsatisfactory the previous directive (92/102/EEC), which appeared inadequate both for management purposes and for health issues. The Council of the European Union considered necessary to create a functional and effective register for sheep and goats, which relying on individual identification and registration of animals, had to be as safe as possible, easy to apply and had to take into account aspects relating to the animal well-being and the cost.

Furthermore, previously, the European Commission had promoted a pilot project for the introduction of new and innovative techniques for the identification of sheep and goats. The project, called IDEA (Electronic Identification of Animals), launched in 1998 and published in April 2002, was useful to show that the introduction of electronic identification in sheep and goats would allow a positive evolution in the identification and management of the registry of these species in production.

Following this study, the EC considered crucial the introduction of an electronic identification system of animals, because it allows the animal recognition uniquely since birth and for the duration of his life.

The EC Regulation no. 21/2004 has been amended by the following EC regulations:

• Reg. 1505/2006 outlined and developed the on-site control systems to verify compliance by keepers with the obligations of identification and registration of animals as provided in Regulation EC 21/2004.

• Reg. 1560/2007 postponed the obligation of electronic identification for all Member States to the 31 December 2009.

• Reg. 933/2008 provided new technical elements concerning the transponders, readers, and ear tags.

• Reg. 759/2009 intervened by providing some clarification on intra-Community trade or export to third countries of animals of the ovine and goat species.

Regulation No. 21/2004 has been adopted by the Italian Government with the circular 28/07/2005, which reports as general characters those required by the Community Regulation and implements them with technical-explanatory subsequent circulars (notes of the Ministry of Health Prot. No. 1110 of 25/01/2010 and Prot. No. 19781 of 06/11/2010).

The general legislative framework currently provides that the basic components and essential to manage a system of identification and registration of sheep are the following: 1) identification tools of each animal (conventional ear tags, tattoos, ruminal boluses, etc.); 2) records of loading and unloading kept up-to-date on each holding; 3) transport and destination documents of the animals; 4) central register held by the local health services or a national computerized database (BDN).

The key principles of the Regulation are based essentially on individual identification of animals, farm registration, and the establishment of the National Data Bank extended to all European Union countries.

Every farm, registered at the ASL of competence, is identified by the Health Authority through an unique alphanumeric identification code so composed: the first two letters indicate the member state (i.e. the IT symbol will indicate that the company is based in Italy); the next three digits indicate the ISTAT code of the town, followed by the initials of the province in which it operate; the latest digits indicate a unique sequential number (for example: IT003SS123).

For the purpose of rapid and accurate tracking and tracing of animals, each Member State must have a computerized database known as BDN (National Data Bank), which must include all holdings in its territory and the movements of animals (sale, slaughter

etc.). In Italy the computerized register system is managed by the Ministry of Health that uses, as a technical agency, the National Services Centre at the Institute of Experimental Animal Disease Prevention of Abruzzo and Molise (IZSAM) based in Teramo.

Also according to the decree of the Minister of Agriculture No. 1922 of 20 March 2015, art. 4, paragraph 7, the farmers who receive funds from the grants referred to Article 22 of the Ministerial Decree 18 November 2014 (awards for the sheep and goat sector) and funds and aid for measures of rural development, must complete the individual registration of animals in the BDN. In Sardinia individual registration of the animals in the BDN can be performed directly from accredited breeder or, upon written authorization: 1) by the Veterinary Service of the competent ASL for territory 2) by CAA (Agricultural Assistance Centre) 3) by the AIPA 4) by ARAS.

Artmann already in 1999 described the transponder as a tool not only useful for the management of breeding but also as a mechanism against fraud, as defined by the ISO11784 and ISO11785 standards for a clear and valid numeric coding. he also asserted that any new one must ensure the compatibility of current systems as well as define the conditions for compatibility with future systems.

1.2. Identification devices

Animal identification systems are divided into 3 groups: ear tag; tattooing; and electronic devices.

1.2.1. The ear tag

Technically, the ear tag complies with the following parameters (Circular 28/07/2005):
a) they are made of flexible plastic material, non-toxic and resistant;
b) they are easily readable for the duration of life of the animal;
c) they are not reusable and must be made so that they can only be removed by breaking them;
d) are designed so as to be applied with the least possible stress for the animal and remain fixed without being harmful;
e) they shall carry only indelible caption made by laser printing;
f) each ear tag shall consist of two parts, "male and female", of different size and shape such as to ensure the readability of the characters without effecting the animal's ear seal, with a shape that minimises the possibility of entanglement (Figure 1.1);
g) each part has a height between a minimum of 17 and a maximum of 40 mm;
h) each part has a width between a minimum of 14 and a maximum of 40 mm;
i) in the case of circular marks the diameter must be between 25 and 32 mm;
j) the characters shall have a minimum height of 4 mm;
k) they must be yellow;
l) the total weight of each mark must be between 2.0 and 7.5 grams;
m) the distance between the male part and female part, when closed must be between 7 and 15 mm;
n) the stud of the male part must be flexible, elastic and resistant with a diameter between 4 and 8 mm and in any case less than the diameter of the pin;
o) the pin of the male part (that is, the element that allows you to pierce the tissue to apply the mark) must be constructed in whole or in part of a harder material than the rest of the mark (such as hard plastics, metal) and if established from more parts these

should be assembled in such a way as not to allow the detachment. It is permitted a conformation of the pin that allow sampling of skin tissue (Figure 1.2);
p) the element of the female part which receive the pin should be partially or totally closed. Models intended for the collection of biological samples must still be equipped with a system which prevents the reuse of the mark and highlight attempts of replacement or manipulation, either complete or partial;
q) after 1 year from the application, at visual examination, the surface must be free of welds, bubbles, cracks, fissure and other defects; the color should be uniform and the marking visible.
Ear tags, applied using a special ear tag applicator, enable the perforation of the ear on the part of the pin this on top of tag. In the whole part of the auricle, the plier engages a plastic block that allows the perfect adherence of the ear tag.

1.2.2. Tattooing

The tattoo is imprinted using a special plier which hold cards with small needles depicting letters and numbers (Figure 1.3). The cards are positioned in the way to compose exactly the individual code assigned to the animal. Through closing the pliers the code is transferred on the exterior of the ear in the form of micro-holes. The subsequent application of a dark colored ink on the holes made with the tattooing pliers makes the code visible and indelible throughout the animal's life. Tattoos are usually applied on the ears or under the tail either on the right or on the left.

1.2.3. Electronic device (Transponder)

There are two types of electronic devices: ear tag containing transponder; and bolus rumen containing transponder. Both should respond to specific characteristics (Ministerial Circular of 28/07/2005):
a) animal's read-only passive transponders applying HDX Half Duplex or Full Duplex FDXB technology, complying to ISO 11784 and ISO 11785;
b) electronic identifiers must be readable by reading devices, complying with standard ISO11785, capable of reading HDX and FDX-B;

c) once the animal is identified with the transponder the reading distance for portable readers must be of at least 12 cm for ear tags and a minimum of 20 cm for ruminal boluses; for stationary readers a minimum of 50 cm for both ear tags and ruminal boluses; these minimum distances are to be checked after implantation of the device;
d) the application of the electronic identifier must be done by specially trained personnel in a manner as to operate the appropriate modalities for identification;
e) the electronic identification devices at the end of productive life of animals (death or slaughter) must always be retrieved and made unusable under the control of the competent veterinary services for the area.

1.2.4 The ceramic device (Bolus)

The bolus is a ceramic cylinder with two ends or poles, one of which has a rounded portion to facilitate swallowing. The other pole has a circular opening that, after the transponder is inserted (Figure 1.4), is sealed with commercial silicon resistant to the corrosive action of gastric juice. It can have different weights and dimensions depending on the species on which is going to be used.
The Ministry of Health, through the circular of 30 March 2007 in accordance to the European Commission Decision no. 968/2006 of the 15 December 2006, gave technical provisions regarding the use of ruminal boluses with specific characteristics for sheep and goat. The marketing of ruminal boluses is allowed only for those models that bear the certifications indicated by technical Guidelines of the JRC of the European Commission (JRC) concerning performance testing, thermal and mechanical stress tests, endurance tests.
In the case of ruminal boluses they must clearly display, on the outer surface, the 12 digits constituting the animal's individual identification code, unless the packaging does not allow an immediate coupling between the transponder and its ear tag. Furthermore boluses should be made with biocompatible material, with low porosity and absence of external irregularities, and with rounded edges.
Currently there are three types of bolus available (Table 1.1) for use in sheep and goats: ruminal boluses of about 75 grams that can be applied in lambs and kids of 25 kg live weight, ruminal boluses of 52 grams, compatible with animals at least 15 kg of weight,

and ruminal boluses of 20 grams for lambs of at least 30-35 days old. Special attention shall be placed in the selection of the bolus size which shall be adequately related to the size and weight of the animals.

Administration should be performed by trained personnel and using the "bolus applicator" suitable for small ruminants. The application can be performed by a single operator, if the animals have already been suitably contained (for example in the rack). After measuring the distance between the angle of the jaw and the buccal opening, the "bolus applicator" is introduced in the buccal cavity. The ceramic bolus is then put into the slot with the rounded part outwards in oerder to prevent mechanical damage on the digestive system. Subsequently, rated the positioning of the bolus in the proximity of the base of tongue's root, the bolus is released and swallowed spontaneously by the animal. It is counterproductive to attempt to force the swallowing since this can cause accidents during administration. To verify the correct positioning and functioning of the bolus in the reticulum a reading is carried out on of the left side of the animal in the proximity of the hypochondrium region. The bolus will remain in the reticulum for the entire life of the animal unless is case of accidental loss.

Numerous contributions have been published internationally over the years about field trials on the use of RFID technology for animal identification in Europe with devices in agreement to the ISO Standard 11784 and 11785 specifications (Caja G., 2003; Caja G. et al., 1999, 2014; Cappai M.G. et al., 2013b, Ghirardi J.J. et al., 2006; IDEA Project; Pinna W. et al., 2012, 2010ab, 2005, 2004). The electronic identification can be therefore considered a robust tool that can support different levels of traceability of livestock production chain. Last but not least, by virtue of promising prospectives of practical-application puratical, this technology could also facilitate improvements of the managerial aspects for the breeders (i.e. the flock register) and the operators of the sector (veterinarians LP and of the National Health Service, Technicians) and to attain a higher degree of transparency of the information available to the consumer.

1.2.5 Transponder readers

The reader substantially performs the function of activating the transponder and receiving the entire sequence of numbers that represents the identification code of the

animals, by displaying it on a display in the case of portable players or storing it in a computer in the case of stationery readers.

The portable readers perform a static reading: the electronic code is detected with stationary animal. Portable readers are often powered by a battery, they may have an internal or an external antenna, and may be connected to the reading instrument via cable. This become useful when it is not possible to get close enough to the animals. The antenna transmits the identification code to the reader which show it on its display. Some readers support a software that elaborate the measured data and transfer them via cable or via Bluetooth to a computer.

Stationery readers perform a dynamic reading, as they offer the possibility of detecting the electronic code (EIC) even when the animals are moving. They are have one or more antennas with different shapes and sizes, an electric current power supply and a reader.

The antennas may be placed in forced corridors or passages, with a width that allows the passage of only one animal at a time, through which the animals are forced to pass at a rate suitable for the system to correctly read the codes. Therefore the device identify the animals during their passage. Stationery readers can be movable, i.e. displaceable from a location to another, or unmovable, designed and fixed in a permanent manner to a structures in a plant.

The scientific literature addressing the assessment of reading tools, used for individual electronic identification of animals, is somewhat lacking and we have to go back to 2007 to find a job that has approached the topic according to the Analytical Hierarchy Process (Pinna et al., 2007) of the using three criteria that are adopted by many medical devices: reliability; accuracy and efficiency.

Reliability is described by the following two performance indicators:

a. Repeatability: refers to the individual code identifying the individual animal that maintains its sequence unchanged and unchangeable in each control with the purposes of the case.

b. Univocability: refers to the assignment of the individual code of the transponder to one and only one animal; this indicator is satisfied when each animal has a unique and exclusive individual code.

Accuracy is described by the following performance indicator:

a. Readability: refers to the ability of the operator to detect the individual code possessed by the transponder of the animal, with fixed or portable player, in any field condition. The greater the percentage of the transponder readability for the animals electronically identified on the farm, the greater its Accuracy. The Readability is denoted by R% (readability) and it is expressed as a percentage by the formula: R = 100 x (number transponder read) / (number animal with transponder).

Efficiency is described by the three following performance indicators:

a. Tool Functioning: refers to the ability by the operator to use, without technical problems, readers and transponders.

b. Technician's Activity Number: is based on the comparison of the number of operations performed by an operator making use of electronic identification technology compared to another that performs the same activities without the help of electronic identification technology; this indicator is satisfied when the operator that make use of electronic identification technology performs the planned activities in the shortest possible time.

c. Technician's Performance Time/Head: refers to the time taken by the operator to carry out its activities on a single head; In absolute terms, this indicator is satisfied when the time for action accomplished by the operator, equipped by electronic identification technology, on the single head is the minimum possible.

1.3. Animal identification and animal welfare

The Royal Decree No. 404 of 1898 required to identify the livestock by the master brand, for cattle and horses, and through "sos Sinnos" in the ear, for sheep and goats.

For the first two species the master brand was affixed with a hot iron, at whose end was a symbol (often the initial letters of the owner's name and surname), applied on the rump or thigh of the animal for few seconds. The burn of more or less deep layers of the skin give rise to a lasting and recognizable brand, useful to identify the owner of the animal.

For sheep instead it was common to remove parts of the auricle, "drawing" special shapes, so as to be recognizable and especially indelible. These practices are now classified as mutilation, due to the removal of tissue for no therapeutic purposes. They are now prohibited and therefore almost abandoned. In European countries they are not recognized as an official identification and they are banned by farming practices to promote the principles of respect of animal welfare in farming. These identification practices were certainly a bloody and painful system for animals which could also suffer from various kinds of complications and secondary trauma. Nevertheless, until short time ago, they were the only means available to solve the problem of detection and identification of livestock.

Although the new identification systems are less intrusive and painful than those used in the past, they are anyway practices that, if mismanaged, could still distress the animals, therefore it's essential to respect the need to protect animal welfare.

The meaning of "animal welfare" cannot be described and enclosed in a single statement; several authors have tried to express this concept. Hughes perhaps was the first when, in 1976, defined it as "the physical and mental state of health, in which the animal is in physical and psychological harmony with the body and its environment"; more recently Broom (1986) summarize the concept with the expression "the welfare of an organism is its status in relation to its attempts to adapt to the environment".

Currently the concept of animal welfare focuses on five fundamental freedoms taken by the Farm Animal Welfare Committee (F.A.W.C., 1993): 1) freedom from hunger and thirst; 2) freedom from discomfort; 3) freedom from pain, injury or disease; 4) freedom to express normal behavior; 5) freedom from fear and distress. Research on animal

welfare can be considered as a tool to improve knowledge regarding the animals, in particular with reference to their physical and mental aspects. This tool is dynamically evolving with human society and changes in the understanding of the biological world and it can offer benefits for both animals and humans who breed and care for them (Carenzi and Verga, 2009).

Therefore these principles must be guaranteed at all stages of the livestock productive life, including the individual identification. This practice must not cause pain and discomfort during device's application and in the rest of the animal's life.

The ceramic bolus, which is designed to remain inside the fore-stomachs, usually in the reticulum, should not accentuate any problems that may occur from grazing, especially the one carried out in areas where Mediterranean shrub prevails over the turf.

In 2008 Pinna et al., assessed the impact on health in kids, in relation to the application of three types of identifiers (as specified by Annex A to the Reg. EC 21/2004): electronic identification (EID), ear tag (ET), ear tattooing (ETT). Specifically they analyzed kids ethogram in the first hours after the identification, paying particular attention on infrequent behaviors directly attributable to the identification: relief of pain groans; momentary unconsciousness; tendency to isolation; and shaking of the head. They ascertained that the system which provided less disturbance of the animal behavior was the electronic identifier, in that case, the ruminal bolus of 70 g containing passive transponder. Both the application of the ear tag and the ear tattooing caused further, minor but detectable, abnormal behavior in most of the animals for a few hours after the application (Pinna et al., 2008).

Some complications may arise from the use of inappropriate ruminal boluses to a specific animal category as showed by the research group of Animal Production, University of Sassari, in their study on a herd of Maltese goats. The study pointed out that ruminal ceramic mini-boluses (70x21 mm, 20g), equipped with transponders (32.5 x 3.8 mm) with Half Duplex, were not retained in animals identified at a young age. The study also showed clearly that the use of those boluses had been adopted without any prior testing and not adequately tested in field conditions (Pinna et al., 2010b).

The Sardinian farming system for dairy ovine, goats and beef cattle, is basically based on the day grazing and shelter at night. Hence the need to explore what may be the

practical implications of the use of different identification system in a semi-extensive livestock management system.

The tattoo system will not exacerbate any problems that may occur from grazing on "difficult" pastures since is imprinted in the outer ear (Figure 1.5).

Ear tags, both simple printed and those with the transponder inside, on the contrary present some contraindications. Ear tags are, for the large part, made of plastic on which the animal identification code is printed. They are applied by pliers in the pinna, punching it. This practice causes a significant amount of pain to the animal, evidenced by plaintive bleating, shaking of the head and bleeding. Episodes of non-healing of the wound, resulting in septic inflammation (purulent otitis), myiasis and various complications (Figure 1.6), may also occur compromising the health and welfare of the animal (Manca, 2006).

Loss of ear tags may occur several times per year, due to the traumatic action determined by the branches of Mediterranean shrubs which, caught up in the tags, can cause the lacerations of the ear and in the most extreme cases the loss of the tag itself. These drawbacks occur mostly in semi-extensive farming where grazing plays a key role in livestock feeding.

So it is good practice to consider what the risk factors related to animal identification might be in relation to the farming method and the pasture type. In Sardinia this is of particular interest due to the pasture composition, especially in hilly areas and slopes, where dense shrubbery is the predominant component.

1.4. Double device (electronic and ceramic) and its interactions with animal

Electronic identifiers such as ruminal boluses, apart from having an effect on the animal welfare conditions, may interact with the animal anatomy, in particular with the reticulum where boluses are usually localized as a consequence of gravity.

In 2006 Antonini et al., revealed the presence of a "footprint" left by ceramic boluses on 8 reticula and 1 rumen of cattle. The histological examination of the organs did not detect any specific lesion in the mucosa of dystrophic areas. The in vitro analysis of the reticulum-rumen mucosa subjected to a long exposure to an active transponder reveled changes due to mechanical action. This observation had little biological relevance since in the field the transponder of the boluses is activated only for a few minutes a day during business operations.

The work of Cappai et al. (2013a) showed that the constant presence, for a long period of time, of a ruminal bolus in the reticulum of Sardinian cattle, bred with the extensive system, resulted in significant morphological changes of the forestomach examined, but since this observation was made after slaughter there was no evidence of negative interactions on the functionality of the organ.

The macroscopic analysis and subsequent microscopic analysis showed a thickening of the mucosa which led to a thinning of the lattice ridges in the point of greater permanence of the ceramic bolus.

Therefore electronic identification system proved itself to be reliable, efficient, effective and above all safe in the long run, although it caused some type of bolus-induced morphological alteration.

Garin et al. (2003) studied the modification of the size of the papillae and the keratinization in the rumen using boluses of 5.2 g and 20 g. The study showed an increase of the dimension of rumen papillae and lower keratinizing, probably due to friction and greater stimulation of rumen motility, with not negative effects on health and on fattening of the lambs.

1.5. Positioning and permanence of the electronic device and its support in the animal organism. Ruminants: bolus location in the ruminant body. The reticulum

The reticulum is part of the prestomachs of ruminants, it is located cranioventral from the rumen, where it receives the outlet of the esophagus, and it communicates extensively with it making a functional unit called reticulum-rumen complex. In sheep it has a volume of about 1-2 liters and can be distinguished from the other prestomachs by its particular conformation: the inner surface presents a series of ridges formed by the folds of the mucosa, which delimit square or hexagonal cells called reticular cells. The mucosal epithelium is stratified squamous cornified.

Predominantly the reticulum performs a mechanical function, its importance derives from the fact that it represents the anatomical element of conjunction between esophagus, rumen and omasum. Its contraction represents the first phase of the movement of the gastric compartments. Foods fall directly into the atrium of the rumen at the first intake, with the exception of heavy parts and cereal crops; on the contrary, foods derived from second mastication (rumination), more rich in water and which form a dense solution, fall in the reticulum which conveys them towards the reticulum-omasum orifice. The contraction of the reticulum perform different actions: convey the food towards the esophagus during rumination; make them progress towards omasum after the second chewing; pass a part of the same foods towards the rumen (Barone, 2003).

