Survival of seeds after digestion by cattle

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Introduction

Restoration ecology deals with nature management practices aiming to re-establish plant species, which have disappeared. There are two possibilities for the re-establishment of species; first by extension from plant species already present by means of vegetative structures, and, second by the establishment of new plants from seed (Bakker *et al.* 1996). Seeds can be present in the persistent soil seed bank as a 'memory' of the original plant community (Thompson *et al.* 1997). If the species has been lost from the persistent soil seed bank, it has to be transported to the site by some vector, e.g. wind, water, animals, man, and assimilate into the fresh seed bank. Without the presence or arrival of seeds no re-appearance in the present vegetation will be possible.

Herbivore mammals like cattle or sheep can affect the plant communities of grasslands in three major ways: removal of biomass (defoliation), trampling and dung deposition (Bakker 1989, Dai 1998). Grazers are known for a long time to be potential dispersers of seed over considerable distances (Ridley 1930, Dore & Raymond 1942, Bonn & Poschlod 1998). Cattle ingest many seeds when grazing pastures and consuming hay, or grain products (Dore & Raymond 1942, Blackshaw & Rode 1991). Seeds of numerous plant species ingested by livestock are known to survive passage through the digestive tract and germinate or even have a successful establishment in dung (Dore & Raymond 1942, Burton & Andrews 1948, Welch 1985). The large depositions of cattle may kill vegetation so providing a niche for opportunist species.

Survival of seeds during passage through cattle has been the subject of considerable experimentation's and observations. Observations have been made of the germination and establishment of seedlings on cattle dung in real life (Welch 1985). Feeding experiments have been carried out to examine the effect of digestion by measuring the passage rate and the survival of seeds after passage through the digestive tract of cattle (Janzen 1981, Simao Neto *et al.* 1987, Gardener *et al.* 1993a). Also laboratory tests were done to give a prediction of which seed could survive the exposure to digestive processes. The seeds were subjected to the complete digestion process of ruminants (Simao Neto & Jones 1987, Gardener *et al.* 1993b, Ocumphaugh & Swakon 1993). These tests were appropriate to find out what the effect of various retention times is on the survival of seed.

It was supposed that the longer the seeds were subjected to digestion processes the lower the percentage of seed survived after defecation (Simao Neto & Jones 1987, Blackshaw & Rode 1991, Gardener et al. 1993b). The retention time and also the survival of seeds could possibly be influenced by the following factors; seed characteristics (e.g. specific gravity, size and seed coat) but also the diet in which the seeds are ingested (Ocumphaugh & Swakon 1993) and animal species or size can effect the degree of digestion.

During ingestion, rumination and digestion the seeds are subjected to a range of (damaging) processes (McDonalds 1988, Syllabus 1998-1999). These processes are most of mechanical kind during the first part and chemical during the second part. Seeds can be crushed or abraded during chewing. Once in the rumen the seeds will be attached by micro-organisms and assimilate in the fermentation process. During passage the seed coat plays an important role in protecting the embryo (Bewley & Black 1978). One can imagine that seeds with a soft (soon imbibed) or thin seed coat will be damaged more quickly than seeds with a hard thick seed coat. Seeds can be totally destroyed, germinate while they are in the digestive tract or the dormancy will be broken by scarification (Baskin & Baskin 1998). Most seeds with a hard thick seed coat could have seed coat dormancy. When dormancy is broken the germination of the defecated seeds will be higher than those that have not been ingested.

Simao Neto and Jones (1987) once said that the only way to know if a species could survive the passage through the digestive tract is to "feed it and see". This approach is however, very slow and expensive. The use of a laboratory test instead of feeding and recovering seeds would be less expensive and less time consuming.

In this essay an investigation will be made of which laboratory tests are known to simulate the passage of seeds through the digestive tract of cattle, and relate this to the survival of seeds in real life. The aim is to find out which test simulates the passage through the digestive tract in the best way so it can be used to screen large numbers of plant species for their potention to pass through the digestive system and remain viable. When we know the species potential for the surviving the passage through the digestive tract it would be useful to associate the difference in survival with particular seed characteristics.

In chapter 1, a general description is given about some specific characteristics of plant seed, which are important for understanding the survival of seeds after being subjected to the digestion of cattle. The digestion of ruminants is described in chapter 2. In the following two chapters an investigation of different studies with their results in survival of seeds during and after passage through cattle is made; the different artificial digestion test (Chapter 3) and the feeding tests (Chapter 4). Further in chapter 5 an overview is given from the different factors that can affect the survival of seeds. The survival of seed in "real life" will be given in chapter 6. In the final discussion and conclusion (Chapter 7) the different studies for the survival of seed will be evaluated and an attempt will be made to draw a conclusion on which test will simulate the survival of seed in real life am best.

1. Seeds

1.1 Internal and external structures of seeds

The independence of the next generation of plant begins with the seed. The seed contains a new plant in miniature. A seed is a mature ovule that usually stores food material. A fruit is a mature floral ovary, which may contain one or more seeds. In almost all cases the seed consist of the following components:

1) the testa
2) the perisperm
3) the endosperm
4) the embryo

The degree to which these various components evolve during development or even whether or not they are all retained leads to some fundamental structural differences among various types of seed.

The testa

The testa is generally a hard seed coat. The physiological importance arises from the presence of an outer and inner cuticle, often fatty or waxy, and one or more layers of thickened, protective cells (Leguminosae). These features confer upon the testa some degree of impermeability to water and/or gases, including oxygen. In some cases the testa may be mucilaginous (Cruciferae) and thereby play an important role in water retention. In many species (e.g. Compositae) the testa is lacking, this is because the outer coat is not the testa but the pericarp (Bewley & Black 1978a).

The perisperm and endosperm

The perisperm as well as the endosperm is an important tissue to store food reserves. The relative proportions of the endosperm and perisperm in the seed vary a lot between different plant species. The Gramineae include species with an endosperm that store relatively large amounts of reserves (Bewley & Black 1978a).

The embryo

The embryo is made up of one or more cotyledons, a plume (embryo bud), hypocothyl (stem portion), and a radicle (rudimentary root). The shape of embryos and their position within the seed varies greatly among species (Bewley & Black 1978a). In non-endospermic seeds the embryo is provided with an energy source, the embryo fills the entire seed in Rosaceae, Cruciferae, Fagaceae and Juglandaceae (Kozlowski & Gunn 1972).

