Fresh Produce ©2007 Global Science Books

Forage Quality: Techniques for Testing

Cecilia L. Fulgueira[1*] • Susana L. Amigot[1] • Mónica Gaggiotti[2] • Luis A. Romero[2] • Juan C. Basílico[3]

[1] Centro de Referencia de Micología, Facultad de Cs. Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, S2002LRK Rosario, Argentina
[2] INTA Rafaela Ruta 34 km 227, 2300 Rafaela, Argentina
[3] Facultad de Ingeniería Química, Universidad Nacional del Litoral, Santiago del Estero 2829, S3000AOM Santa Fe, Argentina

Corresponding author: * **cfulgueira@yahoo.com.ar**

ABSTRACT

Forage quality refers to how well animals consume a forage and how efficiently the nutrients in the forage are converted into animal products. Six major factors affecting forage quality: maturity (harvest date), crop species (differences between grasses and legumes), techniques of harvest and storage, environment (moisture, temperature and amount of sunlight), soil fertility, variety or cultivar. Also, weeds, insect pests, plant diseases and presence of bacteria, molds, and/or some of their metabolites, e.g. mycotoxins can negatively affect forage quality. Recommended tests for determining forage quality are: dry matter (DM), pH, crude protein (CP), available protein, amoniacal nitrogen (as % NH_3/TN), acid detergent fiber (ADF), neutral detergent fiber (NDF), lignin and ash. Energy values such as total digestible nutrients (TDN), net energy (NE) and relative feed values (RFV) can be calculated from these core analyses. There are two methods used to analyse such variables: the traditional chemistry analysis and the newer, near infrared reflectance spectroscopy (NIRS) analysis. Currently, the quality of a forage has been evaluated only through those chemico-fermentative parameters. However, recent studies propose to incorporate the analysis of microbiological parameters such as fungal propagule counts, the presence of *Aspergillus fumigatus* and mycotoxins (aflatoxins and deoxynivalenol) as decisive parameters of forage acceptability. Forage quality information is important for formulating nutritionally balanced rations, evaluating forage management practices (growing conditions, timing of harvest, and handling from harvesting to utilization) and marketing and pricing forages.

Keywords: chemico-fermentative evaluation, feedstuffs, fungal contamination, mycotoxins, silages

CONTENTS

FORAGE CONSERVATION SYSTEMS

Current systems of dairy and beef production demand deeper knowledge of the production processes and quality of every available feed (Bruno *et al*. 1998). Although they may vary according to region, cattle-rearing production systems are based upon the direct grazing of forage resources with supplementary feeding, such as: grains, crop by-products, and stored forages like hay or silage, etc. These methods make it possible for the feed management of herds to improve and to become more cost-effective (Taysom 2002; Beltzer 2003).

Forage conservation arises out of the need to rationally profit from the excess of pastures – lucerne, winter soilage, etc. and of annual crops that have been specially grown for ensilage – maize, sorghum, oat, ryegrass, soya, etc. (Romero *et al*. 2003).

These conservation processes had been previously used

Received: 12 July, 2007. Accepted: 23 September, 2007.

Invited Review

to store excess forage so as to resort to it during feed shortage periods (winter). Nowadays, they are used all year round in order to obtain more balanced diets.

Hays

Hay is a stored forage that is essentially characterized by having low percent moisture content (less than 15%). This means it can be stored unharmed by fermentation or mold development (Beltzer 2003; Reboux *et al*. 2006). Although most forage crops can be stored as haylages, the nutritional value of the latter is closely linked to the type of plant or original forage. Among the substantial benefits brought about by this forage conservation system, the following can be mentioned: low farm labor demand (both for forage harvesting and supply), reduced production costs (Lascano 2002; Romero *et al*. 2003).

Silages

Fresh forage crops, such as maize, sorghum, wheat and lucerne, can be preserved by ensiling (Oude Elferink *et al*. 1999a). Ensiling is a forage preservation method based on a spontaneous acid lactic fermentation under anaerobic conditions (Whitlow and Hagler 2002; Seglar 2003b). Silage techniques minimize the loss of nutrients as from harvest time until storage. Moreover, they also improve the quality of feed (Beltzer 2003).

The epiphytic (existing on plants) lactic acid bacteria (LAB) that are present on forage crops are involved in the fermentation of water-soluble carbohydrates to lactic acid and, to a lesser extent, to acetic acid. As a result, the pH level of the ensiled material is reduced so the activity of spoilage microorganisms is inhibited. Once the fresh material has been stored, compacted and covered to exclude air, the ensiling process can be broken down into four phases (Weinberg and Muck 1996; Driehuis and Oude Elferink 2000).

Aerobic phase: In this phase, which only lasts a few hours, the amount of atmospheric oxygen present in the forage is reduced due to the respiration of the plant material and to aerobic and facultative aerobic microorganisms such as yeasts and enterobacteria.

Fermentation phase: It starts once anaerobic conditions are reached in the ensiled material. It can last for several days or for as long as several weeks, depending on the characteristics of the forage material and the ensiling conditions. If the fermentation is successfully carried out, LAB will develop and become the predominant population, while pH will decrease to values around 4.0.

Stable phase: As long as the silo is properly sealed so that air is not allowed to enter, there are relatively few changes. Most microorganisms of the previous phase slowly decrease in number.

Aerobic spoilage phase: It starts when the silo is opened so that oxygen has unrestricted access to the silage. However, it can start earlier if the silage covering is damaged, for instance, by animals or other agents. Deterioration begins through degradation of forage preserving organic acids by yeasts and occasionally by some bacteria. This results in a rise in pH. Then, temperature increases as well as the activity of spoilage microorganisms such as some bacilli and other aerobic and facultative microorganisms such as molds and enterobacteria (Driehuis *et al*. 1999; Driehuis and Oude Elferink 2000).

The advantages of using silage can be summarised as follows (Cowan 2001; Schroeder 2004e; Romero *et al*. 2006):

- As a reserve during times of extreme feed shortage periods, for instance drought seasons, which entails ensiling pasture or crops under optimal conditions and storing them for a period of 1 to 20 years.
- To enhance productivity due to the increase of the amount of feed available to livestock. The storage period takes less than one year.
- To improve pasture or crop managements where the silage enables other management practices to be carried out. For instance, ensiling temperate fodder crops with increased tiller density at the beginning of the season when there is excessive growth enables the earlier planting of a subsequent crop.
- To profit from excess growth. Generally, this excess is considered to be a waste. Ensiling allows excess growth to be stored so that losses due to maturation or decay *in situ* are avoided.
- To balance the nutrient content of the diet. The silage is used to provide nutrients whenever the feeds available are deficient. For instance, the use of legume silage to complement maize silage, or combining the use of maize silage with grazed legume pastures, or resorting to silages of varied fiber contents.
- To enable storage of perishable materials since the ensiling process ensures the feed can be used over an extended period of time, for instance, the ensiling of wet by-products. This method is similar to that of preservation of feeds through the addition of chemical substances or the exclusion of air from high-moisture grains.
- To preserve the dry matter content and keep income potential (palatability, consistency and composition) of the fermented feed.

Haylage or round bale silages

Haylage is a conservation system for wet forages. Feed is preserved in a combined process of hay and silage making. Forage containing around 50% moisture content is rolled up in bales and then wrapped tightly in polythene or bagged in self-adjustable stretch bags (Lascano 2002). In this way, as long as air is not allowed to enter, a bale becomes a small silo where anaerobic fermentation takes place. Although any forage can be baled, it is advisable to use high quality pastures such as lucerne, clovers and grasses that have a high nutritional value since the additional cost associated with packing should to be taken into account (Beltzer 2003).

The most significant advantages brought about by this system are related to agronomic and nutritional aspects, for instance (Schroeder 2004c; Muck and Holmes 2006):

- Weather-related losses are reduced due to shorter air drying time.
- Since this ensiling process uses wet forage, field losses (mainly from leaves) that result from production, distribution and supply are minimized.
- Small pasture areas can be kept. This differs from silages, since they demand larger areas.
- Since anaerobic conditions are created, the fermentation process starts quickly.
- Low farm labor demand at baling time.
- It requires a relatively low capital investment.
- No special storage facilities are required.
- It is easy to handle for rationing and it allows for complete mechanization for operations to take place.
- Storage losses are low (3-7%).

FORAGE QUALITY

Forage quality is defined as an expression of the characteristics that affect consumption, nutritional value, and the resulting animal performance (Amigot *et al*. 2005). In other words, forage quality refers to how well animals consume a forage and how efficiently the nutrients in the forage are converted into animal products (Twidwell and Wegenhoft 1999; Taysom 2002). Thus, the best measure related to forage quality is animal productivity, which can be affected by nutrient intake, digestibility and utilization efficiency.

Factors that influence forage quality

Six biological and technological factors affecting forage quality (not yield) have been traditionally recognized: crop species, soil fertility and variety, maturity stage, harvest and

storage techniques, environment (Frey *et al*. 2004; Schroeder 2004d; Reboux *et al*. 2006).

Crop species: There can be substantial differences in forage quality between grasses and legumes. These distinctions are generally related to differences in fiber and protein content, digestibility, etc., which have a negative impact on consumption and animal productivity (Twidwell and Wegenhoft 1999; Cherney JH 2000).