1.6. The role of calcium (Ca) in lactation ewes and its interactions with the metabolic profile

Calcium (Ca) is an alkaline earth metal essential for its metabolic role. It is a constituent of bone tissue, has a role in the transmission of neuro-muscular impulses, and it is involved in the regulation of cell membrane's permeability, etc. The analysis of the metabolic profiles, carried out on animal blood samples, allows to investigate the concentration of circulating metabolites in the blood.

The presence of calcium in the blood is mainly regulated by three hormones: parathyroid hormone (PTH), calcitonin (CT) and 1,25-dihydroxycholecalciferol (commonly known as calcitriol or vitamin D3), which control and manage the intestinal absorption of calcium. Therefore it's essential to monitor the animal's physiological response in situations where there is greater need for calcium, such as pregnancy and lactation stages (Robinson and Land, 1985).

In pregnancy significant physiological changes are observed, including a higher absorption of calcium, mainly influenced by calcitriol; the upper concentration is apparently due to prolactin (PRL) and placental lactogen hormone (HPL).

Immediately after birth, with the beginning of lactation, the need for calcium grows strongly, as showed by its concentration in sheep milk which is 193 mg/l in comparison to the blood one which is 13 times lower levels (Pulina and Nudda, 2004). As a result of these fluctuations, low values of Ca^{2+} concentrations in serum may be due to very serious metabolic diseases, such as hypocalcaemia and milk fever, caused by insufficient immediate availability of circulating calcium.

This shortage is due to the fact that, during pregnancy, there is a decline of active absorption since the passive one is sufficient (food intake must be well balanced); that plenty of calcium trigger mechanisms for reducing the absorption of calcium from the intestine by inhibiting the production of calcitriol from calcidiol (or vitamin D2). As the demands increase, with lactation, the passive absorption become insufficient (absorption less effective than the active one) so the active absorption must be restored: PTH triggers the conversion of vitamin D2 in the kidney to form calcitriol, which in turn stimulates the production of particular enzymes and proteins (CABP, or calciumbindingproteins) in the intestinal cells. This recovery mechanism of uptake

activity needs few days, this is incompatible with exponential requests indispensable immediately after birth and beginning of milk production.

It is very important to be able to monitor these metabolic aspects during periods of transition from one physiological state to another, since the onset of hypocalcemia after birth may cause a lack of muscle tone, dislocation of the abomasum, rumen stasis, retained placenta, etc. (Horst et al., 2005).

In this phase excesses of sodium and potassium, especially the second one, may adversely affect the amount of cellular receptors for calcitriol, causing a substantial reduction of the stimulating action in the intestine and bone level.

In continuing of lactation, the amount of calcium needed is satisfied by the hypertrophy of intestinal walls, since this guarantees a greater absorbent surface, but especially by the increase of calcitriol, due both to the PTH and probably to prolactin.

Even calcitonin levels increase at this stage, probably for the increased ingestion which in turn results in a greater presence of hormones in the digestive system. The combined effect of the two hormones allows the body to avoid hypercalcemia after meals and in particular to avoid excessive depletion of bone tissue.

The efficiency with which calcium is absorbed in the intestine and bone mobilization are also influenced by the age of the animal, both of them are higher in young animals in the growth stage.

Only data on cattle is available in literature: at first calving there is a significant decrease in serum calcium in the hours following the meal, probably due to the effect of calcitonin released before the absorption of important amounts of calcium (Bertoni et al., 1999).

Given the importance that calcium has at biological level in animals and particularly in animals bred for milk production, such as the Sardinian race sheep, it is important to monitor any possible effect of the ceramic capsule of the boluses on the levels of calcium through the metabolic profiles.

It is also important to assess how the concentration of calcium in serum changes depending on the physiological condition of the animal and how this can be in some way influenced by the presence or not of a ruminal bolus, in order to prevent the onset of metabolic diseases due to the seizure of this element making it less available for the biological maintenance and production functions.

Deficiency syndromes appear more detrimental in developing animals where even if the disease is not so severe to cause bone mineralization, which can lead to rickets (Marcato, 2002), affecting the skeletal development of the animal, it will nevertheless affect the productive potential of the adult animal. Deficiency in the juvenile stage could have economically significant effects even in animals for milk production as it may adversely affect bone calcium mobilization in the stage of lactation.

In particular, it can highlight the nutritional metabolic importance of its subclinical forms, with "simple" events of prolonged loss of appetite, muscle tremors and atony of the rumen (Bernardini, 1998).

Aspects relating to the production of animals can assume considerable importance, because blood calcium deficiency imply a deficient transfer of this element in milk. Being the level of calcium in blood is in close relation with the level of calcium present in the milk, nutritionally it is advantageous to promote its availability for the transfer in the milk itself.

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1.8. Tables

Table 1.1 Technical characteristics of the ruminal boluses

MODELS	Bolus "20" Lambs of 30-35 days old	Bolus "50" Lambs weighing more than 15 kg	Bolus "70" Lambs of 25 kg of live weight
DIAMETER (mm)	11.9 mm	16.8 mm	20.2 mm
LENGTH (mm)	55 mm ± 1.5 mm	66.8 mm ± 1.5 mm	66.6 mm ± 1.5 mm
WEIGHT (g)	21 gr	53 gr	71gr
DENSITY '(g/ cm3)	3.65 g/ cm^3	3.65 g/ cm^3	3.65 g/ cm^3
READING DISTANCE (cm)	until 80 cm	until 80 cm	until 80 cm
PROTOCOL OF READING	ISO 11784 / 11785	ISO 11784 / 11785	ISO 11784 / 11785
OPERATING FREQUENCY (kHz)	134,2 kHz	134,2 kHz	134,2 kHz
DIM. MEMORY (bit)	64 bit	64 bit	64 bit
BIT RATE	8 kbaud	8 kbaud	8 kbaud
ROUGHNESS	< 0.5 micron (Ra)	< 0.5 micron (Ra)	< 0.5 micron (Ra)
TEMP. OPERATING (°C)	- 25°C fino a 70°C	- 25°C fino a 70°C	- 25°C fino a 70°C
PROTECTION CLASS	IP 68	IP 68	IP 68

1.9. Figures

Figure 1.1. Ear tag made of plastic flag male piece

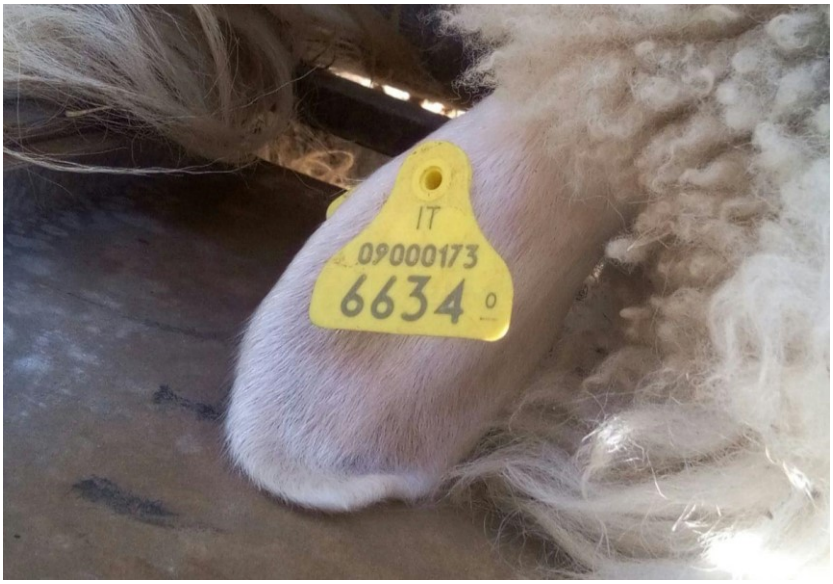

Figure 1.2. Example of device applied. Conventional ear tag used for the identification of dairy sheep

Figure 1.3. Tattooing hammers with special plier.

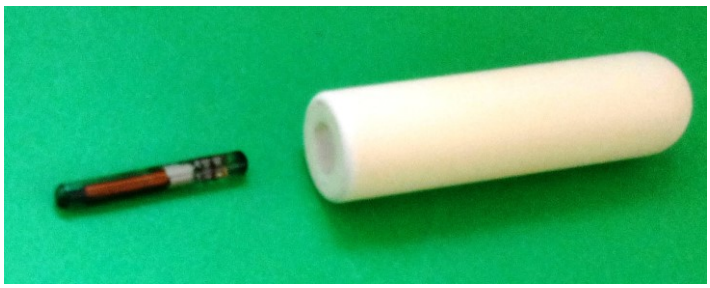

Figure 1.4. Ceramic bolus in which is inserted the transponder

Figure 1.5 The tattoo has no interference being imprinted in the outer ear

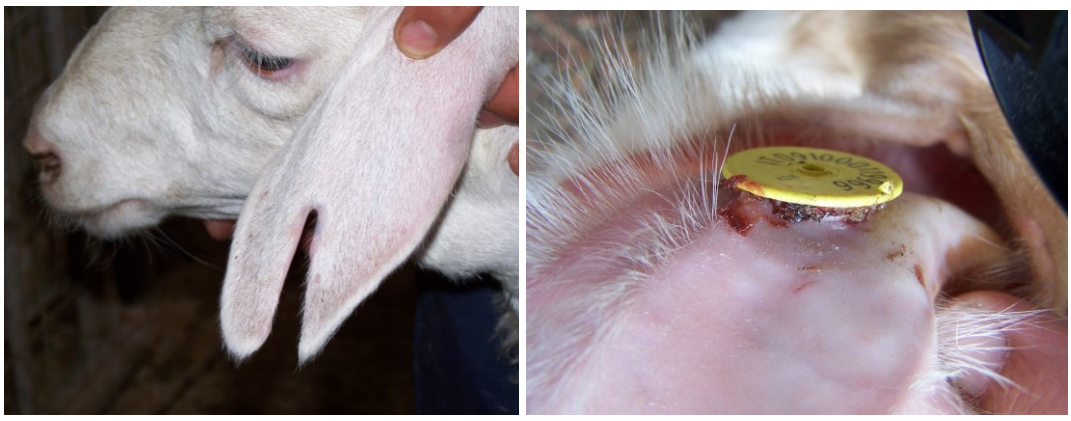

Figure 1.6. Tearing out and healing problems in sheep with plastic ear tags

CHAPTER 2

Part of this chapter was presented at XXII Congresso Nazionale S.I.P.A.O.C. Cuneo 13/16 settembre 2016 (Appendix 1), and Convegno AISSA, Sassari 6-7 novembre 2014 (Appendix 2), and International Workshop "New updates on animal nutrition, natural feeding sources and environmental sustainability", Arzachena 5-6 maggio 2014 (Appendix 3) (see Appendices 1,2 and 3)

Effect of the presence of the ceramic rumen bolus for electronic identification of sheep (RFID) on the reticulum and on the chemical-physical characteristics of the interface surface

2.1. Abstract

Electronic devices used for individual identification of small ruminants are in contact with tissues, inside the animal body, for presumably a long time. To date very little is known about the interactions which may occur between the host and the electronic device though made, as much as possible, of biocompatible and harmless material. In this study we analysed the effect of the permanence on the electronic device, a ceramic bolus, in the reticulum medium and the response of the mucosa of the reticulum to the bolus, in relation to the time to the permanence time inside the body. A total of 222 reticula from slaughtered ewes were analyzed as samples together with the respective boluses for individual identification. The animals came from six extensive sheep farms and the time of permanence of the identification device ranged from a few days to 9 years. For each bolus changes of the surface texture, change of color as well as changes in the elemental composition of the material of the capsules were studied.

The reticulum showed little to modest changes of its internal surface. Microscopically, the mucosa displayed shorted but thicker crests (primary and secondary) right in the place where the bolus most frequently resided (bottom and diaphragmatic side). Those modification could be observed after nine month from the application of the identification device and showed to be affected by the farm. The elemental analysis on the bolus, performed by X-ray fluorescence, detected the presence of calcium, manganese, and zinc salts but no trace of heavy metal. In conclusion the permanence, for long period, of the electronic device induced little changes on the mucosa of the reticulum as they do not seem to impair the organ function. It is interesting to point out the effects on the elemental composition both in terms of mineral compounds sequestered from the diet and as an environmental sentinel of heavy metals contamination.

2.2. Introduction

Radio Frequency Identification (RFID) is a technology based on a low frequency (134.2 kHz) which is now being used for animal identification purposes (ISO standard 11784-11785). When applied to ruminants, the transponder is held in the animal body through its incorporation into a ceramic case (bolus), administered to ruminants *per os* to allow the retaining in the pre-stomachs (reticulum) (Official Journal of the European Union L 5, 1–17 Council of the European Union, 2003. Regulation (EC) No. 21/2004). Despite several investigations were carried out to assess both technological and economical impacts from a massive RFID use (Butler et al, 2009; Caja et al., 1999; Carnè et al. 2011, Garìn et al., 2003, Pinna et al. 2012), an extensive characterization of the devices after long-term permanence in the ruminant body is still missing. To fill this gap, we have used a multi-technique approach for determining the wearing effects on the RFIDs device, with specific regard to the case, in relation to different animal/managerial/environmental parameters. Micro-X-ray fluorescence spectrometry (μ-XRF) and X-ray diffraction (XRD) have been applied to RFIDs boluses, showing different characteristics, after their removal from the carcasses of slaughtered animals (Lasio et al., 2014).

The total appearance of any object is a combination of its chromatic attributes, the color is given by lightness, hue and saturation and its geometric attributes (Eugène, 2008). Colours are studied by colorimetry which is a branch of spectrometry. Spectrometry studies the measurement of light in the visible range. The color is a subjective perception, seeing a color is therefore a sensation. Sensations are not perceived in the same way by each individual, for this reason color variations, in our case of the boluses surface, are analysed with a colorimeter. The aim of the colorimetry is to represent, or to quantify, the human eye response mechanism to the three primary colors.

Each color can be uniquely identified by three parameters: color (or color tone), saturation and relative brightness (or clarity, lightness). Most of the models make use of the color's additivity principle, which is the principle by which cameras, monitors, projectors, etc. work; this principle can be demonstrated by superimposing three primary lights (red, green and blue, or RGB) on a white screen. The secondary colors

are: yellow, indigo and violet. The system used to analyses color variations of the boluses was the Lab system, proposed by the CIE in 1976. This system was born to takle the need to find a method able to express color differences in an objective way. CIELab color space describe all the colors perceived by the human eye (Schanda J., 2007). The Lab space is widely used in color measurement using colorimeters and in color reproduction even in digital form. Furthermore it also allows to define the difference between two colors (ΔE *), in fact, being a three-dimensional, the distance between the chromaticity coordinates can be calculated using the Pythagorean theorem. ($\Delta \mathbf{E}^* = \sqrt{(\text{diff. between values } L^*)^2 + (\text{diff. between values } a^*)^2 + (\text{diff. between values } b^*)^2}$.

Low radio frequency identification devices for animal registration and traceability are an instrument functional to operators in this sector.

To complete previous studies on short and medium term of permanence of boluses inside the animal (Cappai et al., 2013a, 2013b), we extend the timeframe of collection of the experimental evidence conducting the study in Sardinia (Italy). Garín et al. (2003), a studied lambs concluding that neither the M or the S type of boluses affected the dimensions of the reticulorumen, however they found that the earlier presence of M boluses induced a greater papillae size, with no negative effects on health and fattening performances of young lambs.

2.3. Materials and Methods

Animals and farms

For the implementation of the experimental protocol Sarda sheeps from six farms were recruited. The farms are situated in different locations in Sardinia, with altitude above sea level between 280 and 900 meters, which had similar characteristics but different management practices for pastures. Farm A is locatede in the countryside of Arbus, farm B in the countryside of Norbello, farm C in the countryside of Nurallao, farm D in the countryside of Onani, farm E in the countryside of Nuragus, and farm F in the countryside of Isili.

Field activities

The experimental activities for the first test involved the analysis of ceramic boluses recovered from animals slaughtered regularly. At the time of evisceration the

prestomachal package was removed from the carcass after double ligation of the junction esophagus-reticulum and reticulum-rumen. Separately after the dissection of prestomach viscera the contents of the rumen and reticulum were inspected for the recovery of the bolus. On an aliquot (1 g) of the reticulum content, collected immediately after slaughter and diluted in 5 ml of distilled water in a test tube on an analytical balance, the pH of the solution was determined at room temperature (25° C) with a portable pH meter (Cyberscan pH 11/110), in order to determine the acidity of the medium.

Following complete recording of the anatomical margins between the reticulum and the dorsal sac of the rumen, superiorly, and of the margins which separate the reticulum from the omasum, the mean ventrally, the inner surface of the organ was exteriorized, rinsed by immersion of in water and inspected macroscopically. |This evaluation had the purpose to verify the presence of any abnormalities of the mucosa morphology.

The surface of the collected boluses was analysed to identify concretions and colour changes (Minolta CR – 300). Each bolus was verified readability and technical specifications.

Laboratory activities

The analyses were carried out on a total of 222 boluses from the six farms. 99 from farm A (44.6%); 45 from farm B (20.3%); 36 from farm C (16.2%); 17 from farm D (7.6%); 13 from farm E (5.9%); and 12 from farm F (5.4%).

A data set, with the data collected from the recovered boluses, was created for each company containing several variables: EIC (electronic identification code); CIC (Conventional visual identification); manufacturers; Given application; Applied for; Removed from; Type of recovery; External features of the bolus; Bolus type.

Analysis of the concretions and colorimetry on boluses

All data included in the data set were taken into account giving preeminence to those whose permanence time was known. A first assessment of the concretions on the boluses surface was made using a metric scale. This was create to better assess the concretions present in boluses after permanence inside the animal (Figure 2.1). The metric scale goes from 0 to 10, where a score of zero represents a completely white

bolus while 10 represents a bolus with concretions of homogeneous thickness on the entire surface of the bolus (Table 2.1).

Since the perception of color is very subjective, to evaluate the color variations in the boluses in a more objective way we used a colorimeter which works with CIElab color space, similar to the one perceived by the human . The three coordinates of this color space are indicated with the letters: L* (brightness), a* (first coordinate chromatic), and b* (chromatic second coordinate). The brightness "L" ranges from 0 (no light) to 100 (maximum brightness). The second coordinate "a" expresses red when it is positive and green when it is negative. The third coordinate "b" expresses yellow when it is positive and blue when it is negative. Coordinates a* and b* may vary from -∞ to + ∞. For L = 0 and L = 100 a* and b* assume the value of zero. The difference between two colors (ΔE*) is represented in Table 2.2.

To measure the color change in the test bolus a colorimeter Minolta CR- 300 was used. The instrument was calibrated, before measuring the variations in color of the boluses, by measuring the color of a white tile supplied with the instrument. Three measurements were made for each bolus, from left to right, by dividing it into three equal parts (Figure 2.2).

Analysis of the elemental composition of the surface of the bolus

A "random" pool of the recovered boluses (18), representing 6.7% of the total amount, were analyzed for elemental composition. This pool was made up by boluses Z72 and Z50, whose capsules were mainly made of Al2O3, and boluses Z70 and Z20 whose capsules were made mainly of ZrO2.

The analysis was carried out observing the samples along the surface of the ceramic device; on the area of the silicone cup that closes the internal cavity; and, if existent, on the concretions.

For the analysis X-ray fluorescence (XRF) was used, a technique that measure the fluorescent emitted by the elements when they are exposed to a source of high intensity X-rays. To avoid problems of focus of the instrument on the surface of the bolus an ad hoc method was developed.

The samples were positioned on the support and suitably fixed so as not to move and go out focus. Boluses due to their cylindrical shape do not adhere perfectly to the

measurement plane. The analysis was carried out along an ideal line taking twenty spectra (five minutes of each acquisition) equally spaced. The spectra were then averaged to obtain a better quantification of the elements.

The boluses were focused through a microscope, allocated inside the instrument, with goals 10 X 100 X. Then an "Autopoint" was set, through the Tornado M4 software, drawing a line along the length of the sample and the twenty spectra were finally recorded. The analysis was performed with vacuum inside the X-ray tube and a filter was applied to reduce artefacts.

The quantification of the elements was carried out with the help of a dedicated software installed in the instrument. The peaks present in the spectra were first identified the peaks, then the identification/automatic quantification was launched and by comparison with a database the elements present were identified. A series of "Blank samples", that is a series of ceramic devices that had not been applied to the animals, were analyzed to obtain a reference against which compare the results obtained from the boluses recovered from the various farms and at different permanence times (Figure 2.3).

The area of the cup was analyzed by an X-ray fluorescence map to evaluate the cause of the losses of the silicon cup, the presence of concretions and the elemental composition. The maps were obtained analyzing a square area around the cup from 4 to 8 hours. Finally an easily detachable concretion from a Z20 type bolus, coming from an animal of farm C, was analysed by X-ray diffraction to determine the nature of the crystalline phases present in the sample.