Seeds have a number of common characteristics, but there are also a lot of structural, chemical and functional differences. First there are a great number of external variations like size, shape, colour and surface, which are important for seed identification. The appendages have always been of most interest. These appendages include wings, arils, pappus, awns, hooks or spines, tubercles, hairs and elaiosomes. Thompson (1993) summarized the morphological classes in a table. As already mentioned before the internal differences can also vary, e.g. the type, size and placement embryo, the food reserve quantity and quality. Also seed coat, cotyledons and endosperm belong to the internal variation. To the chemical variation belong the differences in percent ash, oil and protein. The last variation is a physiological one, which can differ among plants in sources of carbohydrates and other growth requirements for seed germination and early seedling development (Kozlowski &

Gunn 1972). Gunn (1972) summarized the seed characteristics of 37 families. Here a few examples are given of the external and internal seed coat characteristics for species commonly eaten by herbivores. Leguminosae have seeds with a smooth, rarely rough seed coat. The seed coat is apparently thick and hard. The seed coat in Compositae is thin or absent, when present it is smooth. In Juncaceae the seed coat is reticulate and roughened. The seed has two quite thin seed coats. The Gramineae produces a caryopsis, a one seeded fruit that is usually enclosed in a persistent lemma and palea, rarely naked. The caryopsis is elongated longitudinally and the caryopsis coat of both subfamilies is usually smooth and thin.

1.2 Germination

The seed is a package of energy. Water uptake is the initial step towards germination. Germination in seed plants may be defined as the sequential series of morphogenetic events that result in the transformation of an embryo into a seedling. The process of germination can be divided into the following series of events:

1) imbibition; the physical absorption of water
2) hydration and activation
3) cell division and cell extension
4) protrusion; the physical emergence of the embryo from the seed
5) completion of nonrepetitive morphogenesis; the establishment of the primary plant body (Berlyn 1972, Bewley & Black 1978a).

A major feature of postfertilization seed development is accumulation of nutrient reserves. The greater the supply of stored nutrients in the seed, the greater the vigour of the seedling and its potential for survival. Before the seedling can survive with energy obtained from photsynthetic processes it has to relay on stored nutrients in the seed. The Gramineae contributes more food than any other Family (Berlyn 1972).

1.3 Dispersion

A lot of external characteristics are adaptations that enhance dispersal (Ridley 1930, Fahn & Werker 1972, Stiles 1993, Wilson 1993). The main four types of dispersal are:

1) Zoochory (dispersal by animal)
2) Anemochory (dispersal by wind)
3) Autochory (dispersal by the plant itself)
4) Hydrochory (dispersal by water)

Anemochorous plants often have wings or plumes that increase air resistance and slow the rate of falling. Seeds that are dispersed by the plant itself can disperse their offspring by the explosive opening of the fruits. Special adaptations for hydrochory are less obvious.

The zoochorous plants can be divided into three groups. The first group is the endozoochorous plants of which the diaspores will be eaten by animals, but where the hard seeds or fruit stones pass through the digestive tract without damage, so it may viable after being released. Seeds of epizoochorous plants travel by means of hooks or sticky coatings that adhere to the fleece, coat or feathers of animals. Seeds with a high content of food or edible appendages can be eaten or collected by animals for making stores before the winter are categorized as the last group, the synzoochorous plants.

Animals are said to be important vectors for seed movement, either intern or extern. When seeds are dispersed intern the seeds have to be eaten. For an active internal dispersion

the diaspores must have the means to attract the appropriate animals. This can be achieved by different properties such as colour, odour, and abundance of storage material. The reserve materials of the diaspores are mainly carbohydrates, such as sugar, starch, lipids or proteins (Fahn & Werker 1972). Animals that ingest the seed as an incidental part of some other food they are eating, can transport some seeds. The best examples of this come from the seeds dispersed through the grazing activities of large herbivores, like cattle, sheep or horses (Janzen 1982). These seeds can contribute in a great amount of their necessary nutrients.

Most animal-dispersed seeds are passed through at a least a portion of the digestive tract. To pass unharmed through a digestive tract of an animal first of all the seed must be able to fit into the mouth and throat of the animal. Placement of seeds or fruits on a plant may restrict access to some animals. Internal treatment of seeds is influenced by the physical and chemical processing that takes place in the gut. This is additionally affected by the time the seed spends in the gut before being deposited by the animal. Most highly frugivorous species have short guts and seed passage is very rapid (Stiles 1993). Seed retention in animal guts for longer time may even induce germination while in the gut, and a death of the seed (while still in the gut) follows. Deposition of seeds with faecal material may provide a nitrogen source that increases nutrients for early seedling growth, although large numbers of seeds may be removed from the dung by seed predators (Janzen 1982).

1.4 Dormancy

There are several reasons why a seed, even though it is viable, does not germinate. The first simple reason is that the seed is dry. Another may be that environmental conditions are unsuitable for germination. A third reason is that a seed may be dormant. Embryos of most seeds have a resting stage between development and germination. Germination of viable seeds may be temporarily delayed because of seed dormancy. Embryos that continue to grow within the seed and fruit (lack a resting stage) may be categorised as viviparous. That a seed fails to germinate is caused by block(s) (inhibitors) to germinate (Bewley & Black 1982b, Murdoch & Ellis 1993). There are several external conditions to overcome the block(s), like temperature, oxygen or light. Two-thirds of the species of temperate-zone woody plants show some kind of dormancy (Kozlowski & Gunn 1972), the following classification of types of seed dormancy was described by Crocker in Villiers (1972):

1) immature embryo
2) impermeable seed coats to water
3) mechanical resistance of the seed coats to embryo growth
4) low permeability of the seed coats to gases
5) dormancy resulting from a metabolic block within the embryo self
6) a combination of the above
7) secondary dormancy

Summarizing it can be said that there are basically two types of dormancy. *Embryo dormancy*, where the control of dormancy stays within the embryo itself (1,3 and 5) or *coat-imposed dormancy*, in which dormancy is maintained by the seed coat (2 and 4) a combination of these two (6) is also possible. The first five types can be categorized as primary dormancy, secondary dormancy can be induced in seeds by maintaining them in unfavourable environmental conditions for a time (Baskin & Baskin 1998). Bewley and Black (1982b) gave an overview of the terminology used for primary and secondary dormancy by different authors.

Seed dormancy is sometimes disadvantageous and at other times a distinct advantage. Long postponement of seed germination often makes it difficult to establish plant stands. Seed dormancy prevents germination until favourable conditions are present. A seed dormancy phase is also helpful in natural reseeding of winter annual Legumes, which mature seed in the summer. If seeds germinate in summer the seedlings usually die in the summer heat. However, some seeds germinate in autumn so the seedlings will survive more often. The degree of dormancy varies greatly among species and even within a species (Kozlowski & Gunn 1972a). Seed of some plants may remain dormant in the soil for many years (Thompson 1992).