Soil fertility: Soil fertility exerts greater influence on forage yield than it does on quality. Appropriate soil phosphorus and potassium levels not only contribute to keep legumes in a mixed seeding, but they also reduce weed related problems. It is necessary to balance soil fertility to avoid mineral imbalances in forages. It has been proved that high levels of fertilization in grasses make dry matter production increase. However, as non-protein nitrogen values also increase, unbalanced relationship carbohydrates/protein results. Therefore, the fermentation process may be affected (Schroeder 2004g).

Variety (cultivar): After decades of enhancing forage yield and persistence, attention has recently been aimed at developing or identifying varieties with improved compositional quality. Variety or cultivar can affect the chemical make-up of forages, but not to the same extent as the other factors. In lucerne crops, selection processes to improve quality are being carried out by most commercial companies (e.g. HQ and multifoliated lucerne), and several firms have also started to select improved maize and sorghum hybrids (better stem quality, crops that stay green longer, grains with higher nutritional value, etc.) for ensiling.

Maturity: It refers to the growth stage of a plant at the time that it is harvested. Maturity is the most important factor affecting forage quality. This quality is not static; plants continually change in quality as they mature. In fact, forage plants change so rapidly that it is possible to detect significant declines in forage quality every two or three days. Thus, protein, soluble carbohydrate and vitamin contents of the plant cell wall increase. The amount of lignin, cellulose and hemicellulose increases as well. While cellulose and hemicellulose can be partially digested by livestock, lignin is not digestible. As the amount of structural fiber and lignin increases, digestibility of the forage and its consumption by livestock decreases (Twidwell and Wegenhoft 1999).

Harvest and storage conditions: Inappropriate harvest techniques can seriously reduce forage quality, for instance the loss of leaves in haying. Both storing a forage crop with an incorrect moisture content, and improper ensiling can lower its quality and molds can appear. Fungi generate heat through respiration. This reduces protein content and forage digestibility.

Environment: Climatic conditions (moisture, temperature, and the amount of sunlight) affect both forage quality and its production. When harvesting is delayed due to bad weather conditions, forage crops become overmatured so that their quality is lowered. High temperatures may increase lignin accumulation and decrease quality, but drought stress may increase quality by delaying maturity. The amount of rainfall during the harvest period may bring about losses in forage quantity and quality since dry matter content and soluble nutrients decrease.

In addition, the presence of weeds, the damage brought about by insects, bacteria, molds, and/or their metabolites (mycotoxins) significantly affect forage quality (Cherney JH 2000).

Other important factors that should also be taken into account are: type of silo, filling speed, forage density after packing, sealing technique, feedout speed, amount of forage extracted, use of additives, forage supply techniques (Bolsen 1998; Jahn *et al*. 2000).

Forage quality evaluation

All forage plants are made up of cells that are composed of fibrous cell walls used for support and protection. There are several soluble compounds within the cells. Most of these compounds are highly digestible. Since the material of the cell wall is the primary constituent of forages, one of the main aims of forage analysis is to characterize the cell wall fiber (Cherney JH 2000).

Sensory evaluation

Forages have been traditionally evaluated according to physical parameters such as: color, leaf content, maturity, odor, softness, purity, observations on palatability, etc. Although these parameters are important in determining forage quality, there may be some limitations regarding assessment, since they remain both highly subjective and difficult to standardize (Schroeder 2004a).

Forage sampling

Accurate findings during the quality evaluation of forages depend on the implementation of good sampling techniques, appropriate handling of samples after collection and upon reliable analytical procedures in the laboratory that carries out the evaluation (Schroeder 2004a). So as to conduct a forage quality study, it is important to take into account that the first major obstacle lies in the collection of samples; since it has to faithfully represent the type of feed that will be consumed by livestock (Faithful 2002). Sampling is the major factor affecting the accuracy of forage quality analyses. It has been considered variation from sampling procedures to be 5 up to 10 times higher than that from laboratory procedures (Ferret 2003; Macaulay 2003; Schroeder 2004f). The type of sampling depends upon feed characteristics, establishing a clear difference between hay/haylages and silages.

Whenever hay samples are collected, it is advisable to use a probe that is larger than 1 cm in internal diameter and place it 30 to 45 cm deep. Thus, core samples from the bale can be extracted without opening it so that mistakes during sampling can be avoided. It is advisable to collect 20 subsamples (1 for each bale) and form a pool of 500 to 1000 g (Ferret 2003).

There are two different general aims that influence the collection of silage samples: to make a reasonable prediction of the silage average quality before ensiling or to know the quality of the forage being fed to animals (Muck and Holmes 2006). To achieve the first aim, a probe that allows for the sample to be collected at a certain depth is used. It is also important to seal the holes created by sampling as carefully as possible. For a truly representative sample of silage content to be obtained, it is advisable to take aliquots of the average points from the 4 segments generated at the intersection formed by 2 diagonal lines traced in the upper part of the silage (Faithful 2002; Ferret 2003). So as to achieve the second aim, sampling will be conducted by collecting different subsamples (12 to 15) from the front part of the silage and from the same kind of forage material being fed to animals. This procedure is to be repeated at different times as the content of the silage is used. Moldy or damaged subsamples that are not appropriate to be fed to animals should be avoided. Thus, it is not advisable to collect subsamples in areas that are too near the plastic cover. Between 500 and 1000 g of forage material will be collected during each sampling procedure.

Chemico-fermentative evaluation

Traditional laboratory methods involve various chemical, drying and burning procedures to determine the major chemical components within the forage. Chemical analyses prove to be fundamental to estimate forage quality (Colombatto 2000; Undersander and Moore 2002; Redfearm *et al*. 2004).

Wet chemistry procedures are presently the most widely used for forage evaluation. They are based on sound chemical and biochemical principles and take considerably more time to complete than the newer electronic methods such as

near-infrared reflectance spectroscopy (NIRS) analysis (Schroeder 2004a). This technique combines methods from spectroscopy, statistics and computing and generates mathematical models that relate chemical compositions (presence of active chemical groups) with changes in energy in the area corresponding to the near infrared range (wavelengths between 800 and 2500 nm) (Deaville and Flinn 2000; Cozzolino *et al*. 2003). The advantages of this technique are as follows: it provides information on the nutritional value of feeds within seconds, it is a non-destructive method that only asks for a minimal requirement or even no requirement for sample treatment, it minimizes impact on the environment and it is a multi-analytical technique that allows for various factors to be predicted at the same time. Once the spectrophotometer is calibrated with the same forage crops that come from the same region, the implementation of NIRS methodology can help conducting cost-effective analyses. Thus, this method has been internationally accepted (Reeves and van Kessel 2000; Reeves *et al*. 2002). It is more difficult to interpret analyses obtained by NIRS when feeds are made up of different forage crops (Stokes and Prostko 1998).

The chemico-fermentative parameters that are generally evaluated are as follows:

Dry matter (DM): It corresponds to the percentage of forage which is not water. It has traditionally been determined by drying forages at high temperatures over short periods of time. However, other volatile compounds can also be evaporated during this process. So as to overcome this problem, regression equations have been developed. They correct DM values determined by oven-drying through distillation with toluene (Haigh 1995a, 1995b). The moisture content of forages varies according to crop species, physiological state and season. Thus, all the results should be expressed on a DM basis (as it is the most useful factor to perform comparisons).

pH: It is considered to be the individual parameter that best determines the quality, fermentation and conservation of forages with a high moisture content (higher than 65%). The method for measuring pH is both fast and simple: a pH-meter probe is placed into a sample fluid obtained by pressing or maceration (Ferret 2003; Maculay 2003; Ward 2005a).

Nitrogen values

Crude protein (CP): The term crude protein is used because it represents all of the nitrogen that is in the form of non-protein nitrogen (NPN) such as nitrates, ammonia, urea and single amino acids, as well as the nitrogen present as true protein. The total nitrogen concentration of a feed sample is generally determined by resorting to some variant of the Kjeldhal method (Cherney DJR 2000), but it can also be measured using a total combustion technique by means of an autoanalyser (AOAC 1990). Crude protein is represented by the total amount of nitrogen present when analyzed and then multiplied by a conversion factor of 6.25. This is based on the assumption that true protein contains 16% nitrogen. However, as this is not always the case, Cherney DJR (2000) suggested that when determining crude protein a correction factor for N content should be included. As plants mature, the crude protein usually decreases. Although ruminants can use, to a certain extent, all these types of nitrogen compounds (Schroeder 2004a), a crude protein analysis that follows this criterion proves to be inappropriate in determining the quality of the protein present in the forage (van Soest 1994). The analysis of the protein fraction of a feed should include data on how that protein influences microbial protein formation, on the amount of dietary protein escaping ruminal degradation, etc. (Broderick 1994; Beever and Mould 2000).

Available protein: Available protein is the portion of crude protein that is digestible by the ruminant. It is usually used in describing protein that is 'available'. Because of the feeding rate and rumen retention time, not all of the protein present can be digested. It is usually accepted that only ~70-72% of the protein can be assimilated.