Analysis of the mucosa of the reticula

The reticulum samples analysed belonged to 85 adult Sardinian sheep, breeded in the six farms and identified electronically by a minimum of 4 days to a maximum of 9 years at the time of slaughter. The excision of the sample was made in full-thickness including adventitia, media, and internal, and taking care to include the part of the mucosa in which the visible crests appeared, morphologically different due to the contact with the bolus. The samples were fixed in glutaraldehyde in individual test tubes closed with a screw cap.

The histological samples were picked with the help of tweezers, dried and analysed macroscopically before observing them under a stereo-microscope.

The macroscopic analysis allowed the identification of the crests and papillae modified by the presence of the bolus or unmodified, and consequently to correctly position the specimen so as to facilitate the observation on the stereo-microscope. The diaphragm portions of reticulum was fixed in glutaraldehyde 2.5% (v/v) and analysed with the stereo-microscope (Leica EZ4HD). Images relating to portions of the primary and secondary crests, as well as of the papillae, were acquired and processed.

The images acquired by stereo-microscope were analysed to identify the most representative parts of the sample, these, were measured and 3 values for each processed image, in millimetres, were averaged.

Measurements were performed in the upper margin of the cells, tracing a segment at the free crests both primary and secondary, while for the papillae, the segment was mapped at the base. It was so possible to obtain, for each histological sample analyzed, the averages of the values for the primary and secondary crests, and the papillae in which there was permanence or less of the bolus.

Statistical Analysis

The difference between the ΔE color was analyzed using ANOVA to test whether there are differences between the ceramic bolus Zirconia or Alumina, between farms and the permanent time in the reticulum. The statistical significance was set at $p < 0.05$ while the multiple comparisons were developed using the Tukey test.

The analysis of the elemental composition of the surface of the boluses showed the presence of various minerals (aluminum, calcium, phosphorus, manganese, sulfur and zinc). It has therefore been developed to a Pearson correlation test by setting the statistical significance at $p < 0.05$.

2.4. Results

Concretions and colorimetry on boluses

Ratings concretions scale

The concretions on the boluses were visually inspect to identify which one to subject to elemental composition analysis. A total of 246 boluses were identified in with a permanence time inside the animals ranging from 4 days to 115 months. 44.31% (109) of them were in the White scale; 13.01% (32) were Slightly burnished; 34.55% (85) were Moderately burnished; 3.25% (8) were in Burnished; 12.41% (1) were Slightly burnished with concretions at the ends; 00.41% (1) was Burnished with concretions at the ends; 2.44% (6) had concretions in homogeneously distributed on the surface of the boluses but not in the ends; 12.41% (1) had concretions homogeneously distributed on the surface with silicone plug slightly burnished; 00.41% (1) had concretions homogeneously distributed on the surface with silicone plug dented; 0.81% (2) had homogeneous concretions on the entire surface of the boluses; and 0% (0) had tick homogeneous concretions on the entire surface of the bolus.

Rating colorimetry

In order to correlate the color change of the boluses surface with their time of permanence inside the animals 222 boluses were divided in 3 groups: the first group comprises boluses with a permanence time less than 1 year (9.91%); the second group with permanence time between 2 to 5 years (79.28%); and the third group with permanence time more than 5 years (10.81%). By statistical analysis do not highlight any significance (Table 2.3.). The statistical analysis always on the coloration (ΔE) and the type of bolus used alumina n. 138 (62.16%) or zirconia n. 84 (37.84%), there was a significant statistical analysis with a 95% confidence interval [9 (b) vs. 13.6 (a)]. The statistical analysis between the coloration (ΔE) and the farms from which they were recovered showed a significant statistical analysis between the farm B and the other farms in particular with the farm C as shown in the following table (Table 2.4).

XRF analysis

XRF on the surface of the bolus

The results from the elemental composition analysis on the surface of the boluses from farm B are shown in table 2.6. The data from the ten samples were compared to those from the blank bolus (type Z72). The detected elements were aluminum, calcium, phosphorus, sulfur, zinc and manganese. “Bolus11” showed a high difference in composition compared to the others, this may be explained by the lower permanence time in the reticulum, about three years. In this case the decreasing of the presence in aluminum resulted in an increase in the amount of the others elements. Figure 2.4 shows the different graphs: in light green the trend of aluminum composition showing a decrease for a sample "Bolus11"; red the composition of the calcium. The same sample mentioned above shows an increase of 49.6 Wt.%; manganese, in magenta, you get to 0.35 Wt.%; even phosphorus, in blue, shows an increase for the sample "Bolus11" of 18.6 Wt.%. Considering the elemental composition of the sulfur, in olive green there is a small increase arriving at 1 Wt.%. The change in the elemental composition of zinc, in dark green, you will come to a maximum increase of 1 Wt.%.

XRF analysis it was determined the elemental composition of the boluses and is displayed in Figure 2.5. The composition in aluminum, in light green shows a maximum decrease for the sample "Bolus i" with a value of 65 Wt.%. Comparing the compositions of calcium and phosphorus, respectively in red and blue, it has similar performance for both of coming to maximum levels for calcium of 18 Wt.% While the increase of manganese is very low and reaches about 0.1 Wt.%. For phosphorus and 10 Wt.%. While sulfur in olive green shows a different trend with greater increase for the sample "Bolus d" of 1.2 Wt.%. Shown in dark green the trend of the element zinc, this presents a small increase for the sample "Bolus d" 0.35 Wt.%. We note that these samples show small differences from the blank sample.

XRF on the area of the cap of the bolus

XRF map, in figure 2.6., showed the distribution of the elements calcium, phosphorus, aluminum and silicon in the area of the cap of the bolus. As we can see on the figure the area around the silicone seal is affected by the deposition of the concretion.

XRF on concretion

XRD analysis was performed on the concretion deposited on the sample "Bolus3" coming from the farm B. The sample has retention time on the sheep of three years and based mainly of zirconium oxide (type Z20). The concretion formed on bolus has light green colour and is easily detachable and therefore measurable without further preparation of the sample for to XRD analysis. In figure 2.7 is shown the XRD pattern of the concretion deposited on adhesive tape for the measurement. The concretion, from the XRD analysis, resulted mainly consisting of calcium phosphate hydrated named brushite phase ($CaHPO_4*2(H_2O)$)

Analysis of the effects of the presence of the bolus on the tissues of the grating of long period

Following a long permanence of the bolus in situ, the study of the reticulum mucosa allowed to assess the possible effects on the macro and microscopic morphology.

The macroscopic view shows, in the bottom of the mucosa of the reticulum, a minor modification which is then confirmed by stereo-microscope view.

In point of most frequent bolus permanence of the effect of gravity, the morphology of the free margin of the cells, in fact showed a thickening with reduction of the height of the crests both primary and secondary, already visible to unaided eye.

The morphological differences highlighted for the crests, were found at a careful analysis also in the morphology of papillae in the free margin of the crests; the latter from the conical appeared rounded to effect a decrease of greater axis and a thickening of the side margins and the base in direct contact with the crest (Figure 2.8. and 2.9.).

They revealed no further effect on the morphology of the mucosa in the surrounding parts. The emerging data was represented by the fact that in general the primary and secondary crests directly in contact with the bolus were 2/3 of the height of the crests is not in direct contact with a bolus. There is talk of light to moderate modifications that nevertheless do not seem to compromise functionality organ.

2.5. Discussion

As was to be expected are not highlighted concretions of extensions on the retention boluses in the reticulum for a limited period of time, included between 5 and 7 days. In

fact, the considered permanence time, is not in practice sufficient to allow formation of concretions on the surface of the bolus.

Definitely the most interesting are found to be using the reference scale development by the research group of Animal Production, University of Sassari, the characteristics of the concretions extensions on the ceramic bolus in boluses that were inside of the animal for a time between 33 months and 108 months localization. The presence and extension of the concretion in the bolus after long stay (> 24 months) follows in an interaction between localization internal reticular apparatus and time grazing activities of animals. The latter to be understood in direct relation to the topography and geopedology of land used for grazing but also to practice in each flock feeding technique.

The colorimetric analysis has highlighted several color differences, than the "white" of the same type of bolus reference but had never been applied to animals. The visual differences on the color scale (indicated by the value ΔE), show unambiguously changes between different farms object of the experiment while much smaller results to be the influence between the permanence time and the colorimetric variations of the bolus. This confirm the aforesaid for extensions of the concretions and that is that even the color variations depend on the type of grazing and feeding technique made available to the animals.

The color differences between the bolus "Z72" and "Z70" controlled can be traced back to the fact that the boluses "Z72" being mainly constituted by alumina have smoother surface compared to bolus "Z70" which, however, are made mainly of zirconia and show roughness and surface irregularities.

From the experimental point of view it is interesting to note the records found in samples from the bolus by the Farm A where there has been an increased presence of concretions than the other analyzed farms. These concretions are substantially attributable to the type of mostly sandy soils, on which grazed and from which obtain the hay, compared to other farms considered in this study.

With regard to any structural changes that can be determined on the device as a whole (ceramic bolus + transponder + silicone cap) it has been highlighted after the long presence in the animal around the cap area of the boluses analyzed by XRF maps that the concretions contain calcium and phosphorus tend to accumulate in their site by

creating a "locus minoris resistentiae" capable of compromise over the long time the seal of the cap.

An additional experimental data the composition of concretion formed the bolus from the farm C, which was characterized and found to be brushite, that is calcium phosphate hydrate CaHPO4 * 2H2O.

As regards the effects that are determined on the interface of the animal anatomical which remains in contact with the bolus, confirms that the primary, secondary crests and papillae of the reticulum in which there was permanence of the bolus are lower than the crests and papillae of the control animals. Such detectable differences both direct observation and the stereo-microscope show a change in the morphology the crests and papillae resulting from mechanical effect exerted by the weight of the bolus. In any case, these morphological changes detectable even though they are well have no effect on organ function and, far more importantly, on the state of overall well-being of the animal.

2.6. Conclusions

The long permanence up to 108 months of complex device used (ceramic bolus + transponder + silicone cap) not prejudiced in any way the functionality of the electronic device component allowing constantly reading the EIC in vivo and post mortem.

The interaction between permanence time, diet, color, and extension of the concretions present in bolus, not be caused by the permanence time in the reticulum but the type farm feeding.

The concretions present on boluses have a mineral origin, with prevalence of Calcium, Manganese, Zinc and Phosphorus, in the form of calcium hydroxide phosphate (CaHPO4 * 2H2O).

It is a valence of zootechnical interest is environmental, in perspective the elemental analysis of the ceramic bolus interface surface, conducted in animals in reference to the geolocation of the pasture, may represent a method for the detection of environmental contamination. For example through research of heavy metals in the concretions of sentinel animals.

2.7. References

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2.8. Tables

Table 2.1. Values and significance attributed in the subjective scale of the ceramic boluses

Value	Trial
0	White
1	Slightly burnished
2	Moderately burnished
3	Burnished
4	Slightly burnished with concretions at the ends
5	Burnished with concretions at the ends
6	Concretions inhomogeneous distributed on the surface of the bolus but not in the ends
7	Concretions homogeneous distributed on the surface with silicone plug slightly burnished
8	Concretions homogeneous distributed on the surface with silicone plug dented
9	Concretions on homogeneous entire surface of the bolus
10	Concretions homogeneous thickness on the entire surface of the bolus

Table 2.2. Values and meaning attributed to it in color scale to ceramic boluses

ΔE VALUE	MEANING
<0,2	The difference is not perceptible
From 0,2 to 0,5	The difference is very small
From 0,5 to 1,5	The difference is small
From 2 to 3	The variation of color is discernible
From 3 to 6	The variation of color is quite discernible
From 6 to 12	Real color difference
>12	The colors are different

Table 2.3. Comparison between the various classes of the bolus permanence

Classes	N. boluses	Permanence (years)	Mean ΔE	Grouping
1	22	<1	13,1	a
2	176	2-5	11.5	a
3	24	>5	9.4	a

Table 2.4.. Values of ΔE and their significance for farm

Farm	N. boluses	Permanence (months)	Mean ΔE	Grouping
A	99	8-115	13,2	b
B	45	12-35	21,4	a
C	36	19-108	7,0	c
D	17	< 12	8,4	b c
E	13	12-72	7,6	b c
F	12	14-67	10,4	b c

Means that do not share a letter are significantly different, *different letters means p<0.01

Table 2.5. Elemental composition on the surface of the bolus

Sample	**Al**	**Ca**	**P**	**S**	**Zn**	**Mn**
Z72 White	86,4	3,62	0	0,04	0,05	0
bolus11	27,6	49,6	18,6	0,05	0,1	0,34
bolus12	83,9	5,82	0,94	0,3	0,07	0,11
bolus13	80,4	7,3	1,91	0,88	0,32	0,08
bolus14	86,9	3,39	1,02	0,44	0,06	0,03
bolus15	62,7	19,7	9,17	0,27	0,08	0,2
bolus16	75,8	6,96	3,32	4,91	1,03	0,06
bolus17	83,1	4,84	0,83	1,69	0,32	0,05
bolus18	75,5	9,34	7,45	0,86	0,1	0,07
bolus19	75	9,53	4,89	0,98	0,26	0,11
bolus20	83,1	5,04	0,65	0,46	0,1	0,06

Table 2.6. The analysis of the elemental composition in the surface of the boluses vs farm

	Mean	St.Dev	P
Al	74.73	5.26	0.840
	73.41	17.51	
Ca	10.54	3.99	0.756
	12.15	13.93	
P	5.576	2.45	0.749
	4.874	5.66	
S	0.620	0.34	0.383
	1.084	1.42	
Zn	0.2050	0.09	0.725
	0.2440	0.29	
Mn	0.0600	0.02	0.153
	0.1110	0.09	

2.9. Figures

Figure 2.1. Particular of the concretion in a ceramic bolus after permanence in reticulum

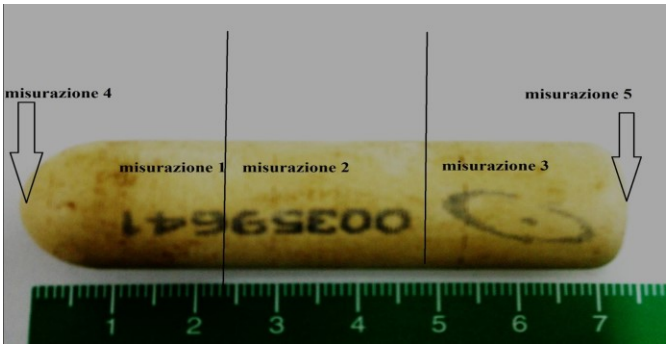


Figure 2.2. Subdivision of the ceramic bolus for measurements of colorimetry

Figure 2.3. Comparison of two ceramic boluses: a) ceramic bolus after permanence in reticulum b) non applied ceramic bolus (control)

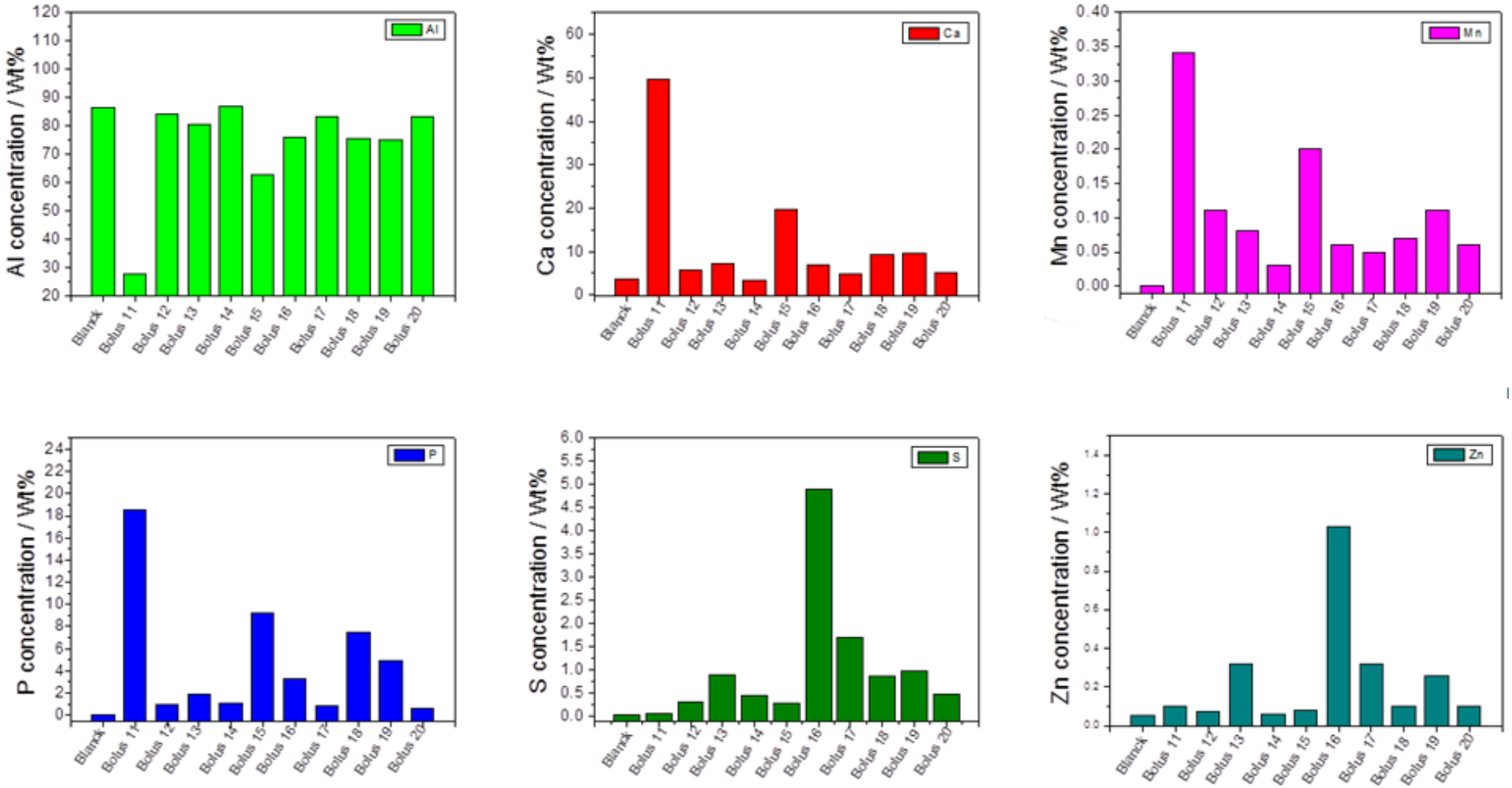


Figure 2.4. Elemental compositions in weight percentage determined by XRF of the blank sample and the boluses (Type Z72) farm A

