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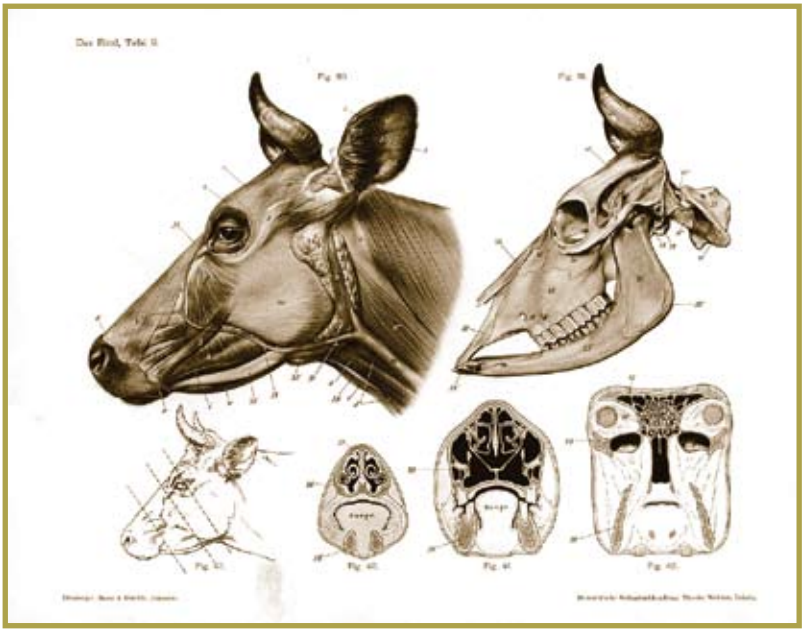

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PROPRIOCEPTIVE INNERVATION OF THE POPLITEUS MUSCLE OF THE SHEEP: ITS ROLE IN THE BIOMECHANICS OF THE KNEE JOINT

L'INNERVAZIONE PROPRIOCETTIVA DEL MUSCOLO POPLITEO DI PECORA: IL SUO RUOLO NELLA BIOMECCANICA DEL GINOCCHIO

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SUMMARY

Aim of this study was to evaluate the proprioceptive innervation of sheep popliteus muscle (PM) to know its involvement in the biomechanics of the knee joint.

The research was carried out on two right and two left limbs of slaughtered sheep and from these pelvic limbs PM was removed. Each muscle was divided in six parts and then each part was treated with the gold chloride method according to Ruffini.

Our research allowed us to document a conspicuous proprioceptive innervation in the sheep PM, in particular muscle spindles and Golgi's tendon-organs.

The high number of proprioceptors lead us to assert that PM has an important role either during joint movements of flexion and extension or during static phase, acting like a stabilizer of the knee.

KEYWORDS

Sheep popliteus muscle, proprioceptive innervation, muscle spindles, Golgi's tendon-organs, stabilizer of the knee.

INTRODUCTION

Biomechanical study of the knee has always created special interestin both human and veterinary medicine.

The popliteus is a muscle of the pelvic limb situated on the lateral part of the knee joint and on the caudal side of the leg. On the basis of its topography and its relations, all anatomical treaties place it in the group of the leg flexor muscles (Getty, 1982; Nickel et al., 1991; Pelagalli and Botte, 1999; Barone, 2004; König and Liebich, 2006; Dyce et al., 2009), even if it is already strengthened that the popliteus muscle (PM) has extensor functions (Fürst, 1903; Basmajian and Lovejoy, 1971; Mann and Hagy, 1977; Fuss, 1989). In particular, Basmajian and Lovejoy (1971) have found the "flexor activity of the popliteus muscle negligible". Mann and Hagy (1977) have observed, in an electromyographic study, only a "trace of activity" during stifle joint flexion, while a strong activity was observed by them when "squatting and then standing" consequently during joint extension.

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Moreover, some authors have supposed, in many species like dog, pig, sheep, horse and also man, that it acts like stabilizer of the knee (Basmajian and Lovejoy, 1971; Fuss, 1989; Poliacu Prosè et al., 1991; Scherer et al., 1996; Ulrich et al. 2002; Nyland et al., 2005; Schinhan et al., 2011), while other authors demonstrated that it intra-rotates the tibia on the femur (Basmajian and Lovejoy, 1971; Lovejoy and Harden, 1971; Herley, 1977; Mann and Hagy, 1977; Fuss, 1989, 1992; Bo Minelli et al., 1993; Ferrari et al., 2003; Palumbo Piccionello et al., 2006; Schinhan et al., 2011). In particular, Palumbo Piccionello et al. (2006) have demonstrated, after complete resection of the tendon, a decrease of the intra-rotation, from an average of 22° to 15°.
Therefore we think that the PM could play a complex role in the biomechanics of the knee. So we have carried out a basic anatomical study to evaluate the presence of a proprioceptive innervation in the PM of the sheep. In fact the goal of our research was to evaluate the involvement of PM in the biomechanics of the knee joint on the basis of its proprioceptive contingent. We have chosen the sheep because it is considered a valid model for the orthopedic studies in man (Bosh et al., 1995; Moeller et al., 1995; Durselen et al., 1996).
In the sheep, the PM originates with a tendon from the lateral femoral condyle, then it runs under the lateral collateral ligament, on the lateral side of the knee joint and it turns to the popliteal incision of the tibia. Muscular fibers have different courses: in the lateral portion of the belly, they are rectilinear, in the medial part, it is possible to distinguish three areas, a proximal area where the fibers are rectilinear, a central area where they are oblique and a distal area where they are vertical (Palumbo Piccionello et al., 2006) (Fig. 1). This muscle organization is taken in consideration in our research.

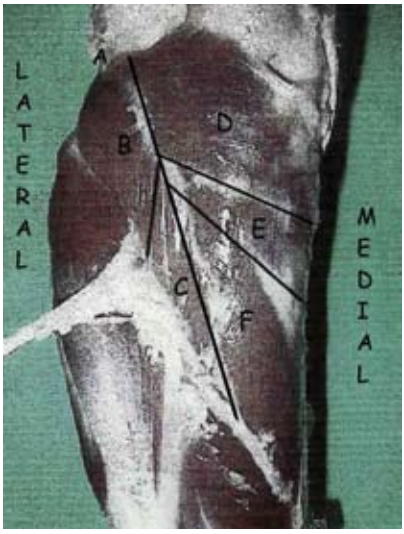

Figure 1: left PM of sheep

MATERIALS AND METHODS

The research was carried out on two right and two left pelvic limbs of slaughtered sheep. From these pelvic limbs, PM was removed. The weight of the 4 muscles ranged from a minimum of 13,9 g to a maximum of 17,5, with an average of 15,8 g. Each muscle was divided in 6 parts according to the disposition of the muscular fibers (Fig. 1). Each part was treated with the gold chloride method according to Ruffini (Ruffini, 1897) and then the samples were observed at the microscope to highlight the eventual presence of proprioceptors.

RESULTS

In the PM of the sheep we found a conspicuous number of muscle spindles, Golgi's tendon-organs and few Pacini's corpuscles.

The muscle spindles observed had the typical spindle shape. These proprioceptors consist of intrafusal muscle fibers, connectival sheat, perifascicular space and sensitive and motor innervations (Fig. 2). Muscle spindles exam allow us to classify the majority of them as complex type according to Ruffini's classification (primary sensitive expansion and one or more secondary ones), the other of the intermediate type (one primary and one secondary sensitive expansion) and of simple type (only one primary sensitive expansion) (Ruffini, 1897, 1898).

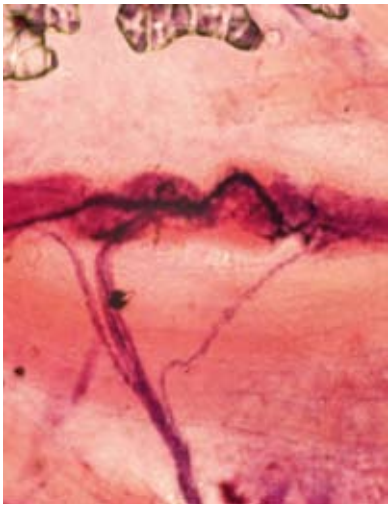

Figure 2: sheep PM, B section, a typical complex muscle spindle, gold chloride method according to Ruffini (X 120).

Golgi's tendon-organs were found isolated or organized to form flowersprays. They originate from the subdivision of one or more myelinic nerve fibers which divide in secondary or tertiary branches. From these branches thin amyelinic stamens originate. Golgi's tendon-organs were wrapped by a capsule made by connective laminae (Fig. 3).

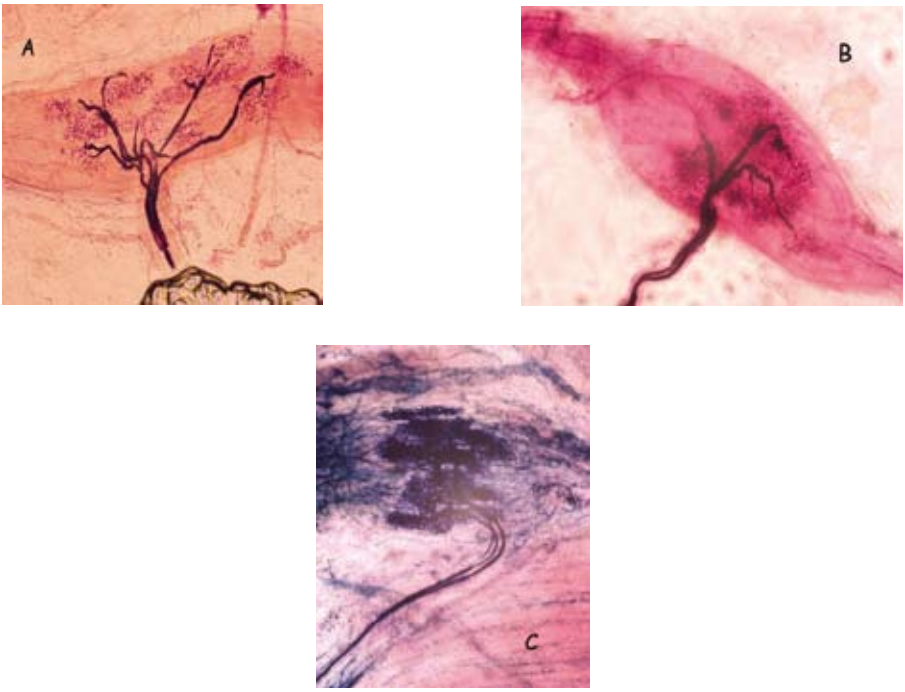

Figure 3: sheep PM, Golgi's organ-tendons, A: section B, B: section D, C: section E, gold chloride method according to Ruffini, (X120)

We also found a few Pacini's corpuscles with the typical elliptical shape. These corpuscles were provided with a well-developed capsule with a lamellar organization and their expansional axon showed a rather various morphology. In fact, it can appear undivided or differently ramified (Fig. 4).

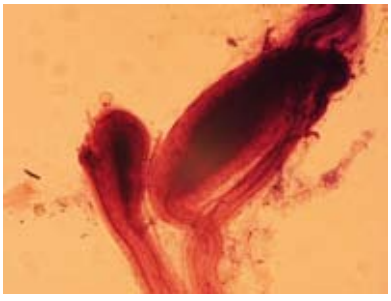

Figure 4: sheep PM, Pacini's corpuscles, D section, gold chloride method according to Ruffini (X190)

It was calculated the total number of proprioceptors. The relative number of muscle spindles ranged from a minimum of 94 to a maximum of 112, with an average of 99, while the relative number of Golgi's tendon-organs ranged from a minimum of 39 to a maximum of 52, with an average of 47. So the number of Golgi's tendon-organs was always lower than that of muscle spindles, in particular, in the four popliteus muscles studied, their number was always about the half respect to muscle spindles. Either muscle spindles or Golgi's tendon-organs were regularly distributed in the whole parts of the muscle, even if in the tendinous part (part A) the number was lower or they were even absent.

SECTION OF THE MUSCLE	MUSCLE SPINDLES	GOLGI'S TENDON - ORGANS
A	9	1
B	31	10
C	12	10
D	24	10
E	14	10
F	18	6

Tab. 1: total number of muscle spindles and Golgi's tendon-organs. Average of the four limbs.

PART OF THE MUSCLE	MUSCLE SPINDLES	GOLGI'S TENDON -ORGANS
LATERAL	52	30
MEDIAL	56	26

Tab. 2: total number of muscle spindles and Golgi's tendon-organs in the lateral part of the muscle and in the left one. Average of the four limbs.

DISCUSSION

Our research allowed us to document a conspicuous proprioceptive innervation in the PM of the sheep. This innervation consisted in capsulated nerve endings (muscle spindles and Golgi's tendon-organs) that were homogeneously distributed in the muscle belly. The total number of proprioceptors found are reported in the table number 1. The muscle spindles were the most frequent proprioceptors but also the Golgi's tendon-organs were present in very high numbers. In fact in the sheep popliteus muscle we documented that the ratio between muscle spindles and Golgi's tendon organs was 2:1. We did not found significant differences between muscles of right and left limb regarding the type and the quantity of receptors. We also did not found any significant differences between the lateral part of the sheep muscle and the medial one (Tab. 2).

Previous analysis of the PM (Basmajian and Lovejoy, 1971; Fuss, 1989; Poliacu Prosè et al., 1991; Scherer et al., 1996; Ulrich et al. 2002; Nyland et al., 2005, Palumbo Piccionello et al., 2006) and this microscopic study lead us to say that the sheep PM, besides a normal mechanical role, could have an important role in the control of nervous activity of the stifle joint, either during joint movements of flexion and extension, or during the static phase when the patellar mechanism manifests.
Therefore the high number of proprioceptors, in particular the relative high number of Golgi's tendon-organs in this muscle, lead us to assert that it has not only a primary role of stabilizer of the knee and internal rotator of the tibia on the femur but also that it carries out an important neurological control of the joint. We think that the PM could be considered a peripheral nervous center that with its conspicuous nervous contingent controls and regulates the knee joint during the static and motor phases (myotatic and inverse myotatic reflexes).

REFERENCES

1. Barone R. (2004): "Anatomia Comparata dei Mammiferi Domestici", Vol. 2, Edagricole, Bologna.
2. Basmajian J. V., Lovejoy J. F. (1971): "Function of the popliteus muscle in man", J. Bone and Joint Surgery, 53 A, 3: 557-562.
3. Bo Minelli L., Sanna L., Gazza F., Acone F., Panu R. (1993): "Muscolo popliteo del cavallo: aspetti morfologici, innervazione sensitiva ed ipotesi funzionale", Atti S. I. S. Vet. 47: 115-117.
4. Bosch U., Decker B., Moller H. D., Kasperczyk W. J., Oestern H. J. (1995): "Collagen fibril organization in the patellar tendon autograft after posterior cruciate ligament reconstruction. A quantitative evaluation in a sheep model", Am. J. Sports Med. 23: 196-202.
5. Durselen L., Claes L., Ignatius A., Rubenacker S. (1996): "Comparative animal study of three ligament prostheses for the replacement of the anterior cruciate and medial collateral ligament", Biomaterials 17: 977-982.
6. Dyce K. M., Sack W. O., Wensig C. J. G. (2009): "Textbook of Veterinary Anatomy", Ed. Saunders, London.
7. Ferrari I. A., Wilson D. R., Hayes W. (2003): "The effect of release of the popliteus and quadriceps force on rotation of the knee", Clinical Orthopaedics and Releated Research, 412: 225-233.
8. Fürst C. M. (1903): "Der musculus popliteus und seine sehne", Lunds Universitets Arsskrift, Band 39 Afdeln. 2 n. 1, Kongl. Fysiografiska Sallskapets Handlingar Band 14 n. 1, E. Malmstroms Buchdruckerei, Lund.
9. Fuss F. K. (1989): "An analysis of the popliteus muscle in man, dog and pig with a reconsideration of the general problems of muscle function", Anat. Rec., 255: 251-256.
10. Fuss F. K. (1992): "Principles and mechanism of automatic rotation during terminal extension in the human knee joint", J. Anat., 180: 297-304.
11. Getty R. (1982): "Anatomia degli animali domestici", Vol. 1; Ed. Piccin, Padova.
12. Herley J. D. (1977): "An anatomic-arthrographic study of the relationships of the

lateral meniscus and the popliteus tendon", Am. J. Roentgenol., 128: 181-187.
13. König H. E., Liebich H. G. (2006): "Anatomia dei Mammiferi Domestici", Vol. 1, Ed. Piccin, Padova.
14. Lovejoy J. F., Harden T. P. (1971): "Popliteus muscle in man", Anat. Rec., 169: 727-730.
15. Mann R. A., Hagy J. L. (1977): "The popliteus muscle", J. of Bone and Joint Surgery, 59A: 924-927.
16. Moeller H. D., Bosch U., Decker B. (1995): "Collagen fibril diameter distribution in patellar tendon autografts after posterior cruciate ligament reconstruction in sheep: changes over time", J. Anat., 187: 161-167.
17. Nickel R., Shummer A., Seiferle E. (1991): "Trattato di Anatomia degli animali domestici", Vol. 1, Casa Editrice Ambrosiana, Milano.
18. Nyland J., Lachman N., Kocabay Y., Brosky J., Altun R., Caborn D. (2005): "Anatomy, function and rehabilitation of the popliteus musculotendinosus complex", J. Orthop. Sports Phys. Ther., 35 (3): 165-179.
19. Palumbo Piccionello A., Bo Minelli L., Gazza F., Botti M., Volta A., Panu R., Del Bue M. (2006): "Morphology and biomechanics of the sheep (Ovis Aries) popliteus muscle: preliminary observations", Annali Facoltà di Medicina Veterinaria di Parma, Vol. XXVI, 111-118.
20. Pelagalli G. V., Botte V. (1999): "Anatomia Veterinaria Sistematica e Comparata, Vol. 1, Edi-Ermes, Milano.
21. Poliacu Prosè L., Steketee A. Van Zutphen M. H. (1991): "The popliteus muscle – The architecture, innervation and insertions", Proceeding of the 148th Meeting Anat. Soc., 108-109.
22. Ruffini A. (1897): "Observations on sensory nerve endings in voluntary muscles", Brain, 20: 368-374.
23. Ruffini A. (1898): "On the minute anatomy of the neuromuscular spindles of the cat and on their physiological significance", J. Physiol., Lond. 23: 192-202.
24. Scherer M. A., Metak G., Anatzberger H., Muller-Gerbl M., Raschke M., Herfeldt K. (1996): "Lesions of the popliteus system and their significance for the stability of the knee joint", Zentralbl. Chir., 121 (7): 591-598.
25. Schinhan M., Bijak M., Unger E., Nau T. (2011): "Electromyographic study of the popliteus muscle in the dynamic stabilization of the posterolateral corner structures of the knee", The American Journal of Sports Medicine, Vol. 39, n. 1, 173-179.
26. Ulrich K., Kruidwig W. K., Ulrich W. (2002): "Posterolateral aspects and stability of knee joint. Anatomy and functions of the popliteus muscle tendon unit: an anatomical and biomechanical study", Knee Sur. Sports Traumatol., Arthrosc. 10: 86-90.

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ETHOLOGY AND VETERINARY MEDICINE
ETOLOGIA E MEDICINA VETERINARIA

Grasselli Francesca[1]; Bracchi Pier Giovanni[1].

SUMMARY
The Authors present a brief review of the historical background of contemporary ethology and its applied branches, which may help in understanding which questions this area seeks to answer. It is argued that ethological concepts, together with anatomy and physiology, give a comprehensive and complete picture of the biology of an animal. Finally, this review explains how ethology and animal protection became examination subjects from academic year 1985/86 in the Italian Veterinary Medicine curriculum.

KEYWORDS:
ethology, animal needs, veterinary medicine curriculum.

INTRODUCTION
Konrad Lorenz (6) was the first to observe that young birds and mammals all possess 'infant features', such as a rounded forehead and large eyes, which can be easily recognized; from an ethological perspective, their main function is to trigger parental care (fig. 1). Since these features are non specific and universal, their secondary function is to bring a message to individuals outside the family of origin and even belonging to different species; thus, not only a possible aggression is inhibited, but protective behavior is also elicited, sometimes resulting in adoption of the young. Man has always displayed a great sensitivity to these infant features. In Mesolithic age, hunters kept their preys in pens and killed only the most dangerous and aggressive animals, while the tamest ones were bred and gradually became less and less frightened by man. This represents the beginning of the process of domestication.

Fig. 1 tratta dal sito http://alfiosironi.files.wordpress.com)

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SCIENCE OF BEHAVIOR AND THE IMPROVEMENT OF ANIMAL WELFARE

Domestication represents a fragile process of selection, adaptation and breeding; first as a hunter, later as a breeder, man has understood animal behavior long before ethology was born.

Rural populations had knowledge of animals' habits and behavior and this represented the basis for the development of ethology or the biology of behavior.

The behavior of adult animals is a blend of innate and acquired components; innate behavior consists of simple reflexes, composite responses and complex behavioral features. Acquired components comprise conditioned reflexes and learned responses. All these components can interact with one another resulting in many different types of behavior (fig. 2).

Fig. 2 (tratta dal sito http://www.abc.net.au)

Since the 1922 study of Schjlderup-Ebbe (8) on 'peck order', ethology studies have greatly developed.

In 1973, the 'pure ethology' pioneers Lorenz, Von Frisch and Timbergen were awarded with the Nobel prize. In the past, behavioral science included two different schools of thought: European researchers studied instinctive behavior by observing wild animals in nature, while American researchers were mainly interested in studying animal behavior by means of controlled experiments in laboratory environments. Both approaches acknowledged Lorenz as the founding father of modern ethology. In 1927 Bertand Russell wrote: "All animals have displayed the national features of the observer. Animals studied by the Americans rush about frantically, with an incredible display of hustle a pep, and at last achieve the desired result by chance. Animals observed by Germans sit still and think, and at last evolve the solution out of their inner consciousness". In summary, Lorenz founded a new school of biological research which was based on his belief that complex animal behavior such as adaptation is essential for survival. As a matter of fact, Lorenz was awarded with the Nobel prize for "….his studies on imprinting….." , a process which was commonly believed to be uncovered by Lorenz himself, who named it.

However, it was not Lorenz who uncovered the process of imprinting, and he was well aware of that. In the nineteenth century Spalding (9), an English roofer who was

interested in ethology, had already observed this process in chicks; also Heinroth (4), at the beginning of the nineteenth century, had studied the behavior of chicks hatched in an incubator and then exposed to.
The Brambell Committee (2) in England was the first to emphasize the relationship between the behavior of farm animals and welfare; the members of the committee, established in 1965 and directed by prof. Roger Brambell, had different approaches to animal breeding. In its final report, the Committee pointed out the lack of behavioral studies focused on animal welfare in intensive livestock farming (fig. 3); moreover, it was underlined that a reliable evaluation of animal welfare should not only include productivity parameters but also farm animal behavior. The knowledge of ethology of the species under examination should represent the basis for studying animal behavior (3). In particular, since suffering is similar in animals and humans it is important to evaluate vocalizations, facial expressions, reactions and abnormal behaviors.

Fig. 3 (tratta dal sito http://crittertips.com)

At last, the committee clarified that animals display signs of pain, exhaustion, fear, frustration, anger and other emotions, which reveal different degrees of suffering. Farm animals such as poultry,horses, cattle, pigs, sheep and goats represent the most studied species (fig. 4, 5, 6).

Fig. 4 (tratta dal sito http://www.animalwelfareintergroup.eu)

Fig. 5 (tratta dal sito http://www.murphybrownllc.com)

Fig. 6 (tratta dal sito http://media.nowpublic.ne)

The improvement of technology in farm animal breeding has required a better knowledge of ethology in breeders, mainly as regards to animal welfare and protection. Thus, animal welfare has acquired much relevance not only in livestock breeding but also for the consumers of animal products and people involved in international legislation.

ETHOLOGICAL RESEARCH APPLICATION

In the last 50 years, biological studies have taken different directions and influenced many different disciplines, thus becoming reliable means of investigation; as a consequence, application of basic knowledge has been developed in order to solve various problems regarding, for example, commercial breeding , wild animal domestication or human psychology.

ETHOLOGY AND VETERINARY MEDICINE

In order to highlight the importance of environmental conditions in the management of health risks in intensive stock farming, some veterinary clinicians (1, 7) proposed also to include, among the risk factors, the problem of animal welfare. In fact, it is assumed that the 'well-being' of animals represents a prerequisite for higher productivity and, thus, for better income (fig. 7, 8).

Fig. 7 (tratta dal sito http://www.petcustomer.com)

Fig. 8 (tratta dal sito http://www.veganoutreach.org)

In Great Britain, good animal welfare is seen as a result of the development of husbandry systems which guarantee both adequate health conditions of animals and the fulfillment of their behavioral needs.
In order to objectively evaluate the welfare of animals, the veterinary clinician must be aware of their behavioral needs; this knowledge requires the basic concepts of anatomy, physiology and applied ethology (fig. 9). Unlike classical ethology, in which animals are observed in wild conditions, applied ethology studies domestic animals in the specific environment which has became familiar as a result of domestication. The environment can vary on the basis of the species, breed, and also the individuals; this depends on specific animal features and also on animal breeding goals, in that the farmer often makes an appropriate selection of animals (5).

A deep knowledge of animal physiology and ethology represents the basis for recognizing abnormal behaviors and understanding their causes and also for planning a strategy to solve them. Health problems and abnormal behavior are often intermingled.

THE CURRICULUM OF VETERINARY MEDICINE

With a sentence of the Justice Court of European Community (September, 18th 1984), Italy was declared in default on the obligations stated by EC Directives 78/1026 and 78/1027 as regards to the teaching of 'Ethology and animal protection' and 'Hygiene

and food techonology' in the Veterinary Medicine curriculum. Following the D.P.R. of April, 5th 1989, both disciplines became compulsory for veterinary medicine students beginning from academic year 1985/6 and therefore many students were required to make up this missing topic.
In fact, the legislative decree published in 'Gazzetta Ufficiale della Repubblica Italiana' on September, 13th 1989 n. 214, decided that:

1. The Italian degree in Veterinary Medicine is recognized by European countries for education courses beginning only after 1980/81;
2. The Board of European Community should put forward the proposal to the Council of Ministers of the EC to solve this aspect by law as regards to students who had begun their education courses before 1985, January, 1st.

Obviously, after 1985/6 the two disciplines became compulsory in the Italian curriculum in Veterinary Medicine.
At the moment, the new magisterial course in Veterinary Medicine, initiated in 2009/10 and regulated by DM 270/04, includes the course of 'Physiology I and Ethology' for second year's students (fig. 10).

Fig. 10 (tratta dal sito http://www.ct.gov)

Since1980 ethology has been included in the Zoology Course at the University of Parma Veterinary School in order to provide the basic knowledge of this science for future veterinary surgeons. The teacher of the course worked for many years in the Zoology Institute of the Faculty of Biological Sciences of Parma University and was greatly influenced by the well known school of Prof. Danilo Mainardi; his research interests are focused on basic ethology .

BIBLIOGRAPHY

1. Ballarini G. 1985 - Patologia dell'ambiente. Atti S.I.S.Vet., XXXIX, Parte I, 187-204.
2. Brambell F.W.R. 1965 - Report on the Technical Commitee of Enquire into the Welfare of Animals Kept under Intensive Livestock Husbandry Systems, Cmnd. 2836. H.M. Stationery Office, London.
3. Craig J.V. 1981 - Domestic animal behavior: cases and impications for animal care and management. Prentice-Hall, New Jersey, 364 pp.

4. Heinroth O. 1910 - Beiträge zur Biologie, namentlich Ethologie und Psycologie der Anatiden. Verh. 5 Int.Orn. Kong., 589-702.
5. Jensen P. 2011 - Etologia degli animali domestici. Edizione italiana a cura di P.G. Bracchi, F.Grasselli, Giuseppe Zannetti. McGraw Hill.
6. Lorenz K. 1990 - L'etologia: fondamenti e metodi. Ed. Bollati Boringhieri.
7. Monti F. 1985 - Benessere animale. Atti S.I.S.Vet., XXXIX, Parte I, 126-136.
8. Schjelderup-Ebbe D.A. 1922 - Contributions to the social psycology of the domestic chicken. Z. Psycol., 88, 225-252.
9. Spalding D.A. 1873 - Instinct with Original Observation on Young Animals, in << McMilian's Mag.>>, 27, 282-283.

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MIND AND FREE WILL
MENTE E LIBERO ARBITRIO

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SUMMARY
A probabilistic system can successfully move towards a different state or a target when the reciprocal affinity between the system and its target are deterministically established a-priori. Our brain behaves like a probabilistic machine so that any decision-making process is always conditioned by a deterministic goal-seeking which, in principle, is incompatible with free will existence. Then, why we deceive ourselves of free will existence yet? According to "Bignetti" theoretical model (http://www.freewill.unipr.it/freewill/home_page.html) all these questions seem to be clarified. The main idea is that our actions are predetermined by the unconscious mind; the conscious one, instead, emerges few milliseconds later, i.e. only when the actions are executed, thus leading us to claim the responsibility of our actions: this is the pillar for any learning and memory process. In conclusion, in "Bignetti" model, free will might be a congenital mind illusion apt to attain cognitive processes. In order to verify the truth and the accuracy of this model a preliminary psychometric test investigating the timing of a voluntary action in relation with a food choice contest, is discussed in the experimental section.

KEYWORDS
Free will, probabilistic mind, decision making, psychometric test, food choice

INTRODUCTION

1. Structure and function of the brain

One of the most fascinating scientific issue is to understand how mind can emerge from a tangle of cells crossed by electrical impulses and hit by chemical signals. It follows on one hand the development of more and more advanced instruments, which allow to unveil the deep mechanisms of the brain, on the other hand a proliferation of research groups characterized by scientific cross-competences that find a fertile ground in neuroscience area.

Thanks to the considerable progresses in neurosciences, an aspect of the mind in particular is in debate, i.e. the capacity to make decisions, to perform voluntary actions and consequently the existence of a Self provided with free will.

To describe clearly the issue, we can start from the famous "paradox of Buridan's ass", a paradox which is incorrectly attributed to Buridan, since it has been formulated by others as logical extrapolation of his thought: in front of a hungry donkey there are two identical meadow or two identical hay sacks, however the donkey cannot decide which one to eat and therefore it starves to death.

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This example, which is absurd even considering the most thickhead of all donkeys, has been utilized to support the thesis that if the mind is strictly "deterministic" or "mechanistic", it would stop in front of two perfectly identical situations and with no preference indication. In reality, the donkey would eat first from a sack and then from the other, apparently at random. This is the point: obviously the mind is not "strictly deterministic"!

In order to better understand the matter, we assume that the relationship between brain and mind is the same as structure and function, like in any other organ of our body. Analyzing the different structural and organizational levels of the brain from the lower molecular one to anatomical structures, it is possible to conceive a model of cognitive function which is complex but acceptable. Isolating the single molecular components or submicroscopic fragments of the nervous system (for example ion channels, membrane receptors or synaptic boutons, which are fragments of the synapses, where transmission of electrical signals from a neuron to the other takes place) it is possible to see that their functioning is unpredictable, i.e. random (as if we were throwing the dice). If this behaviour would be valid also for the mind, we would have serious problems to satisfy our hunger! First of all it would be difficult to understand the meaning of hunger, then it would be hard even to decide how to eat. In other words, a casual behaviour would not explain decision-making and action coherence.

If we take the distance from the molecular dimension of the matter and enlarge our observation angle to supramulecular organization, we realize that things are noticeably changing. First of all we note that the same elements that we observed individually shortly before, can sometimes synchronize according to predictable dynamics, i.e. probabilistic or stochastic laws. Furthermore we observe that these collective events appear in particular conditions, that is in the presence of physiological stimuli evoked by external environment or internal stimuli coming from the organism. It seems that the mind, when is included in an overall relational context, acquires more predictable rules which are more comprehensible also to the context itself. We should not forget anyway that this possibility of dialog with the world is not a different and brand new characteristic of the brain, but it is a surplus value that the brain gain from the integration of different random systems. As a consequence, different events become possible, such as the signal transmission along the single neuron, the synaptic transmission of signals from a neuron to the other, the processing of these signals in the different nerve centre and so on. From these events derive complex and coherent actions, which underlie the common cognitive functions.

A coherent and functional mind, which emerges from the union of casualness and probability, can be considered as a product of a probabilistic-deterministic brain, in the sense that the brain answers to given stimuli become predictable and that the stream of thought is "deterministically" ruled by the probability laws (Koch 1999; Bignetti 2003; Deco et al 2009).

To better understand these concepts, we can look to an analogy, considering the fluid dynamic in a closed space. The fluid molecules move because of thermal perturbation in every direction, at random, without any target. However, if they find an open bulkhead, they spill over in the empty space in a spontaneous and irreversible way and so forth from a bulkhead to the other. An outer observer might think that the fluid net flow

in a specific direction depends from a "a priori collective will" of the fluid, but for the fluid the motion is unconscious, it is the result of a fortuitous cooperation of two factors: the random motion of each molecule of the fluid and the probabilistic direction of the molecules in the newly-formed empty spaces. Similarly the thought is like a fluid, ready to expand in every direction when some stimuli arrive, such as the hunger and the sight of hay that, reminding food, activate some preferential pathways and thought become apparently coherent with a clear goal to pursue. Thought, thanks to its intrinsic "desire" to think, will continuously move through the most probable pathways (open bulkheads) to turn off the initial stimulus (Bignetti 2001; Bignetti 2003).
To conclude, we can go back to the initial paradox of Buridan's ass and, in particular, to the objection that a person could not starve to death because of a so stupid situation. A strictly deterministic brain would not have the means to decide how to satisfy the need to eat. Otherwise we saw that even completely random motions would bring to problems of coherence and adequacy, therefore an individual would not feel neither hunger nor coming to death. The idea of a brain responding to probabilistic-deterministic rules is the last hypothesis explaining how it is possible to feel hunger and to solve the problem of the choice between the two hay sacks. First of all, the perception of hunger would certainly open some bulkheads towards a final target: eating. Secondly, the mind would not spend the whole life to decide what to do if the two hay sacks are identical, rather it would stuff itself with the first sack it comes across, the first "more probable" one.
A deterministic brain stops in front of a fork, a choice, while a probabilistic-deterministic brain would swing from a solution to the other, allowing the thought to go through with even ambiguous situations and to find coherent answers to the initial stimuli. This issue refers both to stimuli such as the hunger, i.e. physiological stimuli, and to reactions coming from our memory and our personal experience, from the world of emotions and affections (that is from the limbic system of our brain). Since each individual has his own personal history, it follows that his actions will be different from whoever. Therefore let's imagine to complicate the Buridan's paradox bringing two donkeys together in front of the two hay sacks: luckily it is not sure that they will quarrel for the same sack.
A last provocation: for someone it is hard to admit that a brain, built up with matter, may originate a creative, artistic, musical mind. Nevertheless we started from the assumption that the mind is with no dispensation the functional expression of its brain!
Furthermore we would like to conclude underlying the fact that probabilistic behaviours are rather common in biology and are widely considered at every level of complex organizations. To this regard, we suggest you to read two recent papers showing that allostery mechanisms in enzymes (Hilser 2010) and the regulation of flagellar movement in microorganisms (Bai et al 2010) exist on stochastic-probabilistic basis.

2. The issue of free will and the "Bignetti" model

Two questions naturally arouse: the mind, which is conditioned by specific goals, is really free to choose? To what extent it is conscious of its actions? In order to answer

to these questions it is firstly necessary to distinguish between what the subject perceives from what really happens. This is indeed the root problem: whatever belongs to our mind is not necessary the reality.
First of all let's see what mind think of itself, then what scientific research says about it and, at the end, which is the approach of philosophy.
Starting from introspection, from the subject it emerges a sense of "Self" as a thinking and independent organism, although interacting with the environment. In an adult individual, the tendency to obtain a final goal is attributed to the Self as causal agent. The purposes to act are premeditated and we see them as the mental causes of our actions since, while we are consciously doing something, we are pervaded by the sensation to have wanted and caused it. In front of a number of opportunities, our Self believes to be free to choose autonomously, this is the common sense that bring to the idea of the existence of a free will. On the basis of these assumptions, two models have been proposed, a "Hard" and a "Soft" model, that support with different nuances the existence of free will (Gillet et al 2001).
The first one is a model that excludes any conditioning form interfering with the decisional action. From the rational point of view it is definitely unlikely: logics indeed asks us to consider the premeditation of a target as the necessary mind "conditioning" to formulate a choice.
The second model, the "Soft" one, suggests that the decision making is a way to follow rules. Even if we consider the volition as the result of a conscious, consenting and individual choice, the "Soft" model foresees a sort of determinism. As you can easily imagine, the epistemological root of this model develops as a typical unsolved residual complex with religious faith. It exists in countless versions and it is in vogue especially among philosophers and scientists who have to bear weighty social and cultural heritage, most of all if derived from occidental monotheistic faiths.
Bignetti expresses in his model a completely different opinion (Bignetti 2001; Bignetti 2003; Bignetti 2004; Bignetti 2010). He argues that the free will does not exist and that it is just an illusion of the mind. Premeditating and action means to look for a coherent connection between an assumption and its demonstration. The conditioning seems therefore unavoidable and definitely in contrast with both the common sense we have of the free will (see above the common sense of Self as causal agent) and with the strictly literal sense of the word, according to which the principle of the free will is valid provided that "any random action in any circumstance is always possible". However, the "intentionally" paradoxical element of this model is that the individual must believe in the existence of the free will, and it is correct that he does so in order to carry out his normal cognitive functions. To understand this assertion, let's think to the most important function of our intelligence: the learning. We know that the normal learning mechanism is based on the reward/punishment principle, which allows us to record in our memory which decisions were correct and which were wrong. In this way, memory becomes a source of information to decide future actions. So to update memory archive after every performed action, it is necessary that the mind may find a "person in charge" of its own decisions, that is a "causal agent" with the "license" of free will, who owns and gets a reward or a punishment to be emotionally fixed in the memory. To this end we could speculate on the exhaust-

ing philosophic debate on cause-effect relationship which invested many thinker for many centuries. Nevertheless, we can summarize the question stating that in the past they discussed on the validity of a cause-effect relationship on deterministic bases, then, after the quantum mechanics discoveries the discussion shifted to probabilistic correlation of phenomena. Whatever the result of an academic discussion on the existence of a cause-effect relationship is, it is evident that in our mind, as Cartesio said, the existence of this relationship has all the characteristics of an innate idea. The common sense of "Self" seen as a causal agent could perform this function. That's why it is "necessary" and "spontaneous" to believe in the existence of the free will, also if it is an illusion. To summarize, the "Bignetti" model assume that the consciousness never decides but always learns, while the unconscious part of the brain acts on the basis of the most probable scheme that processes considering what is already filed in memory. To be plausible a model must be self-consistent and must not be invalidated by scientific evidences. The learning mechanism on which it is based recalls other evolutionary Darwinian-like models.

Like the previously described analogy between fluid dynamics and the brain functioning, we conclude this second section describing some examples in favor of a mind without free will. The first one regards the experiments on "readiness potentials" carried out by Luder Deecke and Hans Helmut Kornhuber at first and, afterwards, by Benjamin Libet, who demonstrated how the mechanical action carried out by a subject to stop a car is decided by the unconscious part of the mind some hundreds milliseconds before the subject is aware of it. The subject is unaware of this hidden activity, so to presume that his behaviour is voluntary and that he acts only after he decided to do so. Libet tried to justify this temporal gap between unconscious and conscious activity without a great success; perhaps he understood that he would be criticized by the occidental world that does not intend to renounce to free will (Libet 2004). However, these experimental observations are in perfect accordance with the "Bignetti" model and with a mind without free will.

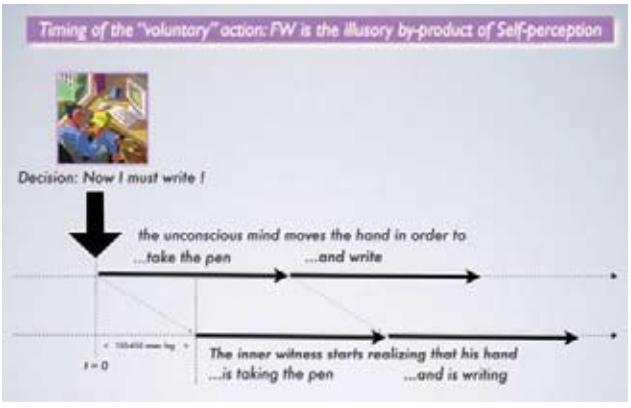

Figure 1: The inner witness is awaken by the events and is immediately pervaded by the feeling to have freely caused these actions (free-will illusion). Feeling responsible for these

actions and the outcomes of these actions, the witness can also learn and memorize the best protocol for the future, so that a repetition of the same actions by the unconscious mind will take a shorter time.

A second example regards the demonstration that also a simple physical-chemical system can show an "intelligent" behaviour. We refer to a scientific paper published few years ago on an eminent journal (Lagzi et al 2010). The authors demonstrated that an oil drop in a water labyrinth after the first attempt finds the shorter way to reach the exit, similarly to laboratory mice after a long specific training. Briefly, the "trick" of this "intelligence without brain" is that the drop and the labyrinth exit have been initially treated with substances with a notoriously high affinity and the drop moves in the labyrinth in a probabilistic-deterministic way. The results of this experiment suggest that only two things are necessary to efficiently reach a target: there must be a certain attraction between the subject and its goal and the movement toward the attraction entity must be carried out by a probabilistic-deterministic system.

3. The eastern philosophy and the "Bignetti" model

The "Bignetti" model draws its inspiration from the eastern philosophic vision of the world, in particular from the Hindu philosophy. Delving into the Indian culture and religion, one discovers a huge and deep philosophical and metaphysical framework regarding the study of knowledge. To this regard, I suggest you to consult the book "La Filosofia Indiana" (1991) written by Sarvepalli Radhakrishnan, who was Professor of Ethics and Oriental Religions at the Oxford University as well as President of the Republic of India in 1965. A long time ago the Indian philosophy felt the necessity to extend the ontological theory of the mind in order to develop the critical theory of knowledge. From the holy literature of epic poems arose different courses of study which are independent but not incompatible with each other: the "Darsana" (Samkhya, Yoga, Vedanta, etc). In order to answer to the common necessity to find sharable solutions to the existential questions, the proponents of the different Darsana analyzed the nature (mind and body) of the individual in depth carrying out a survey that could be considered psychoanalytical ante-litteram. According to the Samkhya of Kapila (600-500 b.C.?), "Prakriti" is a sort of Nature Naturans which acts unconsciously following the rule of spontaneity. "Purusha" sets against Prakriti as the activity of conscious mind of Self and of the environment (a sort thinking spirit or Cartesian Cogito). Purusha awakens dragged by the action of Prakriti and, observing itself, it thinks to be the first and autonomous cause of the action in execution. Therefore the film of our life would be already written, but the veil dimming our mind, Maya, would create the illusion of the free will. This illusion causes a sort of painful dependence. The everyday challenge is to became free from this dependence through meditation, that is revealing the trick of our mind.