Unavailable or bound protein: Unavailable or bound protein is the portion of crude protein that is not usable by the ruminant. This is fundamental in describing heat damaged wet forages, where some of the protein has been rendered unusable due to excessively high temperatures reached during fermentation. It is advisable to perform this analysis in those forages with high-protein content that will be fed to animals. Whenever its value, regarding the total nitrogen content of the sample, is higher than 12%, some overheating of the silage has occurred. Therefore, the digestibility of the available protein in the animal's rumen decreases.

Ammoniacal nitrogen: A substantial part of the forage protein fraction is degraded to peptides, amino acids, amines, and ammonia by plant and microbial enzymes, which reduces the nutritional value of the feedstuff (Schroeder 2004g). Therefore, ammonia concentration (usually expressed as total nitrogen percentage $\%NH_3/TN$) is generally used as an indicator of the silage protein degradation and, consequently, of its bad preservation (Driehuis and Oude Elferink 2000; Ferret 2003). The nitrogen content in a forage sample can also be determined by fluid obtained by pressing or maceration.

According to pH and %NH3/ TN values a forage may be classified as: Very Good ($pH < 4$ and $\% NH_3/TN \leq 5$); Good ($pH \leq 4$ and $\% NH_3/TN$ between 5 and 15), Fairly Good ($pH > 4$ and $\% NH_3/TN \leq 15$) and Bad ($pH > 4$ and $\% NH_3/TN > 15$) (Fahey 1994).

Fibers

The ruminants need a minimum amount of fibers to maintain a good function of rumen. The vegetal fibers include cellulose, hemicellulose and lignin.

Detergent or Van Soest Method of Cell Wall Determination: The detergent analysis system is a wet chemical method that separates soluble cell contents (starches, proteins, sugars, pectins, fats, vitamins, minerals, etc.) from the fiber fraction (structural support of the plant). The fiber fraction of a forage is divided into two components that nutritionists use to prepare feed rations: neutral detergent fiber and acid detergent fiber (van Soest 1994).

Neutral detergent fiber (NDF): It estimates the total fiber content of a forage (cellulose, hemicellulose, lignin). It is the insoluble part of feed in detergent under neutral conditions (Bruno *et al*. 1998). NFD is partially digestible depending on forage crop and maturity stage. NDF levels are used to predict feed intake. High NDF levels in a forage not only decrease intake, but also limit forage effectiveness in, for example, high milk production (Stokes and Prostko 1998; Ward 2005b).

Acid detergent fiber (ADF): It measures cellulose and lignin contents of a plant and shows the animal ability to digest a forage. It is the insoluble part of a detergent in acid conditions. ADF is also partially digestible. When ADF levels increase, forage digestibility usually decreases, so that low levels of ADF are desirable. Some factors that increase ADF in a forage are as follows: maturity, weathering, rain damage, high temperatures and weeds (Stokes and Prostko 1998; Beltzer 2003).

Lignin: It is a non-carbohydrate substance that is the main factor which influences the digestibility of plant cell wall material. It is a fiber component with no energetic value for animals but it can affect the digestibility of other fiber components. Low levels are desirable. When lignin increases, digestibility, intake and performance usually decrease (Stokes and Prostko 1998; Beltzer 2003).

Minerals

Ash: Forage analyses generally report the content of major minerals. The total mineral content of a forage is called ash and it represents 3 to 12% of DM. The minerals typically

determined are calcium and phosphorus. Laboratory techniques used to determine forage minerals are: wet chemistry, colorimetric methods and atomic absorption. Minerals can be divided into two groups: macronutrients (such as Ca, P, K, Mg), and micronutrients (such as Co, Cu, Mn, Fe, Zn and Se (Stokes and Prostko 1998).

Calculated energy values

Accurately predicting the digestible energy of forages for ration formulation and animal performance is important (Bagg 2004).

Measuring the energy content of a feed requires very sophisticated equipment and animal metabolism trials. However, it has been discovered that feed energy content is inversely related to fiber content. Thus, many equations have been developed to predict energy value from fiber content, dry matter, etc. However, there is no one that can estimate it in all forages (Linn and Martin 1999; Rayburn 2002). Moreover, laboratories have not agreed on standardized formulas. This makes it difficult to perform inter-laboratory comparisons.

There are different measures to describe the energy value of a feed. The most popular terms are: net energy (NE), total digestible nutrients (TDN) and relative feed values (RFV) that can be calculated from core analyses (Stokes and Prostko 1998).

Net energy (NE): NE is the energy used for maintenance and for productive purposes, i.e. growth, gestation and lactation. Net energy is derived from animal studies by measuring the gross energy minus fecal energy, minus energy lost in urine and minus combustible gases and heat loss. Net energy (lactation), however, can also be calculated on a dry matter basis for hay, haylage and corn silage using the forage Acid-Detergent Fiber (ADF) analysis (Rayburn 2002).

Total digestible nutrients (TDN): This measure represents the digestible portion of a feed and it can also be used to estimate the energy content of a forage (Beltzer 2003). To calculate TDN contents, previous digestion trials need to be carried out. Forage components can be analysed both from the feed of a group of animals or from their feces, the difference can determine the digestibility of each type of nutrients (Schroeder 2004a). The current formula is: % TDN = % digestible crude protein + % digestible crude fiber + % digestible starch and sugars + % digestible fats x 2.25. (Fats are multiplied by 2.25 because they contain more energy per unit weight). TDN values for hay, haylage and corn silage, however, can also be calculated on a dry matter basis using the forage Acid-Detergent Fiber (ADF) analysis (Rayburn 2002).

As forages tend to lose an important part of energy mainly during ruminal fermentation, the TDN % may be overestimated (Schroeder 2004a). Therefore, it is advisable to use net energy values to formulate rations.

Relative feed values (RFV): A number of factors must be considered to accurately evaluate forage quality. RFV is an index (not units attached) that combines digestibility and potential intake into one number. This term is useful for comparing forages of the same type. It is calculated based on dry matter and dry matter intake. Digestible dry matter is a function of ADF, and dry matter intake is a function of NDF. Therefore, fiber components have an integral effect on RFV.

Generally, nutritionists will require a larger set of analyses to balance rations than what might be required to identify the quality of forage in the marketplace. Many nutritionists are interested in a wide range of analyses, from basic fiber and crude protein to minerals, protein digestion estimates, ash, and sometimes detailed carbohydrate analyses. However, analyses of forage for marketing purposes may only be a subset of these, and should have the following characteristics: must be rapid, be reliable and utilize recognized methods, be repeatable across labs and across time, must not change significantly over time or be subject to different interpretations and must be a relatively powerful predictive tool for nutritionists (Putnam 2004).

Microbiological evaluation

Microbiology of forages: The successful outcome of the conservation process mostly depends upon the microflora present on forages. A wide range of microorganisms are naturally as contaminants found in cereals, oilseeds, their by-products and other components (Driehuis *et al*. 1999). They can be classified into two main groups: desirable micro-organisms and undesirable microorganisms. As mentioned before, the presence of lactic bacteria might be beneficial during forage fermentation. LAB and yeasts have feed probiotic properties, lowering scouring and stimulating animal growth performance.

Undesirable microorganisms from soil and animal feces can contaminate and deteriorate forages (Driehuis and Oude Elferink 2000). They cause anaerobic deterioration (clostridia, enterobacteria) or aerobic deterioration (yeasts, bacilli, *Listeria* sp. and molds). Many of these undesirable organisms (*Listeria* sp., clostridia, molds, and bacilli) not only reduce the nutritional value of the forage, but they may also affect animal health or alter the quality of milk, meat and eggs, or both (Oude Elferink *et al*. 1999b, 2002).

Successful conservation of high moisture forages depends on the control of microbial activity. The preservation process by acidification, dehydration and/or air exclusion early during the storing period should restrict the development of those undesirable microorganisms. However, oxygen can enter the silo through holes in the polyethylene cover or during exposure to air once the cover is open (Driehuis and van Wikselaar 1996). Water activity (a_w) can also increase if hermetical conditions are not kept. In these situations, undesirable microorganisms can develop in the forage (Gotlieb 2002).

Forage bacteria

Lactic acid bacteria. The natural population of lactic bacteria grows significantly between harvest and silage (Oude Elferink *et al*. 2002). Anaerobic conditions should be kept at each stage of the fermentative process to allow LAB to proliferate using endogenous vegetable sugars to produce enough quantities of acid to lower the pH level to 4 (optimum for a successful conservation) (D'Mello 2002). According to sugars metabolism, LAB can be classified as obligate homofermenters, facultative heterofermenters or obligate heterofermenters. Obligate homofermenters, such as: *Pediococcus damnosus* and *Lactobacillus ruminis* produce more than 85% of lactic acid from hexoses (for instance glucose) but they can not degrade pentoses (for instance xylose). Facultative heterofermenters, which include *Lactobacillus plantarum*, *L. pentosus*, *Pediococcus acidilactici*, *P. pentosaceus* and *Enterococcus faecium*, also produce, primarily, lactic acid from hexoses. However, they can also degrade some pentoses producing lactic acid, acetic acid and/or ethanol. Therefore, they constitute the group that converts forage sugars to lactic acid more efficiently (D'Mello 2002). Obligate heterofermenters, which include members of the genus *Leuconostoc* and some *Lactobacillus* such as *L. brevis* and *L. buchneri*, degrade hexoses and pentoses but they degrade the hexoses into equimolar quantities of lactic acid, CO_2, acetic acid and/or ethanol (Schleifer and Ludwig 1995; Oude Elferink *et al*. 2002). LAB are non-proteolytic organisms so they contribute to the preservation of labile proteins and free amino acids in the forage.