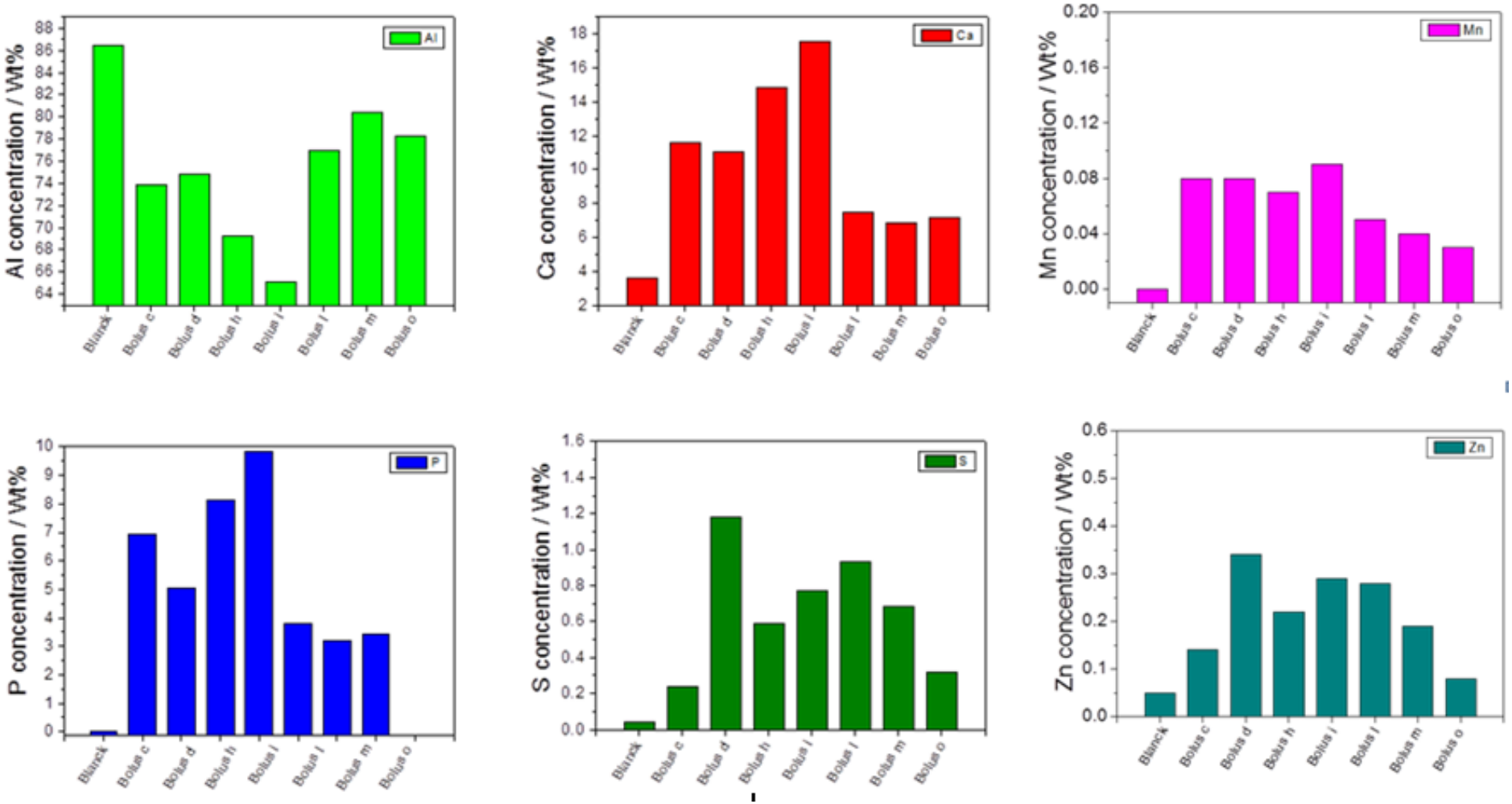
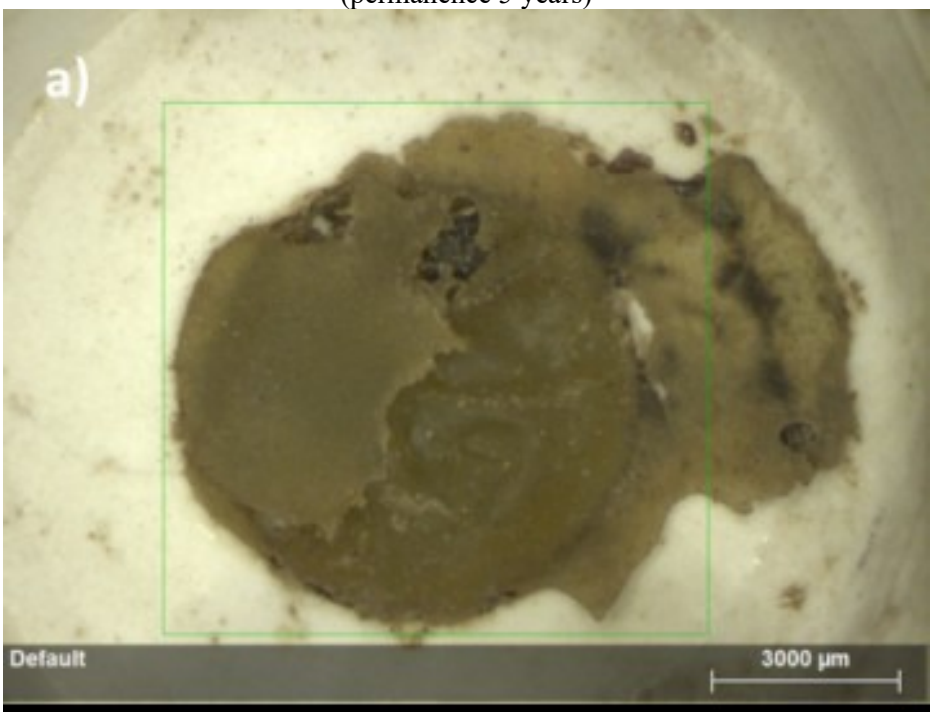
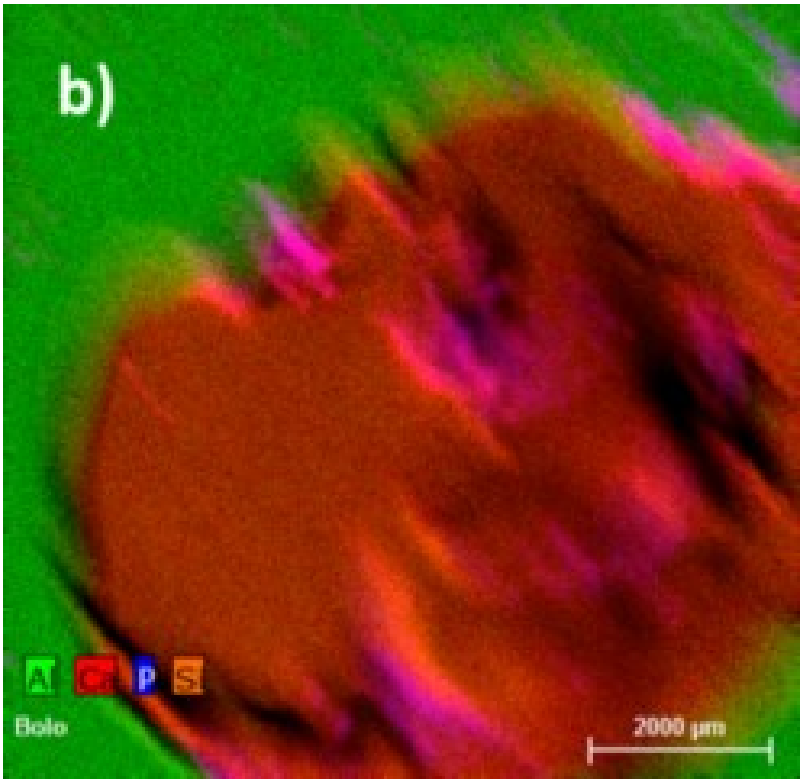


Figure 2.5. Elemental compositions in weight percentage determined by XRF of the blank sample and the boluses (Type Z72) farm F

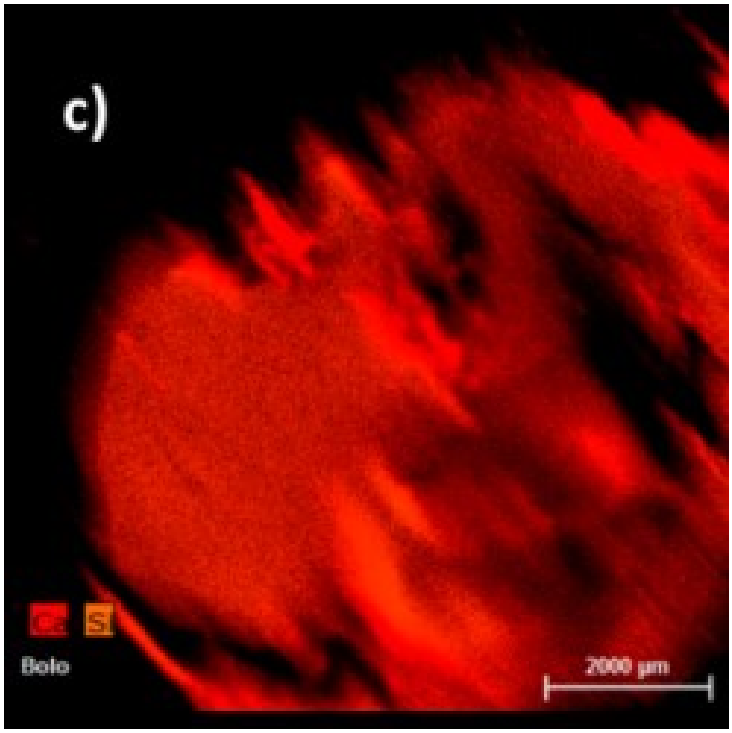
Figure 2.6. XRF map on the area of the cap of the bolus (a, b, c, d)in bolus Z72 (permanence 5 years)



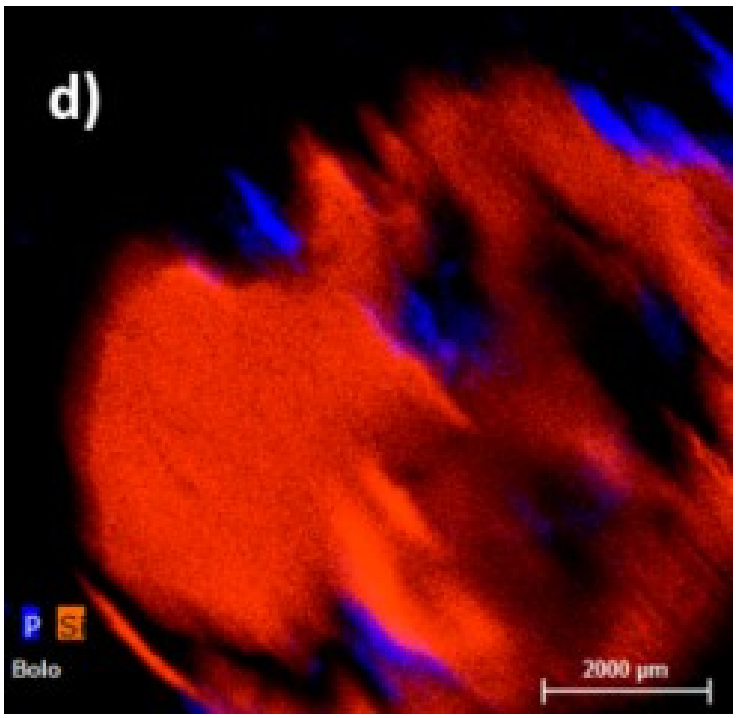
a) 7X optical image of the sample relative to the area of cap considered



b) Map of elements P, Al, Ca and Si (blue, light green, red and orange, respectively) with zoom 10X



c) highlighted the elements Ca and Si zoom 10X



d) highlighted P e S with zoom 10X

a

b

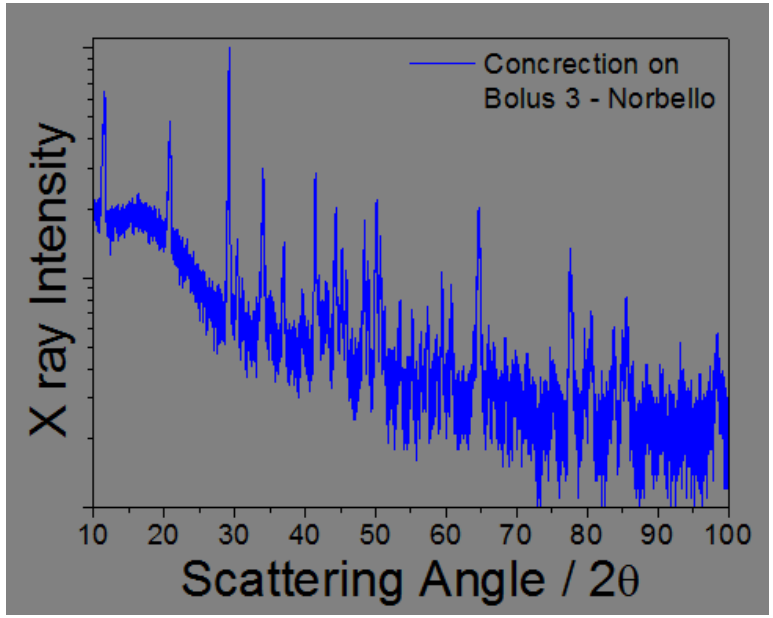


Figure 2.7. Farm B: concretions present on boluses 3.
a) Concretion deposited
b) XRD analysis on the concretion deposited

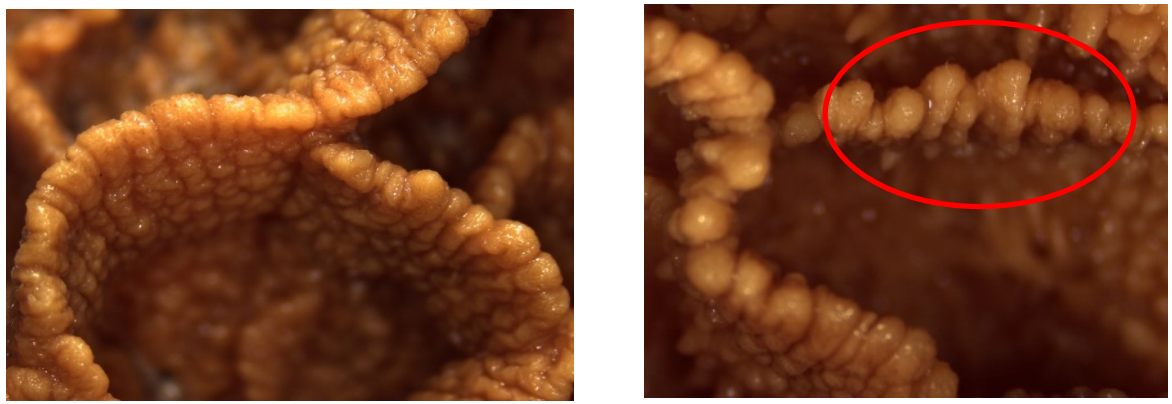


Figure 2.8. Aspect of papillae on primary crests of reticular cells under a stereomicroscope (35x) in fixed in glutaraldehyde 2.5% organ. That the crests interest by bolus permanence (the right pane) appear quite different in morphology with special rounding and thickening of the free margin (red circle). The height was found to be on average 2/3 of that measured for the crests not affected by the presence of the bolus.

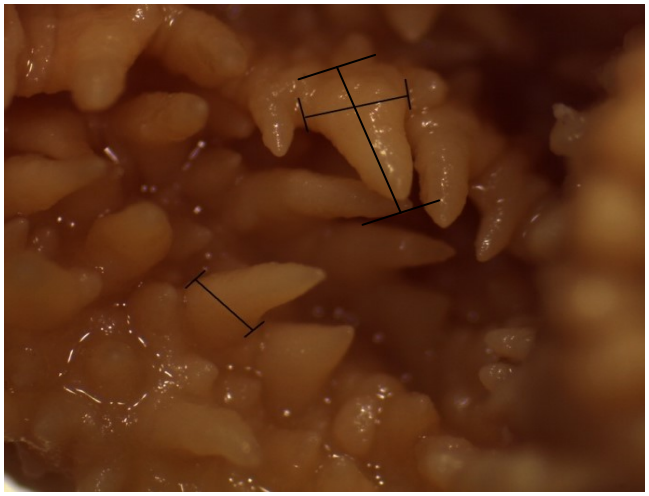


Figure 2.9. Morphometric evaluation of the papillae of the cells and primary and secondary crests (heights measurements expressed in μm).

CHAPTER 3

Comparison of traditional and electronic animal identification in 4 experimental farms

3.1. Abstract

A total of 2461 Sarda ewes were reared in 4 semi-extensive farms, using ceramic bolus with transponder HDX or FDX to perform electronical identification. Tree type of ceramic boluses were used: 70 g (70 mm x 18 mm); 50 g (66 mm x 18 mm); 20 g (58 mm x 12 mm). According to ISO standard 11784 the 32.5 mm x 3.8 mm, 134.2 kHz transponder was used. Electronic identification on livestock is a common practice in all EU countries and various studies were carried out on animals and animal welfare, such as effects on production performance and functionality and readability of the transponder. This study focused on the retention and the readability of both electronic and visual identification.

Greater percentage of the transponder readability is associated with higher reliability. The legibility is denoted by R% (readability), expressed as percentage, using the formula: R = 100 x (number of animals with transponders - number of read transponders); Efficiency (Time/Tech/Head identified electronically: refers to the time ability by the operator to carry out his activities on a single head. When the operator uses the minimum type to identify each head, then the indicator is satisfied). Using electronic identifiers used for a long-term it results higher reliability data related to the individual animals, with reference to their whole of life and productive career. The electronic identification (EID) was more reliable, effective and efficient compared to the ear tag (ET) and ear tattoo (ETt).

3.2. Introduction

The use of radio frequency techniques in the electronic identification has been used since the Seventies (Spahr,1992; Ribò,1996). After various studies and projects FEOGA, IDEA (Caja et al.,1994; Ribò et al.,1994) through European Regulation EC 21/2004; EC 933/2008; EC 759/2009, the electronic identification was used to identify both sheep and goats, in particular for animals born in 2010 or later. It has been also mandatory the use of two identifiers, one visual and one electronic, for sheep and goats intended for replacement (> 6 month of age) or before leaving the farm of origin. In Sardinia (Italy) on 30/11/2016 more than 3413890 sheep and goats, are present and the total number of sheep is greater than 3130741 head (IZS, 2016).

The RIFD is a powerful and versatile technology that allows not only animals but also people and objects to be identified, mapped and managed in various environments. RFID does not require direct contact with the object to be identified and can be read at a distance thanks to the transponder that has a unique identification number (Abecia Martinez, 2010).

The electronic identification of animals includes the use of a transponder (the word derives from transmitter and responder) and a reader or transceiver (from transmitter and receiver). The transponders and transceivers have to comply with ISO 11785 standards, to be "ISO compatible" and necessarily using half duplex (HDX) and full duplex (FDX) transmission protocols. The transponder must be read only and the structure of informations stored on the microchip has to conform to ISO 11784 standards (Operating Procedures for the implementation of electronic identification in small ruminants under Regulation (EC) no. 21/2004 of 17 December 2003 and Commission decision 2006/968/EC of 15 December 2006). Each member state of UE chooses the preferred ID device to be used in practice. The combination of the country code (ISO 3166, 2013) and the national animal number ensures a unique number for an individual animal. It can be used for further management applications, such as the recording of animal characteristics (e.g. sex, size and weight) or medical treatments (Hammer et al., 2015). Detailed information on electronic devices for animal

identification and their manufactures can be obtained from the International Committee for Animal Recording website (ICAR, 2014).

Caja et al. (1996) used two brands of different radiofrequency technologies of glass incapsulated transponders (HDX Tiris, 32x3.8 mm; and FDX-A Nedap, 28x3.6 mm). These have been tested inside the bolus (Caja et al., 1996a). High density ceramic material of the bolus manufacturing did not affect the characteristics of the reading signal in both radiofrequency systems used (Caja et al., 1997).

Recently, the ICAR (International Committee for Animal Recording) or the International Committee for the identification and control of animal productions published a procedural guide to approve the use of any type of device as official method of identification raising a retention of over 98%.

Various studies identified the location of the bolus in the reticulum, verified by X-ray phothography and after slaughtering (Caja et al., 1996b, 1997; Cappai et al., 2014; Pinna et al., 2006). In addition studies on the readability of the boluses endoruminal were carried out and comparative experimental data showed greater readability of HDX devices under farm conditions (Ait-Saidi et al., 2013).

For sheep, the possibility of using such variety of ID devices is caused, to a high extent, by the variable readability obtained in different experiments (Capote et al., 2005; Carné et al., 2009; JRC, 2003; Pinna et al., 2006). Retention rate is conditioned both by the animal species and the bolus design (Garin et al., 2005). In Italy 3 types of electronic devices can be used: 1) ruminal bolus; 2) electronic ear tag; and 3) injectable transponder.

To our knowledge, the only review on sheep e-ID was published more than 19 year ago (Caja et al., 1997) and it justifies an updated re-evaluation of the topic (Table 3.1). Nowadays visual identification, based on plastic ear tags, is the reference method for all livestock species. This paper compares the performances of the electronic identification devices boluses with conventional plastic ear tags in sheep. As already reported by Cappai et al. (2014), the use of different typologies of RFID bolus used may arise different technical problems. For this reason a further investigation was carried out on safety, efficacy and reliability of livestock identification to develop an accurate system

for traceability (Caja et al., 1996a, 1999; 2004; Cappai et al., 2008, 2014; Cappai and Pinna, 2012; Pinna et al., 2007, 2010; San Miguel et al., 2005).

Various studies show that the smallest losses occur when using boluses with weight and specific gravity in sheep, goat and cattle (Ribó et al., 1994; Garin et al., 2000). In 1999 Caja et al. have already realized the reduced loss of boluses endoruminal when using alumina boluses, whose specific gravity is above 3.3, and registered a 98.8–100% retention rate in goats, cattle, and sheep (Garin, et al. 2005).

In this study Repeatability, Univocability, Reliability are investigated. Repeatability it refers to the individual code that identifies the each animal whose sequence keeps unchanged and unchangeable in each control); Univocability it refers to the assignment of the transponder individual code to one and only one animal (this indicator is satisfied when each animal has a unique and exclusive individual code); Reliability, and also Readability, refers to the operator's ability to detect the individual code of the animal's transponder, with fixed or portable handheld, in any field condition. The greater is the percentage of the transponder readability of the animals electronically identified on the farm the higher is its reliability. The legibility is denoted by R% (readability) and expressed as a percentage by the formula: R = 100 x (Number transponders read) / number animals with transponders); Efficiency [(Time/Technician/Head identified electronically): refers to the time taken by the operator to carry out its activities on a single head. When the operator uses the minimum type to identify each head, then the indicator is satisfied].