In the following something more will be said of coat-imposed dormancy because the seed coat has an important function in surviving the digestive tract in ruminants.

Coat-imposed dormancy

The structures responsible for imposing and maintaining dormancy vary from species to species. They include glumes, palea and lemma the pericarp, testa, perisperm and endosperm. The covering structures may prevent embryo germination because they interfere with water uptake and gaseous exchange, contain chemical inhibitors, act as a barrier against the escape of inhibitors from the embryo, modify the light reaching embryo or exert a mechanical restraint (Bewley & Black 1982b). The seed coat can prevent water uptake or the exchange of gases so that the embryo stays dormant because the seed coat restricts the entry of water or oxygen. Rupture of the testa can followed directly by swelling of the seed due to uptake of water and germination usually starts immediately. This is the most important cause of dormancy in most hard seeds of the Leguminosae (Villiers 1972). Many seeds contain germination inhibitors and promoters. Dormancy may be considered to be due to the presence of growth inhibitors, the absence of growth promoters or a combination of both. Both can occur within the embryo as well as in the seed coat. The most important inhibitor in both the embryo and the seed coat is abscisic acid and the important promoter is gibberellic acid. When inhibitors are present in the seed coat no germination will occur. When there are inhibitors in the embryo they have to be released before germination and in that case the seed coat can act as a barrier for escaping inhibitors. When none of these above named effects can explain the action of the coat imposing and maintaining dormancy, it can be concluded that the coat must act by exerting a mechanical restraint.

Dormancy breaking

Several artificial methods are known to soften the hard, impermeable seed coat of the hard-coated families like Leguminosae. The methods include treatment with concentrated sulphuric acid or ethanol, freezing, heating, radiation, percussion and pressure (Bewley & Black 1982b), mechanical scarification, after ripening and chilling or stratification (Thompson & Booth 1993). Possible ways to reach softening of the seeds in nature can be mechanical abrasion through the grinding actions of the teeth in the mouth of mammals or by the little stones in the gizzard of a bird. Chemical abrasion of the seeds can occur naturally during passage through the digestion tract of animals.

2. Digestion of ruminants

2.1 General

The group of animal species that is called the Ruminantia is a suborder of the mammalian. These animals engage in rumination, a process in which partially digested food is regurgitated for remastication after being fermented by micro-organisms in the first division of the stomach (Eckert 1988). The stomach of a ruminant is divided in four chambers, the rumen, the reticulum, the omasum and the abomasum (Fig. 1). The first three compartments are collectively known as the forestomach (proventriculus). The rumen and reticulum are so initially related in structure and function that it is described as a combined reticulo-rumen compartment (Syllabus, Dyce *et al.* 1996). The reticulo-rumen acts as fermentation vat that receives unchewed food and reduces the food by processes of microbial fermentation. Only the abomasum is comparable in structure and function to the simple stomach of most other species. The capacity of the stomach of adult cattle measures about 100 litres or more.

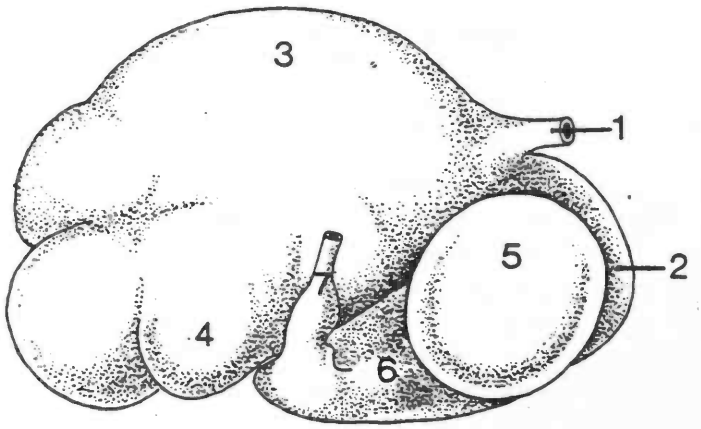

Figure 1. The stomach of a cow, shown from the right.
Legend: 1. Oesophagus; 2. Reticulum; 3. Dorsal sack of the Rumen; 4. Ventral sack of the Rumen; 5. Omasum; 6. Abomasum; 7. Duodenum (From: Syllabus).

The breakdown of food is partly by physical damage and partly by chemical means. During eating and again during rumination the food is diluted with saliva; typical quantities of saliva produced per day are 150 l in cattle and 10 l in sheep (McDonalds *et al.* 1988). Saliva of ruminants contains the enzyme α-amylase, which is responsible for the break down of starch. Phosphate and bicarbonate present in the saliva act as buffers. During rumination the diluted food is drawn back from the rumen to the mouth. Liquid is rapidly swallowed again but coarser material is thoroughly chewed before being returned to the rumen. The time spent by the animal in rumination depends on the fibre content of the food. In grazing cattle it is commonly about 8 hours per day. The food regurgitated is chewed 40-50 times and thus receives a much more thorough mastication than during eating (McDonalds *et al.* 1988). The material with a low specific gravity is most likely to be regurgitated for further mastication and insalivation. Food is mostly regurgitated for remastication for 3 to 6 times, until it is small enough to be pushed further in the digestive tract.

The chemical breakdown of food in the reticulo-rumen is brought up by enzymes secreted, not by the animal itself, but by micro-organisms. The reticulo-rumen provides a continuous culture of anaerobic bacteria and protozoa (and also some fungi). The rumen contents flora (bacteria 10^9-10^{10} per ml) and fauna (protozoa 10^6 per ml). Over 60 species of bacteria have been identified. Table 1 lists a number of the more important species and indicates the substrate they utilise. The products of the fermentation are all acids. The total

numbers of bacteria, and the relative population of individual species, vary with the animals' diet. During dilution of the food in the reticulo-rumen the mechanical strength of the fibres decreases. The gases (methane and carbon dioxide) produced during fermentation are lost by eructation and the volatile fatty acids are mainly absorbed through the rumen wall. The pH in the rumen is under normal conditions maintained at 5.5-6.5. Fermentation demands continuously mixing of the food to prevent accumulation of fermentation products, which can result in a lowering of the pH, and is deadly for the micro-organisms in the rumen. The temperature of rumen remains close to that of the animal (38-42^{0}C). A constant environment is important for the micro-organisms.