4. An experimental approach to the "Bignetti" model

According to the "Bignetti" model, the involvement of the conscious mind is not crucial when deciding an action, but when verifying if the action that the individual is carrying out is correct or not, if it entails a reward or a punishment. This is what

is filed in the operative memory of the conscious mind, giving a demonstration of learning.
To verify this model and to study the modalities, the times and the mechanisms of learning that drive our choices, we had the necessity to develop a suitable instrument to test our hypothesis. We started from the assumption that learning can be demonstrated if a person would react correctly and in a shorter and shorter time during the repetition of identical or similar stimuli. Therefore we developed a psychometric test which has been submitted to some students of the Faculty of Veterinary Medicine. This test consists in the identification of different very short stimuli, which are followed by as many mechanical answers of the subject sitting in front of a computer. Usually the identification of the image occur in few milliseconds. The task that commits the cognitive system for a longer time is the association between an image and a serious of hand actions during the test. Obviously the answers must be different according to the observed stimulus, but the ability of the subject in answering correctly is not as much interesting such as the rapidity of the performance. A repetition of the answer protocol aims to induce a sort of cognitive adaptation of the subject to the test, i.e. to learn the test modality. In conclusion, if during the test the response time decreases and tends to individual reaction time, then one could suppose that the subject is learning thanks to processes foreseen by the "Bignetti" model.

MATERIALS AND METHODS

The test has been realized using a dedicated software developed by M2 Scientific Computing s.r.l. It is organized in three parts. In the first part the subject must assert if he prefers sweet or salty foods. Furthermore some information useful to the execution of the test are given and it is possible to carry out some trials in order to be sure that the demanded task is clear.
The second part of the test is the most important and meaningful. 48 black and white basic pictures representing sweet or salty foods are presented to the subject. Each image is visualized for 30 ms, after which the subject must indicate as quickly and instinctively as he can if the food represented by the previously visualized picture belongs to the preferred category (sweet or salty). In the following step the subject must confirm or modify the given response and only after this indication the test continues with the visualization of a new image. In each of these 48 trials the computer records the answer of the subject (the food I saw belonged/didn't belong to the category I prefer), measures the time necessary to give this indication and records the possible changing of the given answer (I answered that I like it/I don't like it but I wanted to answer that it don't like/like it).
In the third and last part of the test we administered a classical protocol for the determination of the reaction time: the subject must push a button as soon as the red circle become green. It consists of a very simple stimulus which is different from the others visualized during the second part of the test.
Four different versions of the test have been developed. The only difference is in the second part, in particular the complexity of the images increases: in test 1 only 2 images are visualized (one representing a sweet food, the other representing a salty food, they are repeated 24 times each in random sequence), in test 2 4 images are

visualized (2 sweet and 2 salty foods repeated 12 times each in random sequence), in test 3 the images were 8 (4 sweet and 4 salty foods repeated 6 times each in random sequence) and in test 4 the images were 16 (8 sweet and 8 salty foods repeated 3 times each in random sequence).
At the moment tests have been administered to 27 persons altogether.

Figure 2: Diagram showing the structure of the central part of the test. Four versions of the test were created: in the simplest version only two pictures (one representing a sweet food, one representing a salty food) are visualized 48 times in a casual sequence.
The number of pictures increases in the other versions: in test 2 are visualized 4 pictures, in test 3 8 pictures and in test 4 16 pictures.
Each picture is displayed on the monitor for 30 ms and after that the subject must declare if the food he saw belong to the category he prefers (sweet or salty) pushing the space-bar. Then he has to confirm or modify the answer to let the test continue.

RESULTS

From the preliminary experiments of this study interesting results emerged.
As concern test 1 (only 2 picture are visualized 24 times each) the mean time required to answer if observed food was sweet or salty is very close to the reaction time (401.7±51.4 ms vs. 336.6±29.4 ms). In 2 subjects out of 7 as the number of trials increases, the response time decreases (T test $p \leq 0.02$), whereas it is not possible to find a correlation between response time changes and increases of the number of trials in the other subjects. Obviously this test is very simple and is comparable to the protocol used to measure reaction time.
In test 2, 3 and 4 the number of visualized images as well as the complexity increases. The mean response times increase with respect to test 1 (test 2: 534.9±77.4 ms; test 3: 517.2±33.8 ms; test 4: 525.8±61.9 ms) and are always higher than mean reaction times. It is very interesting to notice an increase in the mean response time from test 1 to test 2 but no appreciable changes in mean response time have been observed among the other test versions. It seems to exist a sort of "step" of complexity: below it response times are very close to reaction times, above it the mean response times are higher with respect to the previous level but as complexity increase they do not increase.
In test 2, 3 and 4, as the number of trials increase 11 subjects out of 20 show a significant variation of response time (T test, $p \leq 0.05$). In particular, in all subjects but one

as the number of trials increases, the response time decreases, indicating the presence of a learning mechanism linked to the execution of the test.
As regards the response accuracy, among all the 27 participants, on average 7 errors out of 48 trials of each test have been made, 3 of which have been corrected.

CONCLUSIONS

The preliminary results obtained from the psychometric tests indicate that following a very short stimulus (images were visualized for just 30 ms) our brain not only is able to classify correctly the stimulus, but also it is able to carry on an adequate response in around 500 ms. Furthermore, in half of the subjects who executed the test it is possible to observe a process of "learning" of the execution protocol, as showed by the reduction of the response time during the progress of the test. In general, the response time tends to the reaction time.
These observations represent the experimental basis from which to start to develop more complex experiments. Beyond strengthen the obtained results increasing the number of subjects, we are currently developing a new test that allows us to evaluate which are the processes that interfere with learning mechanisms.

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REFERENCES

1. Bai F, Branch R.W., Nicolau D.V., Pilizota T., Steel B.C., Maini P.K., Berry R.M. (2010): Conformational spread as mechanism for cooperativity in the bacterial flagellar switch. Science 327, 685-689.
2. Bignetti E. (2003): Cervello e mente ovvero casualità e determinismo. Ann. Fac. Medic. Vet. di Parma, Vol. XXIII, 69-78.
3. Bignetti E. (2004): Consciousness can learn but cannot decide. Ann. Fac. Medic. Vet. di Parma, Vol. XXIV, 31-52.
4. Bignetti E. (2001): Dissacrazione della Coscienza. Il Valico Edizioni, Firenze.
5. Bignetti (2010): Free Will is the Illusionary By-product of Self-perception. Proceedings at the IVth Intl. Nonlinear Science Conference of the Society for Chaos Theory in Psychology & Life Sciences. March 15-17, Palermo, Italy, 5.
6. Deco G., Rolls E.T., Romo R. (2009): Stochastic dynamics as a principle of brain function. Prog Neurobiol., 88, 1, 1-16.
7. Gillet G.R., McMillan J. (2001): Consciousness and Intentionality. John Benjamins Publishing Co.
8. Hilser V.J. (2010) An ensemble view of allostery. Science 327, 653-654.
9. Koch C. (1999) Biophysics of computation. Oxford University Press, NY Oxford.
10. Libet B. (2004): Mind time. Raffaello Cortina Edizioni, Milano.

11. Lagzi I., Soh S., Wesson P.J., Browne K.P., Grzybowski B.A. (2010): Maze solving by chemotactic droplets. JACS 132, 1198-1199.
12. Radhakrishnan S (1991): La filosofia Indiana. Ed. Asram Vidya, Roma.

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EVALUATION STUDY ON MORPHOFUNCTIONAL ASPECTS OF THE DIGITAL AXIS IN THE BARDIGIANO HORSES

STUDIO PER LA VALUTAZIONE DEI CARATTERI MORFO-FUNZIONALI DELL'ASSE DIGITALE DEL CAVALLO BARDIGIANO

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Zanichelli Stefano[2]

STRUCTURED SUMMARY

The aim is to determine the morpho-functional parameters of the digital axis in a homogeneous group of horses.

Methods

We divided the horses in 2 groups, the shod (F) and the unshod (nF). For each limb 2 radiographic views, the Dorso-Palmar (DP) and the Lateral-Medial (LM), were taken. Using EponaTechMetron software® were measured 38 bone parameters on each radiography.

Results

We analyzed 10 parameters and found statistically significant differences between the 2 groups; the most interesting ones are the distance from P3 to the toe and the ratio percentage toe/support.

Clinical significance

The data of our study provided a lot of new knowledge about the bone digital axis parameters today unknown of healthy bardigiano horse. The statistical records previously treated are in accord with the most recent scientific literature in particular regarding the values of P1/P2 and P2/P3 joint angle and the results of the center rotation projection of the AIFD on the ground.

KEYWORDS

Bardigiano Horse, Digital axis, Radiographic evaluation, equine bone parameters

INTRODUCTION

In the last few years the interest for the management of the equine foot has grown.
Our aim is to analyze useful tools for the veterinarians through the investigation and the examination of some morpho-functional bone data of the equine digital axis.
The subject of the study is represented by the baridgiano horse. This breed of horses originally from the Emilia Romagna region lives in the Appennino Tosco-Emiliano area. The mountain environment and steep, rough terrain of the area have contributed to produce a robust and hardiness breed.
The stud book was established in 1977, and is held by the Associazione Provinciale Allevatori (A.P.A.), the regional animal breeders' association, of Parma (1).
Some selected horses were to participate to the performance test realized in the 2009

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by the A.P.A. "Cavallo Bardigiano" Section of Parma.
We examined a total of 18 bardigiano horses, including 10 females and 8 males , three years old. The horses from different owners were transferred to two riding schools to start the three months training.
Horses considered in our study were found to be suitable to start the training activities. Each horse underwent medical and orthopedic visit; X-ray screening to verify the complete absence of OCD lesions; evaluation of biometric measurements (height, chest and metacarpal region circumference).
In none of the animals selected for our study were found systemic or musculoskeletal diseases. At the same time of the clinical evaluation we performed X-ray examination.

MATERIALS AND METHODS

Data collection

We arranged an interview with the owners to know the breeding management, the clinical history of each horse and foot management.
The horses were screened by performing radiographic projections of the forelimb. We performed latero-medial (LM) and dorso-palmar (DP) view of the digital axis. Six horses were radiographed with the presence of horseshoes, the remaining 12 horses were not.
We used a portable high frequency X-ray machine (Multimage® 16 mAs and 80 kV), a digital indirect imaging system (Kodak CR 140®) and 35 cm x 24 cm fluoride plate.
We standardized the method of radiographs:

- the horse was positioned with both forelimb on a block with a flat surface of 10 cm of height, with the metacarpal bones perpendicular to the ground, so that the weight was distributed uniformly on both limbs; a marker of metal with a 2mm diameter had been placed on the block, implanted in the middle for the entire length of the side of the block; The foot, after cleaning, was placed with the marker in the middle of the sole on the sagittal and frontal plane;
- on the plate was placed a marker that supported at 5 cm apart two metal detectors applied on a small level (photo 1). The plate was positioned perpendicular to the ground closely to the fetlock in the DP view, while adherent to sole's distal medial margin in the LM view. For each projection, it was necessary to measure the bulbs-plate distance in the DP view and the fetlock-plate distance in LM view;
- we used constant exposure factors of 76 kV and 1.6 mAs and a focus-plate distance of 100 cm;
- to obtain DP view the x-ray beam was centered on the coronary band; to obtain LM view the x-ray beam was centered 2 cm below the coronary band in the midway between the bulbs of the heels and the dorsal aspect of the wall (4, 5);
- all radiographs were analyzed with the program EponaTechMetron® version 3.1. The views were magnified to 150% to minimize the errors. This system uses a scale of markers and reduces the magnification obtained by the X-ray (2).

Statistical Analysis

We used the statistical system NCSS 2007 (Copyright© 2007 by Jerry Hintze, All Rights Reserved) in order to identify significant differences with a $P < 0.05$ (5%). We calculated the averages and the standard deviations in the different groups with Excel (Microsoft® Corporation). We used the T-student to calculate the probability level (P) after verifying the normal distribution of the data.

Photo 1: LM and DP view. Note the markers point.

RESULTS

We reported biometric values in tables 1 and 2. We named the subjects of our study with letters from M1 to M8 for stallions and F1 to F10 for females:

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	Mean	ST. Dev.
Height	143	141	142	143	141	143	141	141	137	139	141,1	1,91195072
Chest circunferen.	173	182	183	183	192	181	181	165	164	187	178,4	8,996295534
Metacarpal circunferen.	18	18,5	18,5	18	19	18	19	18,5	18	18	18,35	0,411636301

Table 1: Biometric values of females.

	M1	M2	M3	M4	M5	M6	M7	M8	Mean	St. Dev.
Height	140	142	139	142	140	142	143	141	141,125	1,356202682
Chest circunference	180	187	176	180	179	182	192	186	182,75	5,2030211
Metacarpal circunference	20	21	19	21	20	22	22	20	20,625	1,060660172

Table 2: Biometric values of stallions

The results of the interviews showed that the breeding management is analogous for all the population. Almost all the subjects were bred at the farm of origin (only two horses were purchased); As for the medical history only 1 horse had an episode of dermatitis with spontaneous healing; regarding therapies performed, we recorded only deworming and vaccination protocols.

The feeding type of the horses used is absolutely similar to Bardigiano horse's breeding that consists in a breeding in the wild method during the spring and the summer, where the animals are fed with polyphite grass and a modest integration of

hay, from October instead the horses stay usually is in the boxes at the stable and fed with hay (1).
Our survey shows that:

- the altitude at which the horses spend most of their lives is between 400-700 m. above sea level, reaching 1000 m during the warm season; the land consists of wooded areas represented by beech forests with soft and dry soils, except for 3 subjects that lives on a rocky terrain and only 1 that lives on a clayey soil;
- the age of weaning was between 6th and 8th months, except for 1 horse that was weaned at 12 months;

The stabling type is one of the parameters that shows a lot of variations:

- some horses were born in a paddock or in multiple box, where they lived until weaning or until 18 months of age before being transferred to single boxes;
- Some horses always lived in the paddock with other subjects of different ages;
- a common aspect of the management was that all horses lived in single or multiple boxes during the winter and in the paddock with other horses during the spring and the summer.

X-Rays results
The EponaTechMetron® system evaluates some parameters and compares the results to preset average values of different horse breeds. We considered only the bone parameters. In our study we performed a total of 72 radiographic views of the equine digital axis, of which 36 of the right forelimb and 36 of the left forelimb. For each finger we made 2 radiographs (DP and LM) and we calculated 38 (tables 3 to 8) parameters:

- 16 in the DP view;
- 22 in the LM view

We considered only few parameters according to recent scientific literature. We used the data that described the position of the third phalanx (P3) in the hoof. The data that evaluate the alignment of the digital axis are important to know the effect of the barefoot trimming. Thus, we divided our horses in 2 groups:

- The shod horses (group F);
- The unshod horses (group nF).

	F1	F2	F3	F4	F5	F6	F7	M6	M5	M8	M7	F8	F9	F10	M1	M2	M3	M4	MEDIA	DEV. ST.
P3/P2-Joint width (cm)	5,39	5,48	5,57	5,83	5,76	5,62	6,08	5,96	6,24	6,47	5,96				6,13	5,62	5,57	5,7	5,825333333	0,307637697
P1/P2-Joint width (cm)	5,06	5,14	5,57	5,31	5,41	5,5	5,72	5,97	5,66	6,09	5,78				5,71	5,57	5,48	5,62	5,572666667	0,27752649
C/P1- Joint width (cm)	5,39	5,13	5,5	5,29	5,4	5,53	5,56	6,21	5,8	6,12	5,87				5,94	5,64	5,69	5,75	5,654666667	0,301895598
P2 Tilt (deg)	2,48	-3,95	3,12	2,91	-0,55	2,29	0,33	1,45	-0,84	0,17	6,05			Z	2,75	0,29	2,83	3,85	1,545333333	2,38608426
P1 Tilt (deg)	-4,34	2,96	2,4	-3,28	1,89	-2,63	4,16	-2,45	-2,71	-1,66	4,06				2,97	1,08	3,84	4,1	0,692666667	3,149173285
P1 Min half-width (cm)	1,85	2,06	1,87	2,17	2,03	1,83	2,05	2,16	2,43	2,16	2,17				2,02	2,07	1,86	1,83	2,037333333	0,169472571
P2 Min half-width (cm)	2,63	2,52	2,58	2,81	2,7	2,84	2,78	3,02	3,16	3	2,82				2,9	2,99	2,76	2,69	2,813333333	0,177951304
P2/P3 Joint height (cm)	5,59	5,53	5,07	5,95	4,97	5,63	5,79	6,06	6,76	7,24	6,75				7,98	6,09	7,02	6,98	6,227333333	0,859139658
P2/P3 Joint tilt (deg)	0,78	-1,54	1,46	4,08	-1,6	1,53	-3,3	0,78	0,85	0,04	2,78				3,84	-3,3	3,14	1,94	0,765333333	2,346465021
Outer wall angle (deg)	97,6	105,01	108,95	85,92	110,3	106,08	107,7	108,95	106,1	111,9	106,2				105,6	103,67	105,08	105,71	104,9793333	6,203891022
Outer wall deviation (cm)	0,32	0,46	0,33	1,47	0,36	0,8	0,33	0,27	0,15	0,64	0,23				0,1	0,25	0,18	0,23	0,408	0,346517367
Outer wall lenght (cm)	6,77	8,27	7,55	6,59	6,91	8,11	7,24	10,1	7,94	9,23	9,77				10,52	9,19	10,32	9,53	8,536	1,357359832
Inner wall angle (deg)	103,6	101,08	110,82	82,66	113,5	108,04	108,3	105,84	100,6	107,8	106,2				107,56	105,94	105,3	104,86	104,8073333	6,964478618
Inner wall deviation (cm)	0,45	0,19	0,2	0,5	0,6	0,47	0,16	0,13	0,44	0,29	0,68				0,62	0,25	0,1	0,6	0,378666667	0,196673543
Inner wall lenght (cm)	6,66	6,69	7,14	7,22	6,73	7,54	7,6	9,06	7,41	8,6	7,84				9,79	9,24	8,35	7,67	7,836	0,973622103
Foot width (cm)	13,07	14,18	16,03	14,38	16,16	15,53	15,89	16,61	15,65	17,95	16,57				16,82	15,11	14,05	14,49	15,49933333	1,287031283
Lenght of P3 (cm)	5,98	5,36	5,48	5,19		5,33	5,94	5,42	5,88	5,81	5,83	5,48		5,22	6,11	5,5	5,29	5,34	5,5725	0,301252939
Lenght of P2 (cm)	3,64	3,83	4,2	4,03		4,1	4,03	4,69	4,05	4,62	3,97	4,12		3,9	4,02	4,45	4,04	3,97	4,10375	0,274684668
P2-P3- Joint radius (cm)	1,46	1,38	1,29	1,69		1,56	1,52	1,63	1,72	1,86	1,94	1,71		1,4	1,66	1,9	1,42	1,41	1,596875	0,196786946
P1-P2- Joint radius (cm)	1,52	1,83	1,68	2,02		1,63	1,59	2,01	1,79	2,11	1,92	2,52		1,63	1,76	1,76	1,86	1,8	1,839375	0,244825618
Navicular width (cm)	1,87	1,69	1,71	1,52		1,73	1,77	1,77	1,81	1,92	1,87	1,75		1,75	1,62	1,64	1,77	1,63	1,73875	0,10404326
P3 Descent (cm)	0,56	0,34	0,56	0,07		-0,5	0,36	1,66	0,19	0,32	1,19	1,28		0,65	0,98	1,79	0,76	0,31	0,6575	0,601093448
P3 Angle (deg)	53,32	52,49	48,75	59,97		50,55	49,32	51,05	55,74	50,42	54,68	49,98		54,63	49,92	50,76	50,4	49,42	51,9625	3,010812736
P3 Dist. to ground (cm)	1,96	2,38	1,83	2,42		2,47	2,02	2,75	2,47	2,95	3,03	3,33		3,13	4,07	2,55	3,69	3,8	2,803125	0,669753375
P3 Dist. to toe (cm)	2,93	3,59	3,14	3,23		3,97	3,34	3,8	3,36	4,82	3,86	4,18		4,01	3,6	3,99	3,69	4,6	3,756875	0,511634228
P3/P2 Joint height (cm)	5,63	5,61	5,01	5,96		5,81	5,37	5,9	6,59	6,41	6,71	6,47		6,59	7,72	5,85	7,09	6,88	6,225	0,704102265
Navicular angle (deg)	44,99	40,84	44,47	44,72		50,16	41,27	44,06	56,66	43,57	50,74	43,57		53,2	43,91	47,85	47,85	44,25	46,381875	4,372355915
Support lenght (cm)	13,42	12,73	13,74	12,3		14,07	13,77	14,9	13,69	15,45	14,17	12,82		13,51	12,92	13,87	12,38	13,33	13,566875	0,852046311
Toe/support %	62,07	66,79	59.90	62,77		62,48	65,18	60,22	59,76	66,83	65,05	73,96		62,96	71,77	66,67	67,33	72,23	65,738	4,304476408
Function. Hoof Ang. (deg)	68,72	73,01	67,65	76,14		69,86	66,44	67,61	73,76	70,82	70,4	65,98		73,13	68,88	69,05	69,38	67,66	69,905625	2,841684873
Hoof angle (deg)	52,37	52,01	51,89	56,95		47,4	49,04	51,9	55,65	50,38	54,81	51,73		51,22	55,67	53,1	53,54	49,22	52,305	2,612763543
Hoof deviation (cm)	0,41	0,22	0,47	0,41		0,34	0,48	0,11	0,32	0,27	0,13	0,51		0,45	0,45	0,4	0,43	0,66	0,37875	0,142869404
Hoof/P3 distance (cm)	1,41	1,74	1,3	1,32		1,36	1,52	1,85	1,53	1,73	1,8	1,52		1,57	1,49	1,71	1,55	1,33	1,545625	0,177387288
Hoof/P3 angle dif. (deg)	0,95	0,48	-3,14	3,02		3,14	0,28	-0,85	0,08	0,04	-0,13	-1,74		3,41	-5,75	-2,34	-3,15	0,2	-0,34375	2,477417674
Dorsal lenght (cm)	9,27	9,34	8,86	8,74		9,03	9,68	11,62	9,48	10,71	11,43	11,92		10,72	12,4	11,1	11,35	11,29	10,43375	1,210894298
P3 Palmar angle (deg)	11,13	11,46	8,31	16,83		10,2	8,86	7,62	18,51	12,48	11,49	10,03		11,01	9,48	6,7	6,83	10,29	10,701875	3,216006258
P2/P3-Joint angle (deg)	10,32	11,92	14,72	-3,44		12,89	2,39	1,52	-0,44	5,16	-6,51	0,09		9,39	3,12	4,85	10,05	7,5	5,220625	6,126910552
P1/P2-Joint angle (deg)	6,46	6,86	7,3	2,57		10,63	2,81	8,8	3,16	-0,6	7,12	1,28		4,33	4,39	1,31	4,77	12,43	5,22625	3,555965645

		F1	F2	F3	F4	F5	F6	F7	M6	M5	M8	M7	F8	F9	F10	M1	M2	M3	M4	MEDIA	DEV. ST.
P3/P2-Joint width (cm)		5,66	5,51	5,9	5,71	5,72	5,88		5,94	6,26	6,52	5,86	5,69	5,71	5,23	5,85	5,55	6,33	5,66	5,822352941	0,315228675
P1/P2-Joint width (cm)		5,05	5,19	5,62	5,15	5,4	5,57		5,88	5,66	6,23	5,76	5,46	5,39	4,84	5,81	5,52	5,58	5,69	5,517647059	0,335773909
C/P1- Joint width (cm)		5,44	5,32	5,55	5,43	5,31	5,44		6,09	5,81	6,01	5,92	5,43	5,67	5,05	6,02	5,57	5,7	5,81	5,621764706	0,291488321
P2 Tilt (deg)		2,29	0,28	1,7	0,19	-0,44	0,85		-5,36	1,67	3,11	-0,42	0,87	0,24	2,24	3,22	1,03	1,46	2,54	0,91	1,973236554
P1 Tilt (deg)		-0,63	-1,47	0,94	-3,3	-1,35	-2,19		-0,5	6,65	1,69	-3,23	2,3	-1,02	3,89	-2,9	0,77	0,52	4,54	0,277058824	2,836744976
P1 Min half-width (cm)		1,85	1,96	1,93	1,84	1,99	1,93		2,17	2	2,2	1,98	2	2,09	1,94	2,21	2,15	1,89	2,23	2,021176471	0,128592494
P2 Min half-width (cm)		2,81	2,66	2,83	2,72	2,84	2,9		3,09	3,11	3,24	2,9	2,78	2,8	2,69	3,12	2,85	2,76	2,74	2,872941176	0,168551354
P2/P3 Joint height (cm)		5,85	5,47	5,44	6,66	4,97	5,92		6,11	6,73	7,11	6,9	6,52	6,7	6,36	7,8	6,72	7,14	7,04	6,437647059	0,728032704
P2/P3 Joint tilt (deg)		1,27	2,2	-0,25	1,48	1,94	0,48		6,01	-0,47	2,4	3,21	3,24	3,86	2,14	3,19	-3,89	3,98	0,49	1,84	2,220855691
Outer wall angle (deg)		98,29	107,3	111,02	102,28	116,74	107,05		110,56	109,95	107,24	102,84	108,24	103,81	108,98	112,09	101,3	107,98	108,76	107,3194118	4,502486078
Outer wall deviation (cm)		0,01	0,16	0,21	0,27	0,81	0,5		0,1	0,45	0,65	0,41	0,04	0,26	0,63	0,44	0,35	0,43	0,38	0,358823529	0,220025065
Outer wall lenght (cm)		7,13	7,37	7,71	7,18	6,98	7,85		7,65	7,73	8,95	9,17	9,09	8,51	9,62	11,26	9,51	10,78	8,8	8,546470588	1,267674747
Inner wall angle (deg)		104,5	106,55	109,94	106,76	108,35	111,46		108,75	103,98	108,21	109,09	98,46	100,11	104	108,97	107,57	103,79	107,99	106,3811765	3,475508744
Inner wall deviation (cm)		0,21	0,63	0,25	0,56	0,51	0,78		0,31	0,13	0,56	0,42	0,22	0,36	0,31	0,85	0,32	0,79	0,15	0,432941176	0,23018375
Inner wall lenght (cm)		6,7	7,25	8	7,52	6,11	7,4		7,45	7,81	8,84	8,17	8,31	7,89	8,8	10,76	10,07	11,07	8,76	8,288823529	1,339850189
Foot width (cm)	13,47	14,92	16,57	15,2	16,3	16,35		17,1	16,28	17,57	16,67	14,81	14,79	14,65	16,68	15,07	13,92	15,43	15,63411765	1,155216748	
Lenght of P3 (cm)	5,6	5,22	5,3	5,2	5,63	5,3	5,66	5,43	5,94	5,84	5,86	5,32	5,84	5,12	5,96	5,38	5,23	5,29	5,506666667	0,284832252	
Lenght of P2 (cm)	3,92	3,85	4,15	3,97	3,86	3,94	4,14	4,42	3,91	4,39	3,9	3,87	3,84	3,86	4,26	4,23	4,04	3,93	4,026666667	0,190664473	
P2-P3- Joint radius (cm)	1,38	1,27	1,67	1,48	1,31	1,7	1,33	1,5	1,73	1,41	1,65	1,43	1,23	1,34	1,41	1,79	1,54	1,52	1,482777778	0,167571076	
P1-P2- Joint radius (cm)	1,79	1,76	2,12	1,53	1,5	1,6	1,51	1,8	1,77	1,63	1,99	1,92	1,4	1,7	1,84	1,72	1,85	1,44	1,715	0,195756451	
Navicular width (cm)	1,81	1,61	1,91	1,38	2,02	1,71	1,67	1,72	1,64	1,98	1,93	1,8	1,82	1,67	1,66	1,7	1,67	1,76	1,747777778	0,152568425	
P3 Descent (cm)	0,94	0,34	0,79	0,34	-0,15	0,54	0,32	1,55	1	1,07	1,02	0,96	0,62	0,66	1,02	1,56	1,13	1,19	0,827777778	0,441781894	
P3 Angle (deg)	54,55	52,16	48,24	59,92	49,69	50,11	49,29	52,66	53,97	52,16	57,54	52,62	55,38	52,38	46,45	48,42	50,04	46,42	51,77777778	3,632689096	
P3 Dist. to ground (cm)	2	2,27	2,41	2,42	2,29	2,34	2,06	2,68	2,6	2,76	2,85	3,44	3,36	3,09	3,93	2,75	3,64	3,67	2,808888889	0,58626323	
P3 Dist. to toe (cm)	3,1	3,59	3,54	3,35	3,86	3,43	3,09	3,65	3,21	4,05	4,27	3,86	3,3	4,06	3,96	4,26	3,79	4,12	3,693888889	0,38813489	
P3/P2 Joint height (cm)	5,63	5,48	5,33	6,05	5,54	5,54	5,24	5,95	6,35	6,42	6,94	6,68	7,2	6,24	7,19	5,74	6,91	6,33	6,153333333	0,641780611	
Navicular angle (deg)	46,64	41,31	47,55	39,38	49,54	45,96	35,09	38,34	41,05	43,72	52,97	33,45	38,35	43,29	44,4	40,7	44,35	41,16	42,625	4,924651376	
Support lenght (cm)	12,3	13,7	13,33	12,96	14,32	13,22	13,12	14,18	13,09	14,37	15,2	12,33	12,29	13,3	13,01	14,21	12,25	12,56	13,31888889	0,855637972	
Toe/support %	64,79	60,61	66,07	59,16	63,69	63,81	64,26	61,31	65,57	64,33	59,92	70,78	67,09	65,5	74,36	67,5	69,92	75,66	65,79611111	4,581051946	
Function Hoof Ang. (deg)	70,35	74,15	69,2	77,2	68,13	69,64	66,94	70,34	68,94	70,77	73,94	70,56	69,86	73,19	64,51	69,3	69,41	63,08	69,97277778	3,338997394	
Hoof angle (deg)	55,96	52,25	51,48	59,33	47,97	51,45	50,96	54,66	55,81	53,03	55,77	53,45	57,35	51,89	50,94	51,25	53	47,73	53,01555556	3,042112699	
Hoof deviation (cm)	0,07	0,19	0,35	0,3	0,69	0,4	0,04	0,43	0,27	0,61	0,2	0,39	0,28	0,23	0,56	0,66	0,82	0,75	0,402222222	0,232190478	
Hoof/P3 distance (cm)	1,52	1,75	1,49	1,43	1,27	1,46	1,54	1,65	1,41	1,72	1,82	1,51	1,25	1,73	1,55	1,74	1,56	1,46	1,547777778	0,161811639	
Hoof/P3 angle dif. (deg)	-1,42	-0,09	-3,24	0,59	1,72	-1,34	-1,67	-1,99	-1,84	-0,87	1,76	-0,83	-1,97	0,49	-4,49	-2,83	-2,96	-1,31	-1,238333333	1,670988612	
Dorsal lenght (cm)	9,5	8,92	9,75	8,82	9,3	9,46	9,3	11,04	10,7	11.06	11,11	11,27	10,92	10,44	12,78	11,12	11,52	12,3	9,928472222	2,624034996	
P3 Palmar angle (deg)	13,34	11,21	4,82	14,05	9,59	11,44	9,2	11	11,12	12,85	13,61	12,96	13,81	9,76	4,12	6,97	8,48	5,49	10,21222222	3,172266875	
P2/P3-Joint angle (deg)	1,6	13,43	5,87	-5,73	9,23	7,52	-1,62	-3,48	-13,49	2,77	-1,96	3,84	1,82	6,94	1,61	16,58	9,28	7,93	3,452222222	7,188142651	
P1/P2-Joint angle (deg)	5,05	5,64	6,01	6,22	9,89	10,13	7,58	8,28	-2,02	2,84	7,64	5,67	1,56	2,41	4,69	5,32	5,91	8,6	5,634444444	3,041012258	

Table 4 Left forelimb data of males and females

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	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	MEDIA	DEV. ST.
P3/P2-Joint width (cm)	5,39	5,48	5,57	5,83	5,76	5,62	6,08				5,675714286	0,234012617
P1/P2-Joint width (cm)	5,06	5,14	5,57	5,31	5,41	5,5	5,72				5,387142857	0,235068379
C/P1- Joint width (cm)	5,39	5,13	5,5	5,29	5,4	5,53	5,56				5,4	0,151437556
P2 Tilt (deg)	2,48	-3,95	3,12	2,91	-0,55	2,29	0,33				0,947142857	2,560317131
P1 Tilt (deg)	-4,34	2,96	2,4	-3,28	1,89	-2,63	4,16				0,165714286	3,457310405
P1 Min half-width (cm)	1,85	2,06	1,87	2,17	2,03	1,83	2,05				1,98	0,13
P2 Min half-width (cm)	2,63	2,52	2,58	2,81	2,7	2,84	2,78				2,694285714	0,122182533
P2/P3 Joint height (cm)	5,59	5,53	5,07	5,95	4,97	5,63	5,79				5,504285714	0,360132251
P2/P3 Joint tilt (deg)	0,78	-1,54	1,46	4,08	-1,6	1,53	-3,3				0,201428571	2,492150534
Outer wall angle (deg)	97,6	105,01	108,95	85,92	110,3	106,08	107,67				103,0757143	8,610789467
Outer wall deviation (cm)	0,32	0,46	0,33	1,47	0,36	0,8	0,33				0,581428571	0,427372927
Outer wall lenght (cm)	6,77	8,27	7,55	6,59	6,91	8,11	7,24				7,348571429	0,656212074
Inner wall angle (deg)	103,63	101,08	110,82	82,66	113,49	108,04	108,27				103,9985714	10,28903855
Inner wall deviation (cm)	0,45	0,19	0,2	0,5	0,6	0,47	0,16				0,367142857	0,178672352
Inner wall lenght (cm)	6,66	6,69	7,14	7,22	6,73	7,54	7,6				7,082857143	0,39919562
Foot width (cm)	13,07	14,18	16,03	14,38	16,16	15,53	15,89				15,03428571	1,172815091
Lenght of P3 (cm)	5,98	5,36	5,48	5,19		5,33	5,94	5,48		5,22	5,4975	0,304196835
Lenght of P2 (cm)	3,64	3,83	4,2	4,03		4,1	4,03	4,12		3,9	3,98125	0,182007653
P2-P3- Joint radius (cm)	1,46	1,38	1,29	1,69		1,56	1,52	1,71		1,4	1,50125	0,148462357
P1-P2- Joint radius (cm)	1,52	1,83	1,68	2,02		1,63	1,59	2,52		1,63	1,8025	0,330010822
Navicular width (cm)	1,87	1,69	1,71	1,52		1,73	1,77	1,75		1,75	1,72375	0,098406954
P3 Descent (cm)	0,56	0,34	0,56	0,07		-0,5	0,36	1,28		0,65	0,415	0,508555377
P3 Angle (deg)	53,32	52,49	48,75	59,97		50,55	49,32	49,98		54,63	52,37625	3,688119682
P3 Dist. to ground (cm)	1,96	2,38	1,83	2,42		2,47	2,02	3,33		3,13	2,4425	0,54142009
P3 Dist. to toe (cm)	2,93	3,59	3,14	3,23		3,97	3,34	4,18		4,01	3,54875	0,46066528
P3/P2 Joint height (cm)	5,63	5,61	5,01	5,96		5,81	5,37	6,47		6,59	5,80625	0,530819784
Navicular angle (deg)	44,99	40,84	44,47	44,72		50,16	41,27	43,57		53,2	45,4025	4,245877833
Support lenght (cm)	13,42	12,73	13,74	12,3		14,07	13,77	12,82		13,51	13,295	0,611905688
Toe/support %	62,07	66,79	59.90	62,77		62,48	65,18	73,96		62,96	65,17285714	4,230420445
Functional Hoof Ang. (deg)	68,72	73,01	67,65	76,14		69,86	66,44	65,98		73,13	70,11625	3,634418552
Hoof angle (deg)	52,37	52,01	51,89	56,95		47,4	49,04	51,73		51,22	51,57625	2,772775492
Hoof deviation (cm)	0,41	0,22	0,47	0,41		0,34	0,48	0,51		0,45	0,41125	0,093417267
Hoof/P3 distance (cm)	1,41	1,74	1,3	1,32		1,36	1,52	1,52		1,57	1,4675	0,148780759
Hoof/P3 angle dif. (deg)	0,95	0,48	-3,14	3,02		3,14	0,28	-1,74		3,41	0,8	2,378072449
Dorsal lenght (cm)	9,27	9,34	8,86	8,74		9,03	9,68	11,92		10,72	9,695	1,092807655
P3 Palmar angle (deg)	11,13	11,46	8,31	16,83		10,2	8,86	10,03		11,01	10,97875	2,607968216
P2/P3-Joint angle (deg)	10,32	11,92	14,72	-3,44		12,89	2,39	0,09		9,39	7,285	6,682337059
P1/P2-Joint angle (deg)	6,46	6,86	7,3	2,57		10,63	2,81	1,28		4,33	5,28	3,092368487

Table 5 Right forelimb data of females

	F1	F2	F3	F4	F5	F6	F7	F8	F9	F10	MEDIA	DEV. ST.
P3/P2-Joint width (cm)	5,66	5,51	5,9	5,71	5,72	5,88		5,69	5,71	5,23	5,667777778	0,200734761
P1/P2-Joint width (cm)	5,05	5,19	5,62	5,15	5,4	5,57		5,46	5,39	4,84	5,296666667	0,256807321
C/P1- Joint width (cm)	5,44	5,32	5,55	5,43	5,31	5,44		5,43	5,67	5,05	5,404444444	0,172199819
P2 Tilt (deg)	2,29	0,28	1,7	0,19	-0,44	0,85		0,87	0,24	2,24	0,913333333	0,967548448
P1 Tilt (deg)	-0,63	-1,47	0,94	-3,3	-1,35	-2,19		2,3	-1,02	3,89	-0,314444444	2,278229088
P1 Min half-width (cm)	1,85	1,96	1,93	1,84	1,99	1,93		2	2,09	1,94	1,947777778	0,076448966
P2 Min half-width (cm)	2,81	2,66	2,83	2,72	2,84	2,9		2,78	2,8	2,69	2,781111111	0,077369963
P2/P3 Joint height (cm)	5,85	5,47	5,44	6,66	4,97	5,92		6,52	6,7	6,36	5,987777778	0,613245012
P2/P3 Joint tilt (deg)	1,27	2,2	-0,25	1,48	1,94	0,48		3,24	3,86	2,14	1,817777778	1,271699432
Outer wall angle (deg)	98,29	107,3	111,02	102,28	116,74	107,05		108,24	103,81	108,98	107,0788889	5,301427743
Outer wall deviation (cm)	0,01	0,16	0,21	0,27	0,81	0,5		0,04	0,26	0,63	0,321111111	0,270852563
Outer wall lenght (cm)	7,13	7,37	7,71	7,18	6,98	7,85		9,09	8,51	9,62	7,937777778	0,936146059
Inner wall angle (deg)	104,5	106,55	109,94	106,76	108,35	111,46		98,46	100,11	104	105,57	4,299235397
Inner wall deviation (cm)	0,21	0,63	0,25	0,56	0,51	0,78		0,22	0,36	0,31	0,425555556	0,203046245
Inner wall lenght (cm)	6,7	7,25	8	7,52	6,11	7,4		8,31	7,89	8,8	7,553333333	0,819115376
Foot width (cm)	13,47	14,92	16,57	15,2	16,3	16,35		14,81	14,79	14,65	15,22888889	1,005975204
Lenght of P3 (cm)	5,6	5,22	5,3	5,2	5,63	5,3	5,66	5,32	5,84	5,12	5,419	0,242140914
Lenght of P2 (cm)	3,92	3,85	4,15	3,97	3,86	3,94	4,14	3,87	3,84	3,86	3,94	0,116045968
P2-P3- Joint radius (cm)	1,38	1,27	1,67	1,48	1,31	1,7	1,33	1,43	1,23	1,34	1,414	0,160222068
P1-P2- Joint radius (cm)	1,79	1,76	2,12	1,53	1,5	1,6	1,51	1,92	1,4	1,7	1,683	0,220758792
Navicular width (cm)	1,81	1,61	1,91	1,38	2,02	1,71	1,67	1,8	1,82	1,67	1,74	0,176194337
P3 Descent (cm)	0,94	0,34	0,79	0,34	-0,15	0,54	0,32	0,96	0,62	0,66	0,536	0,337316205
P3 Angle (deg)	54,55	52,16	48,24	59,92	49,69	50,11	49,29	52,62	55,38	52,38	52,434	3,491482334
P3 Dist. to ground (cm)	2	2,27	2,41	2,42	2,29	2,34	2,06	3,44	3,36	3,09	2,568	0,527653085
P3 Dist. to toe (cm)	3,1	3,59	3,54	3,35	3,86	3,43	3,09	3,86	3,3	4,06	3,518	0,329099782
P3/P2 Joint height (cm)	5,63	5,48	5,33	6,05	5,54	5,54	5,24	6,68	7,2	6,24	5,893	0,641613418
Navicular angle (deg)	46,64	41,31	47,55	39,38	49,54	45,96	35,09	33,45	38,35	43,29	42,056	5,457020351
Support lenght (cm)	12,3	13,7	13,33	12,96	14,32	13,22	13,12	12,33	12,29	13,3	13,087	0,655151382
Toe/support %	64,79	60,61	66,07	59,16	63,69	63,81	64,26	70,78	67,09	65,5	64,576	3,239945816
Functional Hoof Ang. (deg)	70,35	74,15	69,2	77,2	68,13	69,64	66,94	70,56	69,86	73,19	70,922	3,069990228
Hoof angle (deg)	55,96	52,25	51,48	59,33	47,97	51,45	50,96	53,45	57,35	51,89	53,209	3,390979701
Hoof deviation (cm)	0,07	0,19	0,35	0,3	0,69	0,4	0,04	0,39	0,28	0,23	0,294	0,185663974
Hoof/P3 distance (cm)	1,52	1,75	1,49	1,43	1,27	1,46	1,54	1,51	1,25	1,73	1,495	0,163043961
Hoof/P3 angle dif. (deg)	-1,42	-0,09	-3,24	0,59	1,72	-1,34	-1,67	-0,83	-1,97	0,49	-0,776	1,460640651
Dorsal lenght (cm)	9,5	8,92	9,75	8,82	9,3	9,46	9,3	11,27	10,92	10,44	9,928472222	0,833837181
P3 Palmar angle (deg)	13,34	11,21	4,82	14,05	9,59	11,44	9,2	12,96	13,81	9,76	11,018	2,822692962
P2/P3-Joint angle (deg)	1,6	13,43	5,87	-5,73	9,23	7,52	-1,62	3,84	1,82	6,94	4,29	5,545398493
P1/P2-Joint angle (deg)	5,05	5,64	6,01	6,22	9,89	10,13	7,58	5,67	1,56	2,41	6,016	2,75830141