3.3 Materials and methods

Animals and farms

The trial was conducted on Sarda sheep reared in 4 farms, located in different areas of Sardinia (Italy), with altitude above sea level between 40 and 500 meters, and differing for management practices and pasture characteristics. In total 2,461 animals were identified electronically. The number of animals per farm ranged from 228 to 1187. The animals were individually identified by ear tags in accordance with current health guidelines and by ear tattoos (Farms O1, O2, O3). All animals were tracked on the basis of the electronic individual code (EIC). The semi-extensive farming system allowed sheep to graze during the day on green pastures, sheltered during the night and supplemented with hay in the barn and concentrates (feed supplement corn, barley, peas, soybeans, carob) at milking (twice a day) during the lactation period. During summer and early autumn, due to the scarcity of fresh fodder, the diet was composed mainly of hay and concentrate.

Electronic boluses for animal identification

The electronic boluses used to identify sheep were ordered and provided to farmers by the Interprovincial Association Breeders (A.I.P.A), the National health service (SSN) Veterinarian or the breeders. The purchase of batches followed the principle of the cheapest offer on the market. In recent times the farmers themselves ordering based on length of ear tags. On the basis of the boluses currently deployed for small ruminant identification, different mass, size and materials can be found. Independently from the technical differences of the case, the transponder administered through the ceramic endoreticular bolus can be read by RFID reader (ISO11785 compliant) in the reticulum of ruminants, therefore preferably on the left side of the animal, in the bottom area of the rib cage. The bolus is a suitable case for the transponder to ensure both the stability of the material with no transfer or the progressive mass loss (both alumina oxide Al2O3 and zirconia ceramic are currently in use); in general, the bolus is shaped in such as way to be easily swallowed by the ruminant. Each bolus possesses two poles according to the direction of administration: the front has rounded extremities, the back end is

flattened and has a drill-hole in the centre, which allows the insertion of a 32.5 mm × 3.8 mm glass-encapsulated transponder (134.2 kHz, ISO Standard 11784and 11785 compatible) sealed with commercial silicon. No technical differences are reported in the manufacture of boluses for sheep or goats. The technical features in the boluses are: 1) Bolus 70 g (70x18 mm; 71 g, Zirconia/alumina); 2) Bolus 50 g (66x18mm, 50 g, Zirconia); 3) Bolus 20 g (58x12mm, 21 g, Zirconia).

Field activity

The animals were dairy ewes during the lactation season (age range 1 ½-8 years) and lambs (<1 year). The survey of the individual codes of 2 (EID and ET) devices for each animal was made, 2 separate operators (A, B), on the occasion of the planned annual records checks with animals placed in the rack for the feeding of 45 stall for part. The operator A noted the transponders code using one different handheld readers: gesimpex, gallagher and/or datamars. The operator B noted the code of the ear tag by visual reading, transcribing directly on paper document if there were any loss. Periodic monitoring timetabled of whole herd was gathered in an appropriate area equipped with rack of 45 stall.

Hardware/software EID help desk

Three types of handheld readers were used for the reading controls: Gesreader II (Gesimpex, Rumitag, Italia); SmartReader HR3 (Gallagher Europe, Groningen, The Netherlands), GES3S Reader (Datamars, Pianello, Italia).

Two results were obtained: 1) the precise identification of the animal according to anagraphical code EIC; and 2) a code to verify the correct operation of the electronic device (transponder). Reliability, efficiency and readability, registrations performance relative to the 2 individual identification devices were evaluated in comparative terms (EID vs ET).

For all subjects bearing the electronic identifier, the control readings to check the readability of the transponder, and therefore the presence of the bolus, were performed with static reading following this protocol: for the comeback all of EID boluses were read before and after application; 30 days for the heads in farms A and D where there

have being performed blood and milk samples; during the compulsory vaccination protocol organized by the veterinarian in charge (SSN) in farms B and C; on annual basis for anagraphical control in all farms. The readability, referring to the presence of the bolus and in top shape of the transponder, was calculated according to the following formula: R = 100 x (Number transponders read) / number animals with transponders).

Statistical analysis

The data obtained on readability between the EIC and CIC were analyzed by Student's t-test for whether there are differences in the two groups during the period of control of the heads. Statistical significance was set at $p < 0.05$. All analyzes were performed using software R v.3.3.2

3.4. Results

As already mentioned at the time by the research group of Animal Production, University of Sassari, when following some simple protocol rules the correct application of the bolus and electronic identification of sheep and the consequent management of personal data of individual animals it turns out to be a task easy implementation practice. 1 employee to the capture of animals (breeder); 1 technician tattoo or ear tag reading and recording data on paper; 1 technician of the RFID reader management. That team is in fact able to develop a very rapid working capacity but above all to match the double reading of the transponder code EIC and the simultaneous control of the ear tag CIC on sheep in a time of about 6"/head. It means that, with a minimum of operating organization it is possible to check a "formal" anagraphic control of an average flock of 200 herd with a maximum working convenience of about 20 minutes. The different rates of non readability of EIC in sheep have been registered according to the type of bolus used for identification by dimension and mass (Table 3.1).

The Table 3.2 shows the results of readings and loss of the identification of animal obtained during the scheduled checks of the working trials in sheep farms.

In the period considered, for the total of 2461 identified head, permanence of the electronic device vs ear tags on animals was respectively 99.72% vs 93.62% (loss of 157 brands). The remark (readability%) of the EIC code of each individual identification device was found: EID (static reading) 99.72%; vs CIC (ear tag or ear tattoo) 93.62% with p-value = 0.044.

Also for the readability of the electronic identifiers, farms have been monitored. And it showed that some situations about failed remarks of the transponder code at the anagraphic data of 2016 compared to the bolus administration date can be classified as follows: n. 8 in 2010 (24.24%); n. 10 in 2011 (30.30%); n. 3 in 2012 (9.09%); n. 4 in 2013 (12.12%); n. 3 in 2014 (9.09%); n. 3 in 2015 (9.09%); n. 2 in 2016 (6.6%). This leads us to classify it (6.6%) boluses are part of the possible losses in the medium period, while all the other 31 (93.9%) are part loss rate in the long period.

To facilitate a better understanding on the reports it may is useful to remind the classification used in previous works: 1) Medium period loss: episodes that occur in the period between 30 and 210 days. 2) Long-term projection loss: episodes that occur beyond 210 days. For this project these represented a fundamental fact and are, in fact, verifiable with annual check during the sheep census in Ovine Flock Register.
Data on the permanence device (since the application at the long-term anagrapich data) for each animal were analyzed systematically in farm O2. In Table 3.3 the data related to the Annual readability of transponders in the control readings, in the period 2010-2016.

The use of different handheld readers allows the operator to read the transponder code differently. To observe a temporary undetectability EIC using the reader SmartReader HR3 and GES3S Reader, in one case the EIC unread were 20 (1,68%) vs 16 (1,35%) of identifiers 1187 read. Reader efficiency (98,32 vs 98,65%). In addition there have been cases of reactivation of the bolus by the same reader at the second attempt. When electronic identification on controlled farms (the transponder administered through the ceramic bolus by the vet or the breeder himself), with bolus of 50 g in the lambing have not shown adverse effects on health and on the production performance of animals, nor clinical signs attributable to the presence of the bolus and transponder in the animal.

3.5. Discussion

Many authors in the last twenty years highlighted that the bolus is easy to apply and does not pose a risk to health and performance production (Caja et al., 1999; Cappai et al, 2014; Garín et al., 2003, 2005) and the result of our study is in accordance with them also in the long term (2010-2016). The best disposal was the bolus with the specific gravity (>3.3 g/cm^3) that allowed the optimal bolus retention, in agreement with Cappai et al. (2014), and successfully detectable EIC.
In bibliography various works on the identification of animals and the identifiers that can be used and their functionality. Studies readability (%) of electronic ruminal bolus show values between 99 - 100% retention, higher than our results which have

nevertheless achieved a minimum retention rate > 98% as recommended by ICAR (2016).

A recent state of the art has been written for goats (Caja et al., 2014) but not for sheep and even less information on the efficiency of conventional ear tags in the sheep and the only comparison found in the bibliography on the loss of the conventional ear tag is Garin et al. 2005 (Table 3.4; 3.5), finding less than our control, but in agreement for an annual loss rate of 6% (Technical Series ICAR - No.15). To notice, however, that in all species the retention rate of ear tags is extremely variable, ranging from 60-98% (Technical Series ICAR - No.9).

3.6. Conclusions

The electronic identification has no negative effects on health and productive performance of the animals. It also shows a clear superiority with regards to the anagraphical performance (required to farmers, controllers and the Veterinary Service of the NHS) than the other two anagraphic systems in use: the ear tag and the ear tattoo (ear base armpit, rear flank the base of the tail). Electronic identifiers used for the long-term have been reliable concerning about the full enjoyment of the anagraphic data related to the individual animals, in reference to the whole of life and productive career of animals. The electronic identification (EID) was more reliable, effective and efficient than the ear tag (ET) and the ear tattoo (ETt).

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3.8. Tables

Table 3.1. Readability of 3 ceramic boluses typology used in experimental farms

Electronic ID device	Number of boluses present	Total readability of different typology at the last control EIC (%)	% Readability EIC of applied bolus
Bolus 70 g	41	41 (1.67)	100
Bolus 50 g	2335	2308 (93.78)	98.84
Bolus 20 g	85	79 (3.21)	92.94

Table 3.2. Results of the readings and loss of the identification in sheep in the 4 farms in last check experimental (2016)

Farms	Total animals (%)	Without identification (%)	Total animals identify (%)	EIC read (%)	EIC not read (%)	CIC read (%)	CIC not read (%)
O1	1187 (100)	0	1187 (100)	1171 (98.65)	16 (1.35)	1098 (92.5)	89 (7.50)
O2	670 (100)	3 (0.45)	667 (99.55)	654 (98.05)	13 (1.95)	648 (97.15)	19 (2.85)
O3	383 (100)	4 (1.04)	379 (98.96)	375 (98.94)	4 (1.06)	350 (92.35)	29 (7.65)
O4	228 (100)	0	228 (100)	228 (100)	0	208 (91.23)	20 (8.77)
Total (%)	**2468 (100)**	**7 (0.28)**	**2461 (99.72)**	**2428 (98.66)**	**33 (1.34)**	**2304 (93.62)**	**157 (6.38)**

Table 3.3 – Multi-year electronic identification performance in our best practice farm

Date control	Total animals	EIC read	EIC not read	Readability (%)
2010	556	556	0	100
19/1/2011	249	249	0	100
26/5/2011	452	452	0	100
30/9/2011	483	483	0	100
2013	507	507	0	100
2015	542	530	12	97.79
2016	667	654	13	98.05

Table 3.4. Performances of identification devices (v = visual, e = electronic, ET = ear tag, IT = injectable transponder, RB = rumen bolus) in sheep

	Losses, %					Breakages and failures, %					Readability[2], %			
Reference[1]	v-ET	e-ET	e-IT	e-RB[3]		v-ET	e-ET	e-IT	e-RB[3]		v-ET	e-ET	e-IT	e-RB[3]
# 1	–	–	4	-		-	-	44	0		-	-	96.2[4]	-
# 2	-	-	19.2[7]	-		-	-	3.8[7]	0		-	-	76.9[7]	-
# 3	-	8.3	0	0		-	-	-	0		–	–	100	100
# 4	-	2.7	-	0		-	-	-	0		–	–	–	100
# 5	2.1	-	-	-		-	-	-	-		-	-	-	-
# 6	3.2	-	-	-		-	-	-	-		-	-	-	-
# 7	-	-	-	0.001		-	-	-	0		-	-	-	99.99
# 8	-	-	-	0.002		-	-	-	0.0034		-	-	-	99.99
# 9	-	-	-	0		-	-	-	0		-	-	-	100
# 10	-	-	-	0.312		-	-	-	0		-	-	-	99.68
# 11	-	-	-	0.32		-	-	-	0		-	-	-	99.7
# 12	-	-	-	0		3.213	-	-	0		98.87	-	-	100
# 13	-	-	-	2		-	-	-	0		-	-	-	100
# 14	-	-	-	4.2		-	-	-	0		-	-	-	100
# 15	-	-	0	-		-	-	0	-		-	-	100	-
# 16	-	-	-	5.5		-	-	-	-		-	-	-	94.5
# 17	-	-	-	0		-	-	-	-		-	-	-	100
# 18	-	-	-	0		-	-	-	-		-	-	-	100
# 19	0	0.16-1.13	-	0.004-0.28		-	-	-	-		92-97[14]	96-99[14]	7714,7	99-100[14]
# 20	-	-	-	-		-	-	-	-		-	-	93-98[5]	-
# 21	-	-	-	-		-	-	-	-		-	-	95-96[4]	-
# 22	-	-	-	-		-	-	-	-		-	75-95	1005	100
# 23	-	-	-	-		-	-	-	-		-	-	85-95	-
Mean[11] ± SE	2.7 ± 0.8	3.1 ± 2.10	19.2 ± 0	1.4 ± 0.7		3.2 ± 0		3.9 ± 0.1	0.0034±0		96 ± 2.5	91.3 ± 6.3	92.5 ± 2.5	99.6 ± 0.4

Reference number by type of device: **v-ET** (#5 to 6, Garin et al., 2005; #12, Pinna et al., 2007; #19, Caja et al., 2002; **e-ET** (#3 to 4, Caja et al., 1999a; #19, Caja et al., 2002; **e-IT** (#1 to 2, Caja et al., 1998; ((#3, Caja et al., 1999a #15, Pinna et al., 2005; #19 to 23, Caja et al., 2002; (#3 to 4, Caja et al., 1999a #7 to 10, Cappai et al., 2014; #11, San Miguel et al., 2005;#12, Pinna et al., 2007 #13 to 14, Ghirardi et al 2006; #16 to 18, Hertz et al. 2014; #19 and 22, Caja et al., 2002. -[2]Readability, % = (readable devices/applied devices) × 100. ; -[3]Only rumen boluses with specific gravity >3.3 were included; - Subcutaneous injection body sites: [4]ear base, [5]armpit, [6]groin, [7]tail, [8]intraperitoneal, [9]metacarpus or metatarsus, [10]perianal; -[11]Values are unweighed means by type of device; [13]Ear Tattoo; [14] Readabilty Dynamic

Table 3.5. State of the art of performances of identification devices (v = visual, e = electronic, ET = ear tag, IT = injectable transponder, RB = rumen bolus).

	Losses, %					Breakages and failures, %					Readability[2], %			
Reference[1]	v-ET	e-ET	e-IT	e-RB[3]		v-ET	e-ET	e-IT	e-RB[3]		v-ET	e-ET	e-IT	e-RB[3]
# 1	–	–	4	-		-	-	44	0		-	-	96.2[4]	-
# 2	-	-	19.2[7]	-		-	-	3.8[7]	0		-	-	76.9[7]	-
# 3	-	8.3	0	0		-	-	-	0		–	–	100	100
# 4	-	2.7	-	0		-	-	-	0		–	–	–	100
# 5	2.1	-	-	-		-	-	-	-		-	-	-	-
# 6	3.2	-	-	-		-	-	-	-		-	-	-	-
# 7	-	-	-	0.001		-	-	-	0		-	-	-	99.99
# 8	-	-	-	0.002		-	-	-	0.0034		-	-	-	99.99
# 9	-	-	-	0		-	-	-	0		-	-	-	100
# 10	-	-	-	0.312		-	-	-	0		-	-	-	99.68
# 11	-	-	-	0.32		-	-	-	0		-	-	-	99.7
# 12	-	-	-	0		3.213	-	-	0		98.87	-	-	100
# 13	-	-	-	2		-	-	-	0		-	-	-	100
# 14	-	-	-	4.2		-	-	-	0		-	-	-	100
# 15	-	-	0	-		-	-	0	-		-	-	100	-
# 16	-	-	-	5.5		-	-	-	-		-	-	-	94.5
# 17	-	-	-	0		-	-	-	-		-	-	-	100
# 18	-	-	-	0		-	-	-	-		-	-	-	100
# 19	0	0.16-1.13	-	0.004-0.28		-	-	-	-		92-97[14]	96-99[14]	7714,7	99-100[14]
# 20	-	-	-	-		-	-	-	-		-	-	93-98[5]	-
# 21	-	-	-	-		-	-	-	-		-	-	95-96[4]	-
# 22	-	-	-	-		-	-	-	-		-	75-95	1005	100
# 23	-	-	-	-		-	-	-	-		-	-	85-95	-
# 24 NIEDDU	**6.38**	**-**	**-**	**-**		**-**	**-**	**-**	**1.34**		**93.62**	**-**	**-**	**98.66**
Mean[11] ± SE	**3.9 ± 1.6**	3.1 ± 2.10	19.2 ± 0	1.4 ± 0.7		3.2 ± 0		3.9 ± 0.1	**0.7±0.9**		**95.4 ± 1.8**	91.3 ± 6.3	92.5 ± 2.5	**99.5 ± 0.3**

Reference number by type of device: **v-ET** (#5 to 6, Garin et al., 2005; #12, Pinna et al., 2007; #19, Caja et al., 2002; **e-ET** (#3 to 4, Caja et al., 1999a; #19, Caja et al., 2002; **e-IT** (#1 to 2, Caja et al., 1998; ((#3, Caja et al., 1999a #15, Pinna et al., 2005; #19 to 23, Caja et al., 2002; (#3 to 4, Caja et al., 1999a #7 to 10, Cappai et al., 2014; #11, San Miguel et al., 2005;#12, Pinna et al., 2007 #13 to 14, Ghirardi et al 2006; #16 to 18, Hertz et al. 2014; #19 and 22, Caja et al., 2002. -[2]Readability, % = (readable devices/applied devices) × 100. ; -[3]Only rumen boluses with specific gravity >3.3 were included; - Subcutaneous injection body sites: [4]ear base, [5]armpit, [6]groin, [7]tail, [8]intraperitoneal, [9]metacarpus or metatarsus, [10]perianal; -[11]Values are unweighed means by type of device; [13]Ear Tattoo; [14] Readabilty Dynamic

3.9. Figures

Figure 3.1. Administration of electronic bolus in sheep

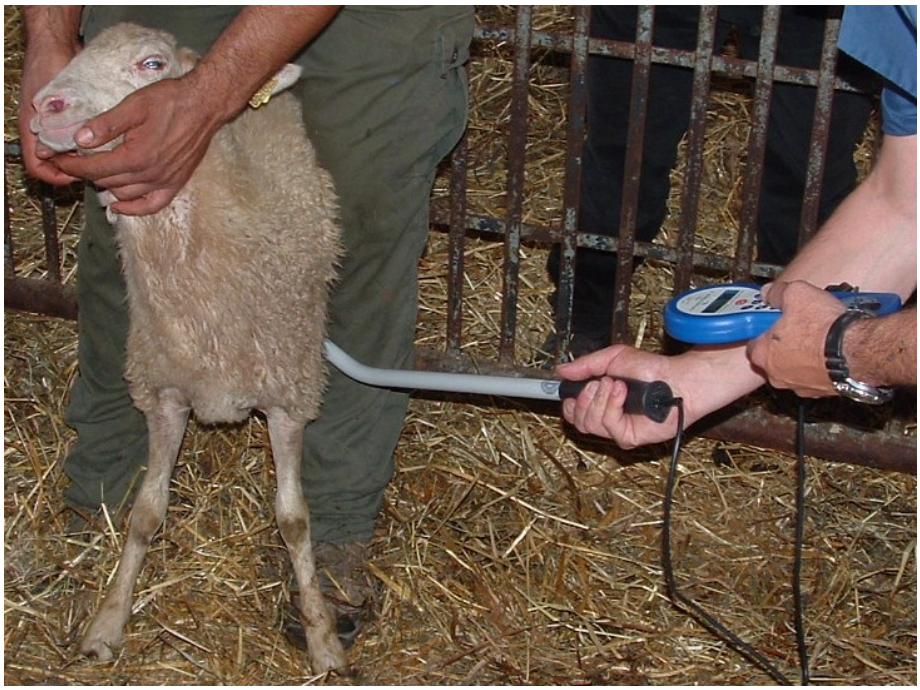

Figure 3. 2. Static reading of electronic bolus in sheep

CHAPTER 4

The metabolic profile and the biological role of calcium (Ca) in relation to the lactation state

4.1. Abstract

Across the different phases of investigations, the bolus mineral profile displayed shallow concretions on bolus surface mainly constituted by Calcium (Ca), phosphorus (P), sulfur (S), manganese (Mn) and zinc (Zn). We therefore meant to further investigated whether any potential effect related to the presence of such mineral concretions in the rumen could be pointed out in the bloodstream as to circulating levels of total calcium in Sarda breed sheep previously electronically identified. Calcium concentration in the bloodstream was determined in different periods: calcemia at different stage of pregnancy lactation; in milk at early, mid and late lactation.