Wet forages are difficult to preserve by acidification and they provide conditions for the development of undesirable bacteria such as clostridia and enterobacteria (D'Mello 2002).

Clostridia are anaerobic bacteria that form endospores. Many of them can ferment carbohydrates and proteins. As a result, they reduce the nutritional value of the silage and, as well as enterobacteria, they produce biogenic amines which

cause several problems. In addition, the presence of clostridia alters milk quality since their spores can survive throughout the digestive tract of animals. As a result, clostridia can be found in feces and may contaminate the milk directly or indirectly when udders are not clean. There are two groups of clostridia: the saccharolytic group (*Clostridium butyricum* and *C. tyrobutiricum*) and the proteolytic group (*C. bifermentans* and *C. sporogenes*). The first group ferments residual sugars such as lactic acid to butyric acid, increasing the pH level; while the second group ferments amino acids to different products (butyric acid and acetic acid, amines, CO_2 and NH_3) and may also increase the pH level (D'Mello 2002). Some types of clostridia can cause serious health problems. The most important species in the dairy industry is *C. tyrobutyricum*, an acid-tolerant organism. It can not only ferment carbohydrates but it can also degrade lactic acid to butyric acid, H_2 and CO_2. Butyric fermentation interferes with the lactic fermentation in silages and cheese and causes gas production (Oude Elferink *et al*. 2002). A usual "clostridial silage" shows large amounts of butyric acid, high levels of pH (>5 in silages with low DM content), ammonia and amines. Ensiling techniques that allow a rapid and significant drop of pH would prevent this problem since enterobacteria and clostridia are inhibited at low pH values. Moreover, clostridia show more susceptibility to the absence of moisture (low aw value) than LAB. Every measure taken to decrease the aw value in a forage, such as inducing wilting to increase the value of the DM content, allows for the selective inhibition of clostridia to take place (Oude Elferink *et al*. 2002).

Enterobacteria are anaerobic facultative organisms. Most of the enterobacteria present in the silage are considered non-pathogenic. Nevertheless, their growth should be avoided since they compete with LAB for sugars, fermenting them to acetic acid, ethanol, CO_2 and H_2. Besides, they can degrade proteins and catabolize amino acids to NH_3, increasing pH (D'Mello 2002). Protein degradation causes a reduction in the nutritional value of the silage and leads to the production of toxic compounds such as biogenic amines and branched fatty acids. Biogenic amines have a negative effect on silage palatability (van Os 1997; D'Mello 2002). The ammonia generated by proteolysis increases the buffer capacity of a silage; this counteracts any rapid pH drop. Moreover, enterobacteria can produce nitrite, nitrogen oxides (NO_2), nitrogen monoxide (NO) and ammonia. NO and NO_2 gases produce lung tissue damage and can cause an illness with symptoms similar to those of pneumonia, known as the "silo filler's disease" (O'Kiely *et al*. 1999).

Escherichia coli O157 belongs to the group of Gram negative bacteria. It is closely associated with human pathologies, for example: hemolytic-uremic syndrome. However, it has not been associated with animal pathologies. O157 is widespread in nature. Besides cattle, it is ubiquitous in birds, deer and other wildlife. Thus, eradication is not possible. Ecological control measures focus on control of bacterial intake in feed and water (Teplitski 2006).

Salmonella also belongs to the group of Gram negative bacteria. It contains many serotypes involved in human and animal pathologies. Among them, *S. typhimurium* is universally distributed and *S. enteritidis* has appeared as a pathogen agent in birds and as egg and chicken meat contaminator. Salmonellosis is one of the most important features in cattle biosecurity. The risks for salmonellosis are minimised if the right practices are implemented when handling feed and following disinfection and vaccination protocols. Cattle feed is frequently contaminated with *Salmonella*. The intensive use of contaminated pastures with infected animal feces and the use of poultry slurry provide additional sources of illness (D'Mello 2002; Winfield and Groisman 2003).

Animals with subclinical infection are more frequent than ill animals and they are more susceptible to other infectious processes. However, asymptomatic carriers eliminate millions of these microorganisms through their feces. Virulent *E. coli* strains can survive for a few months in animal waste, and *Salmonella* can persist in untreated farm waste for up to two years (Winfield and Groisman 2003). Proper utilization and composting of animal wastes are important steps for reducing *Salmonella* and *E. coli* contamination, and breaking the cycle of reinfection (Teplitski 2006).

Listeria monocytogenes is a pathogenic facultative anaerobic organism to several animals and to men. It is widely distributed in nature and can contaminate forages. Animals with temporary inhibited immune systems (pregnant females and neonates) are susceptible to *L. monocytogenes* infections. The *L. monocytogenes* contaminated silage has been associated with fatal cases of listeriosis in sheep and goats and it has been one of the main sources of raw milk contamination by *L. monocytogenes*. The increase in the incidence of listerosis in sheep and cows has been related to the usage of big bale silages, a kind of low density and limited fermentation forage that favours the growth of *L. monocytogenes*. Growth and survival of *Listeria* spp. in the silage are determined by the failure of keeping anaerobic conditions and by the pH value of the silage. *L. monocytogenes* can tolerate low pH levels, between 3.8 and 4.2, for long periods as long as there is oxygen even in minimum concentrations. However, they die in a strictly anaerobic environment with low pH value (Oude Elferink *et al*. 1999a, 1999b). This microorganism can contaminate animal products destined for human consumption.

Fungal contamination of forages

Fungal contamination of cereals, oilseeds and forages represents a major risk for human and animal health in the world. Both yeasts and filamentous fungi can contaminate forages. Yeasts mainly include *Candida* and *Saccharomyces* species. Yeasts are frequently the most numerous isolates. They are eukaryotic, facultative anaerobic and heterotrophic microorganisms. Yeast population can reach 10^7 CFU/g during the first weeks of the ensiling process; however long-term storage gradually reduces the presence of yeasts. Available oxygen facilitates the growth of yeast during storage whereas a high level of formic acid or acetic acid reduces survival (Driehuis and van Wikselaar 1996; Oude Elferink *et al*. 1999b). Under anaerobic conditions yeasts ferment sugars to ethanol and CO_2. The production of ethanol decreases the amount of sugar available to produce acetic acid and affects milk taste (Randby *et al*. 1999). With the introduction of oxygen in the silo, a large amount of yeast species aerobically degrade lactic acid to CO_2 and H_2O. The degradation of lactic acid increases the pH level of the silage and allows the growth of other undesirable organisms such as filamentous fungi (Seglar 2003a).

Molds are eukaryotic organisms that grow in any part of the silo where there is oxygen, even in small amounts. In a good silage that happens at the beginning of the storage period and it is restricted to the surface of the ensiled mass. But during aerobic deterioration all the silage can be invaded by molds (Rankin and Grau 2002).

The most significant factor that determines fungi growth in hays is moisture. Hence, molds are mostly found in hays that are stored wet. Whereas, the factor that determines fungi growth in silages is pH. If the silage is stored too dry or not compacted enough or uncovered, air infiltration will produce microbial activity which, in turn, will degrade the acids of the silage while increasing the pH level, and promoting mold growth (Whitlow and Hagler 2000).

Filamentous fungi more frequently identified in forages belong to the genera *Aspergillus*, *Eurotium*, *Penicillium*, *Fusarium*, *Mu*cor, *Byssochlamys*, *Absidia*, *Arthrinium*, *Geotrichum*, *Monascus*, *Scopulariopsis* and *Trichoderma* (Oude Elferink *et al*. 2002). Molds not only decrease the nutritional value and palatability of the forage but also represent a risk for animal and human health. Inhalation or intake of fungal propagules may cause diseases collectively known as mycosis (Di Costanzo *et al*. 1995; D'Mello 2002). Mold growth in forages expose animals to respiratory problems,

allergies, abnormal ruminal fermentation, diminished reproductive function, reduced production, renal damage, skin and eye irritation (Scudamore and Livesey 1998; Gotlieb 2002). Fungal contamination affects both the organoleptic characteristics and the alimentary value of feed, and exposes animals to the potential risk of toxicosis. Mycotoxins are fungal secondary metabolites that are produced according to a wide range of genetic and environmental factors (D'Mello 2002; Amigot *et al*. 2005).

Mycotoxin contamination of forages and cereals frequently occurs after plants are infected with specific pathogenic fungi or symbiotic endophytes. Moreover, contamination may occur during feed processing and storage, whenever environmental conditions (moisture content and ambient temperature) are appropriate for fungal colonization and mycotoxin production (Rankin and Grau 2002). Fungal growth and mycotoxin production are related to extreme weather conditions, inadequate storage practices, bad forage quality and faulty feeding conditions (Whitlow and Hagler 2000; Lanyasunya *et al*. 2005). Conventionally, toxigenic fungi have been devided into "field" organisms (or vegetal pathogens and "storage" organisms (or saprophytic/spoilage organisms). *Claviceps*, *Fusarium* and *Altrernaria* are usual field fungi; *Aspergillus* and *Penicillium* are examples of storage organisms. When field fungi are isolated from forages they indicate poor preservation conditions because these fungi need a higher a_w to develop, and they are often absent from adequately stored silos (Scudamore and Livesey 1998; Akande *et al*. 2006; Amigot *et al*. 2006).