Table 6 Left forelimb data of females

	M6	M5	M8	M7	M1	M2	M3	M4	MEDIA	DEV. ST.
P3/P2-Joint width (cm)	5,96	6,24	6,47	5,96	6,13	5,62	5,57	5,7	5,95625	0,316811684
P1/P2-Joint width (cm)	5,97	5,66	6,09	5,78	5,71	5,57	5,48	5,62	5,735	0,205287255
C/P1- Joint width (cm)	6,21	5,8	6,12	5,87	5,94	5,64	5,69	5,75	5,8775	0,202537474
P2 Tilt (deg)	1,45	-0,84	0,17	6,05	2,75	0,29	2,83	3,85	2,06875	2,257675401
P1 Tilt (deg)	-2,45	-2,71	-1,66	4,06	2,97	1,08	3,84	4,1	1,15375	3,011397101
P1 Min half-width (cm)	2,16	2,43	2,16	2,17	2,02	2,07	1,86	1,83	2,0875	0,191814643
P2 Min half-width (cm)	3,02	3,16	3	2,82	2,9	2,99	2,76	2,69	2,9175	0,154804024
P2/P3 Joint height (cm)	6,06	6,76	7,24	6,75	7,98	6,09	7,02	6,98	6,86	0,620345526
P2/P3 Joint tilt (deg)	0,78	0,85	0,04	2,78	3,84	-3,3	3,14	1,94	1,25875	2,256548803
Outer wall angle (deg)	108,95	106,07	111,87	106,21	105,6	103,67	105,08	105,71	106,645	2,57459012
Outer wall deviation (cm)	0,27	0,15	0,64	0,23	0,1	0,25	0,18	0,23	0,25625	0,164918811
Outer wall lenght (cm)	10,1	7,94	9,23	9,77	10,52	9,19	10,32	9,53	9,575	0,819773488
Inner wall angle (deg)	105,84	100,55	107,83	106,24	107,56	105,94	105,3	104,86	105,515	2,251336111
Inner wall deviation (cm)	0,13	0,44	0,29	0,68	0,62	0,25	0,1	0,6	0,38875	0,228312912
Inner wall lenght (cm)	9,06	7,41	8,6	7,84	9,79	9,24	8,35	7,67	8,495	0,834112017
Foot width (cm)	16,61	15,65	17,95	16,57	16,82	15,11	14,05	14,49	15,90625	1,314685595
Lenght of P3 (cm)	5,42	5,88	5,81	5,83	6,11	5,5	5,29	5,34	5,6475	0,298460335
Lenght of P2 (cm)	4,69	4,05	4,62	3,97	4,02	4,45	4,04	3,97	4,22625	0,307010353
P2-P3- Joint radius (cm)	1,63	1,72	1,86	1,94	1,66	1,9	1,42	1,41	1,6925	0,204293207
P1-P2- Joint radius (cm)	2,01	1,79	2,11	1,92	1,76	1,76	1,86	1,8	1,87625	0,128167023
Navicular width (cm)	1,77	1,81	1,92	1,87	1,62	1,64	1,77	1,63	1,75375	0,114009711
P3 Descent (cm)	1,66	0,19	0,32	1,19	0,98	1,79	0,76	0,31	0,9	0,617413962
P3 Angle (deg)	51,05	55,74	50,42	54,68	49,92	50,76	50,4	49,42	51,54875	2,33055136
P3 Dist. to ground (cm)	2,75	2,47	2,95	3,03	4,07	2,55	3,69	3,8	3,16375	0,608955487
P3 Dist. to toe (cm)	3,8	3,36	4,82	3,86	3,6	3,99	3,69	4,6	3,965	0,499714204
P3/P2 Joint height (cm)	5,9	6,59	6,41	6,71	7,72	5,85	7,09	6,88	6,64375	0,616254528
Navicular angle (deg)	44,06	56,66	43,57	50,74	43,91	47,85	47,85	44,25	47,36125	4,554797902
Support lenght (cm)	14,9	13,69	15,45	14,17	12,92	13,87	12,38	13,33	13,83875	1,006130317
Toe/support %	60,22	59,76	66,83	65,05	71,77	66,67	67,33	72,23	66,2325	4,59547215
Functional Hoof Ang. (deg)	67,61	73,76	70,82	70,4	68,88	69,05	69,38	67,66	69,695	1,998385062
Hoof angle (deg)	51,9	55,65	50,38	54,81	55,67	53,1	53,54	49,22	53,03375	2,39292969
Hoof deviation (cm)	0,11	0,32	0,27	0,13	0,45	0,4	0,43	0,66	0,34625	0,180549756
Hoof/P3 distance (cm)	1,85	1,53	1,73	1,8	1,49	1,71	1,55	1,33	1,62375	0,177034097
Hoof/P3 angle dif. (deg)	-0,85	0,08	0,04	-0,13	-5,75	-2,34	-3,15	0,2	-1,4875	2,122893914
Dorsal lenght (cm)	11,62	9,48	10,71	11,43	12,4	11,1	11,35	11,29	11,1725	0,83686063
P3 Palmar angle (deg)	7,62	18,51	12,48	11,49	9,48	6,7	6,83	10,29	10,425	3,896947523
P2/P3-Joint angle (deg)	1,52	-0,44	5,16	-6,51	3,12	4,85	10,05	7,5	3,15625	5,103553209
P1/P2-Joint angle (deg)	8,8	3,16	-0,6	7,12	4,39	1,31	4,77	12,43	5,1725	4,186508091

Table 7 Right forelimb data of males

	M6	M5	M8	M7	M1	M2	M3	M4	MEDIA	DEV. ST.
P3/P2-Joint width (cm)	5,94	6,26	6,52	5,86	5,85	5,55	6,33	5,66	5,99625	0,340291367
P1/P2-Joint width (cm)	5,88	5,66	6,23	5,76	5,81	5,52	5,58	5,69	5,76625	0,221161706
C/P1- Joint width (cm)	6,09	5,81	6,01	5,92	6,02	5,57	5,7	5,81	5,86625	0,176872634
P2 Tilt (deg)	-5,36	1,67	3,11	-0,42	3,22	1,03	1,46	2,54	0,90625	2,798192976
P1 Tilt (deg)	-0,5	6,65	1,69	-3,23	-2,9	0,77	0,52	4,54	0,9425	3,392006359
P1 Min half-width (cm)	2,17	2	2,2	1,98	2,21	2,15	1,89	2,23	2,10375	0,128055513
P2 Min half-width (cm)	3,09	3,11	3,24	2,9	3,12	2,85	2,76	2,74	2,97625	0,187230759
P2/P3 Joint height (cm)	6,11	6,73	7,11	6,9	7,8	6,72	7,14	7,04	6,94375	0,478298696
P2/P3 Joint tilt (deg)	6,01	-0,47	2,4	3,21	3,19	-3,89	3,98	0,49	1,865	3,069853417
Outer wall angle (deg)	110,56	109,95	107,24	102,84	112,09	101,3	107,98	108,76	107,59	3,749487584
Outer wall deviation (cm)	0,1	0,45	0,65	0,41	0,44	0,35	0,43	0,38	0,40125	0,151415936
Outer wall lenght (cm)	7,65	7,73	8,95	9,17	11,26	9,51	10,78	8,8	9,23125	1,288137498
Inner wall angle (deg)	108,75	103,98	108,21	109,09	108,97	107,57	103,79	107,99	107,29375	2,165138976
Inner wall deviation (cm)	0,31	0,13	0,56	0,42	0,85	0,32	0,79	0,15	0,44125	0,271737137
Inner wall lenght (cm)	7,45	7,81	8,84	8,17	10,76	10,07	11,07	8,76	9,11625	1,363293674
Foot width (cm)	17,1	16,28	17,57	16,67	16,68	15,07	13,92	15,43	16,09	1,202140947
Lenght of P3 (cm)	5,43	5,94	5,84	5,86	5,96	5,38	5,23	5,29	5,61625	0,311353612
Lenght of P2 (cm)	4,42	3,91	4,39	3,9	4,26	4,23	4,04	3,93	4,135	0,216399102
P2-P3- Joint radius (cm)	1,5	1,73	1,41	1,65	1,41	1,79	1,54	1,52	1,56875	0,141364524
P1-P2- Joint radius (cm)	1,8	1,77	1,63	1,99	1,84	1,72	1,85	1,44	1,755	0,16466416
Navicular width (cm)	1,72	1,64	1,98	1,93	1,66	1,7	1,67	1,76	1,7575	0,128146122
P3 Descent (cm)	1,55	1	1,07	1,02	1,02	1,56	1,13	1,19	1,1925	0,232486559
P3 Angle (deg)	52,66	53,97	52,16	57,54	46,45	48,42	50,04	46,42	50,9575	3,871805596
P3 Dist. to ground (cm)	2,68	2,6	2,76	2,85	3,93	2,75	3,64	3,67	3,11	0,538728662
P3 Dist. to toe (cm)	3,65	3,21	4,05	4,27	3,96	4,26	3,79	4,12	3,91375	0,356608529
P3/P2 Joint height (cm)	5,95	6,35	6,42	6,94	7,19	5,74	6,91	6,33	6,47875	0,503145463
Navicular angle (deg)	38,34	41,05	43,72	52,97	44,4	40,7	44,35	41,16	43,33625	4,423815217
Support lenght (cm)	14,18	13,09	14,37	15,2	13,01	14,21	12,25	12,56	13,60875	1,026303179
Toe/support %	61,31	65,57	64,33	59,92	74,36	67,5	69,92	75,66	67,32125	5,717045784
Functional Hoof Ang. (deg)	70,34	68,94	70,77	73,94	64,51	69,3	69,41	63,08	68,78625	3,473055211
Hoof angle (deg)	54,66	55,81	53,03	55,77	50,94	51,25	53	47,73	52,77375	2,751492777
Hoof deviation (cm)	0,43	0,27	0,61	0,2	0,56	0,66	0,82	0,75	0,5375	0,221278751
Hoof/P3 distance (cm)	1,65	1,41	1,72	1,82	1,55	1,74	1,56	1,46	1,61375	0,143022226
Hoof/P3 angle dif. (deg)	-1,99	-1,84	-0,87	1,76	-4,49	-2,83	-2,96	-1,31	-1,81625	1,830565389
Dorsal lenght (cm)	11,04	10,7	11.06	11,11	12,78	11,12	11,52	12,3	9,928472222	3,968042864
P3 Palmar angle (deg)	11	11,12	12,85	13,61	4,12	6,97	8,48	5,49	9,205	3,479708526
P2/P3-Joint angle (deg)	-3,48	-13,49	2,77	-1,96	1,61	16,58	9,28	7,93	2,405	9,148181708
P1/P2-Joint angle (deg)	8,28	-2,02	2,84	7,64	4,69	5,32	5,91	8,6	5,1575	3,494120572

Table 8 Left forelimb data of males

Statistical results

We analyzed the selected data using statistical methods to identify significant correlations between the two horses' groups (group F and group nF), limiting the probability level (P) to less than 0.05. Before starting the statistical analysis of the results we verified that there were no significant differences between the right foot and left one. The results, which confirmed the preliminary hypothesis, permitted us to divide the horses in two groups for the evaluation of each selected parameter:

- Shod horses (F6,F8, F9, F10, M1, M2, M3, M4, M6, M7);
- Unshod horses (F1, F2, F3, F4, F5, F7, M5, M8).

The following table summarizes the means, the standard deviations and the "P" of the two groups:

	Unshod group (nF)	Shod group (F)	Probability level (P)
	means+Standard deviation	Means+Standard deviation	
P3/toe distance (cm)	3,5 ± 0,48	3,9 ± 0,31	0,002979*
P3/ground distance(cm)	2,3 ± 0,31	3,2 ± 0,52	0,000003*
P3/P2 joint width (cm)	5,8 ± 0,49	6,5 ± 0,60	0.000551*
Toe/support %	63,4 ± 2,60	67,3 ± 4,89	0,009943*
Hoof/P3 distance (cm)	1,5 ± 0,16	1,6 ± 0,17	0,286799
Hoof/P3 Angle Difference (°)	-0,3 ± 1,71	-1,2 ± 2,34	0,246759
P3/Palmar Angle (°)	11,6 ± 2,96	9,5 ± 2,70	0.059579
P1/P2 Joint Angle (°)	3,5 ± 7,80	4,9 ± 5,77	0,613987
P2/P3 Joint Angle (°)	4,6 ± 3,18	6,1 ± 3,24	0.556966
P2/P3 Joint Height (cm)	6,0 ± 0,75	6,7 ± 0,57	0,010582*

Table 9: probability level (P) for each parameter, means e standard deviation of the 2 group. *significant difference with P<0,05.

We used statistical analysis with the parameters that correlate the following factors:

- P3 with the tip, the ground, and the distance from the wall of the hoof;
- The distance of the distal interphalangeal joint (AIFD) and the proximal interphalangeal joint (AIFP) to the ground (DP and LM views);
- The angle formed by the distal surface of P3 and ground;
- the difference in degrees between the angle of the dorsal wall of P3 and the one that the hoof forms with the surface;
- The angles of the AIFP and the AIFD;
- The percentage ratio toe/support.

DISCUSSION

In our study we examined a lot of factors. At first, we observed that the horses belong to an homogeneous group; in fact, these horses were really close in terms

of age, results of biometric measurements (Tab. 1 & 2) farming, management and type of training. Secondly, all the horses were healthy when we conducted the X-ray investigations.
The used diagnostic methodology has proved to be well-repeatable and, except in a few cases, we obtained X-ray views useful for our investigation. The evaluation of the data shows the absence of a few records. This absence is due to the fact that we had some difficulties in positioning some horses on the blocks, not wanting to use physical or pharmacological restraint. This choice derived from our wanting to place the horses on the blocks with the weight distributed in the most natural way.
The results of statistical analysis regarded only some parameters. This choice is in accord with two recent studies (2, 3) that showed how to use bone parameters.
P3 distance to Toe (P3T)
Horizontal distance in centimeters from the tip of P3 to the tip of the hoof. The distance tip-P3 shows a significantly higher value ($P < 0.05$) in the shod horses group. The average value of the group F is 3.9 cm with st. dev.± 0.31 and 3.5 cm with st. dev. ± 0.48 of group nF. We can therefore say that the horses nF have the tip shorter than those of the group F. this difference probably is due to a greater consumption.

Toe/Support percentage (TS %)
The TS% is considered a line perpendicular to the plane that passes through the center of the distal interphalangeal joint. This line intersects the shelf at one point. The distance between this point and the tip of the hoof is the length of our interest. This parameter calculates the ratio as a percentage of the distance on the soil between the heel and the toe. The difference in the two groups is significant at $P = 0.009943$. Specifically, the average value is 67.3% in the group F with standard deviation ± 4.89, while it is 63.4% in the group nF with standard deviation ± 2.60. This finding is consistent with the results of Craig (2, 3) while some authors (8) believed that this ratio should ideally be 50%. In our healthy horses this percentage is in favor of the tip in both shod and unshod groups.
The values of this ratio indicate that the group F has an equilibrium shifted toward the dorsal portion of the foot than in group nF. This result is in line with the previous fact.

P3 distance to Ground: (P3G) (distance in cm from the tip of P3 to the support surface)/ *P3/P2 Joint Height (P3P2JH)* (distance in cm between the distal interphalangeal joint and the support surface) / *P2/P3 Joint Height (P2P3JH)* (distance in cm between the proximal interphalangeal joint and the support surface)
We considered together these 3 parameters and the statistical analysis confirmed the significant differences (in sequence $P=0.000003$, $P=0.000551$, $P=0.010582$) between the values of the two groups. We hypothesized that these results are closely influenced by the presence of the horseshoes during the x-rays examination. We believe it is essential for the evaluation of these data to perform radiographic views without horseshoes in both groups. It should be interesting to do a comparison between the results of our study and those obtained with the barefoot trimming method.

Hoof/P3 Distance (HP3D)
HP3D is the distance in cm from the tip of P3 to the dorsal wall of the hoof. This value does not present a significant difference P>0.05 (P=0.286799) and specifically the average distance is 1.6 cm in the group F with standard deviation ± 0.17, while in the group nF is 1.5 cm with standard deviation ± 0.16.

P1/P2 Joint Angle (P1P2 JA) (measures in degrees the angle formed between a line through the rotation center of the proximal and distal interphalangeal joint and the straight line represents the longitudinal axis of P1) *and P2/P3 Joint Angle (P2P3JA)* (measured in degrees the angle formed between a line parallel to the dorsal wall of P3 that passes through the rotation center of the distal interphalangeal joint and a line passing through the rotation center of the distal and proximal interphalangeal joints) There were no significant differences between the two groups. The values had a P> 0.05 (respectively P = 0.613987 and P = 0.212087). It is interesting to note that these results, according with those found by Craig (2, 3), have an average of 5.4° for the P1/P2 angle with standard deviation ± 3.25 and 4.3 ° for the P2/P3 angle with standard deviation ± 6.67. In our case we can affirm that this angle should not be necessarily close to zero in healthy horses, as believed by some other authors (7).

P3/Palmar Angle (P3PA) (measured in degrees the angle formed by the distal surface of the third phalanx and the support surface) / *Hoof/P3 Angle Difference (HP3AD)* (difference in degrees between the angle formed by the dorsal wall of the hoof and the ground and the angle formed by the dorsal surface of P3 and the ground)
These results did not reveal any statistically significant differences between the two groups subject of the study.
The data of our study provided a lot of new knowledge about the bone digital axis parameters today unknown of healthy bardigiano horse. The statistical records previously treated are in accord with the most recent scientific literature in particular regarding the values of P1/P2 and P2/P3 joint angle and the results of the center rotation projection of the AIFD on the ground.
We believe to have made an important study model and the bardigiano horse represented a good subject for the research. We suppose that this data are an important basic reference for further scientific investigations about the use of this method in equine clinical applications such as the evaluation of the results of therapeutic and corrective shoeing or to discover new methods to improve the care of the horse's foot.

REFERENCE

1. Catalano A. L. (2006), Il cavallo Bardigiano. Origine selezione, morfologia e allevamento, ANACB 2006.
2. Craig J.J., Craig M., Savoldi M.T., Waldsmith J.K. (2005) Locating rotation centers of the equine digit and their use in quantifying conformation, Eponashoe, Inc, 2005 www.eponatech.com.
3. Craig J.J., Craig M. (2005) Hoof and bone morphology of the equine digit:

challange to some common beliefs, The European Farrier's Journal (Issue #114, 2005).
4. Kummer M., Lischer C., Vargas J., Hugelshofer J. (2004) Evaluation of standardised radiographic technique of the equine hoof, Schweizer Archiv für Tierheilkunde 11, 507-514.
5. Kummer M., Geyer H., Imboden I., Auer J., Lischer C. (2006) The effect of hoof trimming on radiographic measurement of the front feet of normal Warmblood horses, The Veterinary Journal 172 (2006) 56-66.
6. Smith M., Barefoot for soundness, www.barefoothorse.com/barefoot_Transition.html
7. Stashak T. S. (2002) Adams' lameness in horses. Lippincott Williams and Wilkins, Fifth edition, 1-13, 98-105.
8. Williams G., Deacon M. (1999) No hoof, no horse, London: The Kenilworth Press Ltd, 1999.

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APPROACH TO THE INTERCHANGEABILITY OF THE CLINICAL VETERINARY LABORATORY METHODS

APPROCCIO ALLA INTERCAMBIABILITÀ DEI METODI DI LABORATORIO NELLA CLINICA VETERINARIA

Fusari Antonella[1], Cacchioli Gianluca[2], Bonati Luca[2], Quintavalla Fausto[2], Ubaldi Antonio[1]

STRUCTURED SUMMARY

Objectives: to evaluate the reliability of a new dry chemistry apparatus (Fuji Dri-chem 4000i, Fujifilm, Japan) in diagnosis decision making in (ambulatory) veterinary laboratory medicine.

Methods: Comparison between results obtained by a traditional (wet chemistry) apparatus (Cobas integra® 400 plus, Roche Diagnostics Ltd., Switzerland) with the results obtained with the "new" dry chemistry apparatus (Fuji Dri-chem 4000i, Fujifilm, Japan).

Results: Results show the importance to determine normal reference range for each technique/apparatus/instrument "*de novo*" introduced in laboratory practice. Comparison between the two methods (not by a "validation" but by a clinical pertinence point of view) stress es that the overlapping of the performance is relating to some test, but not for all the parameters determined.

Clinical significance: In the clinical practice it is important can rely on reliable laboratory results. For this aim is not necessary to validate instrument or methods (the companies already do that), but it is important to know if a new method introduced in the practice perform in the same manner as the old one. In this work, the two instruments utilized gave different results for some parameters: this is only due to the different method employed (wet chemistry *vs* dry chemistry) and are characteristic of the instruments. This means that the instruments work in different manner and not that one is better than the other. Clinician has to know this in order to support a suspected clinical diagnosis with an accurate laboratory diagnosis.

KEY WORDS

decision making, wet chemistry, dry chemistry, laboratory diagnosis, analytical methods

INTRODUCTION

Studies comparing a new method with an established method, to assess whether the new measurements are comparable with existing ones, are frequently conducted in clinical pathology laboratories, especially when new instruments are introduced in the practice of the laboratory.

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Assessment usually involves statistical analysis of paired results from the 2 methods to objectively investigate sources of analytical error (total, random, and systematic). In the review by Jensen et al., (2006), the types of errors that can be assessed in performing this task are described, and a general protocol for comparison of quantitative methods is recommended. The typical protocol has 9 steps: 1) state the purpose of the experiment, 2) establish a theoretical basis for the method comparison experiment, 3) become familiar with the new method, 4) obtain estimates of random error for both methods, 5) estimate the number of samples to be included in the method comparison experiment, 6) define acceptable difference between the 2 methods, 7) measure the patient samples, 8) analyze the data and 9) judge acceptability.
The protocol includes the essential investigations and decisions needed to objectively assess the overall analytical performance of a new method compared to a reference or established method (Jensen et al., 2006).
In this study, comparison between results obtain with a "traditional " apparatus working on the wet chemistry method (Cobas integra® 400 plus, Roche Diagnostics Ltd., Switzerland, afterward called "Cobas") and obtained with a "new" instrument working on the principle of "dry-chemistry" (Fuji Dri-chem 4000i, Fujifilm, Japan afterward called "Fuji") is reported.
The utilization of the so called "dry chemistry" in the veterinary practice has been introduced since '80 years and it is a widespread acceptance. Assuming internal laboratory procedures in veterinary ambulatories is necessary the practitioner to obtain fast results to speed up the diagnostic process and to live up to expectation of the owners of the patients.
Aim of this study is to verify the capability of the new method to ensure that the decision making relating to diagnosis is supported by the new method introduced: for this purpose, it is not necessary to apply validation procedures (already previously performed), but to verify the reliability of laboratory diagnosis deduced.

MATERIAL AND METHODS

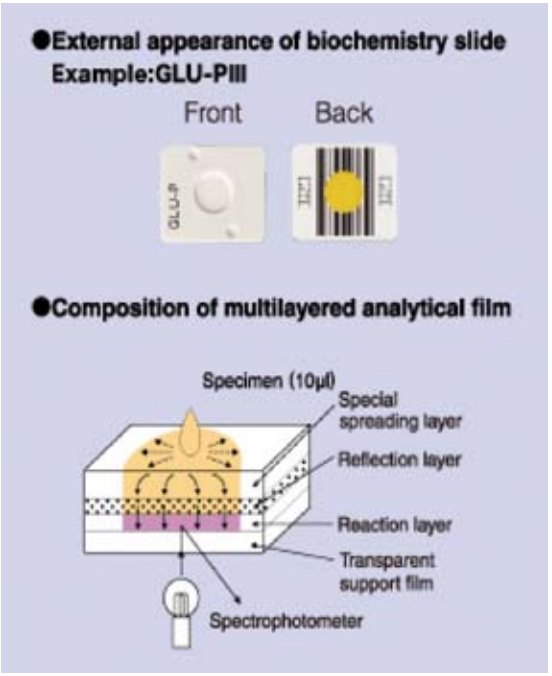
Blood samples: Blood samples were obtained by 32 dogs of different sex and ages healthy and affected by different pathologies. Samples were drained with disposable in tubes containing litio-heparine as anticoagulant. Tubes were centrifuged (1500 rpm) as soon as possible after sampling and plasma frozen (-20°C) until to analysis. Defrosting was realized at room temperature.

Instruments: Compared analytical data were obtained by two instruments: Cobas integra® 400 plus, Roche Diagnostics Ltd., Switzerland, afterward called "Cobas") for the wet chemistry methods, and (Fuji Dri-chem 4000i, Fujifilm, Japan afterward called "Fuji") for dry chemistry method.
The "Cobas" system is utilised in the low workload laboratory and special chemistry testing in medium volume sites (http://www.rochediagnostics.hu/portal/synergy/static/file/synergy/alfproxy//download/1414-ef9509ba52be11dfbf67075f2eb63675/last/integra400_en.pdf.)
Determination of blood parameters utilize different techniques depending on bases of the parameter tested: absorbance photometry to determine enzymes and substrates,

turbidimetry to test specific proteins and drugs of abuse, fluorescence polarimetry to test therapeutic drugs, thyroid tests and ion-selective electrode potentiometry to determine concentrations of Na+, K+, Cl– and Li+. All of these techniques prefigures utilization of wet reagents and trained professional people.
The "Fuji" system is an analytical device designed to measure laboratory clinical chemistry parameters. The device measures 20 parameters in one single run. 24 parameters including the electrolytes sodium, potassium and chloride are available as single reagents. Because of its flexibility, "Fuji" is a practical device for all in house laboratories. Its utilization does not require specifically trained professional people. As for all the "dry chemistry" apparatus, "Fuji" use ready card as support for dry reagents. For this reason the author evaluated the possibility the "Fuji" to be used directly by clinicians in ambulatory laboratory

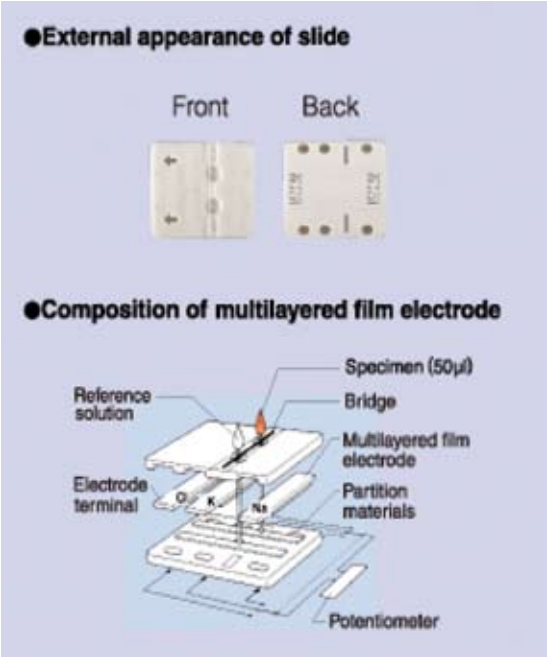
Main specifications for FUJI DRI-CHEM 4000i (http://www.fujifilm.com/products/medical/products/clinical_chemistry_system/fdc_4000i/)	
Measurement tests	Colorimetry: 26 tests Electrolytes: 3 tests
Throughput	Colorimetry: 60 tests/hourElectrolytes: 140 tests/ hourCombined: 77 tests/hour
TAT (Turn around time)	Time required for 6 tests: 8min 42sec.Time required for 12 tests: 16min 37sec.*TAT is the time from when slides are set until final results.
Number of sample racks	1
Number of incubations	Colorimetry: 6, Electrolytes: 1
Measurement time	Colorimetry: 2 to 6 minutes/test,Electrolytes: 1 minute/ 3 tests (Na-K-Cl)
Sample types	Plasma, Serum, Whole blood (NH_3-W,Na-K-Cl)
Sample volume	Colorimetry: 10 µL/ test,Electrolytes: 50 µL/ 3 tests (Na-K-Cl)
Data transmission to PC	USB 2.0 or RS-232C serial D-Sub 9 pin -9 pin cross cable
Data printing	Thermal printer
Electrical requirements	AC 100-240V, 50/60 Hz, 200VA
Dimensions	415 (W) x 390 (D) x 290 (H)mm
Weight	Approx. 20 kg
Operating temperature	15 to 32 °C
Operating humidity	30 to 80 %RH

Principle of measurement of FUJI Dri-chem 4000i The system has two major principles of measurement, one is colorimetric and the other is potentiometric.



- Colorimetric slide (Enzymes, General chemistry, Immunology)

In figure 1 the composition of a multilayered analytical film is reported. For a glucose slide, the actual measurement is conducted as follows. 10 microliters of plasma or serum are spotted on a Fuji Dri-Chem Slide Glu-Pi. After spotting, the sample spreads uniformly on the spreading layer and diffuses into the underlying layer. As the process proceeds, large molecular weight components such as proteins or dye components are filtrated, and only small molecular weight components are able to permeate and diffuse into the reagent layer. Glucoseoxidase (GOD) catalyzes the oxidization of sample glucose to generate hydrogen peroxide. In the presence of peroxidase (POD), hydrogen peroxide reacts with dye precursors and finally forms red dye. The slide is incubated at 37°C for a specified number of minutes in the FUJI Dri-Chem analyzer and the optical reflection density is measured at 505nm. The aforementioned optical reflection density is then converted into the glucose concentration using a calibration curve preinstalled in the analyzer.
http://www.fujifilm.com/products/medical/products/clinical_chemistry_system/fdc_4000i/specifications/



- Potentiometric slide (Electrolytes)
In figure 2 the composition of a multilayered fil electrode is represented potentiometric analysis is based on measurement of the generated voltage difference between ion selective electrodes applied to a reference solution and to the sample. In the FUJI Dri-Chem system, ion selective electrodes for sodium (Na), potassium (K) and chloride (Cl) are set into one slide. After spotting 50 microliters of the sample and of the reference solution on the slide, the slide is incubated for one minute in the FUJI Dri-Chem analyzer and the potentiometric difference between the sample and the reference solution is measured. The potentiometric value is then converted into each electrolyte concentration using a calibration curve preinstalled in the analyzer. http://www.fujifilm.com/products/medical/products/clinical_chemistry_system/fdc_4000i/specifications

Animals: Most authors recommend to include at least 40 patient samples in the method comparison experiment (Jensen et al., 2006). The samples should cover the working range of the methods and should represent the spectrum of diseases expected in routine application of the methods (Jensen et al., 2006). For this reason, animals to be sampled have been chosen randomly among patients of the Veterinary Teaching Hospital of the Veterinary Faculty of Parma.

Table 1 summarizes clinical cases objects of the study: subjects are represented by dogs(female and male) visited in the Veterinary Teaching Hospital of the University of Parma (Italy)

Table 1 Clinical cases (dogs) recorded in the Veterinary Teaching Hospital (University of Parma)

Case number	Progressive number	Breed	Age (month)	Sex
1116	1	English Pointer	71	M
1155	2	Czechoslovakian Wolfdog	47	F
1320	3	Mongrel dog	88	M
1343	4	Dachshund	103	M
392	5	Labrador retriever	160	M
1338	6	Bernese Mountain Dog	28	F
4026	7	Miniature Poodle	15	M
1376	8	Mongrel dog	131	F
1401	9	English Setter	33	M
1396	10	German Shepherd Dog	100	F
1294	11	English Bull terrier	98	M
1530	12	German Shepherd Dog	28	M
1335	13	German Shepherd Dog	167	F
636	14	Mongrel dog	123	M
1552	15	English Setter	154	M
371	16	Mongrel dog	100	F
1958	17	Dalmatian	167	F
1944	18	Kurzhaar	47	M
2075	19	Rhodesian ridgeback	42	M
2073	20	English Setter	5	M
2133	21	Lagotto Romagnolo	160	F
2116	22	German Shepherd Dog	23	M
2117	23	English Setter	55	M
2118	24	Mongrel dog	29	F
2127	25	Mongrel dog	14	F
2219	26	Rottweiler	117	M
2175	27	Mongrel dog	160	F
2426	28	English Cocker spaniel	80	F
3593	29	Rottweiler	76	M
3625	30	Golden retriever	14	M
1294	31	English Bull terrier	99	M
2050	32	Kurzhaar	50	F

M= male F= female

Blood Parameters Blood parameters to be compared have been chosen on the basis of the suspected diagnosis. Plasma were tested for the same parameters with both analytical instruments. Both procedures (wet and dry chemistry) were conducted utilizing quality control serum at the end of the work session. Blood samples analyzed were about 40, but some of them (about 8) were hemolytic or lipemic, and were not included in the study to be sure that the pre-analytical error should not invalidate the results.

Statistical analysis Statistical tests used in this trial and their interpretation can result different respect to studies research conducted in other research areas owint to the high intrinsic variability of the biological contexts. Statistical tests utilized were: t-student test (*t*-test for paired samples, with significance o $p < 0.005$), linear regression, regression line and coefficient of determination (r^2) to evaluate if results obtained with the two different methods overlapping.

The *t* test, like many statistical tests, assumes that you have sampled data from populations that follow a Gaussian bell-shaped distribution. The paired *t* test compares two paired groups. It calculates the difference between each set of pairs, and analyzes that list of differences based on the assumption that the differences in the entire population follow a Gaussian distribution (http://graphpad.com/articles/interpret/corl_n_linear_reg/correlation.htm)

The coefficient of correlation, r, quantifies the direction and magnitude of correlation (http://graphpad.com/articles/interpret/corl_n_linear_reg/correlation.htm) The correlation coefficient, r, ranges from -1 to +1.

Table 2: Interpretation of the value of the coefficient r

Value of r (or rs)	Interpretation
r= 0	The two variables do not vary together at all.
0 < r < 1	The two variables tend to increase or decrease together.
r = 1.0	Perfect correlation.
-1 < r < 0	One variable increases as the other decreases.
r = -1.0	Perfect negative or inverse correlation

The coefficient of determination, R2, is, perhaps the best way to interpret the value of r, squaring it to calculate R2. Statisticians call this quantity the coefficient of determination, but scientists call it r squared. It is has a value that ranges from zero to one, and is the fraction of the variance in the two variables that is shared. For example, if r2=0.59, then 59% of the variance in X can be explained by variation in Y. Likewise, 59% of the variance in Y can be explained by (or goes along with) variation in X. More simply, 59% of the variance is shared between X and Y. It's values determines how closely data conform to a linear relationship and show you how accurate the

regression line is.

The **boxplot (box-whisker plot)** is a quick way of examining one or more sets of data graphically. Boxplots may seem more primitive than a histogram but they do have some advantages. They take up less space and are therefore particularly useful for comparing distributions between several groups or sets of data. For our purpose, the use of the boxplots state and stress the difference between the group of data (and their mean) obtained with the two methods.
Statistical analysis was performed by Ministat (Marco Besozzi, Ministat 2.1 www.bayes.it)

Results

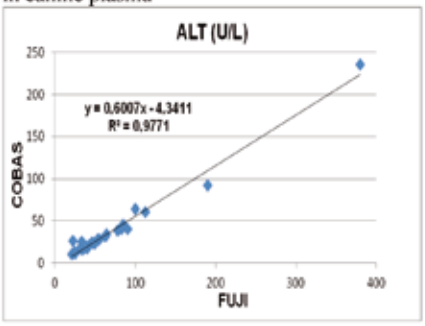
The average age of the dogs which took part in the study (19 males and 13 females) was 81 months.
In Table 3, analytical results obtained in canine plasma processed by both instruments are summarized. Empty fields represent samples in which some trouble has occurred (i.e., breaking tube) or parameter was not requested by the clinician.

Table 3 Comparison between biochemical clinical plasma values in parameters determined by Roche Cobas Integra 400 (Cobas) and Fuji Dri-chem 400 (Fuji) in 32 clinical cases (dogs)

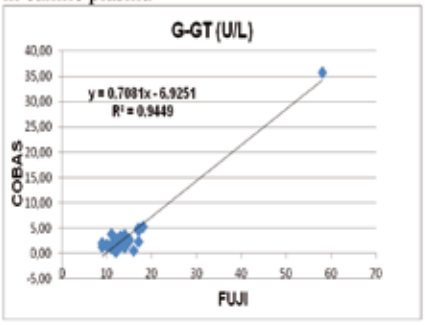
parameter	ALT		G-GT		ALP		Glucose		Urea		Creatinine		Total bilirubin		Total proteins		Albumin	
Units	(U/L)		(U/L)		(U/L)		(mg/dl)		(mg/dl)		(mg/dl)		(mg/dl)		(g/dl)		(g/dl)	
Clinical cases (32 dogs)	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS	FUJI	COBAS
1	36	17	10	1,80	116	24	118	132	31,24	26,80	0,50	0,35	0,50	0,15	5,70	6,37	3,40	2,79
2	90	40	11	3,80	97	22	85	88	41,30	44,30	1,00	0,82	0,50	0,11	6,40	6,78	4,10	3,69
3	190	92	14	3,60	181	35	73	105	30,60	27,10	0,80	0,78	0,50	0,14	5,90	6,27	3,50	3,01
4	40	17	13	1,80	128	20	86	108	25,68	20,40	0,70	0,70	0,40	0,14	6,30	7,35	3,50	3,21
5	100	64	17	4,50	541	105	83	97	78,11	76,90	1,40	1,53	0,50	0,10	6,00	7,41	3,20	2,85
6	63	31	15	2,80	352	67	93	102	33,60	31,50	1,10	1,14	0,50	0,19	6,60	7,18	4,10	3,76
7	37	18	13	3,30	116	22	65	95	60,78	54,10	0,60	0,63	0,40	0,03	5,60	5,95	3,60	3,54
8	55	28	17	2,30	512	100	97	101	44,08	30,20	0,60	0,61	0,70	0,09	6,70	7,30	3,80	3,50
9	24	13	13	1,70	925	245	4	56	17,76	12,80	0,50	0,39	0,50	0,07	7,20	8,64		
10	390	235	56	35,70	1484	450	38	90	29,96	30,00	0,60	0,59	2,30	2,49	5,20	5,82	2,80	2,56
11	33	25	17	4,90	135	22	92	115	42,59	28,50	0,60	0,54	0,50	0,03	6,30	6,95	3,10	2,72
12	22	26	14	2,80	110	24	96	109	54,57	45,80	1,10	0,83	0,60	0,02	4,90	5,37	1,90	1,64
13	63	40	11	1,20	504	135	89	83	91,16	76,40	2,70	1,99	0,90	0,47	6,80	7,22	3,30	2,97
14	112	60	18	5,20	1140	200	81	81	221,92	190,10	1,60	1,22	0,50	0,07	5,80	6,35	3,50	3,28
15	31	17	10	1,40	29	17	118	147	96,09	97,20	0,70	0,54	0,90	0,02	4,30	5,70	2,80	2,95
16	69	22	15	2,50	164	37	40	102	35,31	34,70	1,00	0,83	0,60	0,08	6,90	7,18	3,80	3,27
17	21	10	11	1,00	363	67	60	88	27,39	29,40	0,90	0,87	0,40	0,10	5,10	6,03	3,00	3,24
18	42	21	11	1,80	235	53	84	114	37,45	38,20	0,60	0,59	0,40	0,01	5,40	5,94	3,00	2,93
19	64	34	9	1,30	64	7	77	87	42,37	43,70	0,90	0,87	0,60	0,09			3,70	3,72
20	25	11	12	0,90	239	51	75	119	18,40	17,40	0,50	0,50	0,40	0,01	6,20	7,19	3,80	3,63
21	53	28	12	1,90	34	19	91	95	37,02	33,80	0,30	0,42	0,40	0,10	5,00	6,08	2,90	2,84
22			14	3,10	91	31	77	81	40,23	56,60	0,80	1,03	0,50	0,07	7,30	7,35	4,00	3,24
23	63	32	12	1,40	84	17	51	68	35,52	35,50	0,70	0,79	0,50	0,15	7,80	8,58	4,00	3,76
24			16	0,60	139	19	66	95	53,71	41,00	1,10	0,78	0,60	0,08	6,90	7,71	3,80	3,80
25	46	24	14	2,40	89	23	67	97	63,13	65,40	1,10	1,17	0,50	0,11	6,10	6,63	3,70	3,07
26	85	45	9	2,00	78	19	24	101	19,47	20,30	0,60	0,69	0,80	0,31	6,20	6,76	3,50	3,13
27	83	42	14	1,10	429	85	80	87	32,60	31,00	0,70	0,73	0,50	0,13	7,30	7,70	4,00	3,85
28	38	20	12	2,90	75	14			21,40	20,10	0,70	0,71	0,50	0,21	5,60	6,22		
29	34	15	13	1,50	190	43	91	113	62,49	40,70	1,20	1,27	0,50	0,06	7,80	7,37	3,90	3,99
30	78	38	12	1,10	211	43	104	100	32,31	20,50	0,80	0,82	0,40	0,12	7,80	6,34	3,90	2,75
31									24,18	29,40	0,60	0,60						
32															6,50	7,10	3,70	3,45

In the graphics 1 to 9, linear regression and r squared for the two methods are reported.

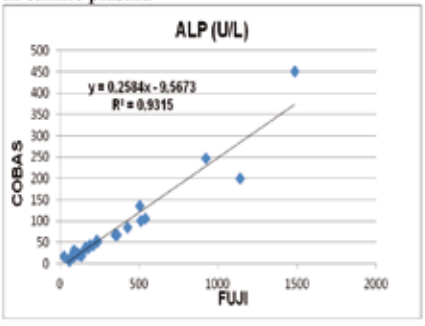
Graphic 1 Regression line and r squared (R^2) of ALT values determined by Cobas and Fuji in canine plasma



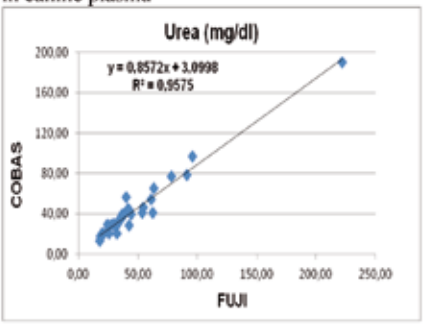
Graphic 2 Regression line and r squared (R^2) of G-GT values determined by Cobas and Fuji in canine plasma



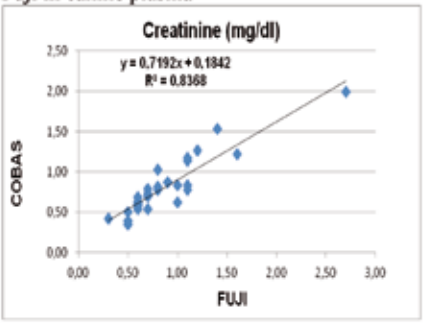
Graphic 3 Regression line and r squared (R^2) of ALP values determined by Cobas and Fuji in canine plasma



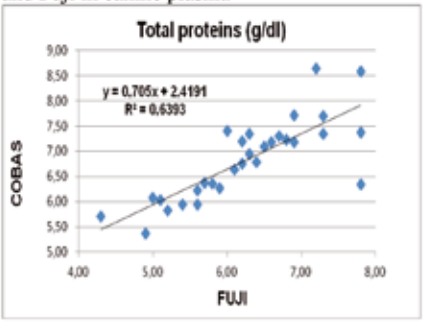
Graphic 4 Regression line and r squared (R^2) of Urea values determined by Cobas and Fuji in canine plasma



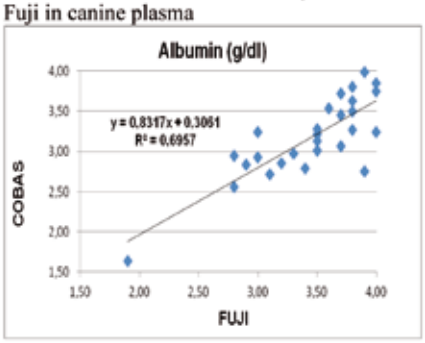
Graphic 5 Regression line and r squared (R^2) of Creatinine values determined by Cobas and Fuji in canine plasma



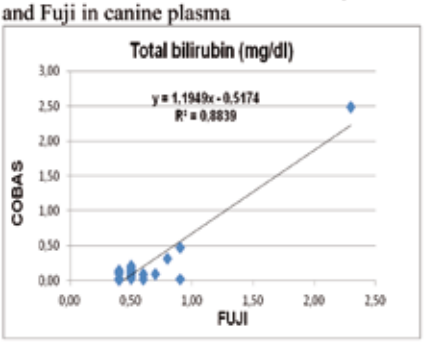
Graphic 6 Regression line and r squared (R^2) of Total proteins values determined by Cobas and Fuji in canine plasma



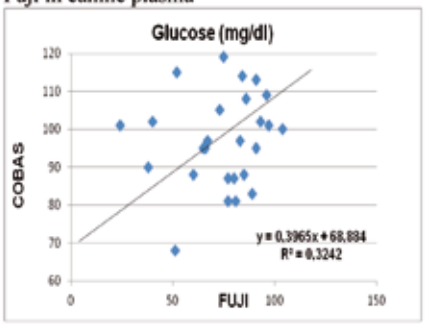
Graphic 7 Regression line and r squared (R^2) of Albumin values determined by Cobas and Fuji in canine plasma



Graphic 9 Regression line and r squared (R^2) of Total bilirubin values determined by Cobas and Fuji in canine plasma



Graphic 8 Regression line and r squared (R^2) of Glucose values determined by Cobas and Fuji in canine plasma

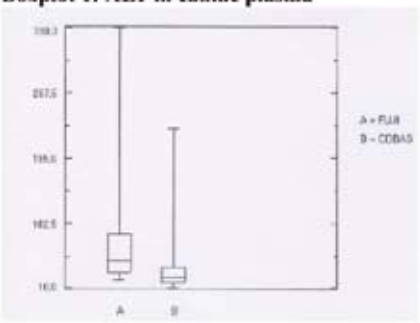


To ameliorate the comprehension of the comparison of the two methods and to stress the statistical significance of the results, in Table 4 expression of regression line and *p* values are reported .