These aspects were considered important in these testing phases. If a potential effect from the presence of such concretions could be seen, the transition period would have represented a crucial moment in which mobilization of Ca from body depots could markedly take place. Furthermore, such scheduled sampling would have highlighted possible events, such as hypocalcemia, due to possible disturbances in the immediate availability of circulating calcium, as it can occur after lambing, loss of muscle tone, displacement of abomasum, rumen stasis and retained placenta.

The two groups, with and without bolus (control), showed averages blood plasma calcium values of 9.32 ± 0.56 and 9.72 ± 0.27 mg/dl throughout the lactation 2016, respectively. No statistically significant differences were registered between heads with *vs*. without bolus.

The average calcium content in milk from animals with *vs*. without ceramic bolus was 1246.81 ± 41.59 and 1213.08 ± 19.31 mg/kg in the lactation 2016, respectively. Again, such resutls did not point to significant differences as to circulating Ca and respective content in milk between electronically identified ewes by means of RFID contained ceramic boluses.

4.2. Introduction

Calcium (Ca) is certainly an important macro-element mineral in its role as a metabolic constituent of bone tissue, and just as importantly in the transmission of neuromuscular impulses and in the processes of membrane permeability and cellular walls of the blood vessels.

The presence of blood calcium is mainly regulated by three hormones: parathyroid hormone (PTH), calcitonin (CT) and 1,25- dihydroxy-vitamin D (calcitriol), which control the intestinal absorption of calcium which can oscillate from 20 - 50% of the ingestion, or reabsorbed share from the bones (Bertoni 1999; Horst 2005; Gaucheron 2013; Pulina and Nudda, 2004).

An increased need for calcium is found during the stages of pregnancy and lactation, and is why it is essential to monitor the physiological response of the animal.

During pregnancy, especially in the terminal phase, there will be an increase in the absorption of calcium, which will increase even more during lactation (1 liter of milk contains from 1 to 1.2 g of calcium), while in the blood content between 1 and 1.8 mmol/L with altered values of P, K and Mg would lead to the metabolite disease known as “milk fever”. It leads to the loss of calcium in milk that restores with Ca absorbed or resorbed by bone tissue (Bertoni 1999).

Immediately after birth, with the beginning of lactation, the need for calcium is strongly growing, the values of mineral content in milk are minor and increase at the end of lactation.

This deficiency is due to the fact that, in pregnancy, with the need for calcium being lower than during lactation, after which the bone resources have been guaranteed, there is a drop in active absorption which is compensated by passive absorption (the fundamental assumption is that the food inputs are well balanced) such an abundance of calcium generates mechanisms to reduce the absorption of the calcium level in the bowels through the inhibition of the production of calcitriol by calcidiol (or vitamin D2).

It is very important that we monitor these metabolic aspects during periods of transition from one physiological state to another, since the onset of hypocalcemia after giving birth may cause less muscle tone (i.e. due to microorganisms access by the udder and

possible mastitis), displacement of abomasum, rumen stasis, retained placenta, etc. With the continuation of lactation the amount of calcium needed is met with hypertrophy of the intestinal walls, as they guarantee a greater absorbent surface, but mainly by the increase of calcitriol, due both to the PTH and probably to prolactin.

Even calcitonin levels increase at this phase, probably due to the increase of ingestion which in turn causes a greater presence of digestive hormones. The combined effect of the two hormones allows the body to avoid hypercalcemia after meals and in particular to avoid excessive depletion of bone tissue.

The efficiency with which the calcium in the intestine and bone mobilization is absorbed are also influenced by the age of the animal, resulting in both being higher in young animals during the growth phase. Forms of deficiency in the juvenile phase could have economically significant effects even in animals for milk production, as they may adversely affect bone calcium mobilization in their stage of lactation.

In more considerably important topics, zootechnical interest can assume aspects that relate to the production, because the deficiency of Calcium in blood also causes the transfer of this element which is deficient in milk. In fact, being that the blood calcium is in close relationship with calcium present in milk, nutritionally it's advantageous to favor its availability of being transferred in the milk itself (Bertoni G. 1999).

Regarding the intestinal calcium absorption, different studies provided conflicting results, too.

Although for transcellular calcium, absorption has been demonstrated through calcitriol-regulated structures. If one considers the retention time of the ingesta in the prestomachs for the absorption of calcium, it is rather long (Wilkens et al., 2011).

In general, the animal's diet, the months of the year, the environmental temperature, the stage of lactation, the age of the animal, milking intervals, and diseases of the udder all influence the content of minerals in the milk (Haenlein 2006; Hilali 2011; Tamime et al., 2011).

The hematological indices are generally used as an immediate indication of no deficiency in a normal blood level. The range of calcium is 1700 - 2400 mg/kg (Gaucheron 2013; Haenlein 2006; Pulina G., 2004; Ljutovac R. et al., 2008; Tamime et al., 2011), while the blood calcium level is 13 times lower (Pulina and Nudda., 2004), 11.5-12.8 mg / dl (12.16 ± 0.28) (Kaneko et al. 1997). Various studies have been

performed that in bolus there is an interaction with the animal welfare and its production performance, so it shows that during the animal's life there have been some negative inferences (Caja et al., 2006; Cappai et al., 2014; Cappai and Pinna, 2012; Garín et al., 2005). Given the importance of Calcium (Ca) on a biological level in animals, and particularly in animals bred for milk production, the trial aimed to assess how the concentration of calcium in blood and milk depends on the physiological condition, and how this can be somehow be affected by the presence or absence of the bolus, to prevent onset of metabolic diseases caused by the bolus making calcium less available to the biological functions of maintenance and production in Sarda breed of sheep.

4.3. Materials and Metods

Animals and farms

The trial started in 2014 and ended in September 2016. The field activities were conducted on 2 farms upon which Sarda are bred. Situated in northern Sardinia, with altitudes of between 40 and 500 meters above sea level, they have different management practices and their pastures both have different characteristics. The experiments were conducted upon: 1) Sarda breed sheep of the Department for Research in Animal Production (DiRPA), Regional Agency for Research in Agriculture (AGRIS) in Bonassai, a town in the countryside near Olmedo (Sardinia, Italy). They have been identified within a random homogenous flock of 150 primiparous sheep. 50 sheep were born in December 2013, 25 of which are not electronically identified with ruminal bolus (control group), but with the ear tag and ear tattoo marked with the ID code and farm code. The other 25 were identified by ear tag and rumen bolus of 50 g. The animals are managed with a traditional breeding, with a semi-intensive system, where grazing is the main food component of the flock. The pastures are composed of wild species and grasslands (medical + ryegrass, ladino clover, sulla). The dietary supplement is composed of complementary feed containing fine bran, dehydrated alfalfa meal, ground maize, ground peas, ground field beans, ground barley, soy flour, calcium

carbonate, sodium chloride, sodium bicarbonate, dicalcium phosphate, and magnesium carbonate. 2) Sarda breed sheep on a farm in the countryside near Villanova Monteleone (Sardinia, Italy). 45 primiparous sheep were identified, born in December 2013, all electronically identified with rumen bolus of 50 g. Animals are managed with a traditional breeding, with a semi-extensive system, where grazing is the main food component of the flock. The pastures are composed of wild species and grasslands (corn, vetch-oat, barley, alexandrian clover and lolium). The dietary supplement is composed of complementary feed containing maize, wheat middlings and / or hard flour, hard wheat bran, molasses from sugar cane, soybean flour extracts. Sunflower seeds,. Extr. Partial. decort., Carob, Calcium carbonate rocks from cal. mac, calcium hydrogen phosphate, Sodium chloride, Sodium bicarbonate, Magnesium Oxide, and Sulfur.

Field activities

The experiments were:

1) blood samples with time intervals of about 30 days during the whole lactation. Blood samples were taken immediately after milking, and then blood count analyses were made in the laboratory, if it appeared necessary, using the instrument Mindray BC-5000 vet and calcium analyses with the BS-200. For blood samples, performed by a farm veterinarian, they were used with and without EDTA vacutainer.

2) For the diagnosis of the pregnant sheep, this one performed by a veterinarian, an ultrasound was used, which allowed us to split groups evenly, dividing them according to the various stages of gestation. The instrument used is the MyLab ™ On/Touch: Portable ultrasound system with touch screen technology, high-performance with a touchscreen display of 12", with a convex probe for abdominal ultrasound, obstetric/gynecological, vascular and anesthesia;

3) For the assessment of bone calcium reabsorption, digital radiographs were carried out. In particular, the front right leg, at the level of the carpal, metacarpal and phalanges

was considered with the animal in position, caught in the rack, to be able to control the deposition phase of salts, limestones and calcium mobilization from the long bones. For this purpose, the ratio between bone marrow cavity and the cortex of the long bone has been taken into account , and the radio opacity was measured by the density of the long bone. The computer assisted as the measure of the density of the cortex was performed. Figure 4.2. The tool used is the Compact 30 HF. using the shaft in aluminum and plexiglass, adjustable to 360°, in aluminum cassette 24x30 cm with a phosphor screen., with the PCS technology (Photo-Collecting System). The picture on the fluorescent screen is conveyed to the receiver by a special parabolic mirror system. High-resolution images are thus obtained and ready to be stored in a personal computer. During the tests, anti-x - Pb 0.50/Standard aprons - 120x60 cm were used.

4) at the end of lactation (July 2015) and during the first months of 2016, milk samples were made by heads in the trial for the subsequent control of calcium at a specialized laboratory.

5) The non-electronically identified animals who were a substantial part of our experimental test were identified through the reading of the code of the ear tag, and at first through a band. The latter because of its inadequacy to the needs of livestock has been replaced during work with a colored collar (figure 4.1).

Laboratory analysis

The laboratory activities included blood samples taken in the field, in a vacutainer with and without EDTA, transported to a thermal container, processed with an automatic hematology analyzer (BC-5000Vet) for complete blood counts and the determination of 23 parameters, specific for veterinary use. Factors researched included: WBC (white blood cell formula, leukocytes (white blood cells); Neu (neutrophils); Lym (lymphocytes); Mon (monocytes), Eos (Eosinophils) Bas (Basophils); Neu%, Lym%; Mon%; Eos%; Bas%; RBC (erythrocytes (red blood cells)); HGB (amount in grams of hemoglobin (absolute value)); HCT (Hematocrit % of blood volume occupied by red

blood cells), MCV (average red blood cell volume), MCH (Quantity of hemoglobin in each red blood cell) MCHC (mean hemoglobin concentration (Index corpuscular)); RDW-CV (Amplitude of the red blood cell volume distribution ria); RDW-SD; PLT (Platelets) MPV (mean platelet volume); PDW (Amplitude platelet distribution); and PCT (platelet hematocrit), all of which allowed us to generally control the animal's health status.

Subsequently, the tubes containing EDTA were centrifuged for separation of plasma, while for the test pieces containing no EDTA, the separation of the serum was performed, the rate of which was inserted into 2 ml vials and then to be frozen, pending the carrying out of the analysis on serum levels of calcium with BS200 of Mindray.

Regarding the analysis of the milk samples, they were processed after having been previously frozen, then left to thaw at room temperature before the day of analysis. The samples were then placed in a water bath (Falc, LABOINDUSTRIA spa, Arzergrande) at 40 ° C for 20 minutes. The sample is weighed on an analytical balance Mettler PC 180 by placing it in a vessel, to be then processed in the digester microwave rotor with 15 seats Ethos easy (Advanced microwave digestion system, Milestone) using 500 g of the sample, 2 ml of hydrogen peroxide and 8 ml of nitric acid, the whole of which is brought to 200 degrees. The mineralizing brought to volume to 50 ml with double-distilled water is then read with the atomic absorption of calcium for the search (Aanalyst 200, Atomic absorption spectrometer, Perkin Elmer) leading to volume 1 ml of mineralized into 9 ml of bidistilled water.

Statistical analysis

The difference in the lactation period (early, middle and end) between the mean values in serum calcium concentration, recorded in both the electronically identified and in the control groups, were analyzed using an ANOVA model.

The difference in the lactation period (early, middle and end) between the mean values in milk calcium concentration, recorded in the electronically identified and in the control groups, were analyzed using an ANOVA model.

The difference between the length and the width, ratio bone marrow cavity and the cortex, density of the main bone in sheep, recorded in the electronically identified and in the control groups, were analyzed using Student't test. Statistical significance was set at $p < 0.05$. All analyzses were performed using the package R v.3.3.2.

4.4. Results

The two groups with and without bolus (control), showed calcium levels in the blood, respectively, of 9.28 ± 0.11 e 9.34 ± 0.08 mg/dL (P = 0.679) at the end of lactation 2015, 7.98 ± 0.81 e 9.61 ±0.36 mg/dL (P = 0.068) early lactation 2016, 10.15 ± 0.59 e 9.53 ± 0.08 mg/dL (P = 0.313) mid lactation; 9.87 ± 0.16 e 10.39 ± 0.43 mg/dL (P = 0.196) late lactation 2016, showed no statistically significant differences among the heads with and without bolus.

The values of calcium in milk for animals with the presence of ceramic bolus and without bolus (control) are, respectively, 1537.58 ± 37.9 and 1397.38 ± 36.77 mg/Kg (P = 0.007) at the end of lactation 2015, 1220.3 ± 19.46 and 1229.27 ± 36.77 mg/Kg (P = 0.822) early lactation 2016, 1314.23± 40.28 and 1228.41±22.15 (P = 0.071) mg/Kg mid lactation; 1205.91± 28.71 and 1181.55±37.21 (P = 0.061) mg/Kg late lactation 2016.

Regarding data obtained for the milking season of 2016, the subject of the experimental test showed no significant differences.

The two groups with and without bolus (control) showed the length of the main bone in animals, respectively, of 130.49 ± 2.37 vs 135.77 ± 2 mm (P = 0.09027) and the width, respectively, of 11.88 ± 0.23 vs 12.33 ± 0.21 mm (P = 0.147), showed no significant differences.

The ratio between bone marrow cavity and the cortex of the long bone in sheep with and without bolus, 2.25 ± 0.12 and 2.12 ± 0.11 mm (P = 0.4005), respectively, showed no significant differences.

The density of the long bone result in sheep with and without bolus was 3635.30 ± 96.60 and 3609.31 ± 76.36 HU (P = 0.8272).

4.5. Discussion

These aspects have been considered important in the testing phase, since the concentration of calcium in the blood and in the milk has been evaluated depending upon physiological condition, and how this can be in some way influenced by the presence or absence of the bolus rumen, which could be in charge of making it less available to the biological functions of maintenance and production in sheep. References do not report data on this aspect to the best of my knowledge. From the comparative analysis of the literature about Ca in sheep milk and in blood, in our experimental experience we can put in evidence that the values we found are closer to those of the goats than to those of lactating sheep. goats Calcium (mg) in milk 1260 mg/kg (Raynal-Ljutovac K. et al.) Kaneko et al., 1997 blood analyte values in goats mg/dL 8.9-11.7 (10.3 ± 0.7). Conversely, it has not been possible in works over electronically identified animals with ceramic rumen bolus.

Some of the experimental animals, in the course of test, exhibited mastitis and lameness. And thanks to the studies of other researchers, we know that the milk composition depends on many factors, such as diet composition, types of pastures, the lactation period, seasonality and the age of the animal (Hilali, 2001; and Haenlein Wendorff, 2006; Khana et al 2006).

The radiographical analysis of bone density was carried out on the basis of an assisted computer software on digital images of long bones in EID vs non EID ewes. This parameter was considered necessary to understand whether the presence of the ceramic bolus might have an impact on Ca^{2+} mobilization from bone under the endocrine regulation of calcitonin/paratormon system aiming at the maintenance of circulating physiological levels in the bloodstream.

This analysis allowed to exclude the pathophysiology of demineralized bones under the push of augmented requirements of the ewe during pregnancy and lactation. In fact, due to own previous observations related to Calcium precipitation on bolus surface, it was considered that calcium from the diet and animal secretions of the upper part of the alimentary tract could have been somewhat influenced by the sequestering of minerals

operated by the ceramic bolus at reticulum level. It this was the case, availability of Ca would have been decreased by the precipitating effect of the presence of the ceramic bolus in the reticulum. That way, circulating levels of Ca in the bloodstream as well as concentration of total Ca in milk were considered also in the light of bone density. According to our results, bone density was not apparently affected because no statistically significance were pointed out.

However, it is to emphasize that probably permanence and extent of mineral precipitations on the surface of the ceramic bolus did not achieve critical points to determine macroscopically detectable effects. Further diagnostic analyses on this topic would be carried out in the future in restricted groups of animals instead of numerous samples, due to costs. However, no results from additional analyses are reported in this thesis. In addition, it is also to point out that our results cannot be compared with reference because no other observations are reported earlier, to the very best of our knowledge.

4.6. Conclusion

The variations of milk calcium that emerge from this test seem to be more influenced by different climatic effect food availability.

Although there are fluctuations in the calcium of milk between electronically identified and not electronically identified animals, the effect of the presence of bolus could be of secondary importance. This investigation approach represents in all probability the first experimental contribution on this issue and it hasn't allowed any comparative analysis, but it seems worthy of a further study for an experimental confirmation.

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4.8 Tables

Table 4.1. The values of calcium in blood in animals with the presence or not ceramic bolus

Animals	Fine Lactation 2015 (mg/dL)	Initial Lactation (mg/dL)	Mid Lactation (mg/dL)	Fine Lactation (mg/dL)
Presence Bolus	9.28 ± 0.11	7.98 ± 0.81	10.15 ± 0.59	9.87 ± 0.16
No presence bolus	9.34 ± 0.08	9.61 ±0.36	9.53 ± 0.08	10.39 ± 0.43
P value	0.679	0.068	0.313	0.196

Table 4.2. The values of calcium in milk in animals with the presence or not ceramic bolus

Animals	Fine Lactation 2015 (mg/Kg)	Initial Lactation (mg/Kg)	Mid Lactation (mg/Kg)	Fine Lactation (mg/Kg)
Presence Bolus	1537.58 ± 37.9	1220.3 ± 19.46	1314.23± 40.28	1205.91± 28.71
No presence bolus	1397.38 ± 36.8	1229.27 ± 36.77	1228.41±22.15	1181.55±37.21
P value	0.007	0.822	0.071	P = 0.061

Table 4.3. Control the deposition stadium of salts limestones and calcium mobilization from the long bones

Animals	Length of the main bone (mm)	Width of the main bone (mm)	ratio marrow/cavity (mm)	density of the long bone (HU)
Presence Bolus	130.49 ± 2.37	11.88 ± 0.23	2.25 ± 0.12	3635.30 ± 96.60
No presence bolus	135.77 ± 2	12.33 ± 0.21	2.12 ± 0.11	3609.31 ± 76.36
P value	0.090	0.147	0.400	0.827

4.9 Figures

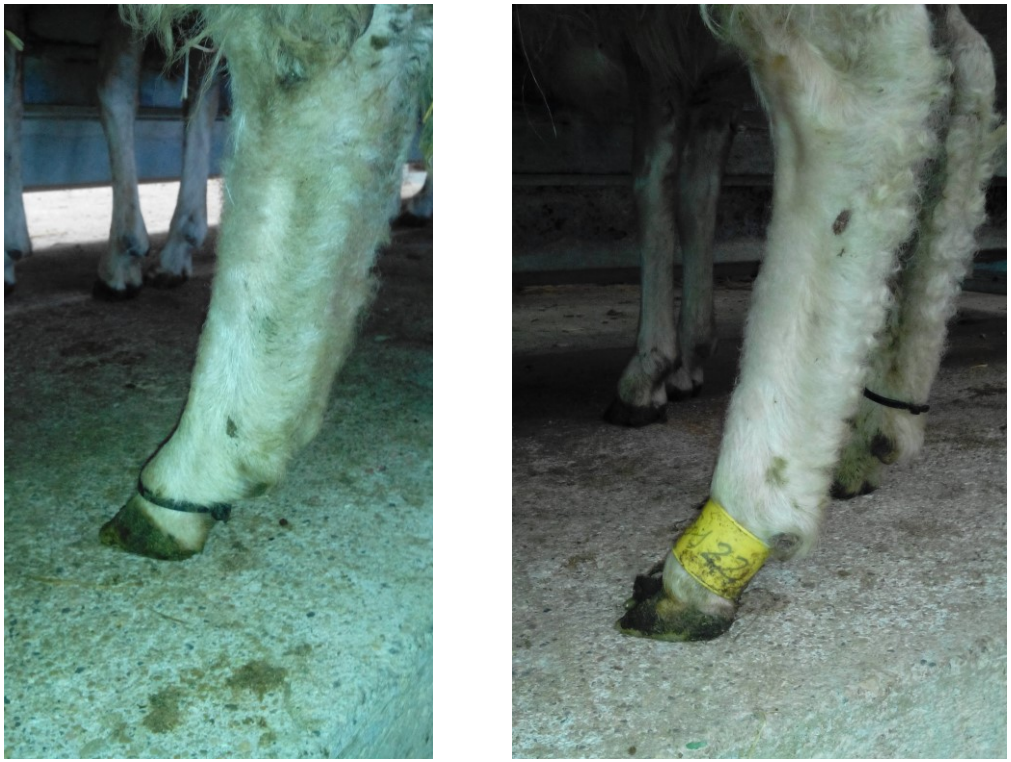

Figure 4.1. Technique resulted inadequate for the identification of individual animals used in visual testing