Although there are over 100000 species of known fungi, the majority of the known toxigenic species fall into three recognized genera. These genera are *Aspergillus*, *Penicillium*, and *Fusarium*. Also, most of the known mycotoxins are elaborated by these genera.

The genus *Aspergillus* is within a large, very diverse family of fungi that are world-wide in distribution but primarily occupy subtropical and warm temperate climates. They are generally regarded as saprophytes that are important in nutrient cycling. Their growth at high temperatures and low water activity allows for their involvement in the colonization of a variety of crops, sometimes with limited parasitism especially under favorable conditions. Some of the most economically important toxigenic species of fungi belong to this genus. Four species are responsible for the production of mycotoxin with larger incidence: *A. flavus* and *A. parasiticus* that synthesize aflatoxins, and *A. ochraceous* and *A. carbonarius* that produce ochratoxins (Moss 2002b). Aflatoxins include: aflatoxins B_1, B_2, G_1 and G_2. Moreover, aflatoxin M_1, the result of the hepatic biotransformation of aflatoxin B_1, may be present in the milk of dairy cows that eat feed contaminated with aflatoxin B_1. When cows consume aflatoxin B1, it can not only be toxic to the cow but also it appears in the milk within 24 hours. Generally, the levels of aflatoxin M_1 appearing in milk are 1 to 2 percent of the aflatoxin B_1 content of the feed. Research data indicate aflatoxins will clear the system of dairy cows within 48 to 96 hours after the contaminated feed is removed from the ration (Waldner and Lalman 1998). Aflatoxin contamination is predominant in maize and tropical feeds such as oilseed by-products derived from groundnuts, cottonseed, and peanut (D'Mello 2002).

Ochratoxins are known to be produced by *Penicillium verrucosum* and species of the *Aspergillus ochraceus* group (Moss 2002b). However it has been recently reported that black *Aspergilli*: *A. carbonarius*, *A. japonicus*, as well as other species that belong to the *A. niger* species complex (Samson 2000) also produce these toxins. Ochratoxins A and B are present as natural contaminators mainly in cereal seeds and in tissues of animals fed with contaminated forage (Heenan *et al*. 1998).

Members of the genus *Penicillium* generally grow and can produce mycotoxins over a wider range of temperatures than those of the genus *Aspergillus* (Ominski *et al*. 1994; Moss 2002b). The *Penicillium* spp. are more abundant in temperate climates. Members of this genus are more commonly associated with storage than with preharvest contamination of grain (CAST 2003).

Fusarium is a large complex genus with species adapted to a wide range of habitats. They are worldwide in distribution and many are important plant pathogens. However, many species are soil borne and exist as saprophytes important in breaking down plant residues. A few species are significant mycotoxin producers and some of them are present preharvest in contaminated grains as well as in other plants. Toxigenic *Fusarium* include: *F. graminearum*, *F. culmorum*, *F. sporotrichioides*, *F. poae*, *F. verticillioides*, *F. proliferatum* (Moss 2002c). These species produce a wide range of mycotoxins. Trichothecenes, fumonisins and zearalenone, are relevant to human and animal health. Trichothecenes are subdivided into 4 basic groups: the most important are groups A and B. Group A trichothecenes include toxins T-2 and HT-2, neosolaniol and diacetoxyscirpenol. Group B trichothecenes include: deoxynivalenol, (also known as vomitoxin or DON), nivalenol and fusarenon-X. Some *Fusarium* produce zearalenone together with some trichothecenes. Fumonisins are synthesized by a particular *Fusarium* group (*F. verticillioides*, *F. proliferatum*). Three related compounds are generally present in maize: fumonisins B_1, B_2 and B_3 (Moss 2002c).

Mycotoxins that are most frequently found in forages are: aflatoxins, zearalenone, ochratoxin, fumonisins, T-2 toxin and deoxynivalenol (Akande *et al*. 2006). Contamination with aflatoxins in cattle feed has been mainly registered in seeds stored in warm climates (Hell *et al*. 2000; da Silva *et al*. 2000; Whitlow 2005). However, most published articles on forage mycotoxin contamination come from mild-cold regions where the use of silages is indispensable to reinforce pastures. In these regions, *Fusarium* mycotoxins prevail. Among them, DON, a *Fusarium graminearum* mycotoxin, is the most commonly reported. The co-occurrence of various mycotoxins (aflatoxin and some *Fusarium* mycotoxins such as DON, T-2 toxin, zearalenone or/and fumonisins) has been also registered in feeds (Dairy Business Communications 2004; Amigot *et al*. 2005, 2006).

The biological effects of mycotoxins depend on the ingested amounts, number of occurring toxins, duration of exposure to mycotoxins and animal sensitivity (D'Mello 2002; Yiannikouris and Jouany 2002a, 2000b). Taking these factors into account, health problems may range from mild digestive disturbances, decrease in feed intake, weight loss, reduced milk production, minor fertility problems and a decrease in natural defenses – generally related to the lack of response to diet change and therapies, to serious damage (even cancer) to the liver, kidney and abortions (Scudamore and Livesey 1998; Moss 2002a; Amigot *et al*.2006). Mycotoxin effects are cumulative over a period of time (di Costanzo *et al*. 1995). Chronic effects on human and animal health are more often noted than acute ones. Often animals do not die or show acute signs early in a mycotoxicity. It may take several days to several weeks to cause market changes in performance or acute symptoms (Adams *et al*. 1993; Bhat and Vasanthi 2003). The presence of more than one mycotoxin may increase their effects. The co-occurrence of several mycotoxins, even in low concentrations (lower than the stipulated limits in the countries with regulations) is of great importance (Rankin and Grau 2002). Due to the possibility of addition, synergism or potentiation, the effect of the mixtures cannot be predicted solely on the basis of the effect of the individual toxins (Yiannikouris and Jouany 2002a). A review of the literature on mycotoxin interactions indicates that additive or less than additive effects were the predominant interactions observed. Synergistic interactions are the least frequent (CAST 2003).

Because of mycotoxin presence in commonly ensiled forages and their potential for affecting dairy cattle production and health, mycotoxin analysis should be part of the routine evaluation of silages (Díaz 2006).

Toxic – fungal analysis

As mentioned before, the quality of a forage is currently evaluated only through chemico-fermentative parameters, among them pH and % NH_3/TN (McDonald *et al*. 1991). Although these parameters are sufficient to evaluate forage nutritional quality and its potential bacteriologic contamination, they fail to predict the presence of fungi and/or mycotoxins.

High fungi concentration records, identification of species pathogenic to humans and animals and/or potentially toxigenic and the identification of mycotoxins in forages destined for animal feed indicate that many times the technology applied during the development of crops, their harvest and the preparation and conservation of forages should be improved. Moreover, this information acts as a warning on the need to evaluate, not only chemico-fermentative parameters but also toxic fungal parameters to determine the acceptability of a forage. Recent studies suggest that a count of fungal propagules, some fungi species in particular (such as *Aspergillus fumigatus*) and some mycotoxins (deoxynivalenol, aflatoxins or both), should be included as decisive parameters to evaluate forage quality and, therefore, its acceptability (Amigot *et al*. 2003, 2005, 2006; Diaz 2006; Gaggiotti *et al*. 2007).

Fungal propagule counts – Identification of isolates

Both yeasts and filamentous fungi can contaminate forages exposing humans and animals to different diseases (Gotlieb 2002). It has been determined that fungal concentrations higher than 10^6 CFU/g in a forage may be the reason for these problems. The following interpretation levels for mold counts in feed have been proposed: $\leq 10^3$ CFU/g - Relatively Safe, 10^3–10^5 – Transition Zone, 10^5–10^6 – Caution Advised, $> 10^6$ – Recommended not to feed (Taysom 2002). Therefore, fungal propagule count should be considered a toxic-fungal parameter to determine forage quality. Although counts of fungi on feed are essential, qualitative investigations that provide additional information about the kind of fungi (harmless or dangerous) contribute to know the product's mycoflora.

Even though mold counts may be low, identification of the isolated mold is highly recommended. These data are indicative of the potential toxicity and pathogenity of forages and they become very important if producers do not pay special attention to proper handling and storage of feed (Whitlow and Hagler 2002). In many studies, it has been reported that potentially toxigenic species represent the larger percentage of the isolated fungi (Amigot *et al*. 2006).

Apart from finding potentially toxic fungi, it is important to evaluate the presence of other species such as *Aspergillus fumigatus* (important human and animal pathogen) which is considered as the pathogenic agent associated with mycotic hemorrhagic bowel syndrome (HBS) in dairy cattle mainly in immunosuppressed animals (Puntenney *et al*. 2003; Tekaia and Latgé 2005). *A. fumigatus* has been found both from dehydrated and fermented forages (Whitlow and Hagler 2002). It produces gliotoxin, a mycotoxin that can suppress immunity, therefore increasing the infectivity of the fungus (Melo dos Santos and Dorner 2002; Whitlow 2005). It has often been associated with forage putrefaction and heating (Scudamore and Livesey 1998). As a result, *A. fumigatus* has been proposed as another forage quality indicator (Amigot *et al*. 2006).