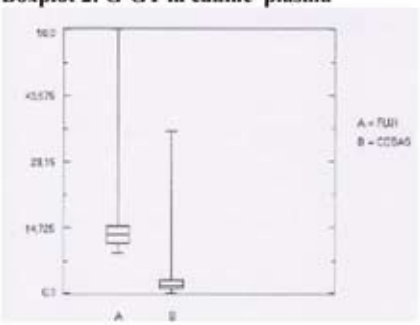
Table 4 Synopsis of the values of R^2 and *p* obtained by the comparison between data by Cobas and Fuji apparatus

Parameter	Linear Regression Line	R^2	*p*
ALT	y=0,6007x -4,3411	0,9771	0,00016
GGT	y=0,7081x -6,9251	0,9449	<0,00001
ALP	y = 0,2584x - 9,5673	0,9315	0,00003
Urea	y = 0,8604x + 2,7587	0,9582	0,02399
Creatinine	y = 0,7185x + 0,1854	0,8348	0,08474
Total proteins	y = 0,704x + 2,4223	0,6383	<0,00001
Albumin	y = 0,8317x + 0,3061	0,6957	0,00042
Total bilirubin	y = 1,1949x - 0,5174	0,8839	<0,00001
Glucose	y = 0,3965x + 68,884	0,3242	<0,00001

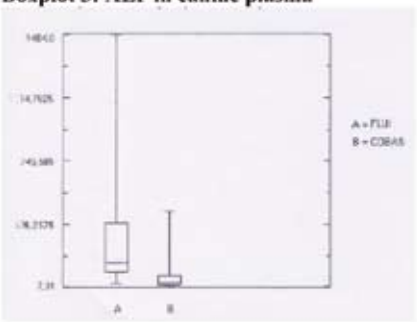
Boxplot 1: ALT in canine plasma



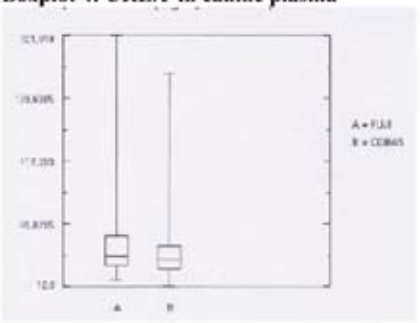
Boxplot 2: G-GT in canine plasma



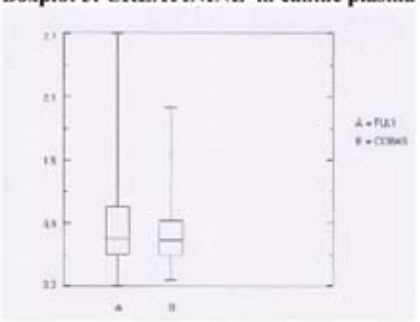
Boxplot 3: ALP in canine plasma



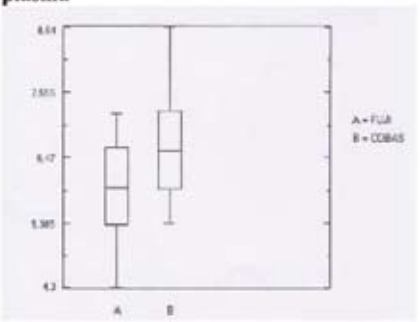
Boxplot 4: UREA in canine plasma



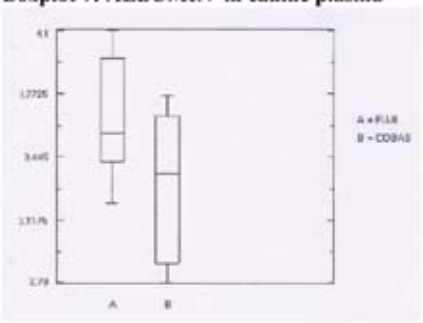
Boxplot 5: CREATININE in canine plasma



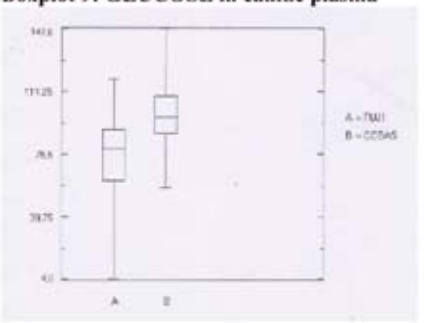
Boxplot 6: TOTAL PROTEINS in canine plasma



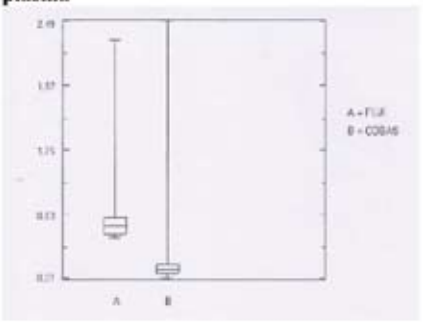
Boxplot 7: ALBUMIN in canine plasma



Boxplot 9: GLUCOSE in canine plasma



Boxplot 8: TOTAL BILIRUBIN in canine plasma



In the boxplots 1 to 9, each parameter, measured with the two methods,is represented by a box with two whiskers: the bottom and top of the box are always the 25th and 75th percentile (the lower and upper quartile, respectively), and the band near the middle of the box is always the 50th percentile (the median). The ends of the whiskers represent the minimum and maximum of all the data.
The boxplot is a rapid way to represent the difference between the "traditional" and the "new" method, to evaluate analytical performances of both methods employed, and the possibility to exchange them without invalidateting the diagnosis.
The use of boxplots demonstrated that results are overlapping for some parameters, while for other, replacing a method with the other can lead to a wrong diagnosis. Moreover, this supports the necessity to determine new reference values for each "new" method introduced in laboratory practice.

DISCUSSION

Afterward, discussion about parameters is reported:

- ALT (Alanine-Aminotranspherase). Measured values of ALT show a good correlation between measured values ($R^2 = 0.9971$). As shown by the boxplot 1, with "Fuji" the enzymatic activity of ALT is higher than the enzymatic activity measured with "Cobas". *p* values equal to 0,00016 indicates that the different is statistical significant.
- G-GT (Gamma Glutamil Transpeptidase). Same observations can be made for the enzyme G-GT. In graphic 2, value of R^2 equal to 0.9449 states for a goodcorrelation between the two methods, but the analytical values are statistical different ($p<0,0001$). In boxplot2 this difference is graphically reported, showing that "Fuji" furnishes G-GT concentrations more elevated and more dispersed respect to "Cobas".
- ALP (Alkaline Phosphatase). In graphic 3, values of ALP enzymatic activity are reported. Value of r^2 equal to 0,9315 states for a good correlation between the two methods, but the analytical values are statistical different ($p=0,00003$). In boxplot3, this difference is drawn, showing that "Fuji" furnishes G-GT concentrations more elevated and more dispersed respect to "Cobas".
- UREA and CREATININE. In graphic 4 and 5, regression line ad values of Urea and Creatinine are reported. Value of R^2 equal to 0,9582 for Urea and 0,8348 for Creatinine states for a good correlation between methods. Otherwise respect to previous parameters, the analytical values are not statistical different ($p=0,02399$ and 0,08474 respectively). Boxplots 4 and 5 show the partial overlapping of analytical results for Urea and Creatinine.
- TOTAL PROTEINS AND ALBUMIN. These parameters, represented in graphics 6 and 7, have lower values of R^2 (0,6383 and 0,6957, respectively) and statistical significant differences ($p<0,00001$ and $p=0,00042$). The exam of boxplots 6 and 7 stresses that "Fuji" furnishes higher plasma total proteins concentrations and lower plasma albumin concentrations. Consequently, concentrations of globulins mathematically obtained (total proteins-albumin) are quite different. On this bases, the accuracy of calculated globulins is insufficient within laboratory diagnosis
- TOTAL BILIRUBIN. "Fuji" measures total bilirubin furnishing results with only one decimal, it is difficult to compare values with the "Cobas", that provides results with two decimals. In pets and ruminants, the methods in dry chemistry for total bilirubin is forcedly approximate and limited in the lesser values of the limit of detection. From this point of view, the comparison between the two analytical methods used is not practicable.
- GLUCOSE. For this parameter, the R^2 and *p* values demonstrate that the results obtained by the two methods are statistical different. To confirm this observation, the boxplot 9 examination stresses that values do not overlap.

CONCLUSION

Close examination of the statistical results of the analytical data shows that the two compared methods perform in the same manner for ALT, G-GT, ALP, Urea and Creatinine. For the other parameters testes (Total proteins, Albumin, Total bilirubin and Glucose) statistical significant differences were observed. Clinicians can easily go over these differences if specific normal interval range for the two methods are available. Laboratory diagnosis should be reliable only if this conditions should be complied. This ensure that laboratory diagnosis should be a reliable tool to confirm clinical diagnosis and to follow up the prognostic process.

REFERENCE

1. L. Jensen, M.Kjelgaard-Hansen. Method comparison in the clinical laboratory Vet. Clin. Path. 35,3, 276–286, September 2006.
2. Ubaldi A., Corbella E. Montanari P. Diagnostica chimico clinica veterinaria Ed. Ambrosiana, 1982.
3. http://www.fujifilm.com/products/medical/products/clinical_chemistry_system/fdc_4000i/
4. Cacchioli G., The laboratory diagnostics in dogs. Comparison between analytical methods and clinical responses. Degree thesis, 29 sett. 2011, Fac. Med. Vet., Univ. di Parma (Italy).
5. A. Fusari, F. Quintavalla, A. Ubaldi. Confronto tra le performance di tre metodiche analitiche in clinica Proc. of XII Congr Naz S.I.Di.L.V., vol XII, 241, Genova 27 - 29 Ottobre 2010.
6. Fusari, F. Quintavalla, A. Ubaldi. Esecuzione del profilo minerale in bovine da latte con una nuova metodica dry chemistry. Proc. of XII Congr Naz S.I.Di.L.V., vol XII, 243, Genova 27 - 29 Ottobre 2010.
7. www.bayes.it

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MOLECULAR TOXINOTYPING OF CLOSTRIDIUM PERFRINGENS CATTLE ISOLATES BY MULTIPLEX PCR

TIPIZZAZIONE MOLECOLARE DI CEPPI DI CLOSTRIDIUM PERFRINGENS ISOLATI DA BOVINI MEDIANTE MULTIPLEX PCR

Zerbini Laura, Ossiprandi Maria Cristina [1]

STRUCTURED SUMMARY

Objectives

Clostridium perfringens may be considered one of the most widespread pathogen, inhabiting the gastrointestinal tract of human beings and animals as well as terrestrial and marine environments. The objective of this study was to investigate the toxin gene profile of cattle samples by PCR assays.

Methods

A total of 104 faecal and intestine specimens were analyzed by culture assay. Overall, 19 samples (18.3%) were positive for *C. perfringens* presence and were screened for the identification of enterotoxigenic strains and the characterization of the toxinotype by (a) a duplex PCR, which amplifies simultaneously the phospholipase C gene (*plc*) and the enterotoxin gene (*cpe*) fragments, and (b) a multiplex PCR, which detect alfa, enterotoxin, beta, epsilon, iota, and beta2 (*cpa*, *cpe*, *cpb*, *etx*, *iap*, and *cpb2*) toxin-encoding genes.

Results

All isolates were type A (*cpa/plc*-positive) and *cpe*-negative by PCR assays. In 2 samples, *C. difficile* was also isolated, resulting *tcdA* and *tcdB* negative. Other 14 samples, *C. perfringens*-negative, resulted *C. difficile*-positive (*tcdA* and *tcdB* negative) by culture assay.

Clinical Significance

We can conclude that the molecular toxinotyping by PCR may provide an useful and reliable tool for *C. perfringens* routine veterinary diagnostics and important information about the distribution of genotypes.

KEYWORDS

Clostridium perfringens, diarrhoeic/non-diarrhoeic, toxin gene profile, PCR assays

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INTRODUCTION

Clostridium perfringens is a Gram-positive, spore-forming, anaerobe bacterium and an important human and veterinary enteropathogenic agent. Owing to its ability to produce spores under adverse environmental conditions, it is one of the most widespread potential bacterial pathogen in nature as well as in the gastrointestinal tract of most animal species (Marks and Kather 2003; van Asten 2010). It has been associated with outbreaks of acute, often severe diarrhoea in humans, horses, dogs and cats. It causes gas gangrene and food poisoning in humans and several enterotoxaemic diseases in domestic (Rood 1998) and wild animals (Uzal and Songer 2008).

The production of four major toxins alpha (α, CPA), beta (β, CPB), epsilon (ε, ETX) and iota (ι, ITX) allows the discrimination into five types from A to E (Schotte *et al* 2004; Lebrun *et al* 2007). This bacterium can produce at least 15 different toxins, helping to explain its ability to cause a broad array of enteric and histotoxic infections (Table 1) (Songer and Miskimins 2005; Lebrun *et al* 2007).

In veterinary medicine, each types has been linked to specific diseases (Schotte *et al* 2004). *C. perfringens* enterotoxin (CPE) serves as an additional virulence factor which is believed to cause enteritis in pigs, cats and dogs (Schotte *et al* 2004).

In 1997, a formerly unknown toxin of *C. perfringens* and its encoding gene (originating from a strain isolated from a piglet suffering from necrotic enteritis) were described. The 27.6 kDa toxin was initially thought to be a digestion product of the 34.8 kDa β-toxin (Lebrun *et al* 2007) but its amino acid sequence showed no significant homology with the sequence of the β-toxin (only 15% identity) or with any other known protein sequence. The newly described toxin was nevertheless termed β2 and its encoding gene *cpb*2, since the toxin displayed similar biological activity as the β-toxin (Lebrun *et al* 2007).

The CPB2 is postulated to play a role in *C. perfringens* neonatal enteritis in pigs (Gibert *et al*, 1997), typhlocolitis in horses and dogs, cattle enterotoxemia and gastrointestinal disease in man (Lebrun *et al* 2007).

Type A strain, that produces only CPA among the major toxins, is a member of the normal flora of warm-blooded animals and is recovered from environment contaminated by faeces. However, when properly genetically equipped and placed in opportune situations, it can cause gas gangrene, food poisoning, and gastrointestinal illness in humans, necrotic enteritis in chickens, necrotizing enteritis in piglets, and abomasitis, tympany, and haemorrhagic enteritis in calves (Ferrarezi *et al* 2008). Gram-positive bacilli are often found on the mucosa and in the submucosa (Songer and Miskimins 2005).

Strains of toxin type B-E are less common in the intestinal tracts of animals and can occasionally be found in the environment in areas where the diseases produced by these organisms are enzootic. They are, thus, considered frank pathogens (Ferrarezi *et al* 2008). Enteritis and enterotoxemia can be produced in calves by all *C. perfringens* toxinotypes.

Clostridial infections of the gastrointestinal tract of calves remain a common problem, in spite of the widespread availability of effective immunoprophylactic products. Neonatal infection by *C. perfringens* type C is widely recognized, and, like other infections by toxin types of *C. perfringens*, type C may colonize rapidly

in the absence of established normal flora (Songer and Miskimins 2005). Robust, healthy calves, usually aging less than 10 days, develop haemorrhagic, necrotic enteritis and enterotoxemia, often with abdominal pain and nervous signs. Death may be sudden, or following a clinical course of several days. Beta toxin is the prime player in pathogenesis of type C infections. Damage to microvilli, mitochondria, and terminal capillaries precedes adherence of the organism to the jejunal mucosa. Widespread and progressive mucosal necrosis follows, and large numbers of Gram-positive bacilli can be demonstrated across vast areas of the mucosa. Death is due, ultimately, to beta toxaemia. The disease cannot be reproduced with toxin alone, but protection is primarily antitoxic (Songer and Miskimins 2005).

Prophylaxis and control of bovine neonatal diarrhoea is essential in cattle herds, and the primary approach to this has been vaccination. For this to be successful, the vaccine must be based upon strains which are clinically relevant. Thus, identification of *C. perfringens* toxinotypes and subtypes is critical for both epidemiological studies and for development and effective use of preventative measures, including vaccination (Ferrarezi *et al* 2008).

The classical typing method is based upon *in vivo* toxin neutralization, with appropriate antisera in laboratory animal models (Baums *et al* 2004), but it has disadvantages relating to expense, turnaround time, sensitivity, lack of a commercial source for antitoxic antibodies, use of animals (guinea pigs or mice). An alternative approach is toxin detection by immunologic or molecular methods. Moreover, enzyme immunoassays have a certain appeal, in that they eliminate the use of laboratory animals and they are rapid and easy to use (Ferrarezi *et al* 2008). Although the ELISA test allows reliable typing of this *C. perfringens* isolates, it does not detect the CPB2. In addition, high levels of enterotoxin CPE have been shown to be present during sporulation only. Nevertheless, the sporulation needs specific cultivation methods (Duncan and Strong 1986). These problems could be solved by genotyping *C. perfringens* strains. Besides, the major problem in use toxicological methods is due to the variability of toxin production *in vitro* by cultures of *C. perfringens*. Molecular genotyping methods, which are mainly PCR-based, including multiplex PCR assays, have became the standard for toxinotyping of *C. perfringens* (Baums *et al* 2004; Ferrarezi *et al* 2008).

The objective of this investigation was to evaluate by duplex and multiplex PCR assays the molecular toxin gene profile of *C. perfringens* isolates from 104 faecal and intestine cattle samples, collected in different farms of the area of Parma (Italy).

Table 1 Diseases caused by toxin types of Clostridium perfringens

Toxin type	Diseases	Major toxins
A	Myonecrosis, food poisoning, necrotic enteritis in fowl, enterotoxemia in cattle and lambs, necrotizing enterocolitis in piglets; possibly equine colitis, canine haemorrhagic gastroenteritis	Alpha
B	Dysentery in newborn lambs, chronic enteritis in older lambs ("pine"), haemorrhagic enteritis in neonatal calves and foals, haemorrhagic enterotoxemia in adult sheep	Alpha, beta, epsilon
C	Enteritis necroticans (pigbel) in humans, necrotic enteritis in fowl, haemorrhagic or necrotic enterotoxemia in neonatal pigs, lambs, calves, goats, foals, acute enterotoxemia ("struck") in adult sheep	Alpha, beta
D	Enterotoxemia in lambs ("pulpy kidney") and calves, enterocolitis in neonatal and adult goats, possibly enterotoxemia in adult cattle	Alpha, epsilon
E	Enterotoxemia likely in calves and lambs, enteritis in rabbits; host range and disease type unclear	Alpha, iota

Songer J. G. and Miskimins D.W. (2005). Anaerobe 11, 290-294

MATERIALS AND METHODS

Samples. A total of 104 samples (94 faeces and 10 intestines) were collected, using a stratified random sampling, from different farms in the area of Parma (Italy) during the period end of 2008 to end of 2010: 62 faeces belonged to non-diarrhoeic animals (25 dairy cows, 19 pregnant heifers, 13 heifers and 5 calves) and 41 faecal and intestine samples belonged to diarrhoeic subjects (27 calves, 11 cows and 3 heifers). Assays were performed on faecal and intestine specimens within 3 hours from the collection. After analysis, samples were immediately stored at -20°C.

Isolation and identification of *C. perfringens* strains. All faecal and intestinal samples were cultured onto prereduced blood agar plates, containing vitamine K, sheep blood and haemin (Schaedler agar, Oxoid**,** Basingstoke, Hampshire, England), and at the same time inoculated in cooked meat broth (Oxoid, England). Plates were incubated in anaerobic conditions at 37°C for 48-72 hours. After 72 hours of incubation in cooked meat broth, the samples were subjected to heat shock for spore selection and then cultured onto Schaedler agar. Isolates which were anaerobic, Gram-positive, rod-shaped, and produced a double zone of haemolysis on blood were preliminarily considered to be *C. perfringens*.

The species identification was confirmed through the Rapid ID32A assay (bioMérieux SA, Marcy-l'Etoile, France). All isolates were stored on cryopreservation beads (MAST Diagnostics, D.I.D, Diagnostic International Distribution S.p.A., Italy) at -70°C.

Reference strains. *C. perfringens cpa+/cpe+*, kindly provided by Istituto Superiore di Sanità in Rome (Italy), was utilized as positive control for duplex PCR. *C. perfringens* ATCC12917, NCTC8346, ATCC373, and ATCC27324 were used as *cpa+/cpe+*, *cpa+/etx+*, *cpa+/cpb+/cpb2+* and *cpa+/iap+/cpe+/cpb2+* controls, respectively, for multiplex PCR genotyping reactions.

DNA extraction. For each *C. perfringens* strain, a 100-µl suspension of cells in sterile water was vortexed, incubated at 100°C for 5 min., and centrifuged at 12,000 g (Microliter Centrifuge, Hermle Z 233 M-2, Delchimica Scientific Glassware s.r.l.) for 2 min. An aliquot (5 µl) of this extracted DNA was used as template in PCR genotyping.

Duplex PCR for the *C. perfringens* phospholipase C and CPE encoding genes. Two sets of primers, which amplify simultaneously the 283 bp *C. perfringens* phospholipase C gene (*plc*) and the 426 bp enterotoxin gene (*cpe*) fragments, were used as previously described by Fach and Popoff (1997). Amplified products were subjected to 1.5% agarose gel electrophoresis (120 V, 1 h) and visualized by ethidium bromide staining and ultraviolet light exposure.

Multiplex PCR for the *C. perfringens* toxins encoding genes. All *C. perfringens* isolates were also PCR-screened for the detection of alfa (*cpa*), beta (*cpb*), epsilon (*etx*), enterotoxin (*cpe*), iota (*iap*), and beta2 (*cpb*2) toxin encoding genes, as described by Baums et al. (2004). The reaction products were subjected to agarose gel electrophoresis as mentioned above.

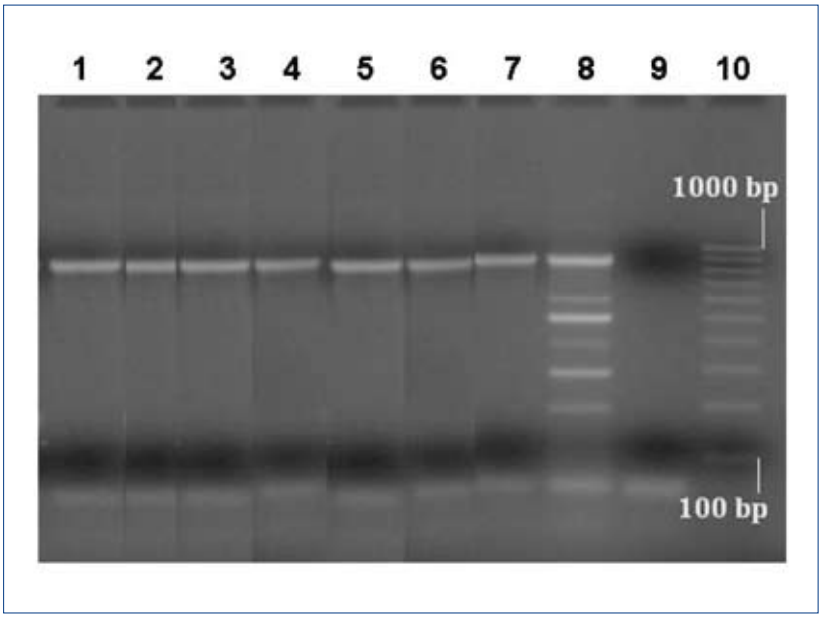
RESULTS

Overall, 19 (18.3%, 95% C.I. 11.7% to 26.6%) of the 104 samples resulted *C. perfringens* positive by culture analysis. In one calf, *C. perfringens* was isolated from the faecal and the intestine sample. All *C. perfringens*-positive specimens belonged to diarrhoeic animals. In 2 faecal samples of calves, *C. difficile* was also isolated. These strains resulted *tcdA* and *tcdB* negative by the duplex PCR, previously described by Spigaglia and Mastrantonio (2002). Other 14 samples, *C. perfringens*-negative by culture assay, were positive for *C. difficile* (*tcdA* and *tcdB* negative). Out of the 16 *C. difficile* isolates, 14 (87.5%) belonged to non-diarrhoeic dairy cows.

All *C. perfringens* isolates were type A (*cpa/plc*-positive) by duplex and multiplex PCRs. All samples (15 faeces and 4 intestines) belonged to diarrhoeic animals (15 calves, 2 cows and 1 heifer). Sixteen of the 19 *C. perfringens*-positive isolates (84.2%) were from calves. In particular, 55.6% of diarrhoeic calves (n=15 of 27) yielded isolates of *C. perfringens*.

None of the 19 strains were enterotoxin-positive (*plc+/cpe-*) by duplex PCR. This result was confirmed by multiplex PCR assay (*cpa+/cpe-*), where other toxin genes weren't revealed (Figure 1).

Figure 1: Multiplex PCR typing of selected *Clostridium perfringens* isolates
Lanes 1-7: type A strains (cpa+); lane 8: *C. perfringens* positive control (cpa+/cpb+/cpe+/etx+/iap+/cpb2+); lane 9: negative control ("0 DNA"); lane 10: molecular size markers (100 bp Molecular Ruler, Biorad, Italy).



DISCUSSION

C. perfringens is commonly found in soil samples and is also readily found in intestinal contents of healthy humans and other animals (Ferrarezi *et al* 2008).
C. perfringens type A, that is often responsible for gas gangrene (myonecrosis) and foodborne illness in humans (Ferrarezi *et al* 2008), produces CPA but can also produce several of the non-typing toxins, including CPE and CPB2. CPE is responsible for human gastrointestinal disease and also has been associated with diseases in dogs, horses, pigs, and several other animal species, including one case of enteritis caused by *C. perfringens* type A *cpe*-positive in a goat (Uzal *et al* 2008). Isolation of non-enterotoxigenic type A strains from a diarrhoeic specimen does not preclude involvement in disease, because there is a plethora of other virulence factors that have not been evaluated. One of these virulence factors is the recently characterized *C. perfringens* CPB2, which has been also associated with both necrotic enteritis in piglets and equine typhlocolitis (Bueschel *et al* 2003; Baums *et al* 2004).
In this study, about 55.6% of diarrhoeic calves (n=15 of 27) yielded isolates of *C. perfringens*, while no isolation was obtained from the 5 non-diarrhoeic calves tested. This difference is statistically significant (one-tailed Fisher's P= 0.031). This high rate of recovery from diseased animals is not surprising, because they had diarrhoea

and strains of type A are found normally in the intestine, even in neonates, and some contend that isolation of these organisms from calves with enteritis should not be given etiologic significance. However, it is useful to view this from the perspective that types B-E are defined by production of beta, epsilon, and iota toxins, while type A is a repository for all those strains which do not produce these toxins. Insufficient attention has been given to the possible action of alpha toxin in the gut. Furthermore, production of other potential virulence factors might set apart groups of isolates associated with specific syndromes which are now considered to be idiopathic. It is also possible that strains normally resident in the gut produce the observed infections into upper small intestine. There is little concrete information on the pathogenesis of type A enteric infections in calves. Disease is sometimes compatible with the action of a haemolytic toxin in the circulation, causing intravascular haemolysis and capillary damage, inflammation, platelet aggregation, shock, and sometimes-fatal cardiac effects.

Alpha toxin can sometimes be found in the intestinal contents of animals with naturally occurring enteric disease, and is an important factor in pathogenesis of myonecrosis. Little is known of the permeability of the intestine to alpha toxin, but it may be a virulence factor in cases of presumed enterotoxemia (Songer and Miskimins 2005).

Moreover, we assessed the toxinotype of the 19 (18.3%) *C. perfringens* isolates from 104 diarrhoeic and non-diarrhoeic faecal and intestine samples.

All 19 *C. perfringens* strains were isolated, as above mentioned, from diarrhoeic subjects and they were type A by multiplex PCR, containing amplicons of *plc*/*cpa*, corroborating the results of others (Ferrarezi *et al* 2008). None of isolates contained *cpe* or others toxin-encoding genes. No strain of *C. perfringens* type A CPB2 toxin gene positive was isolated.

All non-diarrhoeic dairy cow samples resulted *C. perfringens*-negative, but *C. difficile* was frequently isolated (22.6%: n=14 of 62).

In conclusion, multiplex PCR assay for the detection of major toxin-encoding genes of *C. perfringens* is highly specific, and more rapid and considerably less expensive than the mouse neutralization test or other *in vivo* methods. Moreover, the PCR assay eliminates the humanitarian concerns which accompany animal-based testing, and eliminates concerns about lack of availability of standard anti-toxins. PCR genotyping (Uzal *et al* 2008) provides a useful alternative to *in vivo* toxin neutralization tests for typing of *C. perfringens* isolates and can detect the CPB2 gene.

Multiplex PCR may provide an useful and reliable tool for *C. perfringens* genotyping in routine veterinary diagnostics, providing the final piece of information needed to establish a diagnosis. The identification of *C. perfringens* toxinotypes can be critical for epidemiological studies, prophylaxis programs, and to formulate strategies for correct use of vaccines. However, a phenotypic characterization is required before drawing conclusions about the functionality of a particular toxin in the disease.

At present, the most prominent needs are consistent reproduction of the disease, elucidation of virulence attributes, and development and application of prevention and control strategies (Songer and Miskimins 2005).

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REFERENCES

1. Baums C.G., Shotte U., Amtsberg G., Goethe R. (2004): Diagnostic multiplex PCR for toxin genotyping of *Clostridium perfringens* isolates. *Veterinary Microbiology* 100, 11-16.
2. Bueschel D.M., Jost B.H., Billington S.J., Trinh H.T., Songer J.G. (2003): Prevalence of *cpb*2, encoding beta2 toxin, in *Clostridium perfringens* field isolates: correlation of genotype with phenotype. *Veterinary Microbiology* 94, 121-129.
3. Duncan C.L., Strong D.H. (1986): Improved medium for sporulation of *Clostridium perfringens*. *Journal of Applied Microbiology* 16, 82-89.
4. Fach P., Popoff M.R. (1997): Detection of enterotoxigenic *Clostridium perfringens* in food and fecal samples with a duplex PCR and the slide latex agglutination test. *Applied and Environmental Microbiology* 63, 4232-4236.
5. Ferrarezi M.C., Cardoso T.C., Dutra I.S. (2008): Genotyping of *Clostridium perfringens* isolated from calves with neonatal diarrhea. *Anaerobe* 14, 328-331.
6. Gibert M., Jolivet-Reynaud C., Popoff M.R. (1997): Beta2 toxin, a novel toxin produced by *Clostridium perfringens*. *Gene* 203, 65-73.
7. Lebrun M., Filée P., Mousset B., Desmecht D., Galleni M., Mainil J.G., Linden A. (2007): The expression of *Clostridium perfringens* consensus beta2 toxin is associated with bovine enterotoxemia syndrome. *Veterinary Microbiology* 120, 151-157.
8. Marks S.L., Kather E.J. (2003): Bacterial-associated diarrhea in the dog: a critical appraisal. *Small Animal Veterinary Clinic* 33, 1029-1060.
9. Rood J.I. (1998): Virulence genes of *Clostridium perfringens*. *Annual Review of Microbiology* 52, 333-360.
10. Schotte U., Truyen U., Neubauer H. (2004): Significance of β2-toxigenic *Clostridium perfringens* infections in animals and their predisposing factors - A Review. *Journal of Veterinary Medicine* 51, 423-426.
11. Songer J.G., Miskimins D.W. (2005): Clostridial abomasitis in calves: Case report and review of the literature. *Anaerobe* 11, 290-294.
12. Spigaglia P., Mastrantonio P. (2002): Molecular analysis of the pathogenicity locus and polymorphism in the putative negative regulator of toxin production (TcdC) among *Clostridium difficile* clinical isolates. *Journal of Clinical Microbiology* 40, 3470-3475.
13. Uzal F.A., Songer J.G. (2008): Diagnosis of *Clostridium perfringens* intestinal infections in sheep and goats. *Journal of Veterinary Diagnostic Investigation* 20, 253-265.
14. Uzal F.A., Fisher D.J., Saputo J., Sayeed S., McClane B.A., Songer G., Trinh H.T., Fernandez Miyakawa M.E., Gard S. (2008): Ulcerative enterocolitis in two goats associated with enterotoxin- and beta2 toxin-positive *Clostridium perfringens* type D. *Journal of Veterinary Diagnostic Investigation* 20, 668-672.

15. van Asten A.J.A.M., Nikolaou G.N., Gröne A. (2010): The occurrence of *cpb2*-toxigenic *Clostridium perfringens* and the possible role of the β2-toxin in enteric disease of domestic animals, wild animals and humans. *Veterinary Journal* 183, 135-140.

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THE EFFECTS OF SLAUGHTERING METHODS OF RAINBOW TROUT ON ANIMAL WELFARE AND FISH QUALITY: RECENT ADVANCES

EFFETTI DELLE TECNICHE DI MACELLAZIONE DELLA TROTA IRIDEA SUL BENESSERE ANIMALE E QUALITA' DELLA CARNE: RECENTI ACQUISIZIONI

Zanardi Emanuela[1], Soldo Egidio[2], Ghidini Sergio[1], Ianieri Adriana[1]

ABSTRACT

The welfare aspects of the main systems of stunning and killing of farmed rainbow trout, with particular emphasis to the risk assessment carried out by the European Food Safety Authority, and the recent advances of the effects of the slaughter management on product quality are reviewed.

KEYWORDS

rainbow trout; slaughtering methods, animal welfare, fish quality

INTRODUCTION

Italian aquaculture production has grown steadily over the years and in 2004 the total production was estimated at 232.800 t. The mussel segment accounted for around 70% in volume of the total national production, followed by freshwater and euryhaline species sectors which represented about 20% and 9%, respectively (FAO 2006).

Rainbow trout (*Oncorhynchus mykiss*) is native to the Pacific drainages of North America. Since 1874 it has been introduced into the waters on all continents except Antarctica for recreational angling and aquaculture purposes. Production greatly expanded in the 1950s as pelleted feeds were developed (FAO 2005). Rainbow trout is the most important freshwater farmed species in Italy; in 2007 about 40.000 t were produced (API 2007). In some areas of Italy the rainbow trout chain has a significant economic impact. Friuli Venezia Giulia, for example, is an Italian region historically well suited for trout farming thanks to climatic conditions and large water availability. At present Friuli Venezia Giulia houses 70 manufacturing plants and in 2010 it has funded a two-year research project with the aim to characterize and bring out the rainbow trout farmed in the region, and to contribute to the development of a standard of certification (Stefanutto 2011).

Many present commercial methods of slaughtering fish cause stress and aversive behaviour. This is specially true for rainbow trout which are usually slaughtered by removal from water resulting in death by anoxia. In the context of fish farming this

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slaughter method has become less acceptable both from a welfare perspective and in terms of providing consistent and potentially improved flesh quality characteristics. This paper describes the welfare aspects of the main systems of stunning and killing of farmed rainbow trout, with particular emphasis to the risk assessment carried out by the European Food Safety Authority (EFSA), and reviews the recent advances of the effects of the slaughter management on product quality.

THE PRE-SLAUGHTER PROCESS

In Europe trout farming production is performed in a wide variety of production systems, and also in a wide variety of small and medium enterprises. Their annual production ranges from family businesses with less than 20 t of portion-size trout, up to companies producing several thousands of tons. A considerable amount of the trout produced in fresh water is marketed directly, i.e. the fish are killed on site and not in a processing plant (EFSA 2009a).

The slaughtering of freshwater farmed trout may consist of different steps, and these mainly depend on the type of farming system, technical equipment used in the plant, and market demand. There are a lot of different methods for catching trout for stun/kill purposes. In some small and medium enterprises, these procedures are performed on site. In others, trout are transported alive to a separate processing plant. In production systems using fresh water cages, pre-slaughter activities involve cage harvest followed by transport to a holding unit or a processing plant where stunning and killing take place; killing may occur also at the farming site. Holding units are used for direct marketing, where fish often are kept in high densities in small ponds or tanks near the marketing unit. These units may be equipped with a high water flow through and/or aeration/oxygenation. From these facilities, trout are caught by a dip-net, the required number of fish is selected and killed on site (EFSA 2009a).

During harvest, the fish are usually crowded. Depending on the system, crowding lasts from a few minutes to several hours during which the welfare of the fish may be compromised. There is a wide range of crowding techniques used in order to collect only the amount of fish to slaughter which will be put on the market. Such techniques include crowding by feed pellet distribution followed by dip netting, crowding with a seine net or a grid, by lowering the water level or emptying the pond. In fresh water cages crowding is carried out by raising the net.

There are different types of transfer systems depending on type of production. Trout are netted or pumped or flow freely (gravity), depending on the local situation, from the rearing unit or the truck to the place where they are killed. Fish can be netted in air or in water. In freshwater farms, trout are harvested mainly by seine nets of different types of pumping systems: the vacuum pumps (single or twin), Venturi pumps, siphons, air lift or fish elevators (screw type). The pumping distances can range from few meters up to 200 meters. Lifting heights may vary between 0 and 5 meters. Gravity flow systems with or without initial pumping may be used (EFSA 2009a).

When separate slaughter facilities are used, transport is usually carried out by truck. After arrival at the slaughter site, trout are either transferred from the vehicle directly to the slaughter line or to holding facilities. Transfer is carried out as previously

described. The trout may be kept in the holding facility for a few hours, up to a few days, before they are processed depending on market requirements (quantity and size). During holding, trout can recover from transport stress. Feed deprivation is a common practice during the pre-slaughter period (EFSA 2009a).

THE STUNNING/KILLING METHODS

The main methods used for stunning and killing trout are: asphyxia and asphyxia in ice slurry, percussive stunning, electrical stunning, and carbon dioxide stunning. Methods for stunning and killing are applied in combination based on farming systems, size of fish and technical options at the slaughter plant.

Asphyxia, traditionally used for captured fish, consists in leaving fish to die out of the water. This is the oldest fish slaughter method and is characterized by a prolonged suffering period before death depending on the hypoxia resistance of the fish species. For rainbow trout the period necessary to reach unconsciousness is 15 min (Poli *et al* 2005). Fish are left to die and when movement has ceased, they are further processed. The time required for the fish to die is temperature dependent (Robb & Kestin 2002).

In the case of death in ice slurry, after harvesting fish are directly transferred to water/ice slurry tubs. This procedure is used in Mediterranean countries for small-sized species and in UK for rainbow trout. Fish body temperature decreases rapidly as does their metabolic rate and movements (live chilling). Fish oxygen requirements also decrease markedly and the time to death can be prolonged (Poli *et al* 2005). The aim of this system is to simultaneously chill, sedate and kill the fish by asphyxia. The lethal temperature for rainbow trout has been reported as -0,75°C (Fletcher *et al* 1988). Asphyxia in ice does not result in immediate unconsciousness. For trout it takes 9.6 min at 2°C compared to 3 min at 14°C (Robb & Kestin 2002).

Knocking or percussive stunning is frequently used for trout, salmon and other large fish when it is possible to kill single animals. Fish are taken from the water, restrained manually in an adequate place and stunned by one or two blows on the brain using a wood or plastic club. Percussive stun kills instantaneously or renders the fish unconscious: if the energy of the blow is sufficient there is a massive brain disruption and an immediate insensibility in fish whereas a high energy blow can kill the fish immediately. The bleeding that follows assures the death of the fish. Automatic tools such as pneumatic clubs are available (Poli *et al* 2005).

In the case of electrical stunning an electrical current is administered between electrodes which are submerged in a fish holding tank or transport system. While passing between the electrodes, using water and fish as a conductor, the current generates an electric field. The effectiveness of the stunning depends on the strength of the electric field applied, the density and distribution of fish in the tank (EFSA 2009a). For portion size trout, field strength between 3 and 6 V/cm had to be applied for 30 to 60 s in order to achieve permanent insensibility (Lines & Kestin 2004). However, electrical stunning can cause a quick and violent reaction, with mouth and opercula wide open, muscle blood spots and vertebral fractures, according to the duration of the current application. Moreover, the immobility of fish does not assure that fish are unconscious. On the other hand, when well applied, with the optimisation

of the electrical parameters for each species, electrical stunning could really give sudden immobilisation and loss of consciousness and in this way be humane and very practical (Poli *et al* 2005).
Under commercial slaughter conditions of trout, carbon dioxide is bubbled continuously into a tank or bath of water. The pH of water falls and at about pH 4 the water approaches saturation with gas. Fish are transferred into the water and are left in the bath until movement stops. Then they are removed and further processed. Modifications to the process outlined above include cooling the carbon dioxide saturated water to about 1°C by the addition of ice. Nitrogen was experimentally used in one study instead of carbon dioxide resulting in effective stunning with no strong aversive reactions in rainbow trout (Will *et al* 2006).
Bleeding is commonly used for large trout which are bleed immediately after stunning. Smaller trout are usually not bleed but directly eviscerated after stunning. To achieve bleeding, the gills are cut or pulled out, and the fish returned to water to bleed for a period of 10 to 15 min (Wardle 1997).