The abomasum is the compartment were the actual digestion of food takes place. The secretion of gastric juice and the digestive enzyme pepsin (secreted by the animal itself) occurs in the abomasum. Pepsin can break down proteins. Gastric juice consists of an isotone solution of hydrochloric acid and pepsin. The pH of pure gastric juice is 2.1, mixed gastric juice has a pH with a minimum of 3.5 (Syllabus). Through continues secretion of gastric juice the pH of the material excreted from the omasum is lowering.

The passage through the small intestine (duodenum) makes the digestion complete. The pH in the small testine is highered again till a pH of ± 7.6.

Table 1. Typical rumen bacteria and their energy source
(From: McDonalds et al. 1988).

Species	Energy source
Bacteriodes succinogenes	Cellulose
Ruminococcus flavefaciens	Cellulose
Ruminococcus albus	Cellobiose
Streptococcus bovis	Starch
Bacteriodes ruminicola	Glucose
Megasphaera eldenii	Lactate

* Cellobiose is a disaccharide split from cellulose

2.2 The diet

The diet of a ruminant contains considerable quantities of cellulose and hemicellulose, starch and water-soluble carbohydrates mostly in the form of fructants. This food consists mainly of undigestible cellulose or hemicellulose and pectin. Nutrient in the food are enclosed in cell walls with a high content of cellulose. The nutrient become available for digestion enzymes after the cell wall has been broken down. Cellulose is broken down by the enzyme cellulase, a product of symbiotic micro-organisms in the forestomach. The food also contains small amounts of digestible proteins, lipids and saccharides. The extent to which cellulose is digested in the rumen depends on the degree of lignification of the plant material. Lignin appears to prevent the breakdown of cellulose with which it is associated. The breakdown of cellulose and other resistant polysaccharides is undoubtedly the most important digestive process taking place in the rumen. Materials entering the rumen as large particles spend longer time in the rumen than small particles and soluble nutrients, because large particles must be broken down by ruminations and microbial attack before they can leave the rumen. Foods with highly lignified cell walls, such as straws, have long retention times (50-80 hours), whereas more readily digested foods, like immature pasture herbage have a short retention time (30-50 hours) (McDonalds 1988).

3. Laboratory digestion tests

3.1 The two-stage digestion method

During passage through the digestive tract seeds are subjected to a range of different digestive processes. Most of the damage of seeds takes place in the reticulo-rumen by the microbial activities of bacteria and protozoa (Huhtanen *et al.* 1953, Simao Neto & Jones 1987, Dyce *et al.* 1996). In laboratory tests the digestion processes during the passage through the digestive tract of cattle can be simulated. How this can be done can be read in the following chapter.

In 1953 already, a miniature artificial rumen was developed by Huhtanen *et al.* to know more about the factors affecting fibre digestion in the rumen. The miniature rumen consists of a small cellophane sac suspended in a screw-cap jar. The jar contains a solution similar in mineral composition to sheep saliva. The sac placed in the solution contains the substrate and rumen fluid of sheep. Also the efficiency of fibre digestion in cattle was tested with this artificial rumen. It was shown that higher contents of starch, decreases the digestibility from 40% to less than 10%. From this it can be concluded that the diet in which a seed is ingested influences the digestibility.

The two-stage laboratory *in vitro* method was first developed by Tilley and Terry (1963). This method was developed as a simple laboratory technique for forage crops digestion. The digestion was attained by incubation of substrate in solutions of micro-organisms or enzymes which are similar in function to those present in the digestive tract of the ruminant. The method consists of two stages; in the first stage the substrate is incubated in rumen fluid tapped from ruminants, fed with a certain diet, to simulate the exposure to digestive processes in the rumen. A large volume of buffer solution (McDougall's solution) has to be added to maintain the neutral pH level which is the usual pH for digestion in the rumen. To obtain a good digestion it is essential to maintain anaerobic conditions during the first stage. The second stage of pepsin digestion, to simulate the conditions in the abomasum, can be achieved by incubation of the undigested forage in the first stage in a acidified pepsin solution (pH 2.5). Anaerobic conditions were not necessary during this stage. During both stages the temperature should be kept near 38^0C, which is the natural temperature in cattle.

Variations of the two-stage digestion method nowadays consist of an *in sacco* and *in vitro* treatment used to examine the effect of digestive processes on the survival of seeds. The effect of digestion by microbial activities in the rumen on the survival of seed can be examined in the *in sacco* nylon bag technique (Simao Neto & Jones 1987, Blacksaw & Rode 1991, Gardener *et al.* 1993b), or *in vitro* by incubation of seeds in tapped rumen fluid (Simao Neto & Jones 1987, Ocumpaugh & Swakon 1993) or in a cellulase-solution (Simao Neto and Jones 1987, Knevel 1997). The survival of seeds after exposure to acidic conditions in the abomasum and the first part of the small testine (duodenum) were *in vitro* simulated by incubation in solution of acid-pepsin (pH 2.5) (Simao Neto & Jones 1987, Blacksaw & Rode 1991, Gardener *et al.* 1993b, Ocumpaugh & Swakon 1993).

3.2 Stage I

There are three different methods known based on the principle of the first stage of the Tilley and Terry digestion procedure; 1) The nylon bag technique, 2) Tapped rumen fluid technique and 3) The cellulase-test. The first method is an *in sacco* procedure, the two others are *in vitro* procedures.

The nylon bag technique is the first method that can be used to examine the effect of digestive processes on the survival of seeds. With this technique different factors that might affect the survival of seeds during passage through the digestive tract of cattle, such as the time spend in the rumen (Blackshaw & Rode 1991, Gardener *et al.* 1993b) or the diet in which seed are ingested (Simao Neto & Jones 1987, Blackshaw & Rode 1991) can be modified. In this technique about 200 seeds of one species are sealed in bags of closely woven nylon and placed in the ventral sac of the rumen of cattle (see Fig. 1) for different periods. The range of different periods of time spends in the rumen varied from 0 or 24 h to 24 or 241 hours with steps from 2 to 24 hours. A 24-h period of incubation in the rumen is representative of the incubation time of high-grain diets. High-forage diets could result in substantially reduced rumen incubation times and, thus, potentially greater seed survival than indicated in this method (Blackshaw & Rode 1991).