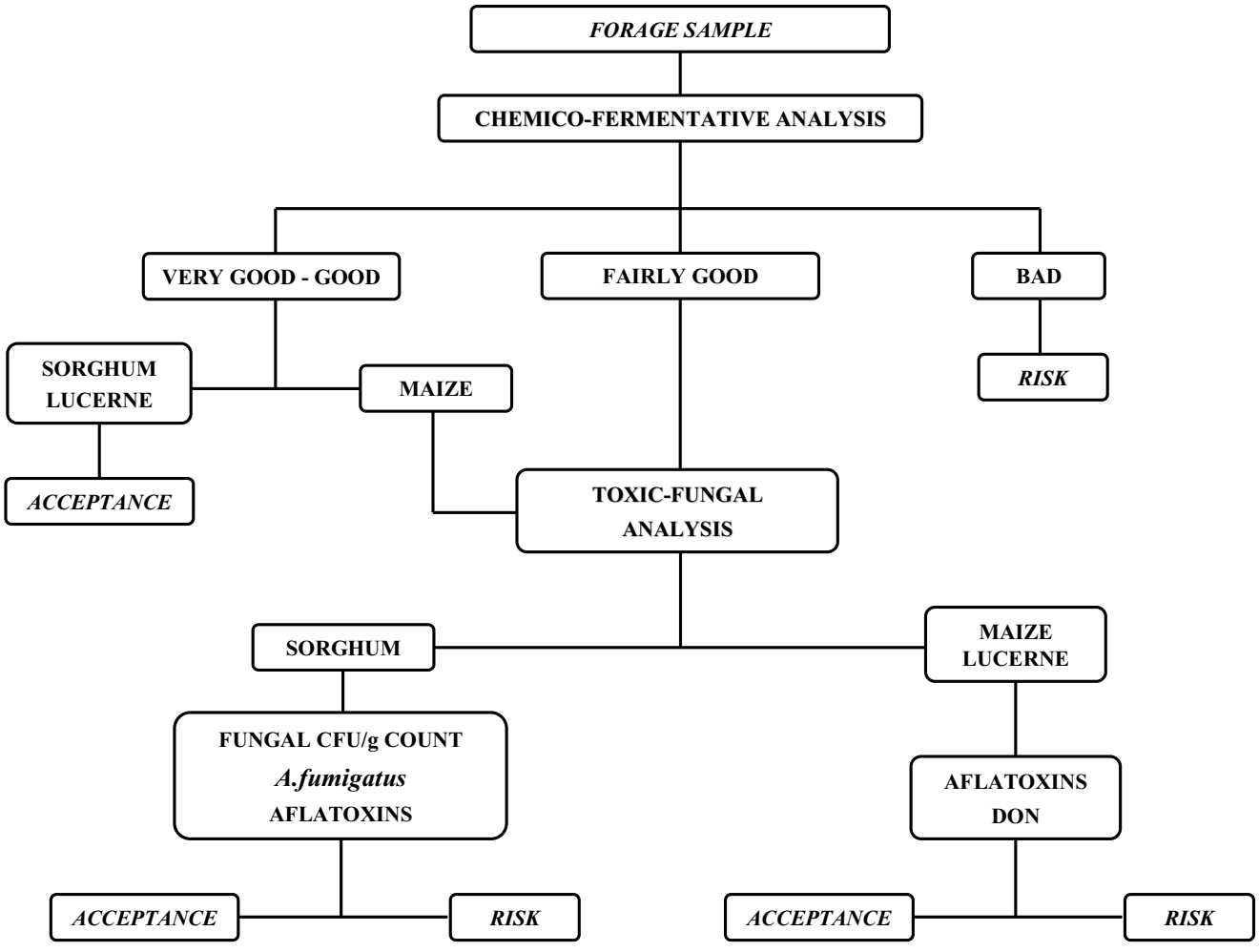

Fig. 1 Protocol to be followed for a quick and safe evaluation of a forage sample.

Mycotoxins

Mycotoxin analysis can be used as an indicator of management problems, and thereby, it may be a useful diagnostic tool (Thomas *et al*. 1998). The isolation of potentially toxigenic fungi cannot be considered a *per se* indicator of forage contamination with those mycotoxins (Magan *et al*. 2003) and, therefore, some toxin analyses must be carried out. Amigot *et al*. (2006) have suggested that the quality of maize, sorghum and lucerne forages should be evaluated as follows: those forages with bad chemical quality would be considered risky, since they do not provide adequate nutritional value for cattle feed regardless of their toxic-fungal evaluation. Those forages with a Very Good, Good, or Fairly Good chemical quality, would need, as a complimentary tool, microbiological assessment, such as fungal propagule counts and the presence of *A. fumigatus* and mycotoxins (aflatoxins and deoxynivalenol). The feed which does not present any altered toxic-fungal parameters would be considered acceptable. If the forage presents some altered toxic-fungal parameters, it would be considered risky (Amigot *et al*. 2006).

However, in order to reduce costs and time while maintaining the integrity of the results, several research studies were driven to determine if one or more of the studied variables (or a combination of them) could be taken as markers of storage quality, without having to evaluate the rest of the variables. Thus, DON has been employed in mild-cold climate regions as a marker of feed exposed to favourable conditions for fungal growth and production of other mycotoxins (Whitlow and Hagler 1998; Seglar 2004). It has been proposed that a positive DON analysis suggests the possible presence of other mycotoxins more toxic than DON itself. However, other research determined that the forage samples which did not contain DON, contained aflatoxins (Gaggiotti *et al*. 2003, 2007). Moreover, studies carried out recently established that for lucerne and maize forages, aflatoxin and deoxynivalenol determination provides enough information to be a marker of feedstuff final evaluation (Amigot *et al*. 2006). However, for sorghum forages, it could be concluded that it is necessary to determine fungal CFU/g counts, the presence of *Aspergillus fumigatus*, and the aflatoxin concentration to evaluate silage quality (**Fig. 1**). **Fig. 1** summarizes the protocol that should be followed to evaluate the quality of maize, sorghum and lucerne forages, using the stated parameters (Amigot *et al*. 2006).

It is important to develop other protocols that allow quick, economic, simple, safe decision-making as regards the acceptability of other forages for their use as animal feed.

For animal production to be both efficient and profitable all the tools and information available should be used. The production of high quality forage is one of the most significant management tools to increase animal performance, reduce feed costs and allow for time/money investment to pay off (Schroeder 2004b, 2004d).

Forage quality is highly variable among and within forage types for nutrient composition as well as digestibility. Routine and accurate forage testing is critical to the success of dairy cattle feeding programs (Allshouse *et al*. 1998; Shaver 2001).

The reports presented in this review also illustrate the importance of monitoring forages in order to have an accurate diagnosis of their quality. The rejection of a forage is related to extreme climatic conditions, inadequate storage practices, low nutritional value, and faulty feeding conditions (Whitlow and Hagler 1998). That is why finding variables that indicate food quality is a very useful tool to perform a rapid evaluation of a production chain (Rayburn 2002).

Both dairy and beef producers as well as forage manufactures should clearly understand how important forage quality analysis is. Dairy-beef producers must know the nutritional content of forages so as to develop the best feed strategy available for them. Forage manufacturers must get acquainted with quality analyses to produce forages dairy-beef producers would be willing to pay for (Stokes and Prostko 1998).

ACKNOWLEDGEMENTS

We would like to address special thanks to Rocío Gómez and Cecilia Martínez, English <> Spanish translators, for their help in the achievement of this work.

REFERENCES

Adams R, Kephart K, Ishler V, Hutchnson L, Roth G (1993) Mold and mycotoxin problems in livestock feeding. Available online: http://www.das.psu.edu/dairynutrition/documents/mold.pdf.