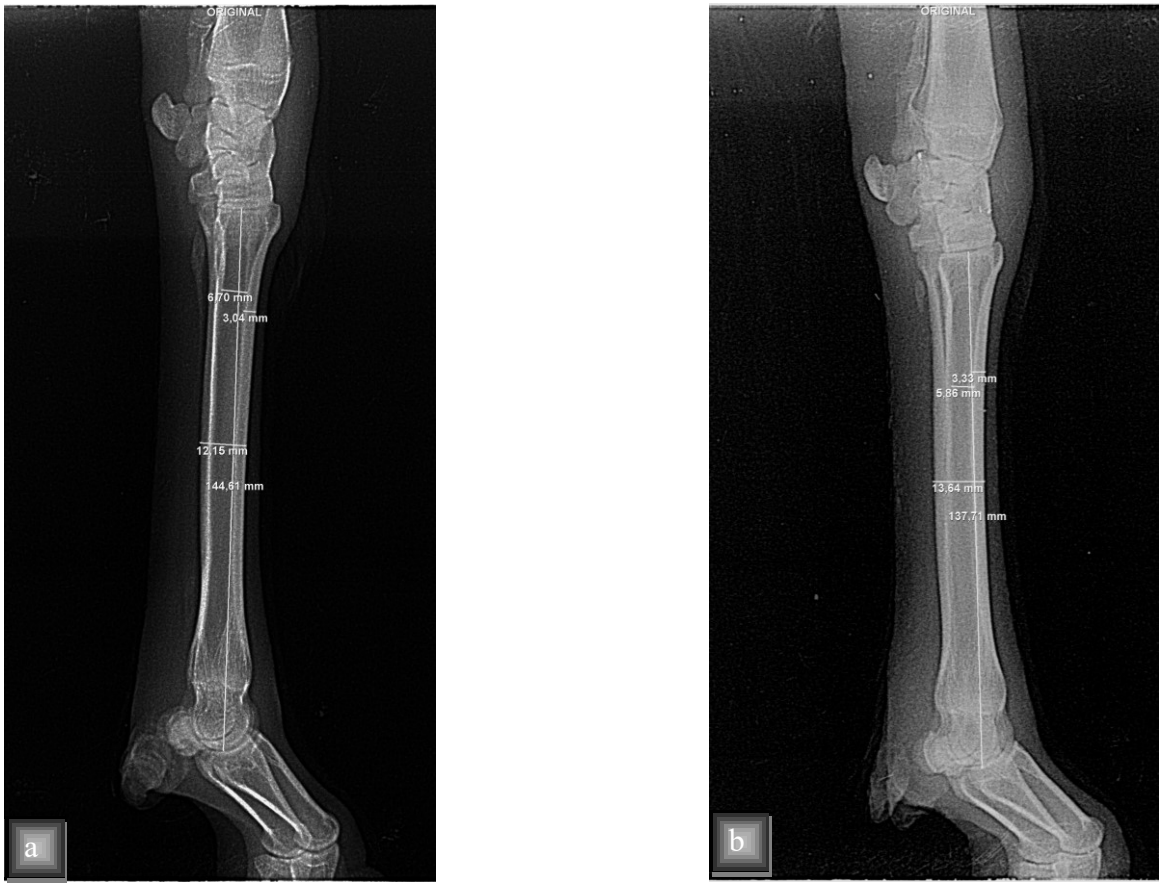

Figure 4.2. Medial-lateral X-rays image of long bone, a) animal identified electronically (EID) b) animal without device (non EID)

CHAPTER 5

Animal readability and data collection efficiencies of an automated milk recording system for dairy sheep and goats

5.1. Abstract

A total of 159 ewes (n = 132) and goats (n = 27) in mid lactation (1.48 ± 0.03 kg/d; 111 ± 34 DIM) were used to evaluate the dynamic reading efficiency (DRE) and milk yield data recording efficiency (MRE) by automated systems (DeLaval, Tumba, Sweden). Animals wore visual ear tags and electronic identification (e-ID) by rumen boluses (e-RB) of half-duplex (e-RB_{HDX}, n = 69) and full-duplex (e-RB_{FDX}, n = 90) technologies. Milking parlor (2 × 12 stalls, Amarre Azul I) had 2 multi-reader walk by panels and 12 free flow milk meters (MM25SG). Data were processed by the AlPro SG v.7.2 software. Despite the panel's nominal multi-readability, e-RB_{FDX} were poorly read (DRE < 20%) and the FDX technology was canceled, switching the panels to only read HDX. Consequently, those animals wearing e-RB_{FDX} were tagged with a supplementary HDX electronic ear tag (e-ET_{HDX}, n = 90) in the left ear. Performances of the system were evaluated by collecting a.m. and p.m. milking data during 30 d (9,540 data). On average, DRE and MRE increased with only HDX devices and were 99.2 ± 0.2 and 97.4 ± 0.2%, respectively. No interference of e-RB_{FDX} or a.m. vs. p.m. differences were detected for DRE (99.0 ± 0.3 vs. 99.5 ± 0.2%, respectively; $P = 0.106$), but MRE values were greater for a.m. vs. p.m. (98.2 ± 0.3 vs. 96.6 ± 0.6%, respectively; $P = 0.023$). This difference was related to the lower milk yield of p.m. vs. a.m. milkings (0.58 ± 0.01 vs. 0.94 ± 0.02 kg/milking, respectively; $P < 0.001$). Low milk yield data were frequently added to the following milk record**.** Corrected MRE values when low milk yields (< 0.4 kg/milking) were discarded, slightly increased and did not differ (99.0 ± 0.3 vs. 98.8 ± 0.3% for a.m. vs. p.m., respectively; $P = 0.577$). Joint readability of whole e-ID devices was assessed by repeated DRE tests (n = 8) of the full flock in a race-way with a universal multi-reader (Datamars F310, Bedano, Switzerland). On average, joint DRE was 94.9 ± 0.9%, varying by technology (HDX vs. FDX, 99.8 ± 0.3 vs. 86.7 ± 2.6%, respectively; $P < 0.001$) at an animal speed of 0.45 ± 0.09 m/s. We concluded on the convenience of only using HDX devices for the automatic milk recording system of DeLaval in sheep and goats. Moreover, we considered unsuitable the use of MM25SG

milk meters and the AlPro Herd Management System for milk recording during the whole lactation of most dairy small ruminant breeds, being only reliable when the individual milk yield is > 0.40 kg per milking.

5.2. Introduction

Automation of small ruminant milk data recording leads to reduced workload and costs in dairy farm management (Ait-Saidi et al., 2007). The milk production of both sheep and goats are increasingly becoming important for the world economy (Milan et al., 2014; Billon et al., 2016), primarily as a dairy consumption. This led to the development of specific milking parlors for goats and sheep (Billon et al., 2016; DeLaval, 2016) and several are available in the market for automated milk recording systems (Afimilk, 2016; DeLaval, 2016).

Since the first machine for milking sheep was born in 1930, machines have evolved over time and become even more expensive, because they have the machine-milking parlor equipment and must suit the number of ewes that can be milked within 2 h by any one operator (Mills, 2011). In 1992, the first automatic milking systems were born, which led to the reduction of work, an improvement. The main reason for the development of automatic milking in the eighties of the last century was the need for improved labor efficacy, due to the growing costs of labor in many dairy countries (de Koning, 2010) exploiting and optimizing the use of electronic identification of animals, which has enabled the creation of new management systems, in particular because it identifies each one (Eradus and Jansen, 1999).

Ait-Saidi et al. (2007) shows that for farmers, the acquisition costs of electronic identification or for the recording of the volume or the milk flow are quite high, but at the same time that the errors are lower than visual identification (Ait-Saidi et al., 2007).

The problem of costs facing farmers is not only a concern of the European Union, but throughout the world. For example, a study in California on electronic identification has found that costs were directly proportional and inversely related to size of operation. Costs appeared higher for smaller operations mainly because of lumpy capital investments such as RFID readers, computers, and software. Visual ID was always less expensive, but was burdened by costs associated with recording information without the benefit of RFID technology. RFID dynamic reading technology was more expensive and appropriate only for large operations. Labor costs were low and relatively insignificant. The largest costs were equipment costs

(Butler et al., 2009).

Another study carried out in India, with a pilot project in 5000 animals, showed that the use of RFID-based identification and recording system in small-hold dairy units offers value-added benefit of data security, and would prevent settlement of fraudulent insurance-related records. The integrated system can be effectively used in providing protocol-based veterinary and animal husbandry services to the farmers and can be used as a cost-effective animal performance recording service. From the economic analysis of the project, it is evident that the cost of investment can be recovered in one year, whereas the fee received from the improved services would make the system sustainable on a long-term basis (Samad et al., 2010).

Still prevailing is what Jansen and Eradus wrote in 1999, where the issue was that the transponders full duplex (FDX) should be compatible with the new generation of players and old players with new generation transponder. Respecting the protocols ISO 11785 and ISO 11784, each transmission protocol has its own pros and cons. Therefore it was decided to include both protocols in the standard number. (Jansen and Eradus, 1999). The principle of the standard on the technical concept ISO 11785 is that two different types of RFID systems (full-duplex, FDX, and half-duplex, HDX) are combined. An FDX transponder sends its telegram during activation by the reader; the HDX system responds during a pause after it was charged with energy during activation. The tag is passive, which means that it does not have a power source of its own. There are two basically different systems for RFID. In the first type, activation by the reader and the response from the transponder occur simultaneously. It is a two way system: while the transponder is being energized by the reader, it sends its code back to the reader. This system is called full-duplex (FDX). The second type is different in that the transponder has the means to store the energy it receives from the activation field. It starts transmitting its code when the reader has stopped generating the activation field. Since, in this system, there is only one-way radio traffic at a certain points in time, this is called half-duplex (HDX). The standard combines the two RFID systems. This means that one ISO reader can read both types of transponders. This is possible because the activation signals can be the same while the responses are separated in time (Kampers et al., 1999).

The objective of this study was to evaluate if the Alpro software meets the needs of the farmers in the daily management of their herd, facilitated by technological innovations that present themselves in the milking parlor, where they can get the data without a manual recording that would require further costs, time and risk of big mistakes. As seen above, the costs that the farmer has to face using manual controls are high. If an automatic reading of the data allows us an optimal efficiency in the management of the milking parlor, the breeder will get the benefits and will be prepared to face a charge that may initially seem higher. For this reason, through the machine software, the following checks were carried out on the efficiency of DRE (Dynamic Reading Efficiency) and MDRc (Milk recording efficiency corrected) in sheep and goats in the milking parlor using the ALPRO software of the DeLaval milk meter MMS25. It has also been made on the control of bolus feature HDX and FDX on the animals of the farmer to control the efficiency of readability.

5.3. Material and Metods

Animals and farm

A total of 159 animals, 67 Lacaune and 65 Manchega dairy sheep, 27 Murciano-Granadina dairy goats, in early-middle lactation, located on the experimental farm of the Servei de Granges i Camps Experimentals (S2GCE) of the UAB in Bellaterra (Barcelona, Spain). All were identified electronically with ruminal bolus and electronic ear tag.

Field activities

The operation control of the electronic identification and production in milking parlor happened at 07.30 a.m. and 17.30 p.m. in a milking parlor's parallel 2 x 12 stalls (Amarre Azul I, DeLaval Equipos, Alcobendas, Madrid, Spain), and had 2 multi-reader walk by panels able to read HDX and FDX technologies and 12 free-flow milk meters (MM25SG, DeLaval, Tumba, Sweden). Data were processed by the AlPro Windows SG 7.2 Herd Management System software (DeLaval). Trials carried out initially under practical milking conditions showed the inability to read e-BFDX (DRE <20%) and e-BHDX (DRE> 90%) boluses together. Therefore, the option to read HDX devices was

selected only as indicated by DeLaval. Consequently, all animals wearing official e-RB_{FDX} were also electronically identified with a supplementary ear tag of HDX technology (e-ET_{HDX}, n = 90) in the left ear.

The DeLaval milk meter MM25SG, is the first electronic milk meter specifically developed for sheep and goat milk recording. The following information was collected for each milking, using AlPro software: identification number, date and time of animal identification, milk yield (l/milking), and milking duration (time between the identification and the last teat-cup detachment, min). The milking parlor had 2 identification portals with multi-reader panels (DeLaval), with only reading warranty for HDX technology e-ID devices.

The experimental period was initiated when the sheep and goats were in early-middle lactation (respectively 45 to 154 d and 16 to 126 d) and consisted of 2 milk recording test-days in 159 sheep and goats for each treatment during 30 d (9,540 data). Milk recording data were collected at random by parlor side and in random groups of 12, before the sheep were milked and after the goats.

All sheep and goats were electronically identified with an electronic bolus (20g; Ø7 mm × L 57 mm, Datamars, Barcelona, Spain, and Ø 08 mm × L 51 mm Azasa-Allflex), which consisted of a high-density ceramic capsule containing an ISO radiofrequency transponder (ISO, 1996) and were used for the milk recording treatment. N. 69 animals were with transponders half-duplex (e-RB_{HDX}) technology and 90 animals were with transponders full-duplex (e-RB_{FDX}) technology, glass encapsulated (32 × 3.8 mm), and marked with country code (0724 Spain, Datamars and Allflex) according to the International Committee for Animal Recording (ICAR, 2007). Animals passed through the hallway before being placed in the milking parlor, in which there was the transceiver allowing the transmission of the electronic identification number to the computer. The identification number was connected to animal productions.

All animals wearing official e-B_{FDX} were also electronically identified with a supplementary ear tag of HDX technology (e-ET_{HDX}, n = 90) in the left ear with a round

plastic electronic button (Ø 25 mm yellow color or Ø 24 mm orange color, Azasa-Allflex, Madrid, Spain) used for mandatory health programs in Catalonia (official electronic identification). Additionally, a second plastic ear tag of a flag type and large size (53 × 55 mm, yellow color; Azasa-Allflex) was inserted in the left ear. These large ear tags were manually marked with 3 digits of 17 × 10 mm each (black plastic ink, Allflex Tag Pen, Dallas, TX) for easy reading in the ID system experimental treatment. Sheep and goats were visually identified in the right ear with an official visual ear tag (polyurethane, 2 yellow flags; male flag = 40 × 38 mm; female flag = 42 × 38 mm; Azasa-Allflex, Madrid, Spain) according to the Spanish legislation.

Dynamic reading efficiency (DRE) was calculated according to Ait-Saidi et al. (2014), using the expression DRE (%) = [(number of read transponders)/ (number of readable transponders)] × 100.

Created by our team particularly for this experiment; Milk recording efficiency (MRE) was calculated using the expression MRE (%) = [(number of read milk production)/ (number of readable milk production)] × 100.

Milk recording efficiency corrected (MREc) was calculated taking into account only the animals that had a volume of milk > 0.40 mL.

In order to assess the potential readability of the e-ID devices used (e-B_{HDX}, e-B_{FDX} and e-E_{HDX}), all animals were also submitted to repeated DRE tests (n = 8) of the full flock in a race-way equipped with a Datamars F310 (Bedano, Switzerland) a universal multi-reader.

Statistical analysis

The data obtained on the dynamic reading efficiency (DRE) and on the efficiency of the milk recording (MRE) were analyzed with a mixed linear model for repeated measurements to control that there were differences in the two groups during the period of checks. Statistical significance was set at $p < 0.05$. All analyzes were performed using the package R v.3.0.2.

5.4. Results

Performances of the automated milk recording system in all animals using either e-RB_{HDX} or e-ET_{HDX} devices were evaluated by collecting a.m. and p.m. milking data during a period of 30 d. On average, DRE and MRE increased with only HDX devices and were 99.2 ± 0.2 and 97.4 ± 0.2%, respectively. No interference of e-RB_{FDX} or a.m. vs. p.m. differences were detected for DRE (99.0 ± 0.3 vs. 99.5 ± 0.2%, respectively; P = 0.106), but MRE values were greater for a.m. vs. p.m. (98.2 ± 0.3 vs. 96.6 ± 0.6%, respectively; P = 0.023) which was related to the lower milk yield obtained in the p.m. vs. a.m. milking (0.58 ± 0.01 vs. 0.94 ± 0.02 kg/milking, respectively; P < 0.001) (Table 5.1). Low milk yield data were frequently added to the following milk record. Corrected MRE values slightly increased and did not differ when low milk yields (< 0.4 kg/milking) were discarded, being 99.0 ± 0.3 vs. 98.8 ± 0.3% for a.m. vs. p.m., respectively (P = 0.577). On average, animal speed was 0.45 ± 0.09 m/s and potential joint DRE was 94.9 ± 0.9%, varying by technology (HDX vs. FDX, 99.8 ± 0.3 vs. 86.7 ± 2.6%, respectively; P < 0.001) (Table 5.2).

5.5. Discussion

The different percentages that are obtained in MRE between the two daily milkings are explained with different milk flows between the morning and evening milkings, and it would be appropriate to analyze the curves separately. In fact, as already demonstrated by Caja et al in 2000, the morning milking is increased by the flow of milk and the milking time, but the alveolar milk emissions can be easily and separately observed in the afternoon. The decision to eliminate some samples of MREc is caused by the use of Minimal scale representation for sheep in ICAR guidelines for milk recording devices and systems is 40 mm/kg or liter (ICAR, 2014).

Data comparable to those obtained in this experiment were obtained in a similar study in dairy cattle demonstrating relatively high readings (99.9% for HDX, 93.8% for FDXB, 97.8% total) (Stewart et al., 2007). However, the animals are of different sizes and move at different speeds. This argument in fact confirms the higher efficiency of the HDX in cattle as well as in sheep. The mean value of DRE (mean pass velocity,

0.45 ± 0.09 m/s) was 94.9 ± 0.9%, although it varied according to devices (HDX vs. FDX, 99.8 ± 0.3 vs. 86.7 ± 2.6%, respectively, P <0.001) and confirmed the superiority of HDX under device mixing conditions, according to Ait-Saidi et al. (2013).

Ait- Saidi et al. in 2014 have also shown the advantages that the use of a semi-automatic milking system can provide to farmers in terms of feasibility and reduction of the number of operators necessary for the recording of data on milk both sheep and goats. It is also confirmed that the data recorded in the control software also allows you to assess what improvements can be developed and how it can be better organized to optimize the business management software.

In regards to the efficiency of the dynamic reading, we have achieved more than 99% results, but 100% efficiency on the dynamic reading of all dairy sheep obtained at milk parlor has not been confirmed.

5.6. Conclusion

In conclusion, the results indicated the low reading of FDX-B devices, according to the indications of the equipment, the need for a production level of > 0.4 L per milking and problems in the data management that make manual correction necessary. Therefore, it seems necessary to improve the DeLaval-AlPro system before being able to recommend it for the automatic milk control of sheep and goats in farm conditions

5.7. References

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5.8. Tables

Table 5.1. Results on evaluation the dynamic reading efficiency (DRE) milk yield recording efficiency (MRE) and corrected MRE by an automated milking system (a.m. and p.m.).

	DRE % (Dynamic reading efficiency)	MRE % (Efficiency of the milk recording)	Corrected MRE %
a.m.	99.0 ± 0.3	98.2 ± 0.3	99.0 ± 0.3
p.m.	99.5 ± 0.2	96.6 ± 0.6	98.8 ± 0.3
p value	0.106	0.023	0.577

Table 5.2. Results DRE: evaluated with an ISO multi reader (Datamars F310) varying by technology (HDX vs. FDX).