FISH WELFARE

The concept of welfare is the same for all the animals, i.e. mammals, birds and fish, used for human consumption and given protection under the Treaty of Amsterdam (1997). However, the welfare of intensively farmed fish has not been investigated to the same extent as that of terrestrial farm animals. Whilst similar measures of welfare developed for other animals are often relevant to fish, clearly defined protocols for fish welfare evaluation are lacking.
Different species of fish have developed highly sophisticated sensory organs to survive in changing environmental conditions. Some of these are absent in mammals, for example electroreceptors and the lateral line system. There is scientific evidence to support the assumption that some fish species have brain structures potentially capable of experiencing pain and fear. However, the knowledge and understanding of manifestations of sentience in fish are limited (EFSA 2009b).

RISK ASSESSMENT

In 2009, the Panel on Animal Health and Welfare of the European Food Safety Authority (EFSA) has ranked the risks of poor welfare associated with different commercially applied stunning and killing methods of rainbow trout. A semi-quantitative risk assessment approach was carried out, mainly based on expert opinions, due to the limited amount of quantitative data and peer-reviewed data published. Pre-slaughter steps which have a direct impact on the welfare immediately before and during killing were included in the risk assessment (EFSA 2009a).
Risk assessment is a systematic process to estimate the probability of exposure to a hazard and the magnitude of the effects of that exposure. In animal welfare risk assessment a hazard may be defined as a factor with the potential to cause a negative animal welfare effect (adverse effect). Risk is a function of both the probability that the hazard and the consequences characterised by the adverse effect occur. Three parameters were scored to assess the importance of a hazard: the intensity of the adverse effect that the hazard causes, the duration of the adverse effect and the

probability of exposure to the hazard. All three factors were included in calculating the final risk score of a hazard. The score for each parameter was standardised by dividing the score by the maximum possible for that parameter, thus all parameters have a maximum value of one. The risk score is the product of the standardised scores multiplied by 100 and thus has a maximum value of 100 (EFSA 2009a).
The formula used by the Panel on Animal Health and Welfare of EFSA for the calculation of risk score was the following:

Risk score: [(*I adverse effect*/3) x (*D adverse effect*/4) x (*P hazard*)] x 100

where:
I adverse effect = the intensity of adverse effect;
D adverse effect = the duration of adverse effect;
P hazard = the probability of exposure to the hazard.

The minimum, most likely and maximum values for *P hazard* were used to generate minimum, most likely and maximum estimates of the risk score. It was also assumed that hazards usually occur independently of each other.

Pre-slaughter hazards
A total of 12 pre-slaughter hazards were identified and categorised as follows: crowding, transfer; holding unit and post-transport. Table 1 shows the risk scores of the pre-slaughter hazards which ranged from less than 1 to 67.

Table 1: Risk scores of pre-slaughter hazards (adapted by EFSA, 2009a).

N°	**Hazard description**	**Risk score**		
		Most likely	Min	Max
	Crowding			
1	Increased density	15.00	12.50	18.75
2	Fish exposed to shallow water	7.50	2.50	10.00
3	Fish exposed to air (> 15 sec)	1.50	0.50	2.50
4	Poor water quality	0.75	0.25	2.50
5	Contact with physical structures	1.33	0.07	3.33
	Transfer			
6	Pumping	66.67	66.67	66.67
7	Poor equipment design	0.33	0.01	0.67
8	Delay in pipe or screw	0.50	0.05	0.90
9	Dip-netting	1.67	0.83	3.33
	Holding Unit			
10	Poor water quality	3.00	1.00	5.00
11	Dip-netting in holding unit	1.67	0.83	3.33
	Post-transport			
12	Poorly performed transport	0.50	0.01	1.00

Within the 12 pre-slaughter hazards, pumping fish ranked as the highest risk, followed by the increased density that occurs when fish are crowded at the start of the process of gathering fish. At the same time fish may be exposed to shallow water, causing stress which was the third highest ranked hazard.

Stunning and killing hazards

Six stunning and killing methods were analysed. The details of hazards and the risk scores are provided in the Table 2.

Table 2: Risk scores of stunning and killing hazards (adapted by EFSA, 2009a).

N°	**Hazard description**	**Risk score**		
		Most likely	Min	Max
	Percussive stunning (semiautomatic)			
1	Laying on table	67.50	60.00	71.25
2	Being handled manually	8.33	8.33	8.33
3	Mis-stun	1.25	0.75	1.75
4	Cut when conscious	0.38	0.38	0.38
5	Evisceration when conscious	0.03	0.03	0.03
	Manual percussive stunning			
1	Laying on table	5.00	3.00	7.00
2	Being handled manually	8.33	8.33	8.33
3	Mis-stun	1.25	0.75	1.75
4	Evisceration	0.25	0.25	0.25
5	Cut when conscious	0.38	0.38	0.38
	Electrical stunning (in water batch system)			
1	Crowding in the stunning tank	16.67	16.67	16.67
2	Poor water quality	1.67	0.83	2.50
3	Mis-stun	1.25	0.75	2.00
4	Mis-cut when conscious (large trout)	3.75	1.50	5.25
5	Evisceration when conscious	2.50	1.00	3.50
	Electrical stunning (pipeline system)			
1	Mis-stun	0.25	0.05	0.38
2	Evisceration when conscious	0.75	0.10	0.75
	Ice slurry			
1	Temperature shock	33.33	26.67	40.00
2	Asphyxia	100.00	100.00	100.00
	Asphyxia (no cooling)			
1	Asphyxia	75.00	75.00	75.00
	Carbon dioxide			
1	Exposure to high levels of CO_2	75.00	75.00	75.00
2	Very low water quality	50.00	50.00	50.00
3	Evisceration if conscious	45.00	42.50	47.50
4	Cut when conscious	67.50	63.75	71.25

Regarding the stunning and killing methods, carbon dioxide, asphyxia on ice and asphyxia are the methods resulting in the poorest welfare. Carbon dioxide has the highest risk score because exposure to the gas causes a strong adverse reaction; in addition, it also does not reliably result in unconsciousness, thus trout may be eviscerated or bled when conscious. Killing trout by asphyxia is judged to be a severe hazard. Asphyxia in ice slurry had a higher score since the temperature shock was considered an additional hazard. Percussive methods and electrical stunning were assessed to reliably cause unconsciousness in the vast majority of trout. The semi-automatic percussive stunning has a higher risk than manual method resulting in poor welfare because fish are being handled in air on a table for some seconds or even minutes (EFSA 2009a).

Recommendations

According to the Panel on Animal Health and Welfare of EFSA pre-slaughter handling directly before stunning is presently unavoidable. Efforts should be made to minimise the stress caused by crowding. Exposing trout to air or shallow water which restricts the movements of the fish should be avoided. Transfer should preferably be performed by free-flowing water or small scale dip-netting. Vacuum pumping of fish should be avoided, as small scale netting and free flow are preferable and cause less harm. Transport of trout from farm to processing plant immediately prior to slaughter should be carried out as gently as possible.

Currently is not possible to identify welfare indicators that could be used to monitor slaughter procedures for trout. Standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced. Validated, robust and practically feasible welfare indicators should be developed (EFSA 2009a).

FISH QUALITY

Animal welfare and product quality are linked aspects of the total quality of fish. Many quality traits can change as affected by the conditions of slaughtering and by the severity of the pre-slaughter and slaughter stresses (Poli *et al* 2005).

In the case of rainbow trout it has been recently shown that the pre-slaughter procedures of crowding and pumping accelerate the onset of rigor mortis of farmed animals; however, these procedures do not affect weight loss in fillets stored on ice or frozen (Merkin *et al* 2010). There is evidence that the slaughter by percussive stunning improves the quality of trout compared to the ice slurry method. Özogul & Özogul (2004) have investigated the effects of percussive stunning and death in ice slurry on the sensory, chemical and microbiological quality of rainbow trout stored in ice or in modified atmosphere packing ($CO_2/N_2/O_2$ 40/30/30). Despite no significant differences were detected in organoleptic traits and total viable count of fish regardless of the two stunning methods, higher concentrations of biogenic amines were found for the ice slurry slaughter procedure. Moreover, the mean K value (parameter correlated with loss of freshness) of rainbow trout slaughtered by percussive stunning was significantly lower than that of trout slaughtered according the ice slurry method.

Advanced live chilling procedure, based on a flow ice system including ozone, has been recently tested for slaughtering and storage of farmed rainbow trout (Ortiz *et al* 2008). The flow ice system, employed in the place of the traditional flake ice, has shown many advantages such as lower temperature, faster cooling, lower physical damage and better heat exchange power in other fish species (Losada *et al* 2004). In trout the ozone presence has shown some profitable effects as leading to an extended shelf life by retention of several sensory parameters; in contrast, some negligible negative effects have been observed on lipid oxidation: the oxidation values reached by the fish kept under flow ice and ozone cannot be considered as particularly high (Ortiz *et al* 2008).

A lower stability of muscle lipids, coupled to a faster ATP depletion, was observed in smoked rainbow trout slaughtered by simple anoxia or bleeding by resection of gill compared to the same product obtained by fish electrically stunned (Giuffrida *et al* 2007).

Both colour and texture are affected by slaughter stress. Lefevre *et al* (2008) have shown a significant decrease in lightness (L*) in fillet from farmed rainbow trout subjected to a minimal stress before slaughter (fish were harvested as fast as possible with a handling net and anaesthetized with eugenol in a separate tank) compared to trout subjected to 15 min confinement stress (the tank water level was lowered till it remained about 20 cm depth; fish remained 15 min and then were harvested with a handling net and treated with the same protocol used for unstressed fish). This result has been related to intense muscle activity before death, as electrical stimulation of carcass leads to similar effects on the colour of flesh in big rainbow trout (Robb *et al* 2000). A lower mechanical resistance of the raw flesh was observed for stressed trout compared to control group of unstressed animals (Lefevre *et al* 2008). For these reason the authors conclude that the control of slaughter stress to a minimum level is necessary to keep the highest quality of the product.

The soft flesh problem in fresh water rainbow trout is associated with an early loss of rigor within 24 h after slaughtering. Although some sites of production seem to be more susceptible to this problem, its occurrence is fairly random, it does not necessarily affect the totality of the fish population of the farm, and its origin is unknown to date. To investigate the soft flesh problem in rainbow trout, Foucat *et al* (2004) have studied the dynamics of water in post mortem white muscle by magnetic resonance imaging measurements. The trout identified as having the soft flesh problem differed from the others by smaller diffusion anisotropy which proved to be the most relevant nuclear magnetic resonance parameter for an early diagnosis of the problem. The authors hypothesized an early and exacerbated tenderization phenomenon because of protein denaturation inducing important modifications in fine connections that anchor the three-dimensional structure of the tissue. The incidence of different slaughtering procedures on rigor mortis were tested and no differences were detected for trout presenting the soft flesh problem.

Conclusions

Aquaculture industry can provide high quality fish to processors and consumers by using improved pre-slaughter handling and slaughter methods. However, specific

welfare indicators to be used to monitor slaughter procedures for trout are not available and standard operating procedures to improve the control of the slaughter process to prevent impaired welfare should be introduced.

REFERENCES

1. API (2007) Associazione Piscicoltori Italiani. http://www.api-online.it/index.cfm?ent=informazioni&p=piscicoltura [accessed 10 May 2011]
2. EFSA (2009a) Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on species-specific welfare aspects of the main systems of stunning and killing of farmed rainbow trout. *The EFSA Journal* 1013, 1-55.
3. EFSA (2009b) Scientific Opinion of the Panel on Animal Health and Welfare on a request from the European Commission on general approach to fish welfare and to the concept of sentience in fish. *The EFSA Journal* 954, 1-26.
4. FAO (2006) National Aquaculture Sector Overview. Italy. National Aquaculture Sector Overview Fact Sheets. Text by Cozzolino M., FAO Fisheries and Aquaculture Department. http://www/fao.org/fishery/country/sector/naso_italy/en#tcNA0140 [accessed 10th November 2010]
5. FAO (2005) Cultured Aquatic Species Information Programme. *Oncorhynchus mykiss*. Cultured Aquatic Species Information Programme. Text by Cowx I.G., FAO Fisheries and Aquaculture Department. http://www/fao.org/fishery/culturedspecies/Oncorhynchus_mykiss/en#tcNA00FE [accessed 10 May 2011]
6. Fletcher G.L., Kao M.H., Dempson J.B. (1988): Lethal freezing temperatures of Arctic char and other salmonids in the presence of ice. *Aquaculture* 71, 369-378
7. Foucat L., Taylor R.G., Labas R., Renou J.P. (2004): Soft flesh problem in freshwater rainbow trout investigated by magnetic resonance imaging and histology. *Journal of Food Science* 69, 320-327
8. Giuffrida A., Pennisi L., Ziino G., Fortino L., Valvo G., Marino S., Panebianco A. (2007): Influence of slaughtering method on some aspects of quality of gilthead sea bream and smoked rainbow trout. *Veterinary Research Communications* 31, 437-446
9. Lefevre F., Bugeon J., Auperin B., Aubin J. (2008): Rearing oxygen level and slaughter stress effects on rainbow trout flesh quality. *Aquaculture* 284, 81-89
10. Lines J. and Kestin S. (2004): Electrical stunning of fish: the relationship between the electric field strength and water conductivity. *Aquaculture* 241, 219-234
11. Losada V., Barros-Velaszquez J., Gallardo J., Aubourg S. (2004): Effect of advanced chilling methods on lipid damage during sardine (*Sardina pilchardus*) storage. *European Journal of Lipid Science and Technology* 106, 844-850
12. Merkin G.V., Roth B., Gjerstad C., Dahl-Paulsen E., Nortvedt R. (2010): Effect of pre-slaughter procedures on stress responses and some quality parameters in sea-farmed rainbow trout (*Oncorhynchus mykiss*). *Aquaculture* 309, 231-235
13. Ortiz J., Palma O., Gonzalez N., Aubourg S.P. (2008): Lipid damage in farmed rainbow trout (*Oncorhynchus mykiss*) after slaughtering and chilled storage.

European Journal of Lipid Science and Technology 110, 1127-1135
14. Özogul Y., Özogul F. (2004): Effects of slaughtering methods on sensory, chemical and microbiological quality of rainbow trout (*Oncorhynchus mykiss*) stored in ice and MAP. *European Food Research and Technology* 219, 211-216
15. Poli B.M., Parisi G., Scappini F., Zampacavallo G. (2005): Fish welfare and quality as affected by pre-slaughter and slaughter management. *Aquaculture International* 13, 29-49
16. Robb D.H.F., Kestin S.C., Warriss P.D. (2000): Muscle activity at slaughter: I. Changes in flesh colour and gaping in rainbow trout. *Aquaculture* 182, 261–269
17. Robb D.H.F., Kestin S.C. (2002): Methods used to kill fish: field observations and literature reviewed. *Animal Welfare* 11, 269-292
18. Stefanutto P. (2011): *La troticoltura in FVG: situazione attuale, criticità, possibilità di sviluppo.* http://www.progettoiridea.it/pdf/stefanutto.pdf [accessed 30 May 2011]
19. Treaty of Amsterdam (1997) http://europa.eu/legislation_summaries/institutional_affairs/treaties/amsterdam_treaty/index_it.htm [accessed 25 May 2011]
20. Wardle C. (1997): Welfare of Farmed Salmon and Impact on Post Harvest Quality. In: Welfare of Fish at Slaughter. Ed. D. Robb. University of Bristol, United Kingdom, pp 25-30
21. Will C.C., Zampacavallo G., Poli B.M., Proctor M.R.M., Henehan G. T.M. (2006): Nitrogen stunning of rainbow trout. *International Journal of Food Science and Technology* 41, 395-398

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BODY MEASURES IN REGGIANA CATTLE AND THEIR CORRELATIONS WITH MORPHOLOGIC EVALUTATIONS

MISURAZIONI BIOMETRICHE E VALUTAZIONI MORFOLOGICHE IN BOVINE DI RAZZA REGGIANA: CORRELAZIONI FRA VALUTAZIONI OGGETTIVE E SOGGETTIVE

Beretti Valentino[1], Simonazzi Davide[2], Paini Valerio[1], Superchi Paola [1], Sabbioni Alberto[1].

STRUCTURED SUMMARY

Objectives – Aim of the study was to evaluate the relationship between objective body measures and subjective morphologic evaluations in Reggiana cattle.

Methods – The research was carried out on 36 primiparous and 10 secondiparous Reggiana cows reared in 7 plain herds of Reggio Emilia province. The cows were submitted to official linear morphologic evaluation based on the Herd Book form (27 traits; points from 1 to 50) at an age of 762 to 1620 d. In the same period (age of 771 to 1391 d) they were submitted to body measurements (height at withers and at rump; rump length and width; chest depth and circumference; rear udder height; teat length). Data were analysed by descriptive, variance, correlation and discriminant analysis.

Results – Significant correlations among body measures and subjective morphologic evaluations have been shown. Moreover the study has shown a significant reduction of body size in relation to the improvement of the final morphologic score. The reduction is related to the improvement of the global efficiency of cows but it is independent from the rump width, so to maintain the calving ease.

KEYWORDS

Reggiana cattle; body measures; morphological evaluations; correlations; discriminant analysis.

INTRODUCTION

Reggiana is an autochthonous Italian cattle breed, reared for the production of milk to be transformed into Parmigiano-Reggiano cheese. The breed was considered in the past as a triple purpose breed, but it is actually specialized in the production of high quality milk, with a particular aptitude to cheese-making and higher cheese yield (Mariani and Russo 1971; Mariani and Pecorari 1987). Since its origin to years ‘50s, the Reggiana breed was the only cattle breed in the province of Reggio Emilia and it was widely spread also in the Parma and Modena provinces, which represent the typical area of Parmigiano-Reggiano cheese production (figure 1).

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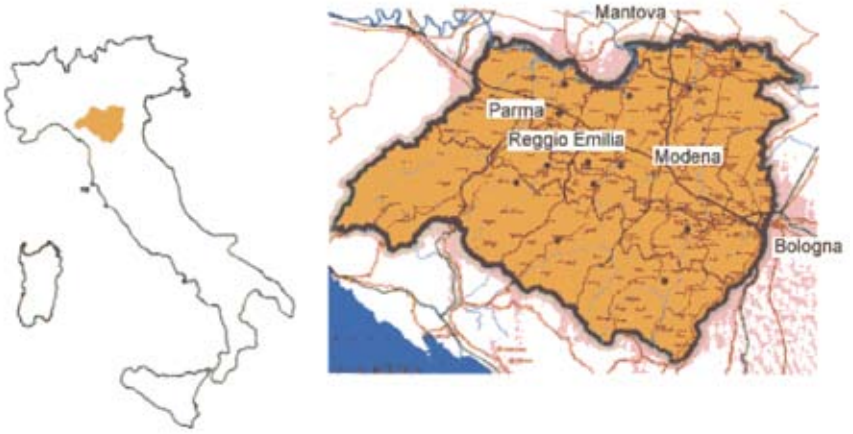
Figure 1: Area of Parmigiano-Reggiano cheese production



More recently the breed has faced a strong reduction of the population size, similarly to other autochthonous breeds in Italy, and it has been substituted in the area of origin with other cosmopolite and selected breeds, more productive and more suited to intensive farming conditions. Among the causes of the number reduction also the changed social conditions of farmers must be accounted, besides the presence of government subsidies for the breeding of more productive animals and the passage from tie stalls to free stalls and from manual to automatic milking. In the latter case the Reggiana breed showed a morphologic limit related to the presence of udders unsuitable for mechanical milking.

During years '60s the population size passed from 60,000 heads to 3000; the lowest population size was reached in 1981 (985 heads, corresponding to 0.6% of cattle reared in the Reggio Emilia province).

From the half of years '90s Reggiana cattle is enjoying a revival (Ronchi 1995) and actually the population size is 2575 heads reared in 177 farms (A.N.A.Bo.Ra.Re. 2011).

Selection in Reggiana cattle is aimed to the production of well conformed animals (good size, height and weight, robust and correct conformation) and with good functional parameters (precocity, fertility, longevity, fitness for a consistently high production of milk suitable for cheese making, good dressing percentage, high ability of exploitation of forages). Previous studies on lactation curves (Sabbioni *et al.* 2003) also showed the ability of Reggiana cattle to produce high quantities of milk (peak yield from 22.17 kg at 1st parity to 28.72 kg at ≥5 parities), fat corrected milk (peak yield from 19.95 kg at 1st parity to 25.33 kg at ≥5 parities), fat (peak yield from 0.75 kg at 1st parity to 0.97 kg at ≥5 parities) and protein (peak yield from 0.70 kg at 1st parity to 0.89 kg at ≥5 parities); persistency of lactation was higher in 1st parity cows (73 d) than in subsequent parities (56-59 d). A further advantage of Reggiana cattle is the high frequency of the casein gene complex (α_{s1}-casein, β-casein and k-casein), that in the period 1971-2003 showed strong variations, with particular reference to

haplotypes *CAB* (+300%), *BAB* (-55%) and *CAA* (-8%) (Caroli *et al.* 2006).
Because low information are present with reference to the morphology, the aim of the study was to evaluate the relationships between objective body measures and subjective morphologic evaluations in Reggiana cattle. The presence of such a correlation could help to a better morphologic classification of cows.

MATERIALS AND METHODS

The study was carried out on 46 Reggiana cows, reared in 7 herd of the plain area of Reggio Emilia province. The cows were primiparous (no.: 36; 78.3%) and secondiparous (no.:10; 21.7%); the age at 1st calving was 697 to 1086 d (mean ± s.e.: 838 ± 16), as the age at 2nd calving was 1046 to 1520 d (1303 ± 42). Cows were submitted to morphological evaluation by experts of the National Association of Reggiana Cattle Breeders (A.N.A.Bo.Ra.Re. 2011) at an age between 762 and 1620 d (1045 ± 35).
The morphological evaluations were performed by experts according to the official form, describing, in agreement to the linear morphological evaluation system (scores from 1 to 50), 27 traits concerning the structure (stature, strength, body depth, dairy form, dorsal line), rump (angle, length, width, tail head), feet and legs (rear legs side view, pastern, claw), udder (fore udder, rear udder width, height rear ligament, udder cleft, udder depth, udder balance), front teat (front teat position rear view, front teat position side view, length), rear teat (rear teat position rear view, rear teat position side view, length), miscellaneous (temperament, milking ability, breed characteristics, score from 1 to 3). A final score was then assigned with scores from 70 to 100.
The body measures were performed by trained personnel, at an age between 771 and 1391 d (1023 ± 20) by means of a Lydtin meter stick or a tape meter. They concerned the height at withers (from the withers top to the ground), the height at rump (from the top of the midpoint between hip bone and pin bone to the ground), the rump length (from the cranial margin of the hip bone to the caudal one of pin bone), the rump width (from a hip tuberosity to the other), the chest depth (from the top of the withers to the bottom edge of the sternum, caudally and tangent to the elbows), the chest circumference (at ribs level, caudally and tangent to the elbows), the rear udder height (between the lower rim of the vulva and the beginning of mammary parenchyma), the teat length (on front left teat).
Data have been subjected to statistical analysis by the package SPSS (ver. 19, 2011) that implemented:

a. Descriptive analysis (mean, standard deviation, minimum, maximum, asymmetry, kurtosis) of body measures and morphologic scores;
b. Simple correlations between body measures and morphologic scores;
c. Analysis of variance of body measures, following a linear model with classes of morphologic scores (3 levels: <82, 82-84, >84), herd (7 levels), parity (2 levels) as fixed factors, age at 1st parity and age at body measures as covariates and 1st order interactions among fixed factors;
d. Discriminant analysis of body measures.

RESULTS AND DISCUSSION

The sample of Reggiana cows subjected to the morphologic evaluations and to body measurements was about the 5% of the total population enrolled in the Herd Book and, in particular the 21% of total primiparous and 8% of total secondiparous cows.

Table 1 reports the descriptive analysis of morphological scores. The sample has shown values higher than 25 for all parameters except rear legs side view (23.00 ± 4.03) and rear teat (24.91±1.15 for rear view; 24.98±1.24 for side view; 24.83±2.76 for length). The highest values were shown by claw (28.59 ± 3.88), rear udder width (28.59 ± 4.84) and rear ligament height (28.59 ± 3.94).

The sample has shown good values for structure, rump and udder and mean values for teats. Feet and legs showed an inconsistent trend, with low scores for side view and good scores for pasterns and claws. Data haven't shown a normal distribution, despite the high number of classes, because of the almost ever high absolute values of asymmetry, with tails sometimes to the left (negative values) sometimes to the right (positive values), and kurtosis, with the presence of distributions both leptokurtic and platykurtic. A similar situation has been observed before by Chen (1994) and by Sabbioni *et al.* (1998) on Red Pied cattle.

Descriptive statistics of somatic measurements have been reported in Table 2. The normality of distribution of descriptive statistics seems higher (lower absolute values of asymmetry and kurtosis) than that observed for morphologic scores. This doesn't agree on what observed by Sabbioni *et al.* (1998) in Italian Red Pied cattle: in the cited study the sample variability in the distribution curve edges looked higher, because of the presence in this breed of animals with strong milking aptitude near others selected for dual purpose.

From the morphologic point of view the Reggiana cattle is a population characterized by a good uniformity level.

The somatic measurements values allow the comparison between the Reggiana cow and other breeds selected for milking (or dual purpose breeds) reared in Italy, at the same age and parity.

Compared to Italian Holstein (Martelli *et al.* 1992; Carnier *et al.* 1994), the Reggiana cattle is higher at withers (+2,77%), longer (+1,11%) and wider at rump (+3,19%) and has a deeper chest (+3,81%) and a shorter chest circumference (-1,97% to -2,77%).

Compared to Italian Red Pied cows (Ricci *et al.* 1994; Sabbioni *et al.* 1998) the Reggiana cattle is higher at withers (+3,27% to +6,55%) and at rump (+6,48%) and has a shorter chest circumference (-1,95% to -4,24%); more similar are the chest depth (-0,74% to +0,32%) and the rump dimensions (length -0,49% to +2,71%; width -3,54% to +0,08%).

The udder size seems to be lower (rear high: + 25,87%) and the teats longer (+ 6,44%).

The Reggiana cow can be defined as an animal with high build, good respiratory capacity (milking type) and rump dimensions suitable for an easy calving.

The correlations between somatic measurements and morphologic score in the sample of the cows under study are shown in Table 3. The animal height is significantly correlated with the stature ($P<0.001$ for height at withers and $P<0.05$ for height at rump), the strength ($P<0.05$), the width of the rump ($P<0.10$ for height at withers and

P<0.05 for height at rump) and the rear udder widht (P<0.10), giving a good help in the definition of animal build and of its productive and reproductive capacity.
Strangely, both the rump length and the rump width are not significantly correlated with their subjective evaluations. Since the measurement point is the same of that of evaluation, this can be interpreted as a limit of evaluation of this region, maybe caused by the relationship between this region and those neighbouring and this leads to look for better define its evaluation parameters.
The chest depth is negatively correlated with the dorsal line score (P<0.10) and it is positively correlated with the rear legs side view score (P<0.10). So, an increasing in chest depth score is associated with a sway dorsal line and sickle-shaped rear legs.
The chest circumference is positively correlated with the rump width (P<0.10) and it is negatively correlated with the position of front teats, both from the rear (P<0.10) and the side (P<0.05) point of view. This shows that the measure is a good indicator of overall size of the animal and indicates that the large rump determines a change in the position of the front teats, which appear more widely spaced and laterally placed. This arrangement is negative, because it leads to an important worsening of milking.
The measurements made on udder (rear height and front left teat length) are significantly and negatively correlated with the final score (P<0.05). This last parameter mostly summarizes information linked to the productive aspect because since these correlations are negative, the final score positively shows the effects of a reduction of both teat length and rear udder height, bound to an increasing in udder volume.
Moreover, the rear udder height is positively associated with the body size (P<0.01), the tail head (P<0.01), the front teats position (from the rear point of view) (P<0.01), the pasterns (P<0.10), and it is negatively correlated with the height of rear ligament (P<0.01), the width of rear udder (P<0.05), the fore udder (P<0.10) and the dorsal line (P<0.10).
The left front teat measurement obviously appears positively correlated (P<0.01) with the teat length evaluation (both front teat and rear teat), as well as the tail head (P<0.05) and, negatively, with the dorsal line (P<0.05) and the rear udder height (P<0.05).
The analysis of variance of body measurements (Table 4) has highlighted the significant effects of the herd and of the score category for many of the parameters, while the parity resulted less important. Between the covariates, only the age at measurement resulted significant (P<0.05), showing that the measurements (and also the morphologic evaluations) have been made in a moment in which the animals are not completely grown.
Table 5 refers the least squares means of body measurements, in relation to the final score class. The animals belonging to the highest class of final score (>84) have shown height at withers and at rump, rump length, rear udder height and teat length significantly lower (P<0.05) than those belonging to the lowest class of final score (<82). The explanation of such results can be searched analysing different aspects. Firstly, it is known that the genetic selection made in the last years in Reggiana cow has lead to an increase in milk yield: since the morphologic evaluation rewards

the animals more oriented at productive function, those with higher morphologic scores will have more probabilities to show also higher productions. This is indicated by the reduction of the rear udder height, which suggests a higher volume of the mammary gland in animals with higher scores. The reduction in the same animals of the teat length is a specific selection target in Reggiana cattle, directed to contrast a common defect of the past years. This trait is important in determining the final score, considering an approximately 10% difference between the two classes.
The significant ($P<0.05$) reduction of body size in relation to the increasing of final scoring could be associated to lower nutrient requirements for maintenance and to a selection goal directed toward an improvement of the global efficiency of animals (Bittante and Gallo 1995). Hansen *et al.* (1999) have shown that a reduction of body size in Holstein cattle is related to increased longevity, lower pathologies, higher fertility at the same production levels; on this basis higher morphologic scores to low sized cows could be justified also in Reggiana cattle. Moreover it is possible that crosses with other red (Danish Red, Angeln) or light fawn (Jersey) cattle, that are characterized by lower body size, have taken place in the past, with the aim to improve milk production and composition of Reggiana cattle.
At last, we have verified if it could be possible to preview the final score class of animals by body measurements. For this purpose we applied the discriminant analysis. Two discriminant functions (Table 6, see also figure 2) were generated on the basis of the linear combination of the dependent variables: the first emphasizes the height measurements (at withers and at rump), the rump dimensions and the udder measurements, the second one emphasizes the chest dimensions. By applying the discriminant functions, we have calculated a probability of 56.5% to correctly classify a cow in its score class by means of body measures. The use of this tool could be checking the results of objective assessment, which must be carried out by experts of the breed anyway. The availability of a function that can assign an animal with a good accuracy to a class of score, based on objective measures, might correct distortions of the subjective assessment due to contingent factors that are not much verifiable. Obviously, these functions should be calculated over a number of animals more representative of the population.

CONCLUSION

The study allowed us to focus the attention on morphologic aspects of the Reggiana cattle breed, highlighting significant correlations between body measurements and subjective animals' evaluations. Moreover the finding of a body size reduction in animals with higher morphologic scores led to interesting implications about the effects of selection on animals' morphology. Such reduction leads to an improvement of global efficiency, but doesn't involve the rump width, allowing so to maintain the ease at calving. At the end the results of the discriminant analysis appear interesting, at least from the methodological point of view. They offer the opportunity to provide an objective method of allocating an individual to a class of final score and are valid to support the action of the body of experts. Finally, the researches about limited sized populations with the purpose of improving their knowledge are essential for their genetic protection and safeguard.

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Table 1 – Descriptive analysis of morphological scores in Reggiana cattle.

Trait	Mean	±	s.d.	Min.	Max.	Asymmetry	Kurtosis
Final score	83.00	±	2.46	77	87	-0.657	-0.470
STRUCTURE							
Stature	26.52	±	4.39	15	40	0.823	3.109
Strength	27.28	±	2.96	20	35	0.124	0.427
Body depth	27.46	±	3.12	20	35	-0.034	0.766
Dairy form	28.22	±	4.31	20	40	0.893	0.899
Dorsal line	27.91	±	2.14	23	32	-0.153	-0.562
RUMP							
Angle	25.17	±	3.38	15	30	-0.926	0.941
Length	27.89	±	3.17	23	38	1.101	1.412
Width	27.54	±	3.15	20	35	0.317	0.783
Tail head	27.17	±	4.46	20	45	2.099	6.370
FEET AND LEGS							
Rear legs side view	23.00	±	4.03	10	30	-1.207	1.408
Pastern	28.54	±	4.89	15	38	-0.037	0.050
Claw	28.59	±	3.88	24	38	0.715	-0.607
UDDER							
Fore udder	28.22	±	3.94	20	36	0.328	-0.094
Width rear udder	28.59	±	4.84	15	40	0.554	1.620
Height rear ligament	28.59	±	3.94	20	40	0.879	1.590
Udder cleft	28.00	±	3.92	20	40	1.138	2.706
Udder depth	27.65	±	3.76	20	37	0.908	0.881
Udder balance	25.61	±	1.04	25	30	2.339	6.502
FRONT TEAT							
Front teat position rear view	25.50	±	1.19	24	28	0.915	0.014
Front teat position side view	25.41	±	0.83	24	27	0.773	-0.131
Length	25.41	±	2.60	21	35	1.284	2.982
REAR TEAT							
Rear teat position rear view	24.91	±	1.15	20	28	-1.559	8.027
Rear teat position side view	24.98	±	1.24	20	27	-1.426	5.464
Length	24.83	±	2.76	20	36	1.503	5.086
MISCELLANEOUS							
Temperament	1.37	±	0.49	1	2	0.559	-1.767
Milking ability	1.54	±	0.62	1	3	0.697	-0.426
Breed characteristics	1.22	±	0.55	1	3	2.525	5.365

Table 2 - Descriptive analysis of body measures (cm) in Reggiana cattle.

Body measure	Mean	±	s.d.	Min.	Max.	Asymmetry	Kurtosis
Height at withers	140.41	±	4.41	131	153	0.240	0.465
Height at rump	148.26	±	4.25	138	157	-0.496	0.407
Rump length	52.76	±	2.57	47	59	0.318	0.062
Rump width	51.78	±	4.16	40	62	-0.280	0.736
Chest depth	72.41	±	2.62	67	79	0.285	0.118
Chest circumference	193.26	±	7.55	180	212	0.059	-0.562
Rear udder height	33.09	±	5.50	20	46	0.165	0.128
Teats length	6.78	±	1.19	4	9	-0.054	-0.227

Table 3 – Correlations between body measures and morphological scores in Reggiana cattle.

Trait	Height at withers	Height at rump	Rump length	Rump width	Chest depth	Chest circum.	Rear udder height	Teats length
Final score	-0.195	-0.164	-0.214	-0.002	-0.048	-0.006	-0.361**	-0.296**
STRUCTURE								
Stature	0.434***	0.339**	0.299**	0.028	0.062	0.188	0.437***	0.031
Strength	0.361**	0.296**	0.070	-0.026	-0.201	0.212	0.211	0.081
Body depth	0.218	0.212	-0.044	-0.136	0.047	0.046	0.198	-0.044
Dairy form	-0.160	-0.053	0.103	0.143	0.196	0.084	0.010	-0.142
Dorsal line	0.131	0.049	-0.068	-0.152	-0.259*	-0.018	-0.283*	-0.339**
RUMP								
Angle	0.106	-0.056	-0.028	0.174	0.062	0.079	0.067	0.037
Length	0.157	0.207	-0.082	-0.170	-0.075	0.083	-0.008	-0.006
Width	0.280*	0.310**	0.162	0.220	-0.006	0.289*	-0.005	0.133
Tail head	0.151	0.123	0.352**	0.327**	0.081	0.232	0.420***	0.292**
FEET AND LEGS								
Rear legs side view	0.284*	0.070	0.009	0.128	0.246*	0.137	-0.131	0.083
Pastern	-0.013	0.171	0.152	0.032	-0.004	-0.012	0.265*	0.093
Claw	-0.099	0.160	0.057	-0.172	-0.118	-0.117	0.168	0.163
UDDER								
Fore udder	-0.201	-0.128	-0.155	0.038	-0.138	-0.032	-0.254*	-0.231
Width rear udder	-0.249*	-0.277*	-0.033	0.106	-0.123	0.146	-0.364**	-0.101
Height rear ligament	-0.127	-0.211	0.032	0.156	-0.125	0.120	-0.400***	-0.323**
Udder cleft	-0.221	-0.033	-0.132	0.059	-0.212	-0.061	-0.232	0.033
Udder depth	-0.192	-0.032	0.143	0.236	0.170	0.007	-0.115	-0.052
Udder balance	0.147	0.379***	0.180	-0.143	-0.005	-0.043	0.250*	0.019
FRONT TEAT								
Front teat position rear view	-0.129	0.053	-0.142	-0.040	-0.225	-0.268*	0.388***	0.220
Front teat position side view	-0.108	-0.138	-0.243	-0.301**	-0.070	-0.308**	0.075	0.048
Length	0.082	0.117	0.065	0.031	-0.009	0.042	0.215	0.460***
REAR TEAT								
Rear teat position rear view	-0.050	0.014	-0.270*	-0.004	-0.091	-0.204	-0.216	-0.047
Rear teat position side view	-0.198	-0.016	-0.064	0.219	-0.100	-0.023	-0.013	0.072
Length	-0.038	0.061	0.125	0.064	0.001	0.095	0.210	0.374**
MISCELLANEOUS								
Temperament	0.237	-0.069	0.019	0.073	0.034	0.160	-0.170	-0.165
Milking ability	0.329**	0.096	0.305**	0.193	-0.004	0.362**	0.044	0.013
Breed characteristics	-0.028	-0.006	0.100	0.214	-0.186	0.140	-0.232	0.073

*: P<0.10; **: P<0.05; ***: P<0.01

Table n.4 - Analysis of variance of body measures in Reggiana cattle.

	d.f.	Height at withers σ^2	Height at rump σ^2	Rump length σ^2	Rump width σ^2	Chest depth σ^2	Chest circum. σ^2	Rear udder height σ^2	Teats length σ^2
Score category (A)	2	60.30*	89.58*	34.21*	9.71	9.08	46.96	79.59*	5.43*
Herd (B)	6	49.46*	49.70*	18.04*	35.11*	13.81*	205.68*	107.34	2.68*
Parity (C)	1	5.27	114.29*	25.70*	20.73	6.89	21.67	14.05	1.63
Age at 1st calving	1	0.49	25.95	8.44	1.61	4.49	164.34*	48.76	1.46
Age at evaluation	1	72.16*	5.15	31.13*	50.44*	0.04	512.58*	10.92	0.38
AxB	8	12.51	13.01	8.01	16.09	19.75*	83.51*	43.09*	2.73*
AxC	2	24.44	124.38*	2.31	1.24	4.99	46.32	23.64	0.03
BxC	1	38.23	43.46*	2.48	3.82	0.26	0.21	7.90	0.01
Error	22	14.07	10.43	4.78	10.03	4.03	30.42	14.04	0.93
R^2	-	0.453	0.564	0.455	0.563	0.557	0.597	0.650	0.505

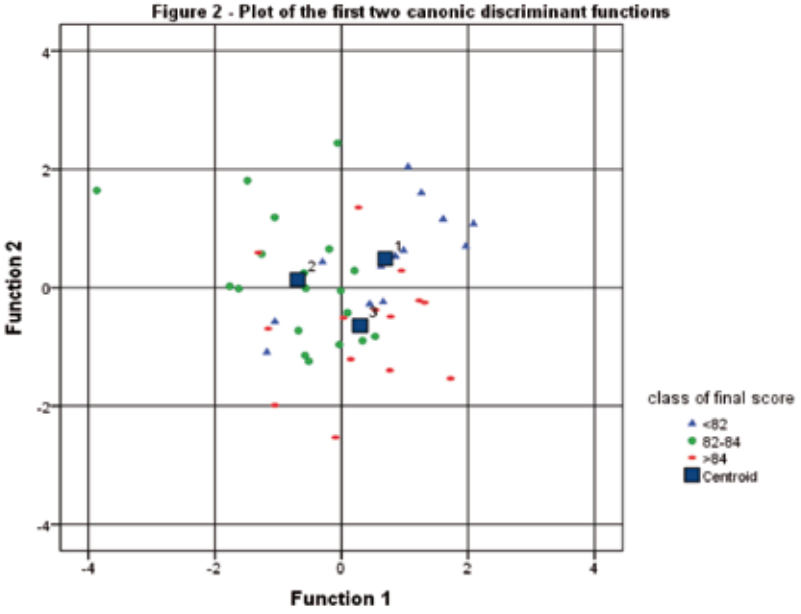
Table n.5 – Least squares means of body measures (cm) in relation to final scores category in Reggiana cattle.

Body measure	Final Score			RSD
	<82	82-84	>84	
Heads no.	15	17	14	-
Height at withers	140.9±1.4 b	141.8±1.2 b	138.1±1.7 a	3.8
Height at rump	149.8±1.2 b	147.7±1.1 a	147.6±1.5 a	3.2
Rump length	53.4±0.8 b	52.8±0.7 b	51.4±1 a	2.2
Rump width	51.3±1.2	52.3±1.1	51.2±1.4	3.2
Chest depth	73.0±0.8	72.2±0.7	73.2±0.9	2.0
Chest circumference	192.8±2.1	193.8±1.9	191.3±2.5	5.5
Rear udder height	36.0±1.4 b	32.0±1.3 a	31.9±1.7 a	3.7
Teats length	7.4±0.4 b	6.5±0.3 a	6.6±0.4 a	1.0

a,b : $P<0.05$

Table n.6 - Coefficients of the canonical discriminant functions for the prediction of final scores category according to body measurements in Reggiana cattle.

Function	1	2
Constant	-11.828	-1.686
Height at withers	0.157	-0.207
Height at rump	0.078	0.012
Rump length	0.159	-0.15
Rump width	-0.042	-0.118
Chest depth	-0.006	0.204
Chest circumference	-0.049	0.086
Rear udder height	0.083	0.114
Teats length	0.331	0.099



REFERENCES

5. A.N.A.Bo.Ra.Re. (2011): http://www.razzareggiana.it [accessed 14 June 2011]
6. Bittante G., Gallo L. (1995): La selezione e l'efficienza della vacca da latte. *Bianco e nero*, 34(9), 43-48.
7. Carnier P., Gallo L., Cassandro M., Mantovani R. (1996): Effetto dell'età, della stagione di parto, dell'ordine di parto e dello stadio di lattazione sulla circonferenza toracica di vacche Frisone e relativi coefficienti di aggiustamento. *Proc 50th Congress of the Italian Society for Veterinary Sciences (S.I.S.Vet.)*, 50, 503-504.
8. Caroli A., Chessa S., Malacarne M., Mariani P. (2006): Variabilità degli aplotipi caseinici nella razza Reggiana: dinamica di un trentennio. *Sci. Tecn. Latt.-Cas.*, 57(6), 599-610.
9. Chen Y. (1994): Test of the normal distribution for 14 linear type traits of Simmental cows. *Acta Veterinaria et Zootecnica Sinica*, 25, 3, 233-236.
10. Hansen, L. B., J. B. Cole, G. D. Marx, A. J. Seykora. (1999): Productive life and reasons for disposal of Holstein cows selected for large versus small body size. *J. Dairy Sci.*, 82, 795–801
11. Mariani P., Pecorari M. (1987): Fattori genetici, attitudine alla caseificazione e resa del latte in formaggio. *Sci. Tecn. Latt.-Cas.*, 38, 286-326.
12. Mariani P., Russo V. (1971): Distribuzione delle varianti genetiche delle caseine e della β-lattoglobulina nelle vacche di razza Reggiana. *Riv. Zoot.*, 44, 310-322.