Akande KE, Abubakar MM, Adegbola TA, Bogoro SE (2006) Nutritional and health implications of mycotoxins in animal feeds: a review. *Pakistan Journal of Nutrition* **5 (5)**, 398-403

Allshouse R, Majewski C, Sniffen C (1998) Investigations in forage quality. Available online: http://research.cas.psu.edu/projects/PEN03965.pdf

Amigot SL, Basílico JC, Bottai H, Basílico MLZ, Fulgueira CL (2005) Estrategias para mejorar la calidad de forrajes. In: *Proceedings of the Xth Argentine Congress of Micology*, Buenos Aires, Argentina, 125 pp

Amigot SL, Caffaratti S, Bottai H, Basílico MLZ, Fulgueira CL, Basílico JC (2003) Búsqueda de nuevos parámetros para evaluar la calidad de forrajes**.** In: *Proceedings of the IInd Argentine Congress of Food Microbiology*, Santa Fe, Argentina, 69 pp

Amigot SL, Fulgueira CL, Basílico JC (2006) New parameters to evaluate forage quality. *Postharvest Biology and Technology* **41**, 215-224

Association Official of Analytical Chemistry (AOAC) (1995) *Protein (crude) in animal feed: Combustion method. Official Methods of Analysis* (Vol 1, 16th Edn), AOAC International, Arlington, Virginia, USA, 30 pp

Bagg J (2004) Determining corn silage digestibility. Available online: http://www.omafra.gov.on.ca/english/crops/field/forages/digestibility_cornsilage.htm

Beever DE, Mould FL (2000) Forage evaluation for efficient ruminant livestock production. In: Given DI (Ed) *Forage Evaluation in Ruminant Nutrition*, CABI Publishing, Wallingford, UK, pp 15-42

BeltzerMP (2003) Estudios de *Byssochlamys nivea* en ensilados de alfalfa y potencialidad de los mismos para la producción de patulina en microsilos experimentales. MSc Thesis Universidad Nacional del Litoral, Argentina, 222 pp

Bhat RV, Vasanthi S (2003) Food safety in food security and food trade. Available online: http://www.ifpri.org/2020/focus/focus10.htm

Bolsen KK (1998) Forage facts. Improving silage quality. Available online: http://www.oznet.k-estate.edu/pr_forage/pubs/97notebook/fora06/pdf

Broderick GA (1995) Desirable characteristic of forage legumes for improving protein utilization in ruminants. *Journal of Animal Sciences* **73**, 2760-2773

Bruno OA, León RJ, Romero LA, Quaino RO (1998) Varieties evaluation of lucerne (*Medicago sativa*) with different winter dormancy class. In: Proceedings of *the XVI International Congress*, Nice, France, pp 463-464

Cherney DJR (2000) Characterization of forages by chemical analysis. In: Given DI (Ed), *Forage Evaluation in Ruminant Nutrition*, CABI Publishing, Wallingford, UK, pp 281-300

Cherney JH (2000) Forage quality in perspective. Available online: http://www.forages.psu.edu/agfacts/agfact30.pdf

Colombatto D (2000) Análisis de alimentos. Aplicaciones prácticas. Available online: http://www.agro.uba.ar/catedras/p_lechera/resumencolombatto.pdf

Council for Agricultural Science and Technology (CAST) (2003) Mycotoxins: Risks in Plant, Animal, and Human Systems Ames, Iowa, USA, 199 pp

Cowan T (2001) Uso de forrajes ensilados en sistemas de producción animal en gran escala. Available online: http://www.fao.org/docrep/005/X8486S/x8486s05.htm

Cozzolino D, Fassio A, Fernández E (2003) Use of near infrared reflectance spectroscopy to analyze corn silage quality. *Agricultura Técnica* **63 (4)**, 387-393

D'Mello JPF (2002) Microbiology of Animal Feeds. Available online: http://www.fao.org/DOCREP/ARTICLE/AGRIPPA/556_EN.HTM

da Silva JB, Pozzi CR, Mallozzi MAB, Ortega EM, Corrêa B (2000) Mycoflora and occurrence of aflatoxin B1 and fumonisin B1 during storage of Brazilian sorghum. *Journal of Agriculture and Food Chemistry* **48 (9)**, 4352-4356

Dairy Business Communications A Multi Ag Media Company (2004) Mycotoxins hurt production and health. Available online: http://www.dairybusinesscom/western/TWDFeatures.htm

Deaville ER, Flinn PC (2000) Near infrared (NIR) spectroscopy: an alternative approach for the estimation of forage quality and voluntary intake. In: Givens DI, Owen E, Axford RFE, Omedi HM (Eds) *Forage Evaluation in Ruminant Nutrition*, CABI Publishing, Wallingford, UK, pp 301-320

di Costanzo A, Johnston L, Windels H, Murphy M (1995) A review of the effects of molds and mycotoxins in rumiants. *Professional Animal Scientist* **12**, 138-150

Díaz D (2006) Mycotoxin contamination in silages. *Western Dairy News* **6** (10), W175-W176

Driehuis F, Oude Elferink SJWH (2000) The impact of the quality of silage on animal health and food safety: a review. *The Veterinary Quarterly* **22** (4), 212-217

Driehuis F, Oude Elferink SJWH, Spoelstra SF (1999) Anaerobic lactic acid degradation in maize silage inoculated with *Lactobacillus buchneri* inhibits yeast growth and improves aerobic stability. *Journal of Applied Microbiology* **87**, 583-594

Driehuis F, van Wikselaar PG (1996) Effects of addition of formic, acetic or propionic acid to maize silage and low dry matter grass silage on the microbial flora and aerobic stability. Available online: www.fao.org/docrep/005/x8486s/x8486s04.htm

Fahey GC (1994) *Forage Quality, Evaluation and Utilization*, American Society of Agronomy: Crop Science Society of America: Soil Science Society of America, Madison, Wiscosin, USA, 998 pp

Faithful NT (2002) *Methods in Agricultural Chemical Analysis: A Practical Handbook*, CAB International, Wallingford, UK, 304 pp

Ferret A (2003) Control de calidad de forrajes. Available online: http://www.etsia.upm.es/fedna/capitulos/03CAP_VII.pdf

Frey TJ, Coors JG, Shaver RD, Lauer JG, Eilert DT, Flannery PJ (2004) Selection for silage quality in the Wisconsin quality synthetic and related maize populations. *Crop Science* **44**, 1200-1208

Gaggiotti MC, Basílico JC, Romero LA, de Basílico MLZ (2003) Eficacia del uso de vomitoxina como indicadora de la presencia de otras micotoxinas en silajes. In: *Proceedings of the IInd Argentine Congress of Food Microbiology*, Santa Fe, Argentina, 63 pp

Gaggiotti MC, Romero LA, Quaino OA, Basílico JC, de Basílico MLZ (2007) Eficacia del uso de vomitoxina como indicadora de la presencia de otras micotoxinas en silajes. Available online: http://www.engormix.com/s_sarticles_view.asp?art=1363AREA=AGR-089

Gotlieb AR (2002) Mycotoxins in silage: a silent loss in profits. Available online: http://www.insurance-portal.com/051502fcsmold.pdf

Haigh PM (1995a) A note on relationship between oven and toluene determined dry matter concentrations in maize silages. *Irish Journal of Agricultural and Food Research* **34**, 63

Haigh PM (1995b) A note on relationship between oven and toluene determined dry matter concentrations in bunker-made grass silages. *Irish Journal of Agricultural and Food Research* **34**, 143

Heenan CN, Shaw KJ, Pitt JI (1998) Ochratoxin A production by *Aspergillus carbonarius* and *Aspergillus niger* isolates and detection using coconut cream agar. *Journal of Food Microbiology* **1**, 67-72

Hell K, Cardwell KF, Setamou M, Poehling HM (2000) The influence of storage practices on aflatoxin contamination in maize in four agroecological zones of Benin, west Africa. *Journal of Stored Product Research* **36**, 365-382

Jahn BE, Vidal VA, Soto OP (2000) Sistema de producción de leche basado en alfalfa (*Medicago sativa*) y maíz (*Zea mays*) para la zona Centro Sur. III Consumo y calidad del forraje. *Agricultura Técnica* **60 (2)**, 99-111

Lanyasunya TP, Wamae LW, Musa HH, Olowofeso O, Lokwaleput IK (2005) The risk of mycotoxins contamination of dairy feed and milk on smallholder dairy farms in Kenya. *Pakistan Journal of Nutritions* **4 (3)**, 162-169

Lascano CE (2002) Caracterización de las pasturas para maximizar producción animal. *Archivos Latinoamericanos de Producción Animal* **10 (2)**, 126-132

Linn J, Martin N (1999) Forage quality tests and interpretations. Available online: http://www.extension.umn.edu/distribution/livestocksystem/DI2637.html

Macaulay A (2003) Evaluating silage quality silage. Available online: http://wwwl.agric.gov.ab.ca/$department/deptdocs.nsf/all/for4909.html

Magan N, Hope R, Cairns V, Aldred D (2003) Post-harvest fungal ecology: impact of fungal growth and mycotoxin accumulation in stored grain. *European Journal of Plant Pathology* **109**, 723-730

McDonald P, Henderson AR, Heron SJE (1991) *The Biochemistry of Silage* (2nd Edn), Chalcombe Publications, Marlow, UK, 340 pp

Melo dos Santos V, Dorner JW (2002) Isolation and toxigenicity of *Aspergillus fumigatus* from moldy silage. Available online: http://www.nal.usda.gov/ttic/tektran/data/000012/72/0000127285.html

Moss MO (2002a) Risk assessment for aflatoxins in foodstuffs. *International Biodeterioration and Biodegradation* **50**, 137-142

Moss MO (2002b) Mycotoxin review 1: *Aspergillus* and *Penicillium*. *Mycologist* **16 (3)**, 116-119

Moss MO (2002c) Mycotoxin review 2: *Fusarium*. *Mycologist* **16 (4)**, 158-161

Muck RE, Holmes BJ (2006) Bag silo densities and losses. *Transactions of the American Society of Agricultural and Biological Engineers* **49 (5)**, 1277-1284

O'Kiely P, Turley T, Rogers PAM (1999) Exposure of calves to nitrogen dioxide in silage gas. *The Veterinary Record* **144**, 352-353

Oude Elferink S, Driehuis F, Gottschal JC, Spoelstra S (1999a) Silage fermentation processes and their manipulation. FAO Electronic Conference on Tropical Silage. Available online: http://www.fao.org/doscrep/05/x8486e/8486e08.htm