	Dynamic Reading	
Animal speed	0.45 ± 0.09 m/s	
DRE	94.9 ± 0.9 %	
HDX	99.8 ± 0.3	P < 0.001
FDX	86.7 ± 2.6	

5.9.Figures

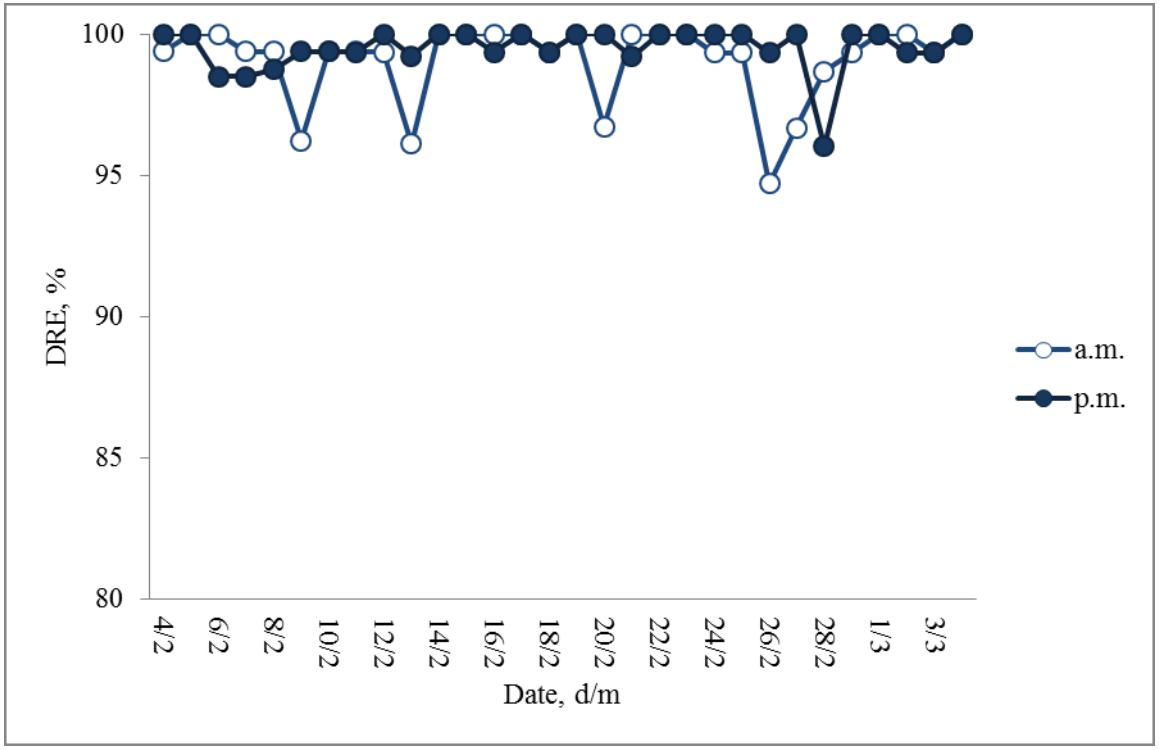

Figure 5.1. Dynamic reading efficiency (DRE) of dairy sheep and goats identified with bolus and HDX ear tag. The FDX reading had a DRE <20% and was discarded

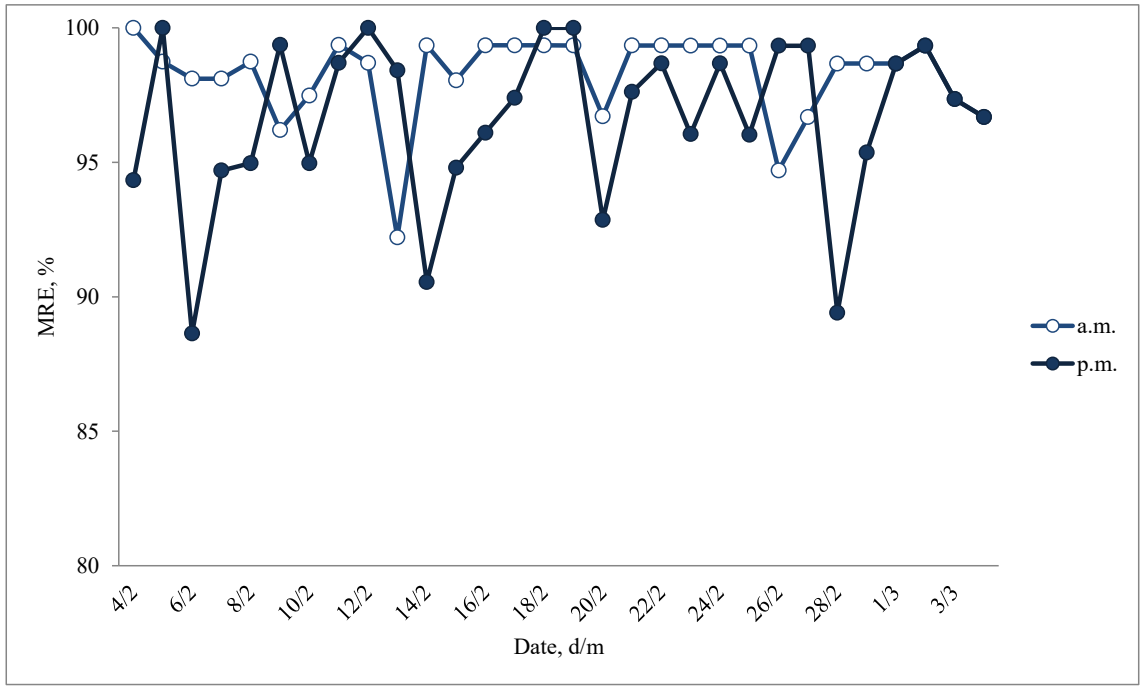

Figure 5.2. Efficacy of individual data collection on milk production (MRE) in dairy control of sheep and goats in the morning and afternoon (uncorrected data)

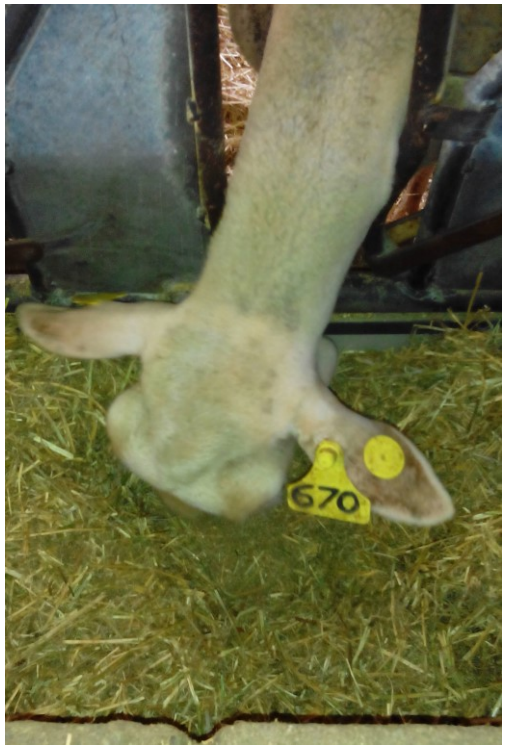

Figure 5.3. Double device (CIC ed EIC), visual and electronic ear tag used for identification of dairy sheep

Figure 5.4. Detail ear tag plier and electronic ear tag before application to animals

Figure 5.5. Detail electronic ID system and DeLaval sorting gate

FINAL CONCLUSIONS

In conclusion, the study of the system of electronic identification (EID) kept into the reticulum for a long period showed the high functionality of the electronic device component. The type of farm, particularly feeding conditions, influenced the color and extension of the concretions present on the bolus. In addition, the elemental analysis of the ceramic bolus interface surface may represent a method for the detection of environmental contamination in the future.

The EID was more reliable, effective and efficient compared to the ear tag and ear tattoo. This system was also better regarding the record performance normally required for farmers and the Veterinary Service of the Italian National Health System (NHS) compared to the other two personal systems used. This thesis also confirmed that the long-term use of EID allows the full utilization of the record data related to individual animals during their whole life and productive career. Therefore, the type of electronic identification examined could be a profitable registry management tool for farmers and their associations. However, although the first experiences on its use date back to fifteen years ago, the use and automatic control of electronic identification in Sardinia is still a goal to be reached more from a practical-operational rather than from a technical-instrumental point of view.
Further studies are needed to confirm that variations in milk and blood calcium are more influenced by different environmental conditions that in turn affect diet composition compared to the presence of bolus in the reticulum.

Based on the assessment of the efficiency of automatic data collection of the identification and milking system currently offered by DeLaval for sheep and goat breeding operations in Spain, it seems that the DeLaval-AlPro system should be improved before recommending it for the automatic milk control of sheep and goats in farm conditions. An optimized production control would be essential for the genetic improvement and profitability of the farms. However, the results showed that the FDX-B device had a lower reading compared to the HDX-B device, and that the record from animals producing less than 0.4 L per milking had to be corrected manually, regardless of the type of electronic device used.

Finally, the EID has been underutilized in Sardinia, where a large number of sheep and goat farms are present, and the widespread adoption of this emerging and reliable technology could help to improve the sheep and goat breeding system and supply chain in the Island.

Conflict of interest

The author declares that there are no conflicts of interest

APPENDICES

APPENDIX 1

Poster: XXII Congresso Nazionale S.I.P.A.O.C. Cuneo 13/16 settembre 2016

Interazione dell'interfaccia di superficie tra mucosa del reticolo-bolo ceramico in ovini identificati elettronicamente con diversi tipi di dispositivi a tecnologia RFID

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PAROLE CHIAVE: Brushite, creste reticolari, papille .

INTRODUZIONE: Successivamente al recepimento e all'applicazione del Regolamento CEE 21/2004 negli Stati Membri in materia di anagrafe ovina e caprina, sono diversi gli aspetti che per natura e caratteristiche stanno interessando le produzioni animali, relativamente all'impiego dell'identificazione elettronica dei capi su larga scala. In particolare, uno degli aspetti che necessitano di essere approfonditi, riguarda l'interazione esistente tra i tessuti dell'animale ed il materiale di contenimento del transponder, ossia il bolo ceramico, che normalmente si trova a contatto con il medium e la mucosa reticolare dell'animale. In base alla nostra conoscenza, infatti, non sono presenti in bibliografia studi che abbiano preso in considerazione l'interazione esistente tra l'interfaccia di superficie della mucosa del reticolo e il bolo ceramico, quest'ultima parte integrante del dispositivo di identificazione. Difatti, sebbene la componente elettronica soggiaccia a rigidi requisiti dettati delle misure ISO 11784 e ISO 11785 (Cappai et al.), il gap conoscitivo è relativo alla composizione di superficie del materiale del *case* e delle sue possibili interazioni con l'ambiente biologico a contatto del quale si suppone permarrà per periodi medio-lunghi. La presente attività di ricerca si è prefissata l'obiettivo di analizzare gli effetti della presenza del bolo ceramico all'interno del reticolo di ovini adulti identificati elettronicamente, provenienti da

diversi allevamenti della Sardegna, regolarmente macellati e sottoposti a prelievo del reticolo e del rispettivo bolo ceramico contenente il transponder.

MATERIALE E METODI: Un totale di 269 reticoli è stato sottoposto ad analisi stereomicroscopica per la valutazione della mucosa assieme al rispettivo bolo ceramico che è stato sottoposto ad una serie di misurazioni per la determinazione delle caratteristiche di superficie. Per ciò che riguarda la procedura analitica della superficie mucosale del reticolo, ogni organo è stato ispezionato visivamente e, verificato il fondo del reticolo come luogo di più frequente permanenza del bolo, è stato effettuato un prelievo autoptico a tutto spessore della parete. I campioni di reticolo sono stati conservati in glutaraldeide al 12.5% (v/v). I pezzi così fissati sono stati osservati allo stereo microscopio e sono state effettuate le misure di altezza e spessore delle creste primarie e secondarie, nonché delle papille di ogni cella. Inoltre è stato misurato il pH del contenuto reticolare mediante pH-metro portatile, a temperatura costante di 25 °C. La superficie di ciascun bolo ceramico, contenente il transponder a tecnologia RFID, associato al reticolo di ogni animale, è stata analizzata secondo i seguenti parametri: colore, concrezione e analisi elementale. Per il colore è stato utilizzato un bolo intonso che rappresentava il campione "bianco" e un colorimetro portatile che restituiva le 3 coordinate cromatiche. Il confronto con il bolo bianco è stato poi utilizzato per la stima della variazione cromatica a carico dei boli applicati agli animali. Mediante fluorescenza a raggi X la presenza delle concrezioni rilevate sulla superficie ha consentito di condurre l'analisi elementale. I dati sono stati analizzati prendendo in considerazione gli effetti sulle caratteristiche della superficie del bolo in relazione a tipologia di bolo, provenienza e tempo di permanenza nel corpo dell'animale.

RISULTATI E CONSIDERAZIONI: In seguito ad una lunga permanenza del bolo *in situ*, lo studio della mucosa del reticolo ha permesso di valutare i possibili effetti sulla morfologia macro e microscopica. La visione macroscopica ha mostrato, nella mucosa del fondo del reticolo, una modificazione di lieve entità che è stata successivamente confermata dalla visione stereo-microscopica. Nel punto di più frequente permanenza del bolo per effetto della gravità, la morfologia del margine libero delle celle, ha mostrato infatti un ispessimento con riduzione dell'altezza delle creste sia primarie che secondarie, visibile già ad occhio nudo.

Le differenze morfologiche evidenziate per le creste, si sono riscontrate ad una attenta analisi anche nella morfologia delle papille presenti nel margine libero delle creste; queste ultime da coniche apparivano arrotondate per effetto di una diminuzione dell'asse maggiore e di un ispessimento dei margini laterali e della base a diretto contatto con la cresta.

Non si sono rilevati ulteriori effetti sulla morfologia della mucosa nelle parti circostanti. Il dato emergente era rappresentato dal fatto che in generale le creste primarie e secondarie direttamente a contatto con il bolo sono risultate 2/3 dell'altezza delle creste non a contatto diretto permanente con il bolo. Si potrebbe parlare di modificazioni di lieve a moderata entità che pur tuttavia non sembrano pregiudicare la funzionalità dell'organo. Interessante è risultata la variazione del colore del bolo a seguito della precipitazione del materiale di superficie, e delle relative concrezioni osservate in stereo microscopia. I boli del tipo Z72, costituiti principalmente da allumina, hanno mostrato una superficie particolarmente liscia rispetto ai boli del tipo Z70, i quali invece erano costituiti principalmente di zirconia e mostravano rugosità e irregolarità di superficie. Inoltre è stato evidenziato che le concrezioni presenti sui boli hanno un' origine minerale, con prevalenza di Calcio, Manganese, Zinco e Fosforo, prevalentemente nella forma di calcio fosfato idrato (CaHPO4*2H2O) o brushite, tipica componente delle concrezioni rilevabili sui denti dell'uomo. Non essendo stati riscontrati metalli pesanti, l'analisi elementale della superficie di interfaccia, così come condotta negli animali al pascolo, può rappresentare un metodo per la rilevazione delle contaminazioni ambientali mediante animali sentinella.

Surface to surface interaction between reticulum mucosa and ceramic bolus in sheep electronically identified by means of different RFID identification devices

Key words: brushite, reticulum crests, papillae.

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APPENDIX 2

Poster: Convegno AISSA, Sassari 6-7 novembre 2014

Tracciabilità nella filiera dell'agnello da latte I.G.P. "Agnello di Sardegna" e sistema innovativo di identificazione elettronica basato sulla tecnologia a radiofrequenza. Vantaggi e limiti di impiego

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Introduzione

Le produzioni IGP rappresentano un settore strategico del comparto agroalimentare in ambito UE (Reg. CE 510/2006). L'Italia presenta un paniere cospicuo di prodotti a marchio IGP (oltre 50) dalle diverse Regioni, tra cui la regione Sardegna detiene il marchio del prodotto "Agnello di Sardegna" IGP, nella filiera della carne. Ciononostante, a fronte di una crescente domanda, soprattutto proveniente dalla grande distribuzione organizzata (GDO), la produzione di agnelli da latte certificati IGP rappresenta una piccola percentuale degli agnelli macellati dalle oltre 13.000 aziende ovine presenti in Sardegna. Come in generale per tutte le produzioni di qualità, anche per la filiera dell'Agnello di Sardegna IGP, una delle esigenze basilari è rappresentata da un'adeguata tracciabilità del prodotto, per poter offrire adeguate garanzie ai produttori, e, soprattutto, ai consumatori.

Materiali e Metodi

Gli AA riportano le evidenze sperimentali emerse da una serie di rilievi *in vivo* e *post mortem* su 216 agnelli da latte, provenienti da allevamenti aderenti al disciplinare di produzione IGP, identificati elettronicamente con tecnologia RFID (applicazione intraperitoneale di transponder iniettabile HDX 32.5×3.8 mm, 134.2 kHz). Le attività

sono state condotte in allevamento e in catena di macellazione, a cui è seguito il recupero del device che deve essere allontanato dalla carcassa in destinazione al commercio.

Ai fini del saggio delle performance del sistema di tracciabilità sono stati valutati:

- Leggibilità del codice individuale del transponder applicato mediante iniezione intraperitoneale.
- Valutazione clinica e del benessere degli animali identificati elettronicamente
- Leggibilità ai successivi controlli per l'identificazione degli animali ai fini della rispondenza con il registro di stalla (prototipo digitale per l'Agnello di Sardegna IGP)
- Valutazione della leggibilità dei codici del transponder in catena di macellazione, velocità della catena per il recupero di ciascun device.
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Risultati

Le prove sperimentali hanno evidenziato: tempo medio di applicazione del transponder 1'51"/capo; nessun caso di mortalità degli animali durante e dopo la stessa applicazione; 3 lievi incidenti di applicazione (1.4%); 100% di leggibilità *in vivo* dei transponders applicati; 100% leggibilità *post mortem*; affidabilità del lettore di transponders 99.15%; recupero dei transponders al mattatoio 99%. Il sistema di identificazione elettronica degli agnelli da latte basato sull'RFID ha mostrato il vantaggio di poter redigere su formato elettronico una certificazione attestante l'identità individuale che accompagna il prodotto dei singoli agnelli dalla partenza dall'allevamento, fino al mattatoio, assicurando un elevato livello di tracciabilità *in vivo*. Allo stato attuale il sistema di identificazione RFID sperimentato ha fatto registrare alcuni limiti nelle fasi *post mortem* in mattatoio: arresto della tracciabilità del singolo capo alla fase di eviscerazione; tempo di recupero del transponder 15"/capo; perdita di transponders in catena 1.4%; notevole rallentamento della catena di macellazione da 240 a 120 capi/h.

APPENDIX 3

Paper: International Workshop "New updates on animal nutrition, natural feeding sources and environmental sustainability", Arzachena 5-6 maggio 2014

µ-XRF Fluorescence and XRD Diffraction characterizations on RFID Radio Frequency Identification Device

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Objective: The Radio Frequency Identification (RFID) is a technology based on a low frequency (134.2 kHz) which is now being used for animal identification purposes (ISO standard 11784-11785). When applied to ruminants, the transponder is held in the animal body through its incorporation into a ceramic case (bolus), administered to ruminants *per os* to allow the retaining in the pre-stomachs (reticulum) [1]. Despite several investigations were carried out to assess both technological and economical impacts from a massive RFID use, an extensive characterization of the devices after long-term permanence in the ruminant body is still missing. To fill this gap, we have used a multi-technique approach for determining the wearing effects on the RFIDs device, with specific regard to the case, in relation to different animal/managerial/environmental parameters. Micro-X-ray fluorescence spectrometry (µ-XRF) and X-ray diffraction (XRD) have been applied to RFIDs boluses, showing different characteristics, after their removal from the carcasses of slaughtered animals.

Animals, material and methods: 60 RFIDs boluses were administered to cattle, goats and sheep from different farms and then recovered from the animals after increasing permanence time in the animal body, from 1 year up to 10 years. The boluses were

administered by experts through a well-established procedure of insertion and removed from the pre-stomachs at slaughter. The boluses are composed by a ceramic capsule, prevalently made by alumina Al_2O_3 or zirconia ZrO_2, holding inside a transponder (passive, Half Duplex, 134.2 kHz, ISO 11784 e 11785) isolated by silicone rubber cap.[2] The characterizations were conducted by using μ-XRF on the bolus surface and XRD on large concretions, previously removed from the RFIDs. To optimize the spectrometer focus conditions, the quantitative elemental analysis of the bolus by μ-XRF was obtained by averaging 20 measures collected from an ideal line crossing the length of the sample.

Results and discussion: The wearing of the RFID device has been evaluated considering the farming area, animal species and permanence time in the *reticulum* of the ruminant. The analyses obtained by μ-XRF and XRD performed on the boluses, revealed that the formation of concretions on the devices can been attributed to the crystallization of inorganic compound on the bolus surface. The elemental composition of the bolus surface, obtained by XRF, has shown an increase of the amount of calcium, phosphorus, manganese, sulfur and zinc and no presence of heavy elements. XRD performed on the concretions deposited on the surface device has allowed identifying different crystalline structure, in particular Brushite, formed by calcium phosphate hydrate. The kinetics of nucleation and growth of the crystalline phase have been followed by comparing the characterization of several boluses after increasing time permanence in several breeding and animal species.

Conclusion: The quantifications of elemental composition have shown that calcium, phosphorus, manganese, sulfur and zinc tend to accumulate on the bolus surface and no presence of heavy metals was detected. The concretions appeared to accumulate preferentially on the top of the device with a progressive loss of the silicone sealing. XRD analysis of the concretions has revealed that the inorganic salts on bolus surface are mainly calcium phosphate hydrate phases.

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