In this technique most of the time adult animals were used. During the experiments the cattle were fed different mixtures of forage. For example with a diet of 50:50 chopped oat straw and Lucerne hay (Gardener *et al.* 1993b) or 80:20 Lucerne hay and milled wheat grain (Simao Neto & Jones 1987) or Lucerne (alfalfa) hay and rolled barley (Blackshaw & Rode 1991). It is important to know the composition of the diet because it influences the digestibility (Huhtanen *et al.* 1953).

The technique with tapped rumen fluid is an other modification of the first stage of the Tilly and Terry (1963) two-stage digestion procedure. The digestion of seeds takes place under *in vitro* conditions. Tubes with tapped rumen fluid from cannulated steers fed with hay mixed with soybean (Ocumphaugh & Swakon 1993) or a mixture of 80:20 Lucerne hay and milled wheat grain (Simao Neto & Jones 1987) are used in this method. Anaerobic conditions can be achieved by flushing the tubes with CO_2. The incubation times differed from 3 to 72 hours. The effect of diet, the times spend in the rumen fluid and the quantity of seed ingested can be examined by this laboratory technique.

The third technique to simulate the digestion caused by rumen enzymes is the *in vitro* cellulase-digestion. Simao Neto and Jones (1987) and Knevel (1997) used this technique. cellulase is the enzyme, produced by micro-organisms, that can break down cellulose and is present in the rumen of cattle. In a cellulase-test the microbial activity of enzymes present in the reticulo-rumen can be simulated by incubation of seeds in a cellulase-solution. During the cellulase-digestion seeds were incubated in a cellulase solution at 40^0C for different periods ranging from 9 to 72 hours.

The species used in the three different experiments mentioned above were mainly grasses, weeds or (tropical) Legumes that are often present in forage fed or eaten by cattle.

3.3 Stage II

The remainder of the seeds used in the first stage digestion-techniques can be further digested in the second stage digestion tests. The second stage consists of an *in vitro* incubation of this remainder in an acidified solution, which simulates the digestion in the abomasum. Mostly a solution of acidified pepsin is used (Simao Neto & Jones 1987, Gardener *et al.* 1993b, Ocumpaugh & Swakon 1993). But Knevel (1997) used incubation in an IADF-solution. The Ingestion Acid Digestible Fibre-test (IADF-test) contains an incubation of seeds in an IADF-buffer (pH 4.6). The incubation of the second stage at 39^0C varied from 3 hours to 14 days.

The tests done by Knevel (1997) were performed to test the role of the different seed attributes in predicting seed longevity. So the species used in this experiment were selected on their seed shape and size to represent the most common seed types.

3.4 Results of the laboratory tests

The results of the survival of seeds found in the different laboratory digestion tests will be dealt with in this paragraph. The terms incubation and digestion have the same meaning in this contexts namely the simulation of digestion by incubation in any kind of solution. Most of the results were given as the percentage seeds tested that germinated, that not germinated but were viable, and the percentage of seeds that were dead (rotten). In all three groups the percentage before and after the digestion test was measured. Assuming that the species tested within one family have more or less the same seed characteristics, the results of different species within one family are averaged. The Poaceae and Leguminosae are families that were frequently used. Also other Dicots were used, but most of the times too less species to evaluate the results for different families within the Dicots. The results of the different tests are divided over two tables. Table 2[a] gives the results of the tests were rumen fluid (plus an *in vitro* incubation in pepsin) was used to test the digestion; either in nylon bags placed in the rumen of fistulated cattle (*in sacco*) or in tapped rumen fluid (*in vitro*). Table 2[b] gives the results of digestion tests using solutions (*in vitro*) that simulate the digestion of cattle. Most authors used different incubation times, for a good comparison between the tests only incubation times of 24 and 48 hours were included.

There is a high variation in percentage germinated seeds of the Dicots before digestion, ranging from 8.8 to 68.3 (Table 2[a]). After a two-stage digestion the germination decreased to about 5%. Higher germination percentages were found after only one stage ranging from 7.6 to 25.1%. The percentage viable seeds of the Dicots was much higher; this ranged from 53.5 to 82.7% before digestion and stayed high after digestion (ranging 43.5 to 87.4%), except for one series of Dicots (Blackshaw & Rode 1991). The Dicots with the highest and lowest percentage of viable seeds before and after digestion belonged to the same series. The percentage of dead seed increased in most of the series, ranging from about 10% before to 50% after digestion, except from the series Dicots tested by Gardener *et al.* (1993b). The following conclusion can be made for the Dicots: The percentage viable seed is high before and after the digestion in rumen (fluid). After digestion the seeds that germinated decreased and the seeds that are dead increased. It seemed that the addition of incubation in pepsin had no great influence on the survival of seeds (Simao Neto & Jones 1987).

For most of the series Poaceae tested either the percentage of seeds that germinated or the viable seeds was presented (Table 2[a]). For convenience the results of the other series of Poaceae the percentage germinated and viable seeds will also be combined and mentioned as Viable seeds. The percentage of Viable seeds before was high (78.5-96.0%) in all series. But after digestion there was a large spreading in percentage of Viable seed ranging from 0.8 to 88.2%. The series with the lowest percentage of germinable seeds before and after digestion belong to the same series. A low percentage of seeds were dead before the digestion test but this increases in one series to 99.2%, only in the series Poaceae tested by Gardener *et al.* (1993b) the percentage of dead seeds stays low (roughly 10%). From these test it seemed that most of the Poaceae seeds die after digestion.

Table 2[a]. The results of the survival of seeds for different families found by different authors. The survival is given as the percentage of tested seeds that were found germinable, viable, or death before and after the digestion. Digestion methods used are the two stage methods were they used either the nylon bag technique or tapped rumen fluid with or without incubation in pepsin.