13. Martelli G., Parisini P., Pezzi P., Sardi L. (1992): Indagine zoometrica su bovine di razza Frisona Italiana allevate nella pianura Padana. *Proc 46th Congress of the Italian Society for Veterinary Sciences (S.I.S.Vet.)*, 46, 1935-1938.
14. Ricci G., Martelli G., Sardi L., Parisini P. (1994): Indagine su alcuni parametri morfo-funzionali di bovine di razza Pezzata Rossa Italiana. *Atti S.I.S.Vet.*, 48, 1747-1751.
15. Ronchi S. (1995): Caratteristiche e miglioramento genetico della razza Reggiana. *L'Informatore Agrario*, 43, 41-43.
16. Sabbioni A., Beretti V., Zanon A., Franceschi P. (2003): Factors affecting the shape of the lactation curve in Reggiana Cattle. *Ital. J. Anim. Sci.*, 2(suppl.1), 278-280.
17. Sabbioni A., Bolognini D., Summer A. (1998): Indagine preliminare sulla possibilità di introdurre nuove classi di valutazione del fattore "taglia" per bovine di razza Pezzata Rossa Italiana. *Pezzata Rossa*, 9(4), 12-15.

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GENETIC FACTORS, CASEIN MICELLE STRUCTURAL CHARACTERISTICS AND RENNET COAGULATION PROPERTIES OF MILK

FATTORI GENETICI, CARATTERISTICHE STRUTTURALI DELLA MICELLA DI CASEINA E ATTITUDINE ALLA COAGULAZIONE DEL LATTE

Marchini Costanza[1], Malacarne Massimo[1], Franceschi Piero[1], Formaggioni Paolo[1], Summer Andrea[1], Mariani Primo[1]

SUMMARY

The qualitative and quantitative changes in milk linked to genetic factors may be considered with regard to the genetic type of caseins. By comparing the effects induced by casein haplotypes with the effect of individual casein variants it is possible to understand the mechanisms that lie at the basis of the relationship between genetic variants and milk composition.

The effect of breed is also important because it impact on presence of a variant and it infuences calcium, phosphorus and colloidal calcium phosphate contents as well. The κ-Cn, β-Cn and αs_1-Cn quantity that enter into the native casein constitution depends on to genetic protein type. In addition it was observed that clotting times and curd firmness change according to the casein micelle composition.

KEYWORDS

Bovine milk, genetic factors, micelle structure, milk composition, coagulation properties.

INTRODUCTION

The milk quality can be evaluated with respect to its ability to provide under normal production conditions a good cheese in terms of chemical, sensory and yield features [1]. A good response of the milk with rennet positively influence the rheological properties of the curd, leading to obtaining a paste with suitable structural properties [2]. In this sense, rennet coagulation aptitude of milk is the basic requirement for hard cheese productions. All physico-chemical, chemical, biochemical and structural variables of milk matrix contribute to the definition of its rennet coagulation aptitude as clotting time, rate of curd formation, texture, permeability, contraction of the curd and, consequently, capacity and rate of syneresis of the cheese mass. In this respect casein, calcium and phosphorus contents, and acidity play an important role.

The content of casein concours to the determination of rheological properties of milk with particular reference to the curd firmness [3-4]. The caseins are large colloidal particles of about 50-600 nm in diameter, called “casein micelles” [5]. According

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to the model proposed by Schmidt (1980) the four caseins (αs_1-Cn, αs_2-Cn, β-Cn and κ-Cn) interact with each other through bonds of secondary nature, to give rise to the submicelle. The submicelle, in turn, aggregate to form the casein micelle itself. The aggregation of submicelles is exerted by an inorganic salt, of amorphous nature, called colloidal calcium phosphate (CCP). In particular, the CCP is able to form electrostatic interactions with the phosphorylated residues of casein αs_1, αs_2 and β present in submicelle (cross bridges submicellar) [6-7]. Recently, the structural organization of casein micelles is explained by dual binding model (Horne, 1998). This includes the existence of hydrophobic interactions between groups of different molecules, where they play the role of "junction" and the presence of ties extending through the CCP. These latter neutralizes the negative charges αs_1-Cn, αs_2-Cn and β-Cn cluster. Since the κ-Cn could lack phosphate groups and being able to bind hydrophobic residues, this casein is distributed on the outer surface of the micelle where it acts as a steric stabilizer [7]. The structure of casein micelles and therefore its lattodynamographic functionality are also influenced by the type of genetic variant of fractions αs_1-Cn, αs_2-Cn, β-Cn and κ-Cn.

The polymorphism of milk protein plays a special role in defining the physical and chemical aspects in the dynamics of milk processing. Numerous scientific articles show a considerable interest in the application of polymorphism of milk proteins, both in livestock and at the level of dairy industry [3-10-11-12]. In that sense, this review want to describe the main changes in the composition of the micelles and milk coagulation in relation to genetic factors.

GENETIC POLYMORPHISM OF MILK PROTEINS

The term genetic polymorphism means that every protein in milk may have two or more genetically forms determined by autosomal and codominant alleles. The term "genetic variant", traditionally used for the mature protein, is now reportedly indiscriminately both at the level of mature protein and coding gene. These are mutations that result in change of amino acid sequence (amino acid substitutions or deletions) determined at the level of DNA by point mutations, insertions or deletions.

The absence of dominance is very useful for count the frequencies. In electrophoresis, the homozygous individuals show only one band, while heterozygotes ones show both bands corresponding to the two alleles. The genetic polymorphism plays a significant role in different application fields, such as animal science and dairy industry. In these areas, the research is focused on the study of possible associations between genetic variants and production traits, reproductive efficiency and adaptability of the dairy cow [13]. In some cases, the effect of these mutations occurs at the level of protein expression. Table 1 shows the nomenclature and characterization of genetic variants of milk casein [14].

Table 1 - Amino acid variation and their position of the different genetic variants of milk casein: amino acids of the variant of reference is highlighted in italics. The phosphorylated residues are indicated with the letter P (Farrell *et al*. 2004; modified by Malacarne *et al.* 2010).

Protein (AA)	Variant	AA position												
		14-26				53	51-58	59		66	192			
αs1-CN	A	deletion									Glu			
(199)	B					*Ala*		*Gln*		*SerP*	*Glu*			
	C										Gly			
	D					ThrP					Glu			
	E							Lys			Gly			
	F									Leu	Glu			
	G(1)										Glu			
	H						deletion				Glu			
		33		47			51-59			130				
αs2-CN	A	*Glu*		*Ala*						*Thr*				
(207)	B	complete sequence is unknown												
	C	Gly		Thr						Ile				
	D						deletion							
		18	25	35	36	37	67	72	88	93	106	122	137/138	152	?
β-CN	A^1						His								
(209)	A^2	*SerP*	*Arg*	*SerP*	*Glu*	*Glu*	*Pro*	*Gln*	*Leu*	*Met*	*His*	*Ser*	*Leu/Pro*	*Pro*	*Gln*
	A^3										Gln				
	B						His					Arg			
	C			Ser		Lys	His								
	D	Lys													
	E				Lys										
	F						His							Leu	
	G						His						Leu		
	H^1		Cys						Ile						
	H^2							Glu		Leu					Glu
	I									Leu					
			10			97	104	135	136		148	155			
k-CN	A		*Arg*			*Arg*	*Ser*	*Thr*	*Thr*		*Asp*	*Ser*			
(169)	B								Ile		Ala				
	C					His			Ile		Ala				
	E											Gly			
	F^1										Val				
	F^2		His												
	G^1					Cys		Ile							
	G^2										Ala				
	H								Ile						
	I						Ala								
	J								Ile		Ala	Arg			

GENOMIC ORGANIZATION OF THE GENES OF CASEINS: HAPLOTYPE AND GENOTYPE

The analysis of allelic segregation at 3 casein loci (αs_1-Cn, β-Cn and κ-Cn) showed how these are closely associated with each other, in linkage disequilibrium (LD). Further observations have shown that also αs_2-Cn coding locus is in LD with the other 3 casein loci. Studies conducted at the DNA level have allowed us to establish the physical association of casein loci. The four casein genes (αs_1-Cn ; αs_2-Cn, β-Cn and κ-Cn) are located on chromosome 6 in a 185-250 kb segment in order *CSN1S1* (αs_1-Cn), *CSN2* (β-Cn), *CSN1S2* (αs_2-Cn) and *CSN3* (κ-Cn) [15-16]. Haplotypes are combinations of casein variants that are inherited together. By comparing the effects induced by casein haplotypes with the effect of individual casein variants it is possible to estimate eventual associations between genetic variants and milk protein composition [17]. This chromosomal region is defined super-casein locus

[18]. For example, the κ-Cn E variant of Dutch Friesian cows is associated with a low concentration of αs_2-Cn. Haplotype frequencies show that κ-Cn E variant is expressed predominantly with β-Cn A^1variant. Other haplotypes (β-κ) containing A^1variant (A^1A and A^1B) are also associated with a low αs_2-Cn concentration. These items suggest that the κ-Cn E variant effect on the αs_2-Cn concentration is not caused by β-Cn A^1variant [17].

These observations prove that the four casein loci behave as a single complex gene, in which allelic combinations at the four casein genes on a single chromosome (haplotype) are closely associated with each other susceptible. In order to estimate the effect of casein variants on production, chemical, physico-chemical and technological-dairy milk characteristics it should be preferable to examine the effect exerted by these combinations at genotype or haplotype level [19-20]. Studies conducted on milk component content of Italian Brown cows has shown that (αs_1_β_κ) BB_AA_AB combination compared to BC_AA_AB combination differs for higher calcium (+10 %), magnesium (+12%) and sodium (+12%) and lower potassium (-5%) content. As for the rennet coagulation parameters the first combination showed higher values of k_{20} (+11%) and, conversely, lower than a_{45} (-4%). The comparison BB_BB_AB combination with BB_AA_AB combination showed that first milk combination is characterised by higher contents of both phosphorus (+3.63%) and potassium (+1.92%) and titratable acidity values (+0.15 ºSH/50mL). Furthermore, this milk is characterized by reduced clotting time (-28%) and curd firming time (-32%) and increased curd firmness, measured both 30 (+97%) and 45 (+28%) minutes after rennet addition. Comparisons underline a positive influence of β-Cn B variant on the milk rennet coagulation properties, this is very important in hard cheeses production.

EFFECT OF BREED AND CASEIN GENOTYPES

The differences between ethnic groups regard the presence of certain genetic casein variants is remarkable. The phenomenon interest all loci that control the synthesis of casein [21-22-23-24]. As for the κ-Cn, the genetic structure of Brown, Modenese and Reggiana populations is quite similar, and characterized by a higher frequency of B variant compared to A. In Friesian cows the B variant is less frequent than the A variant [25-26] (Fig. 1a; 1b). The Red Pied shows an intermediate response, and the Jersey is characterized by having a high prevalence of B variant [27].

Larger are the differences between Friesian and Brown at the level of β-Cn genetic types. The structure of Modenese and Reggiana cattle breeds is similar to that of Brown, that is characterized by a lower frequency A than either B and C variants. In this latter case, the Red Pied follows an intermediate behavior for the presence of the C variant [28].

Figure 1a - Frequency% of β-Cn genetic variants in Friesian, Red Pied, Reggiana, Modenese, Brown and Jersey (Mariani *et al.*, 1978; Summer *et al.*, 2009)

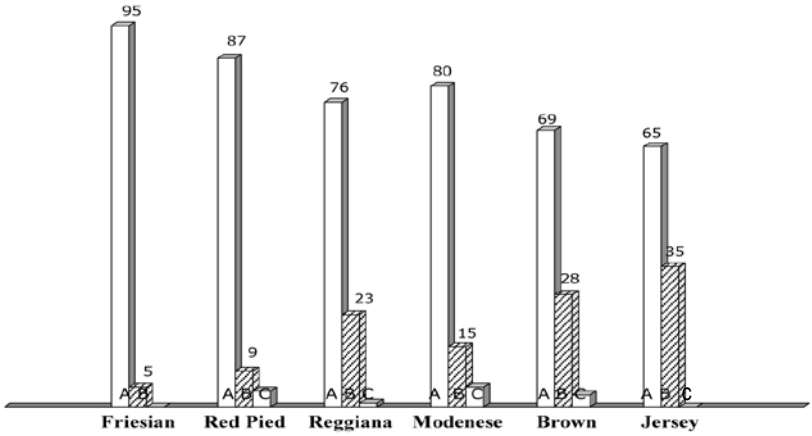
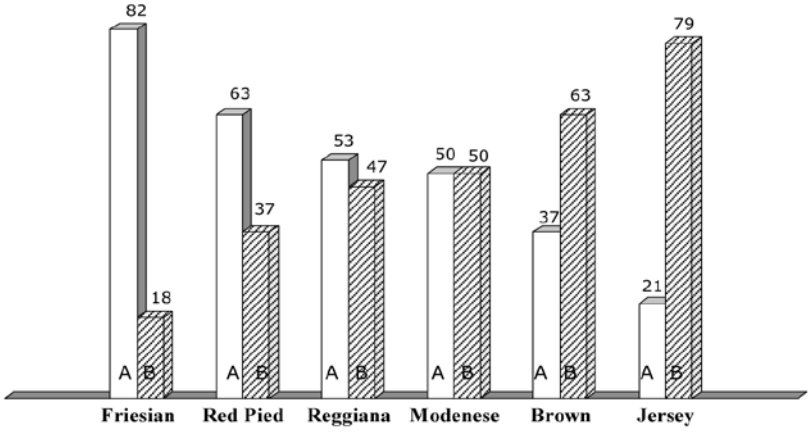


Figure 1b -Frequency% of k-Cn genetic variants in Friesian, Red Pied, Reggiana, Modenese, Brown and Jersey (Mariani *et al.*, 1978; Summer *et al*., 2009)



Electrophoretic analysis of starch and urea at acid pH on the β-Cn A have shown that in Friesian cows prevails A^1variant , while in Brown, Modenese and Reggiana cows the most frequent is A^2 variant [29].

BREED AND CALCIUM AND PHOSPHORUS CONTENT

As 2/3 of total calcium and 1/2 of total phosphorus are constituent of the casein micelle, their contents varied according to protein content. The milk of the breeds differ also for the content of major minerals [30]. The Jersey milk is very rich in calcium and phosphorus (Tab. 2), as shown in U.S., Canadian, and others surveys [31-32]. The Jersey milk contains 20% more calcium and phosphorus compared to Holstein milk. Brown Swiss. Guernsey milks have intermediate calcium values, while phosphorus values are quite high.

Table 2 - Calcium and phosphorus milk contents of American, Canadian and Italian breeds [30]

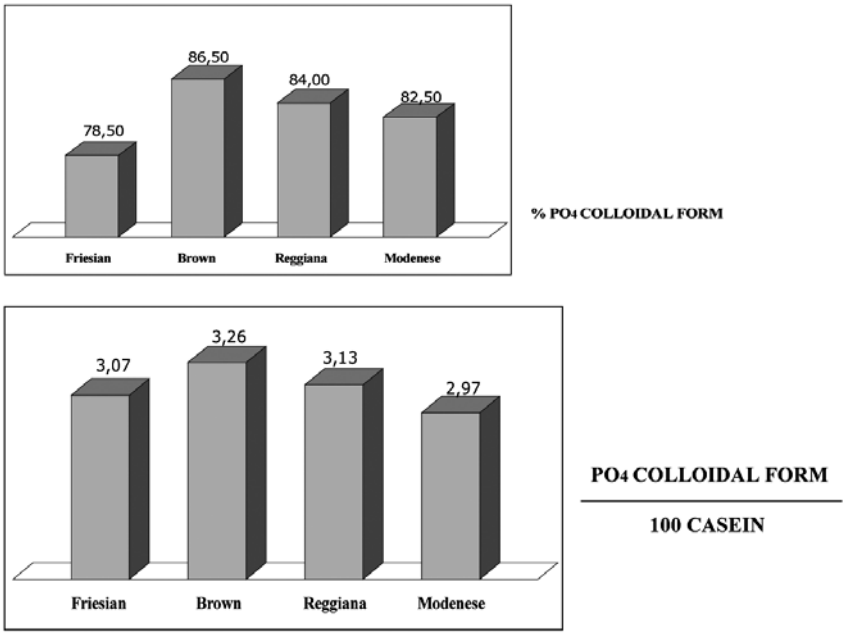
	Calcium	Phosphorus
Holstein Friesian	115	93
Ayrshire	116	97
Brown Swiss	127	111
Guernsey	134	110
Jersey	140	115
Italian Friesian	116	88
Reggiana	129	97
Modenese: Ca/P less than Italian Friesian, Italian Brown and Reggiana		

The differences between Italian cattle breeds are in order of 10% or slightly more. The Reggiana milk have high calcium and phosphorus content than the Italian Friesian milk (Tab. 2). The Italian Brown, Modenese and Reggiana milks are similar among them but they differ significantly from Italian Friesian milk [30]. The Modenese milk is characterized by Ca / P ratio lower than other breeds. This milk has higher phosphorus content and lower calcium content in relation to Italian Brown and Reggiana. The Italian Red Pied milk presents more calcium than Friesian milk and it does not seem to contain significant amounts of phosphorus if compared to those of Italian Brown, Reggiana and Modenese [33].

EFFECT OF BREED AND COLLOIDAL CALCIUM PHOSPHATE

Colloidal contents of both calcium and phosphorus are integral structural components of the micellar complex. Their contents changes with the levels of total casein content, so that each milk tends to express its characteristics [34]. The Brown, Modenese and Reggiana contain milks higher amount of colloidal calcium and colloidal phosphate than Italian Friesian [35]. The same is true for Italian Red Pied. The Modenese milk contains less colloidal calcium per unit of colloidal phosphorus, while this ratio is almost identical for the milk of Italian Brown, Reggiana and Italian Friesian cows [35]. In particular, the Reggiana milk contains large amounts of colloidal calcium phosphate in relation to that of Italian Friesian [36]. Other observations shows that in decreasing order Brown, Modenese and Reggiana milks have more colloidal phosphate and the Italian Red Pied is not much different than milk from Italian Friesian (Fig. 2).

Fig 2 - Colloidal inorganic phosphate in the milk of Friesian-FR, Brown-BA, Reggiana-RG and Modenese-MO with statistical significance of differences: above (* P <0.05), below (** P <0.01) [34]



Such differences, if reported to the casein content, are significantly reduced [37], but they are equally important in terms of milk quality. The Brown and Reggiana milk are proportionally richer in colloidal phosphate [35]. The Modenese milk shows less phosphate per unit of casein when compared to Italian Friesian. In fact, the milk of Modenese is characterised by lower degree of mineralization of the casein micelle. This milk is characterized by lower proportion of inorganic phosphate and that is explained by its high rate of casein. While the analogous phenomenon, which seems to involve the Red Pied , is related to a lower colloidal phosphorus content.

TYPES OF κ-CASEIN AND SIZE DISTRIBUTION OF CASEIN MICELLES

The amount of κ-Cn which enters into the native casein constitution tends to vary according to genetic protein type. The κ-Cn B type contains a higher proportion of κ-Cn than κ-Cn A type. The values reported in the literature (Fig. 3) agree that κ-Cn B type has highest proportions of κ-Cn respect to κ-Cn AB and κ-Cn A [38]. The different percentage casein distribution in relation to the genetic κ-Cn type is confirmed by further research as the ones of Law *et al*. [39] and Walsh *et al*. [40]. The κ-Cn B curd analyzed by electron microscopy (SEM) is like a gel in which paracasein molecules are evenly distributed creating a network. The picture of κ-Cn A type is characterized by a greater number of "large" micelles (2.4% κ-Cn A *vs* 0.8% κ-Cn B) and a significant proportion of submicelles (45.4% κ-Cn A *vs* 30.0% κ-Cn B). On the other hand, κ-Cn

B-casein is richer of small and medium-sized micelles (29.5% κ-Cn A *vs* 43.3% κ-Cn B). Casein κ-Cn B-type has greater total micellar surface [41]. The κ-Cn A and κ-Cn B milks differ for casein content and the casein number. These values are both in favor of κ-Cn B milk. Important differences are also observed for the sialic acid casein content (highest for κ-Cn B type) and for the citric acid content. The κ-Cn B milk contains lower amounts of citric acid compared to that of κ-Cn A type [42].

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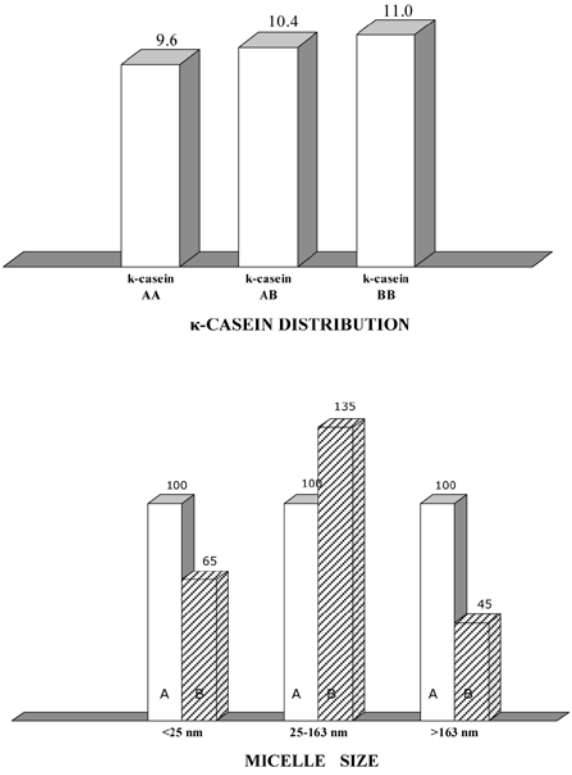
Figure 3 - Types of κ-Cn (above) and proportions of κ-Cn, size of the micelles (below) (from Mariani and Pecorari 1991)



κ-CASEIN TYPES AND RENNET COAGULATION CHARACTERISTICS OF MILK

The κ-casein is the substrate of chymosin action. The hydrolytic enzyme activity triggers the process of micellar system destabilization. This physico-chemical reactions lead to curd formation and syneresis. Its most common variants, A and B, show a different behavior during the micelle construction/ destabilization phases. Various hypotheses have been formulated about the possible mechanisms causing the different behavior of the milk κ-Cn type A and κ-Cn type B. One of the hypotheses is the different expression of two alleles that control the protein synthesis. The different availability of κ-Cn is capable of constraining the micelle size, which, in turn impact

on its reactivity toward chymosin, the clotting enzyme present in rennet [43]. The κ-Cn B milk reacts more readily with chymosin and it shows a clotting time significantly lower than that of κ-Cn A (Fig. 5). κ-Cn AB milk shows an intermediate response. These findings are amply confirmed in numerous investigations whose results were collected by Jakob and Puhan in 1994 and are shown in Figure 5 [44-40].

Figure 4 - Types of κ-casein and milk rennet clotting time (κ-Cn A = 100) (from Mariani and Pecorari 1991)

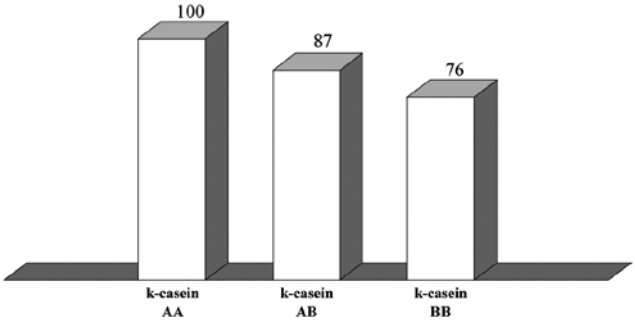
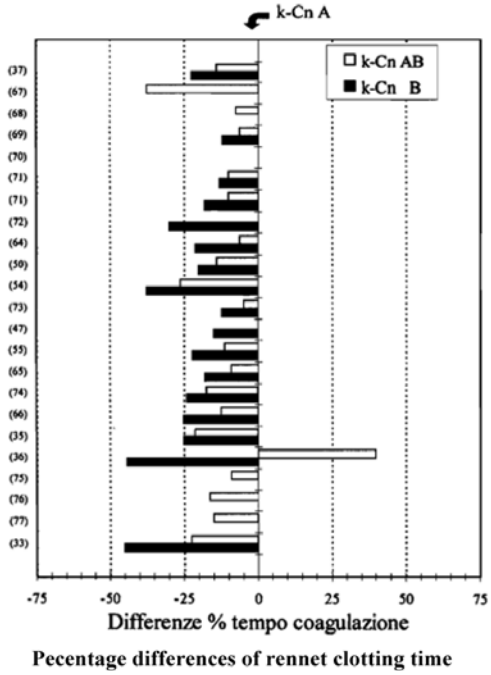
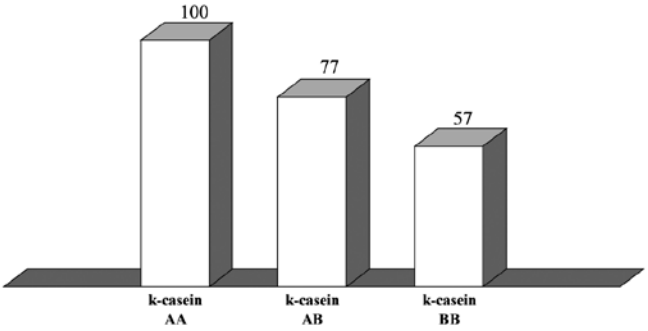


Figure 5 - Types of κ-casein and milk rennet clotting time, according to several authors: percentage differences compared to κ-Cn A (Jakob and Puhan, 1994)



The κ-casein type also has a important effect on the aggregation rate of paracasein micelles. The κ-casein B milk is characterized by a high rate of curd formation [45] (Fig. 6).

Figure 6 - Types of κ-casein and milk firming time (κ-Cn A = 100) (from Mariani and Pecorari 1991)



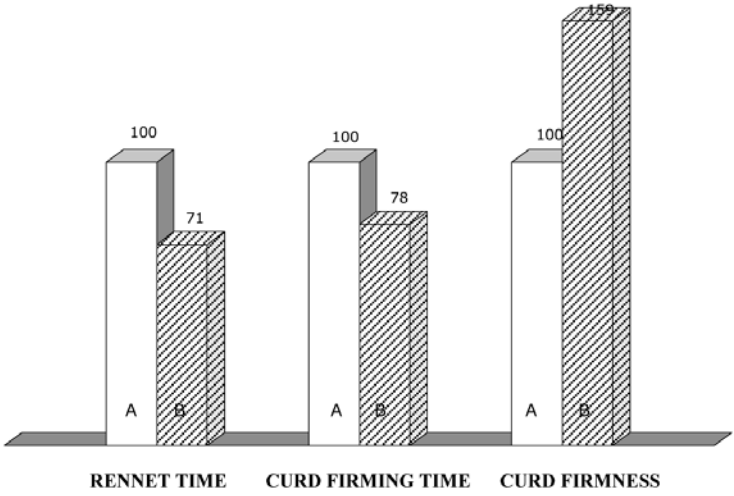
The κ-Cn B milk clots usually in lower times and gives rise to a coagulum that quickly reach a optimal texture for very hard cheese production [46]. The κ-Cn A milk is less favorable for coagulation. This behavior is amply confirmed by gelometric and tensiometric properties of milk before adding of the natural wheystarter. The κ-Cn B curd is more elastic with a network suitable for cheese syneresis, while the κ-Cn A milk gives less consistent curds. The dynamics of curd formation indicate that the κ-Cn B achieves a more contraction force, so it is able to release better and faster the whey. The study of the relationships between genetic types and coagulation characteristics of milk has also concerned other variants of k-casein. According to the survey of Lodes *et al*. [47], κ-Cn AC milk coagulates slowly than all other types. The German authors observed the following trend of clotting time, in order from fastest to slowest: κ-Cn BE <BB <AB <AA = AE <BC <AC. Even κ-Cn BC milk tends to show a rather long clotting time. This is caused by the negative influence of κ-Cn C variant. In fact, its structure is modified such in a way to slow the hydrolysis by chymosin [48-49-50]. The influence of κ-Cn E variant is almost equivalent to that of κ-Cn A: the κ-Cn AE milk coagulates more slowly than the κ-Cn B. Milks containing κ-Cn E variant are characterized by a curd very weak, while the κ-Cn C variant provides strong curd when it is associated with B variant and a coagulum of intermediate consistency with A variant [51].

β-CASEIN TYPES, CHARACTERISTICS OF MILK AND RENNET COAGULATION

The available observations with regard to β-Cn reveal that β-Cn B has effects very similar and of the same sign as those that characterize the κ-Cn B. The β-Cn B micelles are more reactive and more sensitive to the action of chymosin than those containing the β-Cn A. They tend to coagulate more quickly and give rise to a firmness gel [52].

The β-Cn B milk has a curd that hardens faster than A β-Cn type, while the curd firmness does not seem to vary in important measure [53]. An important indication cames from analysis of casein micelles by electron microscopy. The β-Cn A and β-Cn B milks differ in terms of leakage of native casein micelle. The increased of small micelle size frequency present in the β-Cn B milk could explain its different technological behavior especially as regards the better reactivity with rennet. The greater readiness with which the β-Cn B clot is formed can be seen in relation with primary reaction (faster) and the net negative charge (lower) of native casein. These conditions can significantly affect the rate of aggregates formation and of the casein network construction [54]. Curd firmness, adjusted for clotting time, was not different between β-Cn B and β-Cn A. In fact, the best coagulation aptitude of β-Cn B milk is caused by readiness with which the B variant reacts with rennet. Probably the responsiveness is determined by a larger area of native casein (Fig. 7) [55]. The same authors found that the caseine micelles containing β-casein C are characterized by having largest size. Also Delacroix-Buchet and Marie note that the β-Cn C micelles have a diameter significantly larger (+55%) than β-Cn A type [56-57].

Figure 7 - Types of β-casein and rennet clotting time (r), curd firming time (k_{20}) and curd firmness (a_{30}) (β-Cn A = 100) (by Mariani *et al.*, 1992)



TYPES OF A_{S1}-CASEIN, MILK COMPOSITION AND DISTRIBUTION OF CASEIN

Among the different alleles that control the α_{S1} casein synthesis, the G allele, while not changing the specific protein, is able to influence some of the most important milk characteristics, especially the composition of the micellar system.

The changes regarding the composition of casein are essentially linked to the direct quantitative effect of this allele, which manifests itself through a very low α_{S1}-casein synthesis capacity, if compared to α_{S1}-Cn B and α_{S1}-Cn C alleles. This involves sensitive changes in the native casein composition, which affect the glycosylation

(more N-acetylneuraminic acid) and phosphorylation (less phosphorus ester) degree (Tab. 3) [58]. The milk of α_{S1}-Cn G cows is provided with less casein, despite the genetic structure of these cows is favored in κ-casein type. In fact, the α_{S1}-Cn G allele is always in linkage disequilibrium with the k-Cn B allele.

Table 3 - Main characteristics of milk from cows heterozygous for the αS1-Cn G allele: comparison with "αS1-normal" cows (by Mariani et al., 1996).

		α_{s1}-casein low (L) (n=23)[1]	α_{s1}-casein normal (N) (n=23)[2]	Difference (L-N)	N=100
Protein (Nx6.38)	g/100g	3.09	3.26	* - 0.17	95
Casein (Nx6.38)	g/100g	2,41	2.55	* - 0.14	94
Nana casein/casein	g/100g	0,42 [3]	0.35 [3]	** 0.07	120
P casein/casein	g/100g	0,81 [3]	0,84 [3]	** - 0.03	96
pH	unit	6,75	6,73	NS 0.02	—
Tritatable acidity	°SH/50mL	3,07	3.34	* - 0.27	92

[1] Individual milk of α_{s1}-casein BG e CG cows

[2] Individual milk of αs1-casein BB, BC e CC cows

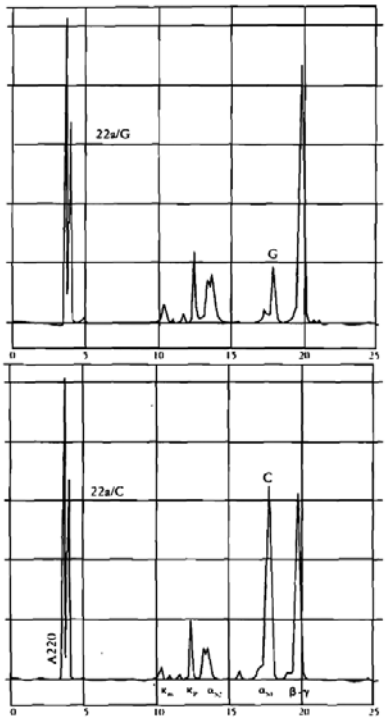
[3] 20 Individual milk samples

Nana casein/casein

NS *P≤0.05; **P≤0.01

The most significant changes relate to the distribution of casein. The casein heterozygous cows for the α_{S1}-Cn G allele (α_{S1}-Cn BG and CG) differs markedly from that α_{S1}-Cn BB, BC and CC cows. It contains 25% less α_{S1}-Cn (27.69% *vs* 36.68%), it is relatively richer of k-Cn (15.10% vs 12.23%), of α_{S2}-Cn (13.26% *vs* 11.62%) and of β + γ-Cn (43.95% *vs* 39.47%). The increase of κ-Cn is caused by minor glycosylated fractions (7.08% *vs* 5.93%) and by main non-glycosylated fractions (8.02% *vs* 6.30%). The quantitative effect of the G allele is perfectly reflected in the characteristic of homozygous cows. In fact, in the milk of these cows, the specific α_{S1}-Cn proportion is reduced by more than half (about 55%). The α_{S1}-Cn drop implies an increase in γ + β-Cn and especially in κ-Cn percentage (Fig. 8) [57].

Figure 8 - RP-HPLC profiles of isoelectric casein α_{S1}-Cn G type (above) and α_{S1}-Cn C (below) (by Mariani *et al*., 1995)



The α_{S1}-Cn drop means a relative β-Cn and β+γ-Cn increase, particularly κ-Cn increasing of 54% (Tab. 4) [58]. The native casein of α_{S1}-Cn G milk has a greater submicelle proportion (34%) and a lower of proportion micelle with a diameter ranging from 76 to 125 nm. The submicelles isolated from α_{S1}-Cn G milk have a greater average diameter those α_{S1}-Cn B [44].

Table 4 - Types of α_{S1}-Cn and percentage distribution of caseins (by Mariani *et al*., 1995)

		α_{s1}-CnG[1]	α_{s1}-CnB[2]	Diff. (G-B)	"B"=100
κ-casein	%	19.05	12.07	6.80	154
α_{s2}-casein	%	16.09	12.01	4.80	140
α_{s1}-casein	%	16.01	35.90	-19.80	45
β+γ-casein	%	47.50	39.30	8.20	121
κ/α_{s1}		1.21	0.35	0.86	346

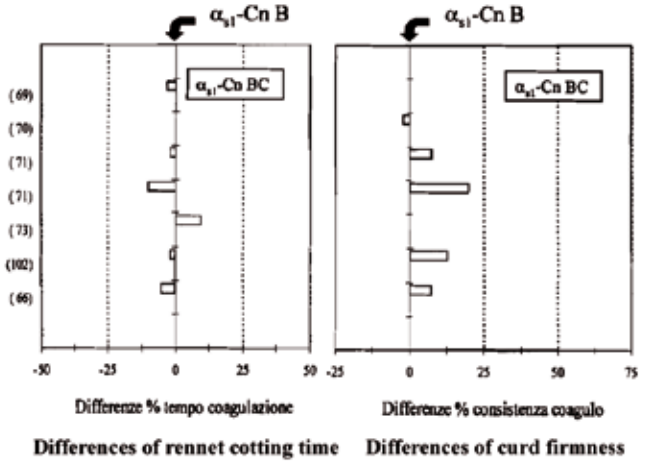
[1] 3 individual milk samples of α_{s1}-Cn cows

[2] 3 individual milk samples of α_{s1}-Cn BB and CC cows

TYPES OF A_{S1} CASEIN AND RENNET COAGULATION OF MILK

The most common α_{S1}-Cn variants, B and C, do not seem able to exert significant influence on the main coagulation characteristics of milk. The α_{S1}-Cn BC combination is better in terms of curd firmness (Fig. 9) [59-60].

Figure 9 - Types of α_{S1}-Cn, clotting time and curd firmness milk:% differences compared to α_{S1}-Cn B (by Puhan and Jakob, 1994)



Relevant is the role of α_{S1}-Cn A, whose presence induces changes of the native casein structure with particular reference to hydration, micellar dispersion degree and solubility in presence of ionic calcium [61-62]. The α_{S1}-Cn A micelles are more hydrated and richer of κ-Cn than α_{S1}-Cn B type. This rare variant gives a different behavior of milk during heat treatment and transformation processes. The milk with α_{S1}-Cn A presents micellar features more heterogeneous, characterized by a moderate large micelle proportion and a high frequency of very small size micelle [63]. Its solubility in presence of ionic calcium is significantly different than that α_{S1}-Cn B

and α_{S1}-Cn C variants, so that his behavior would be more similar to that of β-Cn [64]. The α_{S1}-Cn A milk hardens more slowly than α_{S1}-Cn B and α_{S1}-Cn C milks and also give rise to a soft curd not suitable for cheese production. Conversely, the α_{S1}-Cn BC milk coagulates in lower time and provides a compact curd, better than that obtained with α_{S1}-Cn B milk [65]. The peculiarities of α_{S1} Cn A are largely attributed to the deletion amino acids from 14 to 26, which includes the hydrophobic region located between two hydrophilic regions [65]. Because of the deletion, this protein is more resistant to chymosin and pepsin action. In fact, α_{S1}-Cn A lack the 22-23 peptide bond, which hydrolysis by chymosin give rise to the fragment α_{S1}-I. That makes variant considerably less sensitive against proteolytic degradation compared to α_{S1}-Cn B and α_{S1}-Cn C caseins. Milk contains casein with low α_{S1}-Cn G proportion coagulates in a less time than "α_{S1}-normal" milk (Tab. 5) [66].

Table 5 - Coagulation milk characteristics of heterozygous cows for the α_{S1}-Cn G allele and comparison with "α_{S1}-normal" cows (Mariani *et al.*, 1993)

		α_{s1}-casein low (L) (n=5)[1]	α_{s1}-casein normal (N) (n=5)[2]	Difference (L-N)	N=100
pH	unit	6.77	6.69	0.08	—
Titratable acidity	°SH/50mL	3.07	3.57	0.09	86
Rennet time (r)	min	13.74 [3]	15.36 [3]	0.10	87
Curd firming time (k_{20})	min	6.74 [3]	10.32 [3]	0.11	88
Curd firmnes (a_{45})	min	44.00	36.38	0.12	89
Curd firmness ($a_{1/2r}$) [3]	min	22.68	17.74	0.13	90

[1] Individual milk of α_{s1}-casein BG e CG cows

[2] Individual milk of αs1-casein BB, BC e CC cows

[3] (a) Consistency clot related to coagulation time (1/2r)

The milk of heterozygous cows for the allele α_{S1}-Cn G exhibits a lower coagulation time (about 22%) than α_{S1}-normal milk. The curd of milk with a low α_{S1}-Cn proportion is formed more quickly and reaches a higher final consistency. In addition, the curd is characterised by better rheological features: higher syneresis, greater resistance to cut and resistance to compression.These data suggest that the α_{S1}-Cn G allele is able to influence significantly the main rennet coagulation properties of milk.

CONCLUSIONS

The study of genetic casein variability in bovine milk at the level of haplotype is a crucial element in terms of livestock-industry. The loss or addition of specific genetic combinations may have important effects on milk for quality, nutrition, rheology and technology. It is necessary to develop analysis instruments that allow you to highlight variability in the gene complex of casein with limited cost and with increasing accuracy. In this regard, recent technological advances are pushing for the use of DNA microarray techniques for the characterization of Single Nucleotide Polymorphisms (SNiPs). Through this method it is possible to extract information regarding the existence of mutation points that determine the allelic differences for the αs_1-Cn, αs_2-Cn, β-Cn and κ-Cn caseins.