Oude Elferink S, Driehuis F, Gottschal JC, Spoelstra S (1999b) Controlling silage fermentation. Available online: http://library.wur.nl/WebQuery/wurpubs/310031

Oude Elferink S, Driehuis F, Gottschal JC, Spoelstra SF (2002) Manipulating silage fermentation. Available online: http://library.wur.nl/WebQuery/wurpubs/317834

Puntenney SB, Wang Y, Forsberg NE (2003) Mycotic infections in livestock: recent insights and studies on etiology, diagnostics and prevention of Hemorrhagic Bowel Syndrome. In: *Southwest Nutrition & Management Conference*, Pheonix, University of Arizona, Department of Animal Science, Tuscon, United States of America, pp 49-63

Putnam D (2004) Forage quality testing and markets; where are we going? In: Proceedings of the *National Alfalfa Symposium 2004*, San Diego, USA. Available online: http://alfalfa.ucdavis.edu/symposium/2004/proceedings/Putnam_ForageQualitySYMPOSIUM04FINAL.pdf

Randby AT, Selmer-Olsen I, Baevre L (1999) Effect of ethanol in feed on milk flavour and chemical composition. *Journal of Dairy Science* **82**, 420-428

Rankin M, Grau C (2002) Agronomic considerations for molds and mycotoxins in corn silage. *Focus on Forage* **4 (1)**, 1-4

Rayburn E (2002) Use forage test to diagnose management problems. Available online: http://www.wvu.edu/~agexten/forglvst/foragtst.htm

Reboux G, Reiman M, Roussel S, Taattola K, Millon L, Dalphin JC, Piarroux R (2006) Impact of agricultural practices on microbiology of hay, silage and flour on finnish and French farms. *Annals of Agricultural and Environmental Medicine* **13**, 267-273

Redfearm D, Zhang H, Caddel J (2004) Forage quality interpretations. Oklahoma Cooperative Extension service. Available online: http://www.osuextra.com

Reeves III JB, McCarty GW, Mimmo TV (2002) The potential of diffuse reflectance spectroscopy for the determination of carbon inventories in soils. *Journal of Environmental Pollution* **116** (1001), S277-S284

Reeves III JB, van Kessel JS (2000) Near-infrared spectroscopic determination of carbon, total nitrogen, and ammonium-N in dairy manures. *Journal of Dairy Science* **83**, 1829-1836

Romero LA, Aronna MS, Comerón EA (2003) Evaluación de silaje de sorgo forrajero de nervadura marrón para la producción de leche. *Revista Argentina de Producción Animal* **23(Suppl. 1)**, 10-12

Romero LA, Mattera J, Comerón EA, Gaggiotti, MC, Cuatrin A (2006) Evaluación de silajes de planta entera de sorgo granífero con distintos contenidos de tanino. *Revista Argentina de Producción Animal* **26 (Suppl. 1)**, 169-170

Samson RA (2000) Mycology and mycotoxins. Current Fungal Taxonomy and Mycotoxins. In: de Koe WJ, Samson RA, van Egmond HP, Gilbert J, Sabino M (Eds) *Mycotoxins and Phycotoxins in Perspective at the Turn of the Millennium*, Proceedings of the *Xth International IUPAC Symposium on Mycotoxins and Phycotoxins* Guarujá, Brazil, pp 340-349

Schleifer KH, Ludwig W (1995) Phylogenetic relationships of lactic acid bacteria. In: Wood BJB, Holzapfel WH (Eds) *The Genera of Lactic Acid Bacteria*, Blackie Academic and Professional, London, UK, pp 7-18

Schroeder JW (2004a) Forage nutrition for ruminants AS-1250. Available online: http://www.ag.ndsu.edu/pubs/ansci/dairy/as1250w.htm

Schroeder JW (2004b) Interpreting composition and determining market value AS-1251. Available online: http://www.ag.ndsu.edu/pubs/ansci/dairy/as1251w.htm

Schroeder JW (2004c) Haylage and other fermented forages AS-1252. Available online: http://www.ag.ndsu.edu/pubs/ansci/range/as1252.pdf

Schroeder JW (2004d) Corn silage management AS-1253. Available online: http://www.ag.ndsu.edu/pubs/ansci/dairy/as1253w.htm

Schroeder JW (2004e) Silage fermentation and preservation AS-1254. Available online: http://www.ag.ndsu.edu/pubs/ansci/dairy/as1254w.htm

Schroeder JW (2004f) Search Storage, Sampling and Measuring. AS-1255. Available online: http://www.ag.ndsu.edu/pubs/ansci/dairy/as1255w.htm

Schroeder JW (2004g) Search Stressed-Damaged Crops. AS-1256. Available online: http://www.ag.ndsu.edu/pubs/ansci/dairy/as1256w.htm

Scudamore KA, Livesey CT (1998) Occurrence and significance of mycotoxins in forage crops and silage: a review. *Journal of the Science of Food and Agriculture* **77**, 1-17

Seglar B (2003a) Mycotoxin effects on dairy cattle. Available online: http://www.uwex.edu/ces/forage/wfc/proceedings2001/dairy mycotoxin.htm

Seglar B (2003b) Fermentation analysis and silage quality testing. Available online: http://www.cvm.umn.edu/img/assets/9090/fermentation%20seglar%202003.pdf

Seglar B (2004) Efectos de micotoxinas en ganado lechero. Available online: http://www.asaga.org.ar/magazine/n53/default.asp?reescan=true

Shaver RD (2001) Forage quality variation. Available online: http://www.wisc.edu/dysci/uwex/nutritn/pubs/texasnutrconf404webshare.pdf

Stokes S, Prostko E (1998) Understanding forage quality analysis. Available online: http://animalscience.tamu.edu/main/academics/dairy/L5198_understandingforagequality.pdf

Taysom D (2002) U.S. Dairy Forage Research Center: Managing Forage Resources. Available online: http://www.ars.usda.gov/Research/docs.htm?docid = 11065

Tekaia F, Latgé JP (2005) *Aspergillus fumigatus*: saprophyte or pathogen? *Current Opinion in Microbiology* **8**, 385-392

Teplitski M (2006) *E. coli* and *Salmonella* on animal farms: sources, survival and management. University of Florida, Institute of Food and Agricultural Sciences Extension. EDIS publication SL-239. Available online: http://www.edis.ifas.ufl.edu/SS458

Thomas ED, Sniffen CJ, Gotlieb AR (1998) Forage quality parameters vs. Mycotoxin content of silages at Miner Institute, Chazy, New York. Available online: http://whminer.serverbox.net/research/reports/

Twidwell E, Wegenhoft K (1999) Forage quality and its value. Available online: http://www.agecon.lsu.edu/Extension_Pubs/Forage%20Quality%20and%20Its%20Value.pdf

Undersander D, Moore JE (2002) Relative forage quality. Available online: http://www.uwex.edu/ces/crops/uwforage/RFQvsRFV.htm

van Os M (1997) Role of ammonia and biogenic amines in intake of grass silage by ruminants. Available online: http://library.wur.nl/wda/abstracts/ab2209.html

van Soest PJ (1994) *Nutritional Ecology of the Ruminants* (2nd Edn), Cornell University Press, Ithaca, USA, pp 212-214

Waldner D, Lalman D (1998) Drought brings of aflatoxin contamination of livestock feeds to Oklahoma dairy and beef producers. Available online: http://www.okstate.edu/OSU_Ag/oces/timely/aflatoxi.htm

Ward RT (2005a) Evaluating forage and feed quality – What analyses are the best indicators of quality? In: Proceedings of *Feed and Nutritional Management Cow College*, Virginia, USA. Available online: http://www.dasc.vt.edu/extension

Ward RT (2005*b*) NDF digestibility and its use in prdicting forage quality In: *Proceedings* Proceedings of *Feed and Nutritional Management Cow College*, Virginia, USA. Available online: http://www.dasc.vt.edu/extension

Weinberg ZG, Muck RE (1996) New trends and opportunities in the development and use of inoculants for silage. *FEMS Microbiology* **19**, 53-68

Whitlow L (2005) Mycotoxins In: Proceedings of *Feed and Nutritional Management Cow College*, Virginia, USA. Available online: http://www.dasc.vt.edu/extension

Whitlow LW, Hagler WE (1998) Effects of mycotoxins on the animal: The producer's perspective. In: *Silage: Field to Feedbunk*. NRAES-99. NRAES, Ithaca, New York, USA, pp 222-223

Whitlow LW, Hagler WE (2000) Mycotoxin contamination of feedstuffs – An additional stress factor for dairy cattle. Available online: http://www.cals.ncsv.edu/an_sci/extension/dairy/mycoto~1.pdf

Whitlow LW, Hagler WE (2002) Mycotoxins in feeds. *Feedstuffs* **74 (28)**, 1-10

Winfield MD, Groisman EA (2003) Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*. *Applied and Environmental Microbiology* **69**, 3687-3694

Yiannikouris A, Jouany JP (2002a) Mycotoxins in feeds and their fate in animals: a review. *Animal Research* **51**, 81-99

Yiannikouris A, Jouany JP (2002b) Les mycotoxines dans les aliments des ruminants, leur devenir et leurs effets chez l'animal. *INRA Productions Animales* **15 (1)**, 3-16