Article		Digestion test	Time	Family	Number of Species	% Germinated (Mean) Before	% Germinated (Mean) After	% Viable (mean) Before	% Viable (mean) After	% Dead (mean) Before	% Dead (mean) After
Gardener *et al*. 1993b	*In sacco + In vitro*	Rumen + Pepsin	48 h + 48 h	Tr. Leguminoseae	39	33.7	4.0	47.4	39.8	18.8	59.0
		Rumen + Pepsin	48 h + 48 h	Tmp. Leguminoseae	5	62.2	0.4	2.3	2.6	35.5	97.0
		Rumen + Pepsin	48 h + 48 h	Poaceae	28	29.5	2.2	62.7	86.0	7.7	11.9
		Rumen + Pepsin	48 h + 48 h	Dicots	2	8.8	4.3	82.7	87.4	8.5	8.4
Ocumpaugh & Swakon 1993	*In vitro + In vitro*	Rumen fluid + Pepsin	48 h + 24 h	Poaceae	2	83.5	65.0	#	#	16.5	35.0
Simao Neto & Jones 1987	*In sacco + In vitro*	Rumen + Pepsin	48 h + 24 h	Poaceae	2	*	*	78.5	28.2	21.5	71.8
		Rumen + Pepsin	48 h + 24 h	Dicots	4	32.8	4.4	53.5	50.4	13.8	45.2
	In vitro + In vitro	Rumen fluid + Pepsin	48 h + 24 h	Poaceae	2	*	*	78.5	0.8	21.5	99.2
		Rumen fluid + Pepsin	48 h + 24 h	Dicots	4	32.8	6.3	53.5	43.5	13.8	50.2
	In sacco	Rumen	48 h	Poaceae	2	*	*	78.5	48.8	21.5	51.2
		Rumen	48 h	Dicots	4	32.8	8.1	53.5	51.4	13.8	40.5
	In vitro	Rumen fluid	48 h	Poaceae	2	*	*	78.5	15.6	21.5	84.4
		Rumen fluid	48 h	Dicots	4	32.8	7.6	53.5	45.7	13.8	46.8
Blackshaw & Rode 1991	*In sacco*	Rumen	24 h	Poaceae	5	83.2	11.4	12.8	0.8	4.0	87.8
		Rumen	24 h	Dicots	7	68.3	25.1	25.1	17.9	6.6	57.0

* % Germinated and % viable seeds were not separately measured. In text collectively called as % Viable

% Viable were not presented, only % Germinated seeds were presented. In text collectively called as % Viable

Table 2[b]
before and after the digestion. Digestion methods used are incubations in solutions of Pepsin, Cellulase a combination of Pepsin and Cellulase or IADF.

Article		Digestion test	Time	Family	Number of Species	% Germinated (Mean)		% Viable (mean)		% Dead (mean)	
						Before	After	Before	After	Before	After
Simao Neto & Jones 1987	*In vitro*	Pepsin	24 h	Poaceae	2	*	*	78.5	70.2	21.5	30.3
		Pepsin	24 h	Dicots	4	32.8	11.4	53.5	49.5	13.8	39.1
		Cellulase	48 h	Poaceae	2	*	*	78.5	4.2	21.5	95.8
		Cellulase	48 h	Dicots	4	32.8	7.2	53.5	48.0	13.8	44.8
	In vitro + In vitro	Cellulase + Pepsin	48 h + 24 h	Poaceae	2	*	*	78.5	2.2	21.5	97.8
		Cellulase + Pepsin	48 h + 24 h	Dicots	4	32.8	4.5	53.5	45.1	13.8	50.4
Knevel 1997	*In vitro*	Cellulase	48 h	Leguminosae	3	55.2	5.5	13.6	9.0	31.2	85.5
		Cellulase	48 h	Poaceae	2	79.6	38.1	0.0	38.9	20.4	23.1
		Cellulase	48 h	Juncaceae	2	65.0	53.8	35.0	30.8	0.0	15.4
		Cellulase	48 h	Other Dicots [1]	5	56.4	31.0	41.8	33.4	1.6	35.5
	In vitro	IADF	48 h	Leguminosae	4	55.2	0.3	13.6	2.3	31.2	97.4
		IADF	48 h	Poaceae	3	79.6	32.5	0.0	28.9	20.4	38.6
		IADF	48 h	Cyperaceae	2	5.6	0.3	94.1	94.1	0.0	5.6
		IADF	48 h	Juncaceae	2	65.0	45.8	35.0	37.9	0.0	16.3
		IADF	48 h	Other Dicots [1]	4	70.2	25.1	27.8	30.3	2.0	44.6

* % Germinated and % Viable seeds were not measured separately measured but collectively called as % Viable

[1] Other dicots: Caryophyllaceae, Scrophulariaceae, Compositae

The Leguminosae show approximately the same results as the other Dicots; a low percentage (less than 5%) seed still germinates after digestion, the percentage viable seeds stays the same within the groups of Leguminosae, this is only 2% for the Temperate Legumes and 40% for the Tropical Legumes. The percentage dead seeds increases after digestion, although the percentages viable seeds was lower and the percentage dead higher before and after digestion were higher than was found in the other Dicots.

In Table 2[b] the results of the seed survival in different solutions found by two authors are included. Again the families are particularly divided in Poaceae and Dicots but also something is known about the Leguminosae, Poaceae, Juncaceae and Cyperaceae. In all the tests incubation periods of 24 or 48 h were maintained.

When Poaceae are incubated in pepsin the percentage of viable seeds stays almost the same (roughly 75%) but when incubated in cellulase the viability decreases a lot (from about 80% to 3%). The percentage of dead seeds is much higher when incubated in only cellulase or cellulase plus pepsin than in only pepsin. The incubation in a combination of these two solutions showed only a small difference from incubation in cellulase only. From these two findings it can be concluded that cellulase has much more effect on the digestion of seeds than pepsin. This was obvious for the Poaceae but also in an inferior way found for the Dicots. The percentage germinated Dicot seeds decreases when they stayed in cellulase, pepsin or in a combination of these two solutions. The percentage of viable seeds before and after digestion stays almost the same (roughly 50%). The percentage of dead seeds increased after digestion (13.8 before to 39.1-50.4% after).

Something different was found for the Poaceae in the other series incubated in cellulase or in IADF (Table 2[b]). The germination decreased and a higher percentage of viable seeds were found after digestion. The increase of dead seed was not much (from 20% to about 30%). For the Juncaceae in both tests (cellulase and IADF) almost the same results were found for the germination and dead groups but the percentage of viable seeds stayed constant or even increased a bit. From the Cyperaceae the low number of seeds that germinated were dead after digestion. Most of the seeds stayed viable after incubation in IADF (94.1%). A high percentage (31.2%) of the Leguminosae seeds used in the cellulase and IADF test was dead before digestion. The percentage germinable seeds decreased after digestion with about 50%. A low percentage seemed to be viable both before and after digestion in cellulase and IADF. The germination of the other Dicots reduced after digestion. The percentage of dead seed increased from about 2% to about 40% after incubation in both cellulase solution and IADF solution. The percentage viable seeds stayed more or less the same (roughly 30%).