REFERENCES

1. Mariani P., Pecorari M. (1987): *Fattori genetici, attitudine alla caseificazione e resa in formaggio.* Sci. Tecn. Latt.-Cas. 38, pp 286
2. De Kruif C.G. (1998): *Supra-aggregates of casein micelles as a prelude to coagulation* . J. Dairy Sci. 81, pp 3019-3028
3. Losi G., Mariani P. (1984): *Significato tecnologico del polimorfismo delle proteine del latte nella caseificazione a formaggio gran.* Ind. Latte 20, pp 23-53
4. Mariani P., Summer A., Formaggioni P., Malacarne M., Battistotti B. (2001): *Rilievi sui principali requisiti tecnologico-caseari del latte per la produzione di formaggio grana.* Sci. Tecn. Latt.-Cas. 52, pp 49-91
5. Fox P.F., Brodkorb A. (2008): *The casein micelle: Historical aspects, current concepts and significance*. International Dairy Journal 18, pp 677-684
6. Shimdt D.G. (1980): *Colloidal aspects of casein.* Neth. Milk Dairy J. 34, pp 42-64
7. Lucey J.A., Johnson M.E., Horne D.S. (2003): *Perspectives on the Basis of the Rheology and Texture Properties of Cheese*. J. Dairy Sci. 86, pp 2725-2743
8. Corradini C., Pettinau M. (1972): *Possibili riflessi tecnologici del polimorfismo delle caseine.* Sci. Tecn. Latt.-cas. 23, pp 243-253
9. Mariani P. (1983): *Genetica e qualità del latte per la caseificazione.* Ob. Doc. Vet. *4 (*9), pp 25-30
10. Mariani P., Summer A. (1999): *Polimorfismo delle proteine ed attitudine tecnologico-casearia del latte*. Sci. Tecn. Latt.-Cas. 50, pp 197-230
11. Russo V. (1975): *Polimorfismo delle proteine del latte e possibilità di utilizzazione zootecnica.* Il Mondo del Latte 29, pp 296-304
12. Mariani P. (1988): *Fattori genetici, proprietà tecnologico-casearie e resa del latte in formaggio grana*. Atti Convegno "La trasformazione del latte in formaggio grana: tecnologie e qualità", Gonzaga (MN), pp. 9-30
13. Formaggioni P., Summer A., Malacarne M., Mariani P. (1999): *Milk protein polymorphism: detection and diffusion of the genetic variants in Bos genus.* Ann. Fac. Med. Veter. Univ. Parma 19, pp 127-165
14. Malacarne M., Summer A., Mariani P. (2010): *About the characterisation of the genetic variants F^2 and G^1 of cow's milk k-casein*. J. Dairy Sci.93, pp 796-800
15. Ferretti L., Leone P., Sgaramella V. (1990): *Long range restriction analysis of the bovine casein genes*. Nucleic Acids Res. 18, pp 6829–6833
16. Threadgill D.W., Womack J.E. (1990): *Genomic analysis of the major bovine milk proteins genes*. Nucleic Acids Res. 18, pp 6935–6942
17. Heck J.M.L., Schennink A., Van Valemberg H.J.F., Bovenhuis H., Visker M.H.P.W., Van Arendon J.A.M., Van Hooijdonk A.C.M. (2009): *Effects of milk protein variants on the protein composition of bovine milk*. J. Dairy Sci. 92, pp 1192-1202
18. Caroli A.M., Chessa S., Erhardt G.J. (2009): *Invited review: milk protein polymorphisms in cattle: effect on animal breeding and hu man nutrition*. J. Dairy Sci. 92, pp 5335-5352
19. Ikonen T., Bovenhuis H., Ojala M., Ruottinen O., Georges M. (2001): *Associations between casein aplotypes and first lactation milk production traits in Finn-*

ish Ayrshire cows. J. Dairy Sci. 84, pp 507-514
20. Ikonen T., Ojala M., Ruottinen O. (1999): *Associations between milk protein polymorphism and first lactation milk production traits in Finnish Ayrshire cows*. J. Dairy Sci. 82, pp 1026-1033
21. Aschaffenburg R. (1968): *Genetic variants of milk proteins: Their breed distribution*.J. Dairy Res. 35, pp 447
22. Grosclaude F. (1974): *Analyse genetique et biochimique du polymorphisme electrophoretique des caseines* α_{s1}, *β, et κ chez les bovins (Bos taurus) et les Zebù (Bos indicus)*. Thèse Doctorat d'Etat-ès-Sciences, Universitè Paris VII
23. Hossain M.A. (1974): *Genetic variants of milk protein and their importance in dairying*. Kieler Milchwirtschaft, Forschung 26, pp 17
24. Thompson M.P., Farrel $H.J_{R}$., (1974): *Genetic variants of the milk proteins*. in Lactation: A comprehensive treatise vol 3, Acad. Press N. York, pp. 109
25. Russo V., Mariani P. (1978): *Polimorfismo delle proteine del latte e relazioni tra varianti genetiche e caratteristiche di interesse zootecnico, tecnologico e caseario.* Riv. Zoot. Vet. 6, pp 289-365
26. Summer A., Malacarne M., Formaggioni P., Franceschi P., Mariani P. (2009): *Fattori genetici e valorizzazione tecnologico casearia del latte*. Sci. Tecn. Latt.-Cas. 60, pp 415-440
27. Bettini T.M., Masina P. (1972): *Proteine e polimorfismo proteico del latte vaccino.* Prod. Anim. 11, pp 107
28. Corradini C., Battistotti B. (1973): *Indagine elettroforetica della progressiva idrolisi delle proteine durante la maturazione del formaggio Montasio*. Sci. Tecn. Latt.-Cas. 24, pp 11
29. Di Stasio L. (1983): *Indagine elettroforetica sulle razze bovine Modicana e Cinisara mediante l'analisi dei sistemi proteici del latte*. Riv. Zoot. Vet. 11, pp 70
30. Mariani P., Russo V. (1976): *Ricerche sul contenuto di calcio e fosforo nel latte delle razze Frisona, Bruna alpina, Reggiana e Modenese*. Riv. Zoot. Vet. 4, pp 23
31. Reinart A., Nesbitt J.M. (1956): *The composition of milk in Manitoba*. XIV th Int. Dairy Congr. 1 (2), pp 946
32. Robertson N.H., Dixon A. (1967): *The influence of certain milk salts, their relative ratios and their ratios to total nitrogen on the heat stability of milk in the winter rainfall region*. S. Afr. J. Agric. Sci. 10, pp 909
33. Comberg G. (1967): *New investigations on the mineral content of bovine milk.* D.te Tieraztl. Wschr. 74, pp 613
34. Mariani P., Colajacomo A. (1971): *Caseificazione del latte delle razze Reggiana e Frisona: aspetti tecnologici, resa e caratteristiche dei sieri e dei formagg.* Riv. Zoot. 44, pp 207
35. Mariani P. (1985): *Osservazioni sul contenuto e la ripartizione dei principali costituenti del sistema micellare del latte in quattro razze bovine*. Ann. Fac. Med. Vet. 5, Univ. Parma, pp 173
36. Resmini P., Volonterio G., Annibaldi S. (1971): *Alcune carateristiche chimiche e chimico-fisiche di latti destinati alla produzione di formaggio Parmigiano-Reggiano*. Sci. Tecn. Latt-Cas. 22, pp 63
37. Mariani P. (1973): *Correlazione fra le caratteristiche del latte nelle razze Reg-*

giana e Frisona. Riv. Zoot. Vet. 1, pp 459
38. Mariani P., Pecorari M. (1991): *Il ruolo delle varianti genetiche della κ-caseina nella produzione del formaggio*. Sci. Tecn. Latt.-Cas. 42, pp 255-285
39. Law A.J.R., Leaver J., Banks J.M., Horne D.S. (1994): *The effect of κ-casein genotype on the composition of whole casein.* IDF (International Dairy Federation), Brussel, Special Issue no. 9402, pp 134-141
40. Walsh C.D., Guinee T.P., Reville W.D., Harrington D., Murphy J.J., O'Kennedy B.T., FitzGerald R.J. (1998): *Influence of κ-casein genetic variant on rennet gel microstructure, Cheddar cheesemaking properties and casein micelle size*. Int. Dairy Journal 8, pp 707-714
41. Mariani P., Summer A., Rando A., Fossa E., Serventi P. (1996): *N-acetylneuraminic acid content of casein in Italian Brown cows with different genotypes at the casein κ-casein locus*. Ann. Fac. Med. Vet. 16, Univ. Parma, pp 83-95
42. Morini D., Losi G., Castagnetti G.B., Benevelli M., Resmini P., Volonterio G. (1975): *L'influenza delle varianti genetiche della κ-caseina sulla dimensione delle micelle caseiniche*. Sci. Tecn. Latt-Cas. 26, pp 437-444
43. Puhan Z., Jakob E. (1994): *Genetic variants of milk proteins and cheese yield*. IDF(International Dairy Federation), Brussel, Special Issue no. 9402, pp 11-122
44. Pecorari M., Mariani P. (1990): *Caseina, attitudine alla coagulazione del latte, resa e qualità del formaggio.* Sci. Tecn. Latt.-Cas. 41, pp 225-244
45. Lodes A., Krause I., Buchberger J., Aumann J., Klostermeyer H. (1996): *The influence of genetic variants of milk proteins on the compositional and technological properties of milk.* Milkwissenschaft 51, pp 543-548
46. Jakob E. (1193): *Relationships between genetic polymorphism of milk proteins and the rennetability of milk* Thesis ETH, Eidgen. Techn. Hochshule, Zürich, no. 10224, XIII pp 220
47. Plowman J.E., Creamer L.K., Smith M.H., Hill J.P. (1997): *Restrained molecular dynamics investigation of the differences in association of chymosin to κ-caseins* A and C J. Dairy Res. 64, pp 299-304
48. Smith M.H., Hill J.P., Creamer L.K., Plowman J.H. (1997): *Towards understanding the variant effect on the rate of cleavage by chymosin on κ-casein*. A and C. Proc. IDF Seminar, Palmeston North, New Zealand, International Dairy Federation, Brussel, pp 185-189
49. Grosclaude F. (1988): *Le polymorphism gènetique des principales lactoproteines bovines. Relations avec la quantitè, la compositionet les aptitudes fromagères du lait*. INRA Prod. Anim. 1, pp 5-17
50. Mariani P., Meazza M., Resmini P., Pagani M.A., Pecorari M., Fossa E. (1986): *Osservazioni sui tipi di beta-caseina e caratteristiche di coagulazione del latte* L'Industria del Latte 22, pp 35-58
51. Mariani P., Zanzucchi G., Bonatti P., Fossa E., Pecorari M. (1992): *Caratteristiche di coagulazione del latte in rapporto al tipo genetico della beta-caseina in vacche di razza Bruna*. Sci. Tecn. Latt.-Cas. 43, pp 7-17
52. Lodes A., Krause I., Buchberger J., Aumann J., Klostermeyer H. (1996): *The influence of genetic variants of milk proteins on the compositional and techno-*

logical properties of milk. Milchwissenschaft 51, pp 368-373

53. Delacroix-Buchet A., Marie C. (1994): *Comparaison des variants A et C de la casèine β des laits de vaches Tarentaises en modèle fromager de type Beaufort. I. Aptitudes fromagères et rendements en frais.* Lait 74, pp 343-360
54. Mariani P., Zanzucchi G., Summer A., Senese C., Rando A., Masina P., Pecorari M. (1996): *Effects of the α_{s1}CnG allele on the main characteristics and nitrogen fractions of milk from Italian Brown cows.* Sci. Tecn. Latt.-Cas..47, pp 245-260
55. Sala G., De Noni I., Pagani M.A., Mariani P., Rando A. (1995): *Alcune caratteristiche delle micelle caseiniche in latti individuali con bassa proporzione di α_{s1}caseina.* Sci. Tecn. Latt.-Cas. 46, pp 290-300
56. Mariani P., Bonatti P., Pecorari M. (1988): *Rennet coagulation properties of cow milk in relation to α_{s1}-casein genotype*. Sci. Tecn. Latt.-Cas. 39, pp 431-438
57. Mariani P., Summer A., Anghinetti A., Senese C., Di Gregorio P., Rando A., Serventi P. (1984). *Effetti dell' allele α_{s1}-Cn G sulla ripartizione percentuale delle caseine $\alpha_{s1,}$ $\alpha_{s2,}$, β e κ in vacche di razza Bruna*. L'Industria del Latte 31 (4), pp 3-13
58. Losi G., Mariani P. (1995): *Significato tecnologico del polimorfismo delle proteine del latte nella caseificazione a formaggio grana.* L'Industria del Latte 20, pp 23-53
59. Dewan R.K., Chudgar A., Bloomfield V.A., Morr C.V. (1974): *Size distribution and solvation of casein micelles in milk containing α_{s1}-casein A*. J. Dairy Sci. 57, pp 394-398
60. Thompson M.P., Gordon W.G., Boswell R.T., Farrell H.M.Jr. (1969): *Solubility solvation and stabilization of α_{s1} - and β-caseins.* J. Dairy Sci. 52, pp 1166-1173
61. Salder A.M., Kiddy C.A., McCann R.E., Mattingly W.A. (1968): *Acid production and curd toughnes in milks of different α_{s1}- casein type.* J. Dairy Sci. 51, pp 28-30
62. Sherbon J.W., Ledford R.A., Regenstein J., Thompson M.P. (1967): *Variants of milk proteins and their possible relation to milk properties.* J. Dairy Sci. 50, pp 951
63. Mariani P., Morini D., Losi G., Castagnetti G.B., Fossa E., Russo V. (1979): *Ripartizione delle frazioni azotate del latte in vacche caratterizzate da genotipo diverso nel locus β-lattoglobulina*. Sci. Tecn. Latt.-Cas. 30, pp 153-176
64. Creamer L.K. (1974): *Preparation of α_{s1} -casein* A. J. Dairy Sci. 57, pp 341-344
65. Coker C.J., Creamer L.K., Burr R.G., Hill J.P. (1997): *The action of chymosin or plasmin on α_{s1} -casein A, B and C*. Proc. IDF Seminar, Palmerston North, New Zealand, International Dairy Federation, Brussel, pp 175-181
66. Mariani P., Anghinetti A., Serventi P., Fossa E. (1993): *Frazionamento della caseina mediante RP-HPLC: sulla osservazione di alcuni latti individuali caratterizzati da una bassa proporzione di α_{s1} -caseina in vacche di razza Bruna*. L'Industria del Latte 29, pp 75-85

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DIVERSIFICATION OF FARMING ACTIVITIES: NEW INCOME OPPORTUNITIES

LA DIVERSIFICAZIONE DELLE ATTIVITA' AZIENDALI: NUOVE OPPORTUNITA' DI REDDITO

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ABSTRACT

The survey aims at monitoring the behavior of the farmer face of the agrarian crisis. It examines the activities' diversification strategy in particular.

The recent modifications of art. 2135 of the Civil Code allow the farmer to expand his competences and to increase his income opportunities.

The detailed list of the related activities, approved by ministerial decrees, defines the new boundaries of the activities that can take advantage of the agricultural income tax rules.

The business survey and the drawing up of the transitional balance sheet, from traditional farming to pluriactivity, have allowed us to highlight the business strategies aimed at broadening the farm's budget which are reflected in the high value added activities' choice without the need to expand the business' land area.

For many small-scale agricultural enterprises located in difficult areas, the only perspective of survival is related to their ability to benefit from the new arising opportunities.

KEYWORDS:

business strategies; multifunctionality, agricultural income

1. INTRODUCTION

Agricultural diversification is an issue of particular actuality, in view of the sector crisis and the opportunities offered by recent legislation.

This income crisis is putting a strain on the survival of many farms with the risk of marginalization and over spilling from the primary sector (Romano 2010; Van Der Ploeg 2007).

The diversification strategy, as a privileged path to deal with the income crisis, is a rapidly spreading phenomenon that highlights the farmers' enterprise abilities seeking the most appropriate solutions for the business' and family's specificities (Casini 2002; Ellis 2003; OECD 2009; Rete Rurale Nazionale 2010).

What emerges is a mixed picture of different solutions that give an idea of a site in progress, seeking the most efficient combination and taking into account the opportuni-

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ties and the constraints of non-agricultural origins (European Commission 2008).
The work consists of the analysis of the various diversification forms, the dynamics of legislation and the study of the transitional economic balance of a business case.

2. MULTIFUNCTIONALITY, PLURIACTIVITY & PRODUCTION DIVERSIFICATION

The agriculture multifunctionality is one of the strategic keys to valorize and develop the sector. The European Union defines the term multifunctionality as "the fundamental link between sustainable agriculture, food safety, territorial balance, preservation of landscape and environment, and food supply securing" (Van Der Ploeg *et al* 2003). Recently, the Commissioner for Agriculture and Rural Development, Cioloş, has expressly stated that he is working towards maintaining a strong Common Agricultural Policy even after 2013. In a discussion paper about the future of the CAP, the DG Agriculture has supported the idea of keeping a slightly fixed sustain to the farmers' income enabling them to ensure the production of "public goods" related to agricultural activities. Therefore, the production of the so-called positive externalities is the discriminating factor that can justify the financing of agricultural activities with subsidy payments arising from the EU's budget. Thus, the revival prospects of European agriculture seem to be oriented for after 2013 towards the achievement of two objectives, the first one is aimed at valorizing the public goods and services produced by agriculture through appropriate and proportionate terms of payment and the second one is bound to revive the competitiveness of agricultural and food systems in the EU (De Filippis 2010).
According to Frascarelli (2010), the objectives of competitiveness and public goods care cannot be adequately faced unless under a cross action.
Environmental goods, biodiversity, landscape, sustainable management of water resources and contrasting climate changes are public goods that interest the EU's collectivity.
For the production of such European public goods, it's indispensable to have agriculture in the marginal areas where the market is not able to guarantee its subsistence unless in conditions of high prices, generally not detected in the markets (Aguglia *et al* 2008; Nazzaro 2008)
Analyzing the evolution of multifunctional farms, it is noticeable how significant it has been in Italy. The number of farms that performed agritourism has reached 18,480 in 2008, registering an increase of 90.2% in the last decade (INEA 2010).
The farms that carry out organic farming are increasing at both Community and National level. In 2008, the EU has known an increase of 3.9% in the number of organic farms and 5.8% in the number of the areas devoted to organic farming. In this context, Italy is in a leading position in Europe and is ranked fifth in the world for organic in-conversion area with over 1.1 million hectares. The organic production techniques have had a very important and recognized role in the valorization of agriculture as a producer of positive externalities for the whole collectivity. In fact those produces are obtained by using only natural products and do not involve synthetic chemical inputs (fertilizers, phytosanitary products) regulated by Community law (EC Regulations No 834/2007 and No 1804/1999).

Among the agrarian activities that determine the benefits to European consumers, there is also the production of quality products identified by the marks, Protected Designation of Origin (PDO), Protected Geographical Indication (PGI) and Traditional Speciality Guaranteed (TSG). It is a voluntary certification (standardized by the EC Regulations No 510/2006 and No 509/2006) by which the manufacturer seeks to guarantee certain characteristics regarding the source and processing of his products to the consumer. The number of recognized designation of origin to Italian products continues to grow, and has reached 210 in August 2010 confirming Italy's primacy in Europe (more than 22.6% of the total denominations).
Falling within multifunctionality, the so-called direct selling, that gives the possibility for individual or associated producers to sell by retail the products of their own farm, has been included among the agricultural activities since 2001 by the farmwork law (*legge di orientamento*, LD 228/2001). In 2009, more than 63 thousand farms have approached the market directly with an increase of 4.7% and representing 7.4% of the total productive units registered at the Chambers of Commerce (INEA 2010). Even in this case, besides the producer's advantage to realize the consumption prices of his production, emerges an advantage for the final consumer who can purchase the product directly from the producer at sometimes a lower price.

3. THE FARMWORK LAW

The so-called farmwork law (*legge di orientamento*) retakes the multifunctionality of agriculture concept, though not explicitly mentioned, in connection with the guidelines of the common agricultural policy.
This law rewrites art. 2135 of the civil code, which dates back to 1942 and defines the farmer's competences, extending the scope of action to activities that formerly were precluded from the primary sector.
In particular, it includes some activities in agriculture that once were exclusive to secondary and tertiary sectors since they were related to the agriculture's main activities including those addressed for providing goods and services.
The bond with the main activities should be subjective, same subject, and objective, the prevalence. New activities must always keep a connection with the farm, with features already included in the traditional production process, but also with functions that could be added such as the contracts of collaboration with the public administration.
The legislation of related activities may be considered a "construction site in progress" because of the regular updates of the July 11th, 2007 decree. In fact, the last decree of August 5th, 2010 updated the old table with the significant addition of bread, grappa and beer production.
In practice, regarding all activities provided by the decree, the taxation is going to be applied under the agricultural income law and not on the difference between proceeds and costs.

4. RELATED ACTIVITES

Related activities are those that have a connection with the agriculture's main activities and that justify the changes brought to art. 2135 of the Civil Code and subsequent executive orders.

Table 1 shows the list of related activities for the year 2010. It is possible that in the near future there may be some changes and further additions.
In bold font are pointed out the changes and the most recent additions that clarify the competences expansion to the activities that are more and more incisive in enhancing the agricultural enterprise's budget. The legislator's intention is to acknowledge that the growth of the agriculture business volume cannot fit in the traditional agricultural activities, restricted by the rigidity of farmland area but investing work and more flexible capital factors in released activities from constraint, with high value added.
Besides specialization processes and scale economies proposed by the productivity paradigm, there are diversified and non univocal forms of development (Van Der Ploeg *et al* 2003 and 2006).

Table 1 – Related activities

Number	**Related activities description**	*Codes**
1	Meat production and produces of its **slaughter** (except smoked meat and cold cuts)	*10.11.0, 10.12.0*
2	Dried, salted or smoked meat production (speck, ham, bresaola), sausages and salami production	*10.13.0*
3	Potatoes processing and preserving, **not including the production of dehydrated mashed potatoes, potato based snacks, potato chips and industrial potato peeling**	*10.31.0*
4	Fruit and vegetable juices production	*10.32.0*
5	**Production** and preservation of fruits and vegetables	*10.39.0*
6	Olive oil and oilseeds production	*01.26.0, 10.41.1 10.41.2*
7	Corn oil production	*Ex 10.62.0*
8	Milk hygiene practice and milk derivatives production	*01.41.0, 01.45.0 10.51.1, 10.51.2*
9	Grain processing	*10.61.1 , 10.61.3*
10	**Flour production or leguminous meal, roots or tubers or comestible nuts**	*Ex 10.61.4*
11	**Production of fresh bakery products**	*10.71.1*
12	Wine production	*01.21.0, 11.02.1 11.02.2*
13	**Grappa production**	*Ex 11.01.0*
14	Vinegar production	*Ex 10.84.0*
15	Cider **and other fruit based wines** production	*11.03.0*
16	**Malt and beer production**	*11.06.0, 11.05.0*
17	Lucerne dehydration	*Ex 10.91.0*
18	Honey processing, refining and packing	*Ex 10.89.0*
19	Fish, crustaceans and molluscs **production** and preservation by freezing, deep-freezing, drying, smoking, salting, brining and **canning** and the production of fish fillets	*Ex 10.20.0*
20	Manipulation of the products derived from the cultivations **mentioned in classes 01.11.01, 01.12. and 01.13, as well as those derived from the activities listed in the above mentioned groups and classes**	*01.11.01, 01.12 01.13*

* Ateco 2007 classification of economic activities, also used by Istat.

5. THE BUSINESS CASE

5.1 Premise

Multifunctionality's development doesn't imply the abandonment of "productive" farming but, on the contrary, it requires the research of an efficient compromise between the strictly productive objectives and those social and environmental ones. In practice, the multifunctionality of agriculture fulfills itself in a pluriactivity in which the component of services and related activities is acquiring greater importance.
More and more farmers associate other activities to agricultural production, different from primary foodstuff production including accommodation, restoration and educational farms, pushing themselves even in the social field. This roles' expansion stimulates the introduction and the development of new practices related to the main activity of the farm and may represent an additional source of attraction for customers. It is the case of the farm we've selected, in which the donkey-breeding responds perfectly to the model of multifunctionality described above.
In this context, it is particularly interesting to deepen dairy donkey-breeding development opportunities in order to enhance the farms' budget, consent people permanence in the territory, and produce positive externalities for the collectivity.
The business, subject to analysis, has recently undertaken the choice of breeding donkeys for milk production to obviate the economic difficulties of beef cattle-breeding, broadening thus its own productive offer to support the educational farm and the kindergarten activities that were already in function in the farm.

5.2 Farm's structure and dynamics

The farm we surveyed is located in a flat area of the *Piemonte* and it is considered to be in transition between the breeding of *Piemontese* beef-breeding and dairy donkey-breeding, along with other related activities.
Founded in 1984, the farm covers an area of nearly 40 hectares. Only 40% of the land is owned, while 60% is rented. The Utilized Agricultural Area (UAA) is 39 hectares, 5 of which are permanent grassland and 34 are devoted to arable crops.
The farm structures include a cattleshed of 540 m², created for house fattening cattle, but currently since the productive trend of the farm has changed, it is used for donkeys stabling. In fact, the number of bovine heads reared has been remarkably reduced (from over 100 to 16 at present). The external areas, adjacent to the cattleshed are fenced with wooden stakes and sleepers and are used as a pasture. Jennies' milking room is located in a separate area inside the barn. The milking system is a sheep modified milking machine. The barn, the farm machinery shelter and a little byre for the remaining bovines have been drawn out from an old shed.
As mentioned before, the productive trend of the farm has been changed, focusing on diversification with the introduction of donkey-breeding. The owner's intention has been to increase their income by expanding the business' practiced activities and replacing the beef cattle-breeding which is characterized by low value added.
Among the various services supplied, donkey riding and agritourism are not figured, but social and educational activities instead such as kindergarten and summer camp. In addition, the farm offers educational farm facility, and has recently begun to ex-

periment onotherapy.
The enterprise is individual and family managed, full employing a family of four. The staff is qualified as it's formed by agricultural college graduates who have also attended training courses pertaining to the business activities. The family is assisted by two permanent workers and one casual worker, the first two work in the kindergarten and the summer camp for a total of 200 days a year, while the last one collaborates just for two summer months. The farm also makes a regular recourse to a psychologist.

5.3 Transitional balance sheet

The economic data, collected through a business schedule, allows the drawing up of the balance sheet, which we have defined as transitional since it concerns the new activities introduced in the farm, and preserving, even though in limited measure some productions of the previous management were linked to *Piemontese* cattle-breeding (Table 2).
The balance sheet is composed of assets, represented by business' productions and services company, and liabilities that include the costs items differently divided (Bregoli 1987; Torquati *et al* 2003). The Gross Marketable Production (GMP) coincides with proceeds earned during the year. The productions are represented by vegetable products, jenny's milk and beef.
The main crops are ryegrass and clover. When the bovine herd was whole, the farm's forage production was not sufficient; consequently, the farmer had to acquire further fodder to meet the breeding needs. By reducing the number of fattening cattle and the introduction of donkey-breeding to the farm, the forage produced exceeded the farm's needs. The excess is sold to the market, realizing a proceeds of € 6,300. Whereas the share of fodder destined to livestock's feeding is not counted in the GMP as it is an intermediate product that is reused in the production cycle of milk and meat.
The farm's jenny's milk production is approximately 720 l / year. Half is sold while the other part is invested in experiments in collaboration with a cosmetics lab in order to formulate a product to sell under the farm's brand. For the first line of these products containing a high percentage of milk (55%), a fairly high price could me assumed. The milk is sold directly to consumers in the farm at a price of € 15 per liter, and provides € 5,400 of proceeds.
As for beef production, there are slaughtered heads on an average of 11 per year. The *Piemontese* breed has a very good slaughter yield, that guarantees the farm to produce, on an average of 2.500 kg of meat which is sold on order in the farm in family-size packs at approximately 13 € / kg, with an annual turnover of € 32,500.
In part of the farmhouse, a kindergarten is carried out for 15 children, active for about 8 months a year, while in the summer, a camp is set up where, besides the traditional maternal school's activities, the donkeys are used for educational purposes. In addition to the kindergarten's enrolled children, 20 more join during the summer. The farm has also received about 200 visiting students within the educational farm program. These activities exalt the multifunctional bent of the farm and increase its proceeds by € 96,100. Finally, the farm received € 17,000 in CAP payments, obtaining thus a total GMP of € 170,500.

Taking a look at the second part of the balance sheet, the reinstatement costs that represent what has been consumed in the farm to realize the productive process are distinguished in: costs of technical floating capital and quota of technical fixed assets. Altogether, the reinstatement costs amounted to € 62,012, representing 36.4% of gross output. However, the most important expense consists of food for kindergarten and summer camp (€ 25,290).
Significant also the expense on concentrated feed and supplements used in cattle breeding. Depreciation allowance, maintenance and insurance amount to € 10,050.
The net product, obtained by subtracting the reinstatement costs and the non-farming services from the GMP, corresponds to the wealth produced in the production cycle and serves to compensate the farm inputs (land, capital and labor). Veterinary's and farrier's costs are very reasonable, while the taxes and contributions weigh on the business' budget 11.3% of the GMP.
The net income, that is what remains to the farmer after deducting the external inputs fees from the net product, is the remuneration for the entrepreneurial activity and for the inputs he invested in the production cycle (Giacinti *et al* 2002). The most important item regarding the remunerations is no doubt salaries, with an incidence of 14.7% on the GMP. This fact seems quite normal considering that the external labor is employed in the farm's most profitable activity (kindergarten and summer camp). The land rental cost is € 5,000, while the interest amounts to € 6,000 and correspond respectively to 3% and 3.5% of the GMP. Subtractions show that the net income amounts to € 49,588. This result can be considered provisional if we take into account the transitional period of the farm. In any case, it is destined to improve when all the activities will be running in full swing with the perspective of launching further initiatives.

Table 2 – Farm's balance sheet

Gross Marketable Production		***170,500***
Vegetal		***6,300***
Hay	6,300	
Animal		***51,100***
Meat	45,700	
Jenny's milk	5,400	
Services		***96,100***
Educational farm	7,000	
Kingergarten	60,750	
Summer camp	28,350	
Other		***17,000***
CAP payments	17,000	
Costs of floating capital		**51,962**
Specific costs for vegetal productions		***6,010***
Seeds	4,650	
Fertilizers	1,360	
Specific costs for animal productions		***9,912***
Feed and supplements	9,312	
Fodder and bedding	0	
Drugs	0	
Other expenses	600	
Specific machinery operating costs		***5,050***
Fuel and lubricants	4,400	
Other expenses	650	
General expenses		***30,990***
Eletricity, gas, water	4,500	
Telephone, administrative expenses	1,200	
kingerdarten and summer camp food costs	25,290	
Quota		**10,050**
Amortizations	2,750	
Maintenance	5,000	
Insurance	2,300	
Reinstatement costs		**62,012**
Net Product (broadly speaking)		***108,488***
Non-farming services cost		**22,700**
Professional services	3,300	
Rentals		

Taxes and contributions	19,400	
Net Product (strict sense)		***85,788***
Remunerations		**36,200**
Rent	5,000	
Salaries	25,200	
Interests	6,000	
Net Income		***49,588***

6. NEW ENTERPRISE STRATEGIES

The multifunctional role of agriculture in Italy has been corroborated by the issuing of LD n. 228 on May 18th, 2001 that gave, through the implementation of the farmwork law, a new juridical and functional configuration to the agricultural enterprise. In spite of remaining anchored to the agricultural sector, it could cease to be "monosectorial" and become "multisectorial".

Today, multifunctionality is thus seen, from the agricultural sector, as an economic opportunity for farms. In fact, it attempts to translate the new functions attributed to the farmer in remuneration forms capable of allowing the economic sustainability of the sector.

And it is, by expanding the agricultural sector competences, promoted by the reform of art. 2135 of the Civil Code, making it possible to expand the budget of the farm without extending the land area. Traditionally, in the past, agricultural production was represented by crops and livestock. If instead we take a look at the monitored farm's revenues, it is evident that services represent 56% of the GMP, while livestock production is limited at 30% and crop production at only 4%. This farm is definitely oriented towards multifunctionality and pluriactivity where the service component is prevalent.

The examined farm is going through reconversion and new initiatives of the farmer have been added to the traditional agricultural activities to ensure development perspectives.

In this strategy, the donkey-breeding introduction represents a bond between the traditional activities and those allowed by the farmwork law. In fact, the beef cattle herd has been downsized to a level that enables the valorization of the obtained product through direct selling in "family-size packs" that allows the realization of market prices highly competitive compared to traditional selling of live animals. This has been possible because the sale of meat falls under the "related activities" according to the new regulation. This sales channel, called zero-mile, must be sized to the local market potential.

At the same time, the opening of the donkey-breeding for milk production has allowed the opening up of other merchant perspectives: the jenny's milk production for human nutrition and cosmetics, and the use of donkeys' services performance. The presence of donkeys is useful for kindergarten's and summer camp's activities, as for

those of the educational farm.
Those services have increased the level of activity, allowing the employment of six full time working units (four family members and two employees). In practice, 3.5 hectares of arable land per worker would be sufficient. It is the "miracle" of multifunctionality and pluriactivity that would guarantee the continuity of business for many precarious agricultural enterprises due to a reduced land (OECD 2005).
The development perspectives are linked to the farmer's ability to seize the new opportunities offered by multifunctionality and pluriactivity. The good conditions are not lacked if we consider the strategies adopted recently in the radical reconversion of the farm, giving credit also to donkey-breeding.

7. CONCLUSIONS

The study has highlighted the results of new business strategies for coping with the agricultural sector crisis, that is in an income crisis.
The future of agriculture cannot be tied only to the productivity paradigm; there are diversified and non univocal forms of development. Related activities and services' performance provided by the agriculture represents strategic solutions to business' continuity to safeguard the income in difficult areas and with inadequate farm size.
The new initiatives that the farmers are pursuing, thanks also to the adjustment of the regulations and the public financial support, prefigure a new lay-out of the primary sector aimed at ensuring food supplying in line with consumer expectations, along with positive externalities.
The European Union also places among the main objectives of the Common Agricultural Policy, for post-2013, a form of fixed income support for farmers in a position to enable them to continue the "public goods" production.
Therefore, the task of the farmer today is to find the winning strategies for income diversification depending on the resources of the business and the family as well as the surrounding environment and the consumer demand, as the monitored enterprise has demonstrated it is capable to do.

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REFERENCES

1. Aguglia L., Henke R., Salvioni C. (2008) (a cura di): *Agricoltura multifunzionale. Comportamenti e strategie imprenditoriali alla ricerca della diversificazione*. Studi & Ricerche INEA, Edizioni Scientifiche Italiane, Napoli.
2. Bregoli A. (1987): *Bilancio e contabilità nell'azienda agraria*. Liviana editrice, Padova.
3. Casini L. (2002): *Funzioni sociali dell'agricoltura e nuove tipologie d'impresa*, in SIDEA, Nuove tipologie di impresa nell'agricoltura italiana, Atti del XXIX Convegno di Studi, Firenze.
4. De Filippis F. (2010): *La Pac dopo il 2013: una riforma (troppo) annunciata*.

Agriregionieuropa, anno 6, n. 22.
5. Ellis F. (2003): *Farm diversification activities: benchmarking study 2002*, University of Exeter.
6. European Commission (2008): *Other gainful activities: pluriactivity and farm diversification in EU 27*, Brussels.
7. Frascarelli A., Sotte F. (2010): *Per una politica dei sistemi agricoli e alimentari dell'UE*. Agriregionieuropa, anno 6, n. 21.
8. Giacinti R., Tellarini V., Salvini E., Di Iacovo F., Andreoli M., Moruzzo R., Olivieri D. (2002): *Analisi e gestione economico–contabile per l'impresa agrozootecnica*. Franco Angeli, Milano.
9. INEA (2010): *L'agricoltura italiana conta 2010*. INEA, Roma.
10. Nazzaro C. (2008): *Sviluppo rurale, multifunzionalità e diversificazione in agricoltura*, Pubblicazioni Dases, Franco Angeli, Milano.
11. Organisation for Economic Co-operation and Development (OECD) (2005): *Multifunctionality in agriculture.What role for private initiatives?*, Paris.
12. Organisation for Economic Co-operation and Development (OECD) (2009): *The role of agriculture and farm household diversification in the rural economy*, Paris.
13. Romano D. (2010): *L'impatto della crisi economica sull'agricoltura italiana*, in De Filippis F. e Romano D. (a cura di) Crisi economica e agricoltura, Edizioni Tellus, Roma.
14. Torquati B. (2003): *Economia e gestione dell'impresa agraria*. Edagricole, Bologna.
15. Van Der Ploeg J.D., Roep D. (2003): *Multifunctionality and rural development: the actual situation in Europe*, in G. Van Huylenbroeck, G. Durand (eds.), Multifunctional Agriculture. A new paradigm for European agriculture and Rural Development, Ashgate, Burligton, VT (USA) and Aldershot (UK).
16. Van Der Ploeg J.D. (2006): *Oltre la modernizzazione. Processi di sviluppo rurale in Europa*. Rubbettino Editore, Soveria Mannelli (Catanzaro).
17. Van Der Ploeg J.D. (2007): *The third agrarian crisis and the re-emergence of processes of repeasantization*, Rivista di Economia Agraria, LXII, n. 3.
18. Rete Rurale Nazionale (2010): *La multifunzionalità delle aziende agricole condotte da giovani agricoltori* (www.Reterurale.it).

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PROFIT AND CASH FLOW: ECONOMIC AND FINANCIAL APPROACHS ANALYSIS APPLIED TO PARMA PDO HAM FIRMS

PROFITTO E FLUSSO DI CASSA: L'APPLICAZIONE DEGLI APPROCCI DI ANALISI ECONOMICO E FINANZIARIO ALLE IMPRESE DEL PROSCIUTTO DI PARMA DOP

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ABSTRACT

Firms characterized by products long aging period often are not able to sustain financial cycle of production, even in case of good profit level. The aim of the paper is to deepen the analysis in applying an accrual basis profit approach and a financial basis cash flow approach to Parma PDO Ham firms, that are often (Bonazzi *et al.*, 2009) characterized by an high level of capital requirement because of investment in fixed assets (buildings, equipments, machinery) and in working capital assets (credits and stocks).

The basic idea is to analyze the difference in the profit and cash flow of firms in the Parma PDO Ham sector, characterized by long aging period of productions in which the quantification of profit alone could not be sufficient in assessing the sustainability of the business cycle. The paper analyses 2009 data from annual account of 40 companies in the sector, drawn randomly from the joint-stock company registered in the Parma PDO Ham Consortium, to test if there is any significant differences in the profit and cash flow generation, and analyzing if these differences have effects on the firm's management cycle. The analysis shows that there are significant differences between profit and cash flow and that it could be useful to compare profit margins analysis and cash flow margins analysis to assess the sustainability of the business cycle.

KEYWORDS:

Parma PDO Ham, Free cash flow, Ratio analysis, Investment valuation

INTRODUCTION

Businesses operating in the area of Parma Ham are characterized (Bonazzi *et al.*, 2010) by high investment that are related to the fixed assets of production (buildings, plant and equipment) and to the acquisition of the fresh meat of pork, to be aged for a period of not less than 12 months, as required by the product specification of the

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Parma PDO Ham. These firms therefore need to have sources of capital to finance the investments required by the typical production, in type of equity capital or as debt capital. It is to note that an increasing in debt level can decrease firms capital structure strength (Bingsbergen *et al.*, 2008); at the same time it causes an increasing in financial charges. Investment in fixed assets determine costs of amortization that have no effects on financial statement, in which it is to consider the loans mortgage; financial charge only have effect on income statement. It appears that an assessment based on the profit analysis may not be sufficient to evaluate the sustainability of the business cycle, because firms in the Parma PDO Ham sector are also characterized by high investment in working capital cycle and these investments lead to an increase in capital requirements. It is also to consider that an increase in the value of the stock has a positive effect on business profit, where it is regarded as a positive component of profit, while it has the effect of absorption of capital in the analysis of financial flows; a variation in stock (working capital) results, at the same time, as a positive income factor and as a capital liquidity-absorbing voice. So it may therefore be useful to compare the economic performance (profit) and financial results (cash flow) in firms in the Parma PDO Ham sector to determine if these tests give different results that may be useful to companies to take management decisions, even considering working capital cycle.

MATERIALS AND METHODS

Data

The analysis is based on a sample of 40 firms randomly chosen among the 123 companies operating in the sector of Parma PDO Ham in the form of limited companies, analyzing the 2009 annual accounts of firms in the sample, composed by income statement and balance sheet, set out in statutory form on the basis of articles 2423 and subsequent of the national Civil Code. The reclassification of the Civil Code uses the classification of costs and revenues by nature, and does not indicate significant intermediate profit margins as value added, EBITDA and EBIT (Ferrero *et al.*, 2005); in the analysis, following the approach of different authors (Poddighe, 2004; Ceccacci *et al.,* 2008), the income statement are reclassified on a value-added basis that is a reclassification also possible to an outside of the company point-of-view (see Alberici *et al.*, 2003 and Pavarani, 2006). Even more, the reclassification of the income statement on a value-added basis can also be useful to quantify intermediate profit margins (EBITDA and EBIT) expressing the operating income available to cover the cost of debt (EBIT) and to approximate level of creation of the cash flow from operations (EBITDA); EBITDA is a margin that exclude costs that have no monetary effects, and then EBITDA margin is considered an intermediate profit margin that approximates the level of cash generated from operations (Mella, 2001). Moreover, in order to express directly the cash flow generate by firms, it is necessary (Kohli, 1993; Krolick, 1998; Penman, 2004) to analyze the cash flow statement, that is (Brealey *et al.*, 2003) a financial document drawn up on the data available in the income statement and balance sheet, that directly allows to calculate the result of financial management. It is possible, using cash flow statement, to integrate the information on profit and cash flow analysis deriving from income statement that is worded in

accordance with the principle of accrual, under the provisions of the national Civil Code (art 2423 and subsequent) so expressing only indirectly the creation of financial liquidity by firms.

Economic and financial ratio analysis (ICR)

For the purpose to quantify the sustainability of a firm cycle, a possible approach (about this topic see Dallocchio *et al.*, 2004) consider a comparison with the intermediate profit margins and cost of debt:

$$(1) \quad ICR_1 = \frac{EBIT}{I} \quad ; \quad (2) \quad ICR_2 = \frac{EBITDA}{I}$$

Where ICR is interest coverage ratio, EBIT is earnings before interest and tax and EBITDA is earnings before interest tax depreciation and amortization, I is interest that is the cost of debt (explicitly expensive). The implicit cost of debt burden, as the debts to suppliers, already incorporates the implicit cost of deferment of payment in the cost of purchase of inputs (raw materials, services and so on). It is also possible to directly express the cash flows available for debt service, such as CF (cash flow), OCF (operating cash flow), UFCF (unlevered free cash flow). These cash flows, if posed in relation with interest, are to quantify the sustainability of the cost of debt (Iotti, 2009) and is:

$$(3) \quad ICR_3 = \frac{CF}{I} \quad ; \quad (4) \quad ICR_4 = \frac{OCF}{I}; \quad (5) \quad ICR_5 = \frac{UFCF}{I}$$

At the end, if the aim is to quantify the profitability of the firm investment for equityholders, the ratio analysis suggest to quantify return on equity analysis (ROE), even considering a financial approach to ROE. It is possible to compare economic approach and financial approach to ROE having these ratios:

$$(6) \quad ROE_e = \frac{TT}{E} \quad ; \quad (7) \quad ROE_f = \frac{FCFE}{E}$$

Where, in (6) ROE_e is return on equity with economic approach, having Π as profit, then in (7) ROE_f is return on equity with financial approach; in both E is equity (capital given by equityholders).

RESULTS

The analysis of the balance sheet shows an average total asset (TA) of € 16.336 million with € 1.463 minimum and € 51.017 maximum, average WC_tA / TA is 68.11% and FA / TA è 31.89%; WC_iA / TA is 40.85%. In terms of source of capital, the average Leverage is 2.843 and DER is 1.843 expressing that equity is 35.17% of source if capital.

Table 1 – Investment and source (Firm Sample 40 firms – annual account 2009)

Index	Mean	Median	St. Dev.	CV	Min	Max
Inventories (WC_tA)	*6,673,943*	*5,295,221*	*6,192,240*	*1.08*	*7,100*	*27,966,350*
Account receivable	*4,005,653*	*2,634,852*	*3,424,930*	*1.170*	*208,090*	*13,007,191*
Cash	*241,450*	*29,822*	*434,509*	*0.556*	*22*	*2,440,116*
Others	*205,818*	*13,899*	*586,130*	*0.351*	*-*	*3,336,096*
Working capital (WC_tA)	11,126,864	8,693,725	9,133,917	1.218	371,600	41,655,870
Fixed asset	5,210,066	2,955,549	4,938,912	1.055	108,167	24,467,455
Total Asset (TA)	**16,336,931**	**13,547,869**	**12,372,612**	**1.320**	**1,462,954**	**51,017,425**
Equity	5,746,911	4,504,441	5,423,703	1.060	29,290	24,674,954
Financial Debt (s)	3,551,724	3,043,283	3,418,444	1.039	-	15,233,067
Financial Debt (l)	2,834,592	1,500,000	3,414,669	0.830	-	13,155,378
Working Capital (debt)	4,203,705	2,616,927	3,339,965	1.259	321,802	12,492,717
Total Source (TS)	**16,336,931**	**13,547,869**	**12,372,612**	**1.320**	**1,462,954**	**51,017,425**

Source: firm data and elaborations

Analyzing data on table 2 it is possible to note a difference between economic and financial result in the sample. In fact, 26 firms on 40 have a positive profit but 11 firms on 40 have positive FCFE, and 34 firms on 40 have a positive EBITDA but 16 firms on 40 have positive OCF.