3.5 Discussion of the two stage digestion procedure

To preserve the population of the micro-organisms in *in vitro* tests, compared to the reticulo-rumen, it is important to maintain the quality of the diet fed to cattle. The composition of these digestive fluids fluctuates in chemical composition with diets that differ in quality. A disadvantage of the tapped rumen fluid and the cellulase method is that it is difficulty to maintain the conditions constant and comparable to the condition in the living animal. To obtain a good digestion it is essential to maintain anaerobic conditions throughout the first stage and the incubation temperature should be kept constant and as near as possible to 38^{0}C. When gassing the tubes with CO_2 attained anaerobic conditions it is necessary to release the fermentation gasses. Also the pH should be carefully controlled especially because the pH is different in all compartments of the digestive tract. In the *in vitro* experiments, were different tubes are used, hardly a clear comparison can be made when the conditions are not the same

in all tubes. During the *in vitro* incubation, the tubes have to be capable of maintaining true rumen organisms during the whole digestion period in about the same numbers in which they are usually found in the rumen.

The *in sacco* and *in vitro* tests have been expected to overestimate the actual survival of seed, because the seeds were not ingested by ruminants and were not exposed to the damage that can occur during mastication and rumination. The overestimation could be greater for large-seeded species since large seeds are more exposed to regurgitation and damage than small, dense seeds (Gardener *et al.* 1993b). The absence of mechanical damage could be balanced with a longer exposure to chemical damage than occurs in normal circumstances. But the retention of seeds in the abomasum and duodenum is normally only 2-4 hours (Gardener *et al.* 1993b). It was already known that most of the damage occurred in the rumen, the results of the incubations in *in sacco* plus pepsin incubation confirmed that most of the damage of seeds occurs in the rumen (Table 2^a). From those two findings it can be concluded that cellulase has much more effect on the digestion of seeds than pepsin. However some losses in viability after exposure to pepsin occur, either after rumen digestion (Table 2^a) or cellulase incubation (Table 2^b) indicates that some damage occurs in the abomasum. An other reason that an overestimation will be made is the lack of further damage that will occur during passage through the duodenum and after defecation by aerobic micro-organisms in the dung.

The major advantage of the laboratory tests, to simulate passage through the digestive tract, is that they are easy and fast techniques and fairly suitable for screening large numbers of species. Thus it can be done with relatively small number of seeds. Not al enzymes that cause damage to the seed are present in *in vitro* experiments. So the survival of seeds placed in nylon bags in the rumen and then immersed in acid pepsin is the best indicator, compared to the *in vitro* tests, of the actual survival of seeds through the digestive tract of cattle.

The comparison of different families is hard because all authors used seeds of different species in their tests. Most of the variation in percentage viability of seeds before digestion was probably caused by variation in the species used. Variations in seed can also appear due to the way of storage of the seeds or even due to differences in development. What can be said is that Leguminosae show approximately the same results as the other Dicots, the percentage of seed that is viable stays nearly the same after digestion. Most of the seeds that germinated before digestion were dead after digestion even though at forehand Legumes seemed more resistant to the digestive processes than the other Dicots. Most of the Poaceae died after digestion but different results were found for their viability. Sometimes high losses were found in percentage seeds that were viable before digestion and sometimes the percentage of viable seeds seem to increase; probably those seeds that germinated before digestion but did not after digestion.

4. Feeding tests

When cattle eat seeds, seeds are subjected to mechanical as well as chemical damaging processes. The mechanical damage of the seeds caused by the grinding action of teeth during ingestion and rumination and the aerobic break down of organic matter by micro-organisms in moist dung was not included in the *in vitro* or *in sacco* tests. In feeding tests however the seed will be exposed indeed to all mechanical and chemical damaging processes during the passage through the tract. These tests can be used to measure the retention time in or the survival of different species after passage through the digestive tract.

4.1 The feeding test

In the feeding test a known number of seeds are fed to (Burton & Andrews 1948, Janzen 1982, Simao Neto *et al.* 1987, Barrow & Havstad 1992) or placed directly in the rumen of cattle (Gardener *et al.* 1993a). After excretion the survival of seeds was followed in dung pats and seedlings of many species have been recorded in the faeces. The survival of seeds usually falls with the retention time in the digestive tract, so survival should be related to those seed characteristics that affect the retention time. Possible seed characteristics responsible for retention time are specific gravity, amount of hard seed and seed size or weight (Gardener *et al.* 1993a).

Just as in the two-stage digestion procedure also in the feeding test the seeds are subjected to the processes of cattle digestion, which usually has a damaging effect on the seed. But in the feeding tests no simulation has to be made of these damaging processes, as the seed passes through the whole tract *in vivo*. In the feeding experiments seed samples consists of a certain amount of seeds; either the weight or the number seeds is known. The samples can be placed directly into the rumen of cattle through permanent fistulae in the rumen wall (Gardener *et al.* 1993a). Barrow and Havstad (1991) used a method with gelatine capsules, these capsules contained a certain number of seeds and were placed in the rumen of steers. The gelatine capsules dissolved within 45 min after placement in the rumen. The seeds can also be mixed in a certain diet the cattle are feeding on (Burton & Andrews 1948, Janzen 1982, Simao Neto *et al.* 1987).

4.2 Collection of the faeces

The cattle fed with seeds were kept in metabolism cages, the faeces produced after feeding or placing the seeds in the rumen can be collected in metal trays (Gardener *et al.* 1993a) in faecal bags fitted to each steer (Barrow & Havstad 1991) or just from a solid floor. The faecal output is collected at a range of hours after feeding the seed; varying from 22 collections in a time period of 160 h with the first collection 9 hours after feeding, to 4 collections in a time period of 96 hours with the first collection 24 hours after feeding. Or every day one collection.

From each faecal collection subsamples were taken for different measurements. Most of the time dry matter and/or organic matter determinations were made. Gardener *et al.* (1993a) found a dry matter weight of faeces produced per animal over a 160-h period of 22.4 kg. Subsamples were also taken for the recovery and viability of the seeds. Most of the time grass seeds can be extracted from the faeces, because of their size and presence of awns, the seeds can be separated from the organic matter by drying and sieving. The remaining numbers

of seeds can be sorted, counted and tested for their viability. Sometimes it is impossible to extract all seeds from the faecal matter. Then an estimation of the seed survival can be made by counting the seeds that germinate out subsamples of the faeces spreaded in pots filled with sand (Gardener *et al.* 1993a). The numbers of seeds that not germinated are mostly classified as hard (seed coat impermeable to water) or rotten seeds.