Table 2 – Economic and financial margins (Firm Sample 40 firms – annual account 2009)

Index	Mean	Median	St. Dev.	CV	Min	Max	≥0	0<
TURNOVER	11,843,845	8,293,972	10,642,911	1.113	943,025,21	38,644,60	NU	NU
EBITDA	313,394	229,084	921,793	0.340	- 4,168,38	2,750,30	34	6
EBIT	312,280	245,344	839,588	0.372	- 3,442,04	2,107,16	31	9
PROFIT	- 29,434	31,140	748,767	- 0.039	- 4,230,12	1,184,40	26	14
CF	198,435	201,404	830,754	0.239	- 4,168,38	2,539,98	33	7
OCF	- 2,089,222	- 57,173	6,831,918	- 0.306	- 25,787,47	13,977,86	16	24
UFCF	- 2,464,282	- 317,315	10,706,548	- 0.230	- 34,094,83	24,994,15	11	29
FCFE	- 2,699,552	- 420,038	10,835,799	- 0.249	- 35,119,48	24,886,20	11	29

Source: firm data and elaborations
NU: not useful

DISCUSSION

Discussion (Profit / FCFE)

To compare different economic and financial results (table 2), it is useful to compare the sign of profit and FCFE, applying the sign test having a results that is frequency of 29 out of 40 observed + sign differences, frequency of 11 out of 40 observed - sign differences; the theoretical proportion expected for +'s or -'s is 0.5 and the test is for the probability of the +'s or -'s as small or smaller than that observed given the

expected proportion. Z value for Normal Distribution approximation = -2.688 and probability = 0.0036 then there is a significant difference between profit and FCFE sign series.
Comparison between profit and FCFE means, using a t-student paired sample test (parametric approach), shows a t-value (95%) with 39 degree of freedom, having a t-value that is 1.5455 under t-critical value (1 tail) that is 2.022; it is not possible to reject the null hypothesis of equal means having a probability that is 13.03% (2 tails) to have equal means, as it is expressed in table 3. Ratio of standard deviations is 14.472, ratio of variances is 209.43; 95% confidence interval for ratio of standard deviations is 10.524 to 19.899 and for variance ratio is 110.76 to 395.96.

Table 3 – COMPARISON OF TWO MEANS (t-student) – Profit / FCFE

Column	Profit	FCFE	Differences
Sample size	40	40	40
Minimum	-4.2301E6	-3.5119E7	-2.4832E7
Maximum	1.18441E6	2.48862E7	3.51282E7
Range	5.41453E6	6.00057E7	5.99604E7
Mean	-29434	-2.6996E6	2.67012E6
Std. deviation	758305	1.09738E7	1.09266E7

Std. error of mean difference = 1.72765E6 with 39 d.f.

95% conf. interval for mean diff -824375 to 6.16461E6

t value testing mean diff = 0 is 1.5455
Significance level is 0.1303 (13.03%) for 2 sided test

Stat t	1,5455	
P(T<=t) 1tail		0,0651
t critical value 1 tail	1,6849	
P(T<=t) 2 tails	0,1303	
t critical value 2 tails	2,0227	

Source: firm data and elaborations

Moreover, not having information about the normality of the distribution, it could be useful to apply a non parametric approach as Wilcoxon paired two-sample test, having a rank sum with negative differences that is 201.0 and a positive differences 619.0, it is Wilcoxon T = 201, with N = 40, having a H_0: Mean (for T) = 410.00 s.d. = 74.40, hence z = -2.81 there is a significance level of 0.25% for one-sided test and significance level of 0.50% for two-sided test to reject the null hypothesis of equal medians.

Discussion (EBITDA / UFCF)

Considering a comparison between EBITDA and UFCF could be useful in order to analyze two different margin often considered to express the capacity to cover inter-

est charge and serve debt. A comparison between EBITDA and UFCF means, using a t-student paired sample test (parametric approach), shows a t-value (95%) with 39 degree of freedom, having a t-value that is 1.604 under t-critical value (2 tails) that is 2.022; it is not possible to reject the null hypothesis of equal means having a probability that is 11.68% (2 tails) to have equal means, as it is expressed in table 4.

Table 4 – COMPARISON OF TWO MEANS (t-student) – EBITDA / UFCF

Column EBITDA UFCF Differences
Sample size 40 40 | 40
Minimum -4.1684E6 -3.4095E7 | -2.4824E7
Maximum 2.75031E6 2.49942E7 | 3.49762E7
Range 6.91869E6 5.9089E7 | 5.98003E7
Mean 313394 -2.4643E6 | 2.77768E6
Std. deviation 933536 1.08429E7 | 1.09524E7
Std. error of mean difference = 1.73172E6 with 39 d.f.
95% conf. interval for mean diff . -725065 to 6.28042E6
t value testing mean diff = 0 is 1.6040
Significance level is 0.1168 (11.68%) for 2 sided test
Stat t 1.6040
P(T<=t) 1tail 0.0584
t critical value 1 tail
1.6849
P(T<=t) 2 tails
0.1168
t critical value 2 tails
2.0227

Source: firm data and elaborations

It is useful to compare the sign of EBITDA and UFCF, applying the sign test having a results that is frequency of 30 out of 40 observed + sign differences, frequency of 10 out of 40 observed - sign differences; the theoretical proportion expected for +'s or -'s is 0.5 and the test is for the probability of the +'s or -'s as small or smaller than that observed given the expected proportion. Z value for Normal Distribution approximation = -3.004 and probability = 0.0013 then there is a significant difference between EBITDA and UFCF sign series. Ratio of standard deviations is 11.615 and ratio of variances is 134.91; 95% confidence interval for ratio of standard deviations is 8.447 to 15.971 and for variance ratio is 71.352 to 255.07.

Moreover, not having information about the normality of the distribution, it could be useful to apply a non parametric approach as Wilcoxon paired two-sample test, having a rank sum with negative differences that is 183.0 and a positive differences 637.0, it is Wilcoxon T = 183, with N = 40, having a H_0: Mean (for T) = 410.00 s.d.

= 74.40, hence z = 3.05 there is a significance level of 0.11% for one-sided test and significance level of 0.23% for two-sided test to reject the null hypothesis of equal medians.

Discussion (ROE_e / ROE_f)

In table 5 are expressed ratios to analyze profitability (ROE_e and ROE_f) and capacity to cover interest charge (ICR_1, ICR_2, ICR_3, ICR_4, ICR_5).

Table 5 – Economic and financial ratios (Firm Sample 40 firms – annual account 2009)

Index	Mean	Median	St. Dev.	CV	Min	Max	>0	>1
ICR_1 EBIT / I	2,655	1,496	11,325	0.234	- 40,26	893,10	31	28
ICR_2 EBITDA / I	3,103	1,607	8,958	0.346	- 21,73	4,232,81	34	26
ROE_e PROFIT / E	- 0,004	0,010	0,117	- 0.033	- 0,57	0,31	26	NU
ICR_3 CF / I	1,710	1,016	6,347	0.269	- 23,03	3,617,94	33	20
ICR_4 OCF / I	- 0,035	- 0,444	34,856	- 0.001	- 69,24	2,547,27	16	14
ICR_5 UFCF / I	- 1,584	- 4,018	49,455	- 0.032	- 22,723,01	688,07	11	11
ROE_f FCFE / E	- 0,567	- 0,165	2,879	- 0.197	- 14,74	8,68	11	NU

Source: firm data and elaborations

The analysis express a difference in ROE_e and ROE_f, having $ROE_e > 0$ in 26 cases on 40 and $ROE_e > 0$ in 11 cases on 40. A ROE_e and ROE_f values comparison, using a t-student paired sample test (parametric approach), shows a t-value (95%) with 39 degree of freedom, having a t-value that is 1.2090 under t-critical value (2 tails) that is 2.0227; it is not possible to reject the null hypothesis of equal means having a probability that is 23.39% to have equal means, as it is expressed in table 6.

Table 6 – COMPARISON OF TWO MEANS (t-student) – ROE_e / ROE_f

Simple Models - Normal Distribution, Two Samples			
Column	ROEe	ROEf	Differences
Sample size	40	40	40
Minimum	-0.5712	-14.743	-8.6683
Maximum	0.3172	8.6872	15.06
Range	0.8884	23.43	23.728
Mean	-0.00392	-0.56714	0.56322
Std. deviation	0.11862	2.9159	2.9463
Std. error of mean difference = 0.46585 with 39 d.f.			
95% conf. interval for mean diff. -0.37905 to 1.5055			
t value testing mean diff = 0 is 1.2090			
Significance level is 0.2339 (23.39%) for 2 sided test			
Stat t	1,2090		
P(T<=t) 1tail	0.1170		
t critical value 1 tail	1.6849		
P(T<=t) 2 tails	0.2339		
t critical value 2 tails	2.0227		

Source: firm data and elaborations

Moreover, considering a non parametric approach as Wilcoxon paired two-sample test, having a rank sum with negative differences that is 191.0 and a positive differences 629.0, it is Wilcoxon T = 191, with N = 40, having a H_0 : Mean (for T) = 410.00 s.d. = 74.40, hence z = -2.94 there is a significance level of 0.16% for one-sided test and significance level of 0.32% for two-sided test to reject the null hypothesis of equal medians of ROE_e / ROE_f values.

Discussion (ICR_2 / ICR_5)

The capacity to cover interest charge is expressed by an economic coverage ratio, as ICR_2, having a comparison with ICR_5 that is used in a financial approach. In table 5 it is that $ICR_2 > 1$ in 26 cases on 40 where it is $ICR_5 > 1$ in 11 cases on 40. Wilcoxon paired two-sample test, having a rank sum with negative differences that is 190.0 and a positive differences 630.0, it is Wilcoxon T = 190, with N = 40, having a H_0 : Mean (for T) = 410 s.d. = 74.40, hence z = -2.96 there is a significance level of 0.16% for one-sided test and significance level of 0.31% for two-sided test to reject the null hypothesis of equal medians of ICR_2 / ICR_5 values.

CONCLUSIONS

In analyzing the data of the firms are possible to apply two main approaches (Damodaran, 1994); a first approach, that follows the principle of accrual, shall be developed according to general law requirement and schemesthe civil code, under the na-

tional and international accounting standards, considering the principle of economic competence (accrual basis approach); this principle is also enshrined in the European Directive on the annual account (IV UE Directive 78/660/CEE, july 25th, 1978) that has been implemented in Italy by D.Lgs 127/91. Several authors have developed the economic approach of annual account, in order to derive useful information to quantify the level of investment in the company's convenience (Belli *et al.*, 2001; Pezzani, 2004; Ferrero *et al.*, 2005). National legislation, however, shows how economic analysis should be complemented with some indicators of financial status (art. 2423 and subsequent in Civil Code) applying the financial statements that aims to quantify the cash flows generated by operations (Damodaran, 1994; Shireves et al., 2000; Pavarani, 2006). Then appears useful to have a joint consideration of economic and financial aspects of firms business cycle in order to consider, at the same time, convenience and sustainability. Applying these conclusions to the Parma PDO Ham firms sector, the analysis show that there is a statistically significant difference, as shown in particular by non-parametric approach, between economic values and financial values in the sample firms. This analysis suggests that it is necessary to integrate economic analysis with financial analysis, in order to verify the sustainability of the cycle of business management, considering, at the same time, that the capacity to generate cash flows is influenced by the type of sector, the size of the enterprise and the type of production. Companies in high capital intensity sectors are particularly disadvantaged in the generation of financial values (Bonazzi *et al.*, 2010), as Parma PDO Ham sector, characterized by high investment level. Considering the scope of the work, that is to check differences between economic performance and financial results in the sector of Parma PDO Ham, the analysis shows that the sample firms have better economic performance than financial performance (this is comparing ROE and FCFE) having situations in which, even in the face of profit-making, the firms do not have the financial liquidity for new discretionary investments or dividends distributions to shareholders.

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REFERENCE

1. Alberici A., Caselli S. (2003): *La valutazione dell'impresa per i fidi bancari*. Franco Angeli, Milano.
2. Belli P., Anderson J.R., Barnum H.N, Dixon J.A., Tan J.P. (2001): *Economic Analysis of Investment Operations: Analytical Tools and Practical Applications*. World Bank, Washington D.C.
3. Binsbergen J., Graham J., Yang J. (2008): *The cost of debt*. Duke University, Working paper.
4. Bonazzi G., Iotti M., Salatas V. (2010): Financial Analysis of the Parma PDO Ham firms. In *Proceedings of the 7th International Conference on Applied Financial Economics*. 1st jul. - 3rd jul. 2010. National and Kapodistrian University of Athens, Athens.
5. Brealey R.A., Myers S.C., Sandri S. (2003): *Principi di finanza aziendale*. McGraw-Hill, Milano.
6. Ceccacci G., Camanzi P., Rigato C. (2008): *Basilea 2 per piccole e microimprese*. Milano, Edizioni FAG.
7. Dallocchio M., Salvi A. (2004): *Finanza d'azienda*. EGEA, Milano.
8. Damodaran A. (1994): *Damodaran on Valuation*, John Wiley & Sons, New York.
9. Ferrero F., Dezzani F., Pisoni P., Puddu L. (2005): *Le analisi di bilancio. Indici e flussi*. Giuffrè, Milano.
10. Iotti M. (2009): *La valutazione degli investimenti industriali*. Franco Angeli, Milano.
11. Kohli K.N. (1993): *Economic Analysis of Investment Projects: a Practical Approach*. Oxford University Press for the Asian Development Bank, Oxford, UK.
12. Krolick D.L. (1998): *The Relevance of Financial Statement Information for Executive Performance Evaluation and Equity Valuation: Evidence from the Choice of Bonus Plan Accounting Performance Measures*. John M. Olin School of Business, Washington D.C.
13. Mella P. (2001): *Indici di bilancio*, Il Sole 24 Ore, Milano.
14. Pavarani E. (2006): *L'equilibrio finanziario*. MacGraw-Hill, Milano.
15. Penman S. (2004): *Financial Statement Analysis and Security Valuation*. McGraw-Hill, London.
16. Pezzani F. (a cura di), (2004): *Il bilancio d'esercizio nell'informativa esterna d'impresa*. Giuffrè, Milano.
17. Poddighe F. (2004): *Analisi di bilancio*. CEDAM, Padova.

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REGOLAMENTO (CE) 767/2009: NOVITÀ PER I MANGIMI GENERALITÀ

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INTRODUZIONE

Fra gli obiettivi fondamentali della legislazione europea in materia alimentare senza dubbio c'è la ricerca di un elevato livello di protezione della salute del consumatore e della salute degli animali. Sono ormai diversi decenni che tale obiettivo viene enunciato nei consideranda sia delle Direttive che dei Regolamenti. Fra le tante norme che lo riportano, vogliamo ricordare in particolare il Regolamento (CE) n. 178/2002 (GUCE n. L 31, 01/02/2002) che, oltre a stabilire i principi e i requisiti generali della legislazione alimentare, istituire l'Autorità europea per la sicurezza alimentare e fissare le procedure nel campo della sicurezza alimentare, definiva la così detta strategia «dai campi alla tavola», con la quale veniva individuata nell'alimentazione degli animali una fase complessa che si pone all'inizio della catena alimentare ma che può avere comunque ripercussioni sul consumatore finale. Ne consegue che la produzione di mangimi, che da una parte costituisce un importante sbocco per i prodotti agricoli europei e dall'altra costituisce il fattore di costo più importante per i cinque milioni di allevatori della Comunità, deve essere soggetta ad una normativa che garantisca un elevato livello di sicurezza dei mangimi stessi in qualunque forma essi possano essere presentati sia come materie prime per mangimi, che come mangimi composti, piuttosto che come additivi per mangimi, premiscele o mangimi medicati.

Da questo quadro emerge chiaramente l'importanza di un sistema di etichettatura uniforme, coerente, trasparente e comprensibile tanto ai fini relativi all'applicazione della legislazione, alla tracciabilità e ai controlli che a quelli relativi alla possibilità per gli acquirenti di avere le informazioni necessarie per scegliere il prodotto più adatto alle proprie esigenze. Senza dimenticare i vantaggi che un tale sistema di etichettatura offre per la creazione di un contesto commerciale realmente competitivo.

REGOLAMENTO (CE) N. 767/2009

Sulla GUUE n. 229 del 1 settembre 2009 è stato pubblicato il Regolamento (CE) n. 767/2009 del Parlamento europeo e del Consiglio, del 13 luglio 2009, sull'immissione sul mercato e sull'uso dei mangimi, che modifica il regolamento (CE) n. 1831/2003 GUUE L 268, 18/10/2003), che abroga le direttive 79/373/CEE del Consiglio (GUCEE L 086, 06/04/1979), 80/511/CEE della Commissione (GUCEE L 126, 21/05/1980), 82/471/CEE del Consiglio (GUCEE L 213, 21/07/1982), 83/228/CEE del Consiglio (GUCEE L 126, 13/05/1983), 93/74/CEE del Consiglio (GUCE L 237,

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22/09/1993), 93/113/CE del Consiglio (GUCE L 334, 31/12/1993) e 96/25/CE del Consiglio GUCE L 125, 23/05/1996) e la decisione 2004/217/CE della Commissione (GUUE L 67, 05/03/2004). Questo Regolamento ha l'obiettivo di armonizzare le condizioni per l'immissione sul mercato e l'uso dei mangimi con il fine di garantire un elevato grado di sicurezza dei mangimi e, di conseguenza, un elevato grado di protezione della salute degli animali e dei consumatori oltre ad informare in maniera appropriata gli utilizzatori ed i consumatori ed a rafforzare il buon funzionamento del mercato interno nonché tutelare il segreto industriale dei produttori .

Innanzi tutto vogliamo sottolineare che uno degli obiettivi del regolamento è quello di semplificare le norme attualmente in applicazione, in particolare considerando che al non uniforme recepimento di Direttive che contenevano fra l'altro richiami incrociati e sovrapposizioni, conseguiva una non uniforme applicazione delle disposizioni legislative negli Stati membri tanto che si erano determinati ostacoli agli scambi intra comunitari. Non solo, vogliamo anche sottolineare il valore positivo della scelta compiuta dal legislatore comunitario in favore del regolamento, come strumento legislativo, anziché di una nuova direttiva, perché ciò consente in primo luogo un'applicazione uniforme e simultanea dei dettami in esso previsti, evitando così discordanze e discrepanze a livello dei singoli Stati. È inoltre uno strumento che può essere facilmente modificato per aggiornarlo ai progressi tecnico-scientifici in materia, ed è infine di semplice applicazione da parte sia degli operatori comunitari che dei partner commerciali. Tutto ciò, comportando regole uguali per tutti gli operatori interessati, faciliterà un'applicazione armonizzata negli stati dell'Unione Europea.

Il Regolamento (CE) n. 767/2009 è costituito da 39 consideranda, da sei Capi (Capo 1: Disposizioni introduttive; Capo 2: Disposizioni generali; Capo 3: Immissione sul mercato di categorie specifiche di mangimi; Capo 4: Etichettatura, presentazione ed imballaggio; Capo 5: Catalogo comunitario delle materie prime per mangimi e codici comunitari di buona pratica in materia di etichettatura; Capo 6: Disposizioni generali e finali) e da nove Allegati (Allegato I: Disposizioni tecniche relative a impurità, mangimi da allattamento, materie prime per mangimi utilizzate come denaturanti o leganti, contenuto in ceneri e tenore di umidità di cui all'articolo 4; Allegato II: Disposizioni generali in materia di etichettatura di cui all'articolo 11, paragrafo 4; Allegato III: Elenco di materiali la cui immissione sul mercato o il cui uso ai fini dell'alimentazione animale sono soggetti a restrizioni o vietati a norma dell'articolo 6; Allegato IV: Tolleranze ammesse per l'etichettatura riguardante la composizione delle materie prime per mangimi o dei mangimi composti di cui all'articolo 11, paragrafo 5; Allegato V: Dichiarazione obbligatoria per le materie prime per mangimi di cui all'articolo 16, paragrafo 1, lettera b); Allegato VI: Indicazioni di etichettatura delle materie prime per mangimi e dei mangimi composti per animali destinati alla produzione di alimenti; Allegato VII: Indicazioni di etichettatura delle materie prime per mangimi e dei mangimi composti per animali non destinati alla produzione di alimenti; Allegato VIII: Disposizioni specifiche in materia di etichettatura dei mangimi che non sono conformi ai requisiti di sicurezza e commercializzazione stabiliti dal diritto comunitario di cui all' articolo 20, paragrafo 1; Allegato IX: Tavola di concordanza).

Ribadiamo che l'obiettivo del Regolamento, come previsto nell'art. 1, "conformemente ai principi generali stabiliti nel Regolamento (CE) n. 178/2002 (GUUE L 31,

01/02/2002), consiste nell'armonizzare le condizioni per l'immissione sul mercato e l'uso dei mangimi, in modo da garantire un elevato livello di sicurezza dei mangimi e, in tal modo, un elevato livello di protezione della salute pubblica, nonché di fornire un'informazione adeguata per gli utilizzatori e i consumatori e di rafforzare il buon funzionamento del mercato interno"; evidenziamo che (art. 2) "stabilisce le norme in materia di immissione sul mercato e di uso dei mangimi per animali destinati e non destinati alla produzione di alimenti nella Comunità, ivi comprese le prescrizioni relative all'etichettatura, all'imballaggio e alla presentazione" e dispone che "non si applica all'acqua, né assunta direttamente dagli animali né aggiunta intenzionalmente ai mangimi loro destinati" mentre "si applica ai mangimi da somministrare attraverso l'acqua"; segnaliamo che con l'art. 3 fornisce una serie particolareggiata di definizioni relative, ad esempio, ai vari tipi di mangimi, a particolari finalità di utilizzo, all'etichettatura, agli animali destinatari (Tabella 1, parte prima e seconda).
Per quanto riguarda le prescrizioni in materia di sicurezza e commercializzazione, l'art. 4 stabilisce che i mangimi, riservati o meno ad animali destinati alla produzione di alimenti, possono essere immessi sul mercato ed utilizzati unicamente "se sono sicuri", "se non hanno effetti nocivi diretti sull'ambiente o sul benessere degli animali", "sono conformi alle riserve tecniche relative ad impurità e ad altri determinanti chimici" indicati nell'Allegato I (comma 3); dispone inoltre che gli operatori del settore devono garantire che i loro mangimi "siano sani, genuini, di qualità leale, adatti all'impiego previsto e di natura commerciabile", nonché "etichettati, imballati e presentati" conformemente alle disposizioni del regolamento e delle altre disposizioni pertinenti della legislazione comunitaria. L'art. 6 precisa ulteriormente che "i mangimi non contengono o non sono costituiti" da

Tabella 1 – Definizioni (parte prima)

Termine	Definizione	Norma di riferimento
mangime o alimento per animali	qualsiasi sostanza o prodotto, compresi gli additivi, trasformato, parzialmente trasformato o non trasformato, destinato alla nutrizione per via orale degli animali	Reg. (CE) n. 178/2002
impresa nel settore dei mangimi	ogni soggetto pubblico o privato, con o senza fini di lucro, che svolge una qualsiasi delle operazioni di produzione, lavorazione, trasformazione, magazzinaggio, trasporto o distribuzione di mangimi, compreso ogni produttore che produca, trasformi o immagazzini mangimi da somministrare sul suo fondo agricolo ad animali	Reg. (CE) n. 178/2002
immissione sul mercato	la detenzione di alimenti o mangimi a scopo di vendita, comprese l'offerta di vendita o ogni altra forma, gratuita o a pagamento, di cessione, nonché la vendita stessa, la distribuzione e le altre forme di cessione propriamente detta	Reg. (CE) n. 178/2002
additivi per mangimi	sostanze, microrganismi o preparati, diversi dai mangimi e dalle premiscele che sono intenzionalmente aggiunti agli alimenti per animali o all'acqua al fine di svolgere, in particolare, una o più tra le funzioni di cui all'art. 5, paragrafo 3	Reg. (CE) n. 1831/2003

premiscele	le miscele di additivi per mangimi o le miscele di uno o più additivi per mangimi con materie prime per mangimi o acqua, utilizzate come supporto, non destinate ad essere somministrate direttamente agli animali	Reg. (CE) n. 1831/2003
coadiuvanti tecnologici	tutte le sostanze non consumate direttamente come alimenti per animali utilizzate deliberatamente nella trasformazione di alimenti per animali o materie prime per mangimi per conseguire un determinato obiettivo tecnologico durante il trattamento o la trasformazione, che possono dar luogo alla presenza, non intenzionale ma tecnicamente inevitabile, di residui delle sostanze stesse o di loro derivati nel prodotto finale, a condizione che questi residui non abbiano un'incidenza negativa sulla salute degli animali, sulla salute umano sull'ambiente e non abbiano effetti tecnologici sul prodotto finito	Reg. (CE) n. 1831/2003
razione giornaliera	la quantità totale di mangimi, nella base di un tasso di umidità del 12 %, necessaria in media al giorno ad un animale di una specie, di una categoria d'età e di un rendimento determinati, per soddisfare tutti i suoi bisogni	Reg. (CE) n. 1831/2003
stabilimento	un'unità di un'impresa nel settore dei mangimi	Reg. (CE) n. 183/2005
Autorità competente	l'autorità di uno Stato membro o di un paese terzo designata per effettuare i controlli ufficiali	Reg. (CE) n. 183/2005
operatore del settore dei mangimi	persona fisica o giuridica responsabile del rispetto delle disposizioni del presente regolamento nell'impresa nel settore dei mangimi posta sotto il suo controllo	Reg. (CE) n. 767/2009
alimentazione degli animali per via orale	introduzione di mangimi nel tratto gastrointestinale attraverso la bocca, con l'obiettivo di soddisfare i requisiti nutrizionali dell'animale e/o mantenere la produttività di animali sani	Reg. (CE) n. 767/2009
animale destinato alla produzione di alimenti	qualsivoglia animale nutrito, allevato o detenuto per la produzione di alimenti destinati al consumo umano, ivi inclusi animali che non sono destinati al consumo umano, ma appartengono alle specie che possono essere normalmente destinate al consumo umano nella Comunità	Reg. (CE) n. 767/2009
animali non destinati alla produzione di alimenti	qualsivoglia animale nutrito, allevato o detenuto, ma non destinato al consumo umano, ad esempio animali da pelliccia, animali da compagnia e animali detenuti in laboratori, giardini zoologici o circhi	Reg. (CE) n. 767/2009
animali da pelliccia	qualsivoglia animale non destinato alla produzione di alimenti nutrito, allevato o detenuto per la produzione di pellicce e non destinato al consumo umano	Reg. (CE) n. 767/2009
animale da compagnia o animale familiare	qualsivoglia animale non destinato alla produzione di alimenti appartenente ad una specie nutrita, allevata o detenuta, ma normalmente non destinata al consumo umano nella Comunità	Reg. (CE) n. 767/2009
materie prime per mangimi	prodotti di origine vegetale o animale, il cui obiettivo principale è soddisfare le esigenze nutrizionali degli animali, allo stato naturale, freschi o conservati, nonché i derivati della loro trasformazione industriale, come pure le sostanze organiche o inorganiche, contenenti o meno additivi per mangimi, destinati all'alimentazione degli animali per via orale, in quanto tali o previa trasformazione, oppure alla preparazione di mangimi composti oppure ad essere usati come supporto di premiscele	Reg. (CE) n. 767/2009

Tabella 1 – Definizioni (parte seconda)		
Termine	Definizione	Norma di riferimento
mangimi composti	miscele di almeno due materie prime per mangimi, contenenti o meno additivi per mangimi, destinati all'alimentazione degli animali per via orale sotto forma di mangimi completi o complementari	Reg. (CE) n. 767/2009
mangimi completi	mangimi composti che, per la loro composizione, sono sufficienti per una razione giornaliera	Reg. (CE) n. 767/2009
mangimi complementari	mangimi composti con contenuto elevato di talune sostanze, ma che, per la loro composizione, sono sufficienti per una razione giornaliera soltanto se utilizzati in associazione con altri mangimi	Reg. (CE) n. 767/2009
mangimi minerali	mangimi complementari contenenti almeno il 40 % di ceneri grezze	Reg. (CE) n. 767/2009
mangimi d'allattamento	mangimi composti somministrati allo stato secco o diluiti in una determinata quantità di liquido, destinati all'alimentazione dei giovani animali come complemento o in sostituzione del latte materno postcolostrale o destinati a animali giovani, come vitelli, agnelli o capretti da macellazione	Reg. (CE) n. 767/2009
supporto	sostanza utilizzata per sciogliere, diluire, disperdere o altrimenti modificare fisicamente un additivo per mangimi allo scopo di facilitarne la manipolazione, l'applicazione o l'impiego, senza alterarne la funzione tecnologica o senza esercitare essa stessa alcun effetto tecnologico	Reg. (CE) n. 767/2009
particolare fine nutrizionale	il soddisfacimento delle esigenze nutrizionali specifiche di animali il cui processo digestivo, di assorbimento o il cui metabolismo sono o rischiano di essere alterati temporaneamente o in forma irreversibile e che, di conseguenza, possono trarre giovamento dall'assunzione di mangimi adeguati al loro stato	Reg. (CE) n. 767/2009
mangimi destinati a particolari fini nutrizionali	mangimi in grado di soddisfare un particolare fine nutrizionale in virtù della loro particolare composizione o del particolare metodo di fabbricazione, che li differenzia chiaramente dai normali mangimi. I mangimi destinati a particolari fini nutrizionali non includono i mangimi medicati ai sensi della direttiva 90/167/CEE	Reg. (CE) n. 767/2009
materiali contaminati	mangimi contenenti un tenore di sostanze indesiderabili superiore a quella tollerata dalla direttiva 2002/32/CE	Reg. (CE) n. 767/2009
durata minima di conservazione	periodo durante il quale la persona responsabile dell'etichettatura garantisce che un detto mangime, in condizioni di conservazione appropriate, conserva tutte le sue proprietà dichiarate; solo una durata minima di conservazione può essere indicata per ogni mangime nel suo complesso e viene determinata in base alla durata minima di conservazione di ciascuno dei suoi componenti	Reg. (CE) n. 767/2009

partita o lotto	una quantità identificabile di mangimi che possiedono caratteristiche comuni come l'origine, la varietà, il tipo d'imballaggio, l'identità dell'imballatore, quella dello speditore o l'etichettatura e, nel caso di un processo produttivo, un'unità di produzione prodotta in un singolo impianto applicando parametri di produzione uniformi o più unità di produzione, se prodotte in ordine continuo e immagazzinate nello stesso impianto	Reg. (CE) n. 767/2009
etichettatura	attribuzione di qualsiasi dicitura, indicazione, marchio di fabbrica, nome commerciale, immagine o simbolo forniti con qualsiasi mezzo quale imballaggi, contenitori, cartoncini, etichette, documenti commerciali, anelli e fascette o in Internet, che accompagnano un dato mangime o che ad esso fanno riferimento, anche per finalità pubblicitarie	Reg. (CE) n. 767/2009
etichetta	ogni cartellino, marca, marchio commerciale, illustrazione o descrizione di altro tipo, scritta, stampata, stampigliata, marchiata, impressa in rilievo o a impronta sull'imballaggio o sul recipiente contenente mangimi o ad essi attaccata	Reg. (CE) n. 767/2009
presentazione	la forma, l'aspetto o il confezionamento e i materiali di confezionamento usati, il modo in cui i mangimi sono disposti, il contesto in cui sono esposti	Reg. (CE) n. 767/2009

materiali, riportati nell'Allegato III, "la cui immissione sul mercato o il cui uso ai fini dell'alimentazione animale sono soggetti a restrizioni o vietati". Non solo, gli operatori del settore, come già avviene per i prodotti alimentari, sono indicati come i responsabili della tracciabilità e rintracciabilità dei mangimi in tutte le fasi della produzione, trasformazione e distribuzione in quanto sono le figure in grado di identificare i fornitori di un mangime, di un animale destinato alla produzione alimentare o di qualsiasi sostanza destinata o idonea ad essere contenuta in un mangime: ne consegue che tutti i mangimi che sono o potranno essere immessi sul mercato europeo hanno un sistema di etichettatura o di identificazione tale da agevolarne la tracciabilità.

In generale, secondo quanto disposto dall'art. 11, l'etichettatura e la presentazione dei mangimi non dovranno indurre l'utilizzatore in errore né "riguardo all'uso previsto o alle caratteristiche dei mangimi, in particolare, alla loro natura, al metodo di fabbricazione o di produzione, alle proprietà, alla composizione, alla quantità, alla durata, alle specie o alle categorie di animali cui sono destinati" né "attribuendo ai mangimi effetti o proprietà che non possiedono oppure lasciando intendere che i mangimi possiedono caratteristiche particolari benché tutti i mangimi comparabili posseggano queste stesse caratteristiche" e neppure "riguardo alla conformità dell'etichettatura al catalogo comunitario e ai codici comunitari".

Tuttavia, l'etichettatura e la presentazione delle materie prime dei mangimi e dei mangimi composti, commercializzati sfusi o in imballaggi o recipienti non sigillati, sono corredati di un documento recante tutte le indicazioni obbligatorie di etichettatura e possono richiamare l'attenzione, in particolare, sulla presenza o sull'assenza di una data sostanza nei mangimi, su una caratteristica o su un processo nutrizionale specifico

o su una funzione specifica correlata con uno di questi aspetti purché l'indicazione sia oggettiva e verificabile dalle autorità competenti e comprensibile per l'utilizzatore dei mangimi e che la persona responsabile dell'etichettatura fornisca, su richiesta, "una prova scientifica della veridicità dell'indicazione". Tuttavia, gli acquirenti hanno il diritto di portare all'attenzione delle autorità competenti i loro dubbi quanto alla veridicità dell'indicazione.

Le prescrizioni obbligatorie generali in materia di etichettatura sono elencate nell'art. 15. Si stabilisce che le materie prime per mangimi o i mangimi composti potranno essere immessi sul mercato solo se l'etichetta riporta il tipo di mangime ("materia prima per mangimi", "mangime completo" o "mangime complementare"); il nome o la ragione sociale e l'indirizzo dell'operatore del settore dei mangimi responsabile dell'etichettatura; il numero di riconoscimento, se noto, dello stabilimento della persona responsabile per l'etichettatura; il numero di riferimento della partita o del lotto; il quantitativo netto; l'elenco degli additivi per mangimi preceduti dalla dicitura "additivi"; il tenore di umidità.

Il Regolamento stabilisce anche prescrizioni supplementari obbligatorie specifiche in materia di etichettatura delle materie prime per mangimi (art. 16), dei mangimi composti (art. 17), dei mangimi destinati a particolari fini nutrizionali (art. 18), degli alimenti per animali da compagnia (art. 19) e dei mangimi non conformi (art. 20). Più specificatamente per i mangimi composti l'etichetta deve riportare la specie animale o le categorie di animali di destinazione; le istruzioni per un uso corretto che indichino l'esatta destinazione; l'indicazione della data di conservazione minima ("da consumarsi entro …" oppure "da consumarsi preferibilmente entro …" seguita dall'indicazione della data, giorno, mese e anno nel primo caso per i mangimi molto deperibili a causa dei processi di deterioramento e mese ed anno per gli altri mangimi); l'elenco delle materie prime che compongono il mangime, recante la dicitura «composizione» e il nome di ogni materia prima, elencandole nell'ordine decrescente di importanza ponderale, calcolata in base al tenore di umidità del mangime composto ed in aggiunta la percentuale in peso. Devono essere indicati "il nome e la percentuale in peso di una materia prima per mangimi se la sua presenza è evidenziata nell'etichettatura in parole, immagini o grafici" e "se le percentuali in peso delle materie prime contenute nei mangimi composti per animali destinati alla produzione di alimenti non sono indicate nell'etichettatura, la persona responsabile dell'etichettatura mette a disposizione dell'acquirente, su richiesta, informazioni sui dati quantitativi relativi alla composizione del prodotto, in un intervallo percentuale ± del 15 % del valore, secondo la formulazione del mangime", fatta salva la Direttiva n. 2004/48/CE sul rispetto delle proprietà intellettuali. A questo principio si fa riferimento anche nei casi in cui, per qualsiasi emergenza relativa alla salute umana e animale o all'ambiente, l'autorità competente fornisca all'acquirente questo tipo di informazione "a condizione che, dopo aver valutato i rispettivi legittimi interessi dei produttori e degli acquirenti, abbia concluso che la fornitura di tali informazioni è giustificata. Se del caso, l'autorità competente fornisce tali informazioni previa sottoscrizione di una clausola di riservatezza da parte dell'acquirente".

Nell'etichettatura dei mangimi per animali non destinati alla produzione di alimenti, ad eccezione degli animali da pelliccia, le materie prime specifiche possono essere

sostituite dalle categorie di appartenenza mentre l'etichettatura dei mangimi destinati a particolari fini nutrizionali, oltre alle prescrizioni obbligatorie, deve includere anche la qualifica di "dietetici", esclusivamente nel caso di mangimi destinati a particolari fini nutrizionali, oltre alla denominazione del mangime, le indicazioni riguardanti l'uso previsto; l'avviso di consultare un esperto in nutrizione o un veterinario prima dell'uso del mangime o prima di prolungare il periodo di impiego.
Infine sull'etichetta degli alimenti per animali da compagnia sono indicati un numero di telefono gratuito o altri mezzi di comunicazione idonei a consentire all'acquirente di ottenere altre informazioni, oltre a quelle obbligatorie, riguardo agli additivi per mangimi contenuti nell'alimento ed alle materie prime per mangimi in essi incorporate, classificate per categoria.
Il Regolamento istituisce, al Capo 5, il Catalogo Comunitario delle materie prime per mangimi "quale strumento per migliorare l'etichettatura delle materie prime per mangimi e dei mangimi composti. Il catalogo facilita lo scambio di informazioni sulle proprietà del prodotto ed elenca le materie prime per mangimi in modo non esaustivo". Per ciascuna voce dell'elenco figura almeno la denominazione, il numero di identificazione, una descrizione delle materie prime e, se del caso, informazioni riguardanti il processo di produzione e un glossario con la definizione dei diversi processi e delle espressioni tecniche utilizzate. La prima versione del Catalogo Comunitario doveva essere adottata entro il 21 marzo 2010 e le voci da riportare sono quelle elencate nella parte B dell'Allegato della Direttiva n. 96/25/CE e nelle colonne da 2 a 4 dell'Allegato della Direttiva n. 82/471/CEE. Viene comunque precisato che l'uso del Catalogo da parte degli operatori del settore dei mangimi è facoltativo. Tuttavia, la denominazione di una materia prima per mangimi figurante nel Catalogo potrà essere utilizzata soltanto a condizione che siano rispettate tutte le pertinenti disposizioni del Catalogo.
L'art. 25 afferma che la Commissione incoraggia la preparazione di due Codici Comunitari di buona pratica in materia di etichettatura, uno per gli alimenti per animali da compagnia e l'altro per i mangimi composti per animali destinati alla produzione di alimenti, che può comprendere una sezione riguardante i mangimi composti destinati agli animali da pelliccia, volti a migliorare l'adeguatezza dell'etichettatura in quanto contengono disposizioni relative alla presentazione delle indicazioni di etichettatura (art. 14), all'etichettatura facoltativa (art. 22) e all'utilizzo delle allegazioni (art. 13). Così come l'uso del Catalogo Comunitario delle materie prime per mangimi, anche l'uso dei Codici di buona pratica in materia di etichettatura da parte degli operatori del settore dei mangimi è facoltativo, anche se è stabilito che l'utilizzo di uno dei Codici può essere indicato sull'etichettatura solo a condizione che tutte le pertinenti disposizioni di tale codice siano rispettate.

CONCLUSIONI

Vogliamo concludere questa prima nota sottolineando che il provvedimento emanato con lo scopo di garantire la salute del consumatore persegue anche altri obiettivi e fra questi ricordiamo:

- adeguare le norme a quelle approntate per i prodotti alimentari destinati al consumo umano;

- aggiornare la legislazione sui mangimi, destinati sia agli animali produttori di alimenti sia agli animali da compagnia che ai selvatici, stabilendo le norme in materia di immissione sul mercato e di uso;
- semplificare e consolidare il diritto comunitario abrogando ben sette direttive ed una decisione e sostituendole con un unico regolamento: in questo modo da una parte risponde alle esigenze di certezza giuridica degli operatori e dall'altra riduce il numero delle norme già esistenti in materia, in quanto la non uniforme applicazione, in particolare delle direttive (spesso contenenti riferimenti incrociati e sovrapposizioni), ha creato limiti ormai inaccettabili agli scambi intra-comunitari;
- statuire le prescrizioni relative all'etichettatura, all'imballaggio e alla presentazione per assicurare un'informazione appropriata agli utilizzatori e ai consumatori;
- riconoscere il diritto di proprietà intellettuale anche sui mangimi preservando le esigenze di tutela del segreto industriale dei produttori poiché, in conseguenza delle emergenze determinate dalla BSE e dalla diossina, la legislazione era divenuta ingiustificatamente penalizzante del diritto dei produttori a mantenere segreta la formula dei propri composti;
- armonizzare e rinforzare il buon funzionamento del mercato interno.

Il Regolamento, che era entrato in vigore nella Comunità Europea il 21 settembre 2009, ha trovato effettiva applicazione dall'1 settembre 2010.

Le sanzioni applicabili in caso di violazione, "efficaci, proporzionate e dissuasive" a norma dell'art. 31, dovevano essere stabilite entro il 1 settembre 2010 dagli Stati membri che dovevano anche adottare le misure necessarie ad assicurarne l'applicazione. Questo aspetto, come spesso accade nel nostro paese, rappresenta la maggiore criticità; infatti, ad oggi, le sanzioni non sono state ancora stabilite, creando una *vacatio legis* che quasi vanifica l'opera di controllo effettuata dagli organi preposti, i quali devono utilizzare sanzionatori di norme parallele, come ad esempio il 178 del 2002.

RIASSUNTO.
Gli Autori prendono in considerazione il Regolamento (CE) N. 767/2009 del Parlamento Europeo e del Consiglio del 13 luglio 2009 sull'immissione sul mercato e sull'uso dei mangimi, che modifica il regolamento (CE) n. 1831/2003 e che abroga le direttive 79/373/CEE del Consiglio, 80/511/CEE della Commissione, 82/471/CEE del Consiglio, 83/228/CEE del Consiglio, 93/74/CEE del Consiglio, 93/113/CE del Consiglio e 96/25/CE del Consiglio e la decisione 2004/217/CE della Commissione e sottolineano i seguenti obiettivi:
- armonizzare le condizioni per l'immissione sul mercato e l'uso dei mangimi per garantire un elevato grado di sicurezza dei mangimi e quindi un elevato grado di protezione della salute degli animali e dei consumatori;
- semplificare le norme attualmente in applicazione;
- informare in maniera appropriata gli utilizzatori ed i consumatori;
- rafforzare il buon funzionamento del mercato interno
- tutelare il segreto industriale dei produttori .

Parole chiave: mangime; immissione sul mercato; uso dei mangimi

SUMMARY.
The Authors take into consideration the Regulation (EC) No 767/2009 of the European Parliament and of the Council of 13 July 2009 on the placing on the market and use of feed, amending European Parliament and Council Regulation (EC) No 1831/2003 and repealing Council Directive 79/373/EEC, Commission Directive 80/511/EEC, Council Directives 82/471/EEC, 83/228/EEC, 93/74/EEC, 93/113/EC and 96/25/EC and Commission Decision 2004/217/EC. They underline the following targets:
- to harmonize the conditions for the placing on the market and the use of feed, in order to ensure a high level of feed safety and thus a high level of protection of animal and public health;
- to simplify the rules actually in application;
- to strengthen the effective functioning of the internal market;
- to provide adequate information for users and consumers.

Key words: feed; market; use

Animal anatomical engraving from *Handbuch der Anatomie der Tiere für Künstler*
Hermann Dittrich, illustrator, 1889