The measurement of seedling emergence in dung is either directly done from subsamples taken from moist faeces (Simao Neto *et al.* 1987) or dung pats made from the remainder of the faeces, after the subsample has been taken away. Gardener *et al.* (1993a) rewetted the remainder of the faeces to the moisture content of freshly voided faeces and dung pats were made by placing 70 g dry weight into a PVC ring on a bare soil surface. The seedlings emerging on the dung pats in the field were counted for 8 month. Simao Neto *et al.* (1987) measured the seedling emergence from faeces by spreading subsamples equivalent to 20 g dry weight on the surface of filled pots in a glasshouse (temp. 35^0C).

4.3 Survival of seeds after *in vivo* digestion

The seeds that were retrieved from the faeces were either in tact, germinated or rotten. The fraction of seeds, which survived the passage through the digestive tract and emerged as seedling ranged from 0 to 64% for the grasses (Gardener *et al.* 1993a). The same percentages (0 to 62%) of recovered seed were found from the encapsulated grass seeds of four species (Barrow & Havstad 1992). The percentage of grass seeds recovered from 6 species by Burton & Andrews (1948) ranged within the same percentages. These germination percentages of the recovered seeds ranged from almost zero to 50%. From one grass species even 17% of the seeds retrieved was recovered as seedling in the faeces. The number of intact seeds recovered from the faeces (expressed as the fraction of the number of seed fed) varied from 6 to 80% for the Legume seeds (Gardener *et al.* 1993a). In most feeding experiments retention times of 2 or 3 days were found, but sometimes small amounts of seeds were found after 160 h (Gardener *et al.* 1993a).

More about the retention time and the factors that affect the retention time can be read in Chapter 5.

4.4 Discussion of the feeding test

Seeds can be fed either by placing the seeds directly in the rumen or fed in a mixture of forage. When the seed are fed in a mixture of forage, no seed must be present in the feed itself. When seeds were directly placed in the rumen they suffer no mechanical damage during ingestion, however most of the damage is caused during rumination rather than during ingestion (Gardener *et al.* 1993a). But mechanical damage of the seed can occur during rumination. When a mixture of seed with forage is fed it is possible that the animal consumes not all of the seed.

Compared to the *in sacco* and *in vitro* tests the *in vivo* feeding tests always contains more or less the mechanical damage of the grinding teeth. In most of the feeding test the seeds are, after excretion, exposed to the activities of micro-organisms in dung although there is some difference in placing the dung on a bare soil surface or in pots filled with sand in the glasshouse. You can imagine that the conditions in the glasshouse are far more controlled than the condition outside. Concluding, seeds digested in feeding tests are exposed to more different damage processes compared to the seed digested in *in sacco* and *in vitro* test. The

advantage of the *in vivo* feeding test is that no simulations have to be made for the digestion processes in the digestive tract, and since the seeds will pass through the whole tract, it is unlikely that overestimation on seed survival will be made. The disadvantage of the feeding test compared to the *in sacco* or *in vitro* tests, is that more seeds are needed and the tests are more time and space consuming. In the *in sacco* and *in vitro* test tens or hundreds of seeds but in the *in vivo* ten thousands of seeds have to be used.

When seeds were used to relate seed characteristics with the retention time, many characteristics can be measured. Gardener *et al.* (1993a) found that respectively specific gravity, amount of hard seed and seed size account for the passage rate through the digestion tract. When seed characteristics were used to find a relation between these characteristics and the retention time or survival, it is necessary that the same kind of seed were used, meaning that seeds of one species only can be compared when it is used in different experiments with or without the same appendages.

5. Factors affecting survival of seeds

It is generally known that factors such as, retention time in the digestive tract, damage to the seed during ingestion or the quality of the diet in which the seed is fed are known to affect the survival of seeds after passage through the digestive tract of cattle. But to what extent they effect the survival is not yet well defined. How these factors can be determined or measured and in which ways or to what extent the digestion of seeds is influenced by these factors will be dealt with in the following chapter.

5.1 Retention time

The retention time of seeds in the digestive tract is one of the factors mentioned by many authors to effect the survival of seeds (Simao Neto & Jones 1987, Blackshaw & Rode 1991, Gardener *et al.* 1993b). It is generally known that the retention time effects survival by the following; the longer the seed is exposed to digestion processes the more the seed is digested and higher the change that a seed not survives the passage through the digestion tract. The retention time of seeds can be measured in the *in vivo* feeding test described in chapter 4. The effect of different retention times on the survival of seed can be determined in *in sacco* or *in vitro* tests described in chapter 3.

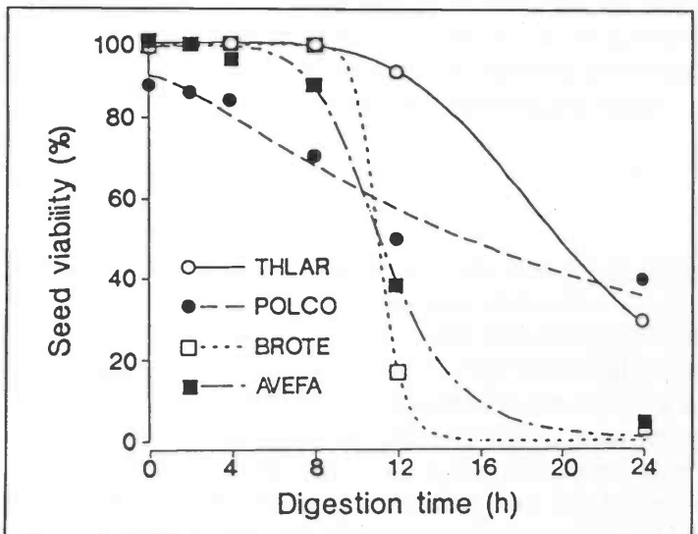

Figure 2. Viability of *Thlaspi arvense* (THLAR), *Polygonum convolvulus* (POLCO), *Bromus tectorum* (BROTE) and *Avena fatua* (AVEFA) seeds after varying periods of rumen digestion by cattle (From: Blackshaw & Rode 1991)

Different authors found different retention times. Janzen (1982) found for the relatively large seeds, from a large native mimosaceous tree, *Enterolobium cyclocarpum* (guanacast) that the bulk of seed was defecated between 5 and 10 d after feeding. He suspected that the cows might carry a few dormant hard guanacast seeds for as much as months or more. In experiments with tropical grasses and Legumes, the seed content of the faeces was highest between 48 an 72 hours after ingestion and fell after 96 h (Simao Neto *et al.*1987, Burton & Andrews 1948). The retention times found for other tropical Legume seeds, varying from 35 to 51 h, did not correspond to the former retention times found (Gardener *et al.* 1993a). Gardener *et al.* (1993a) found for the tropical grass seeds a shorter retention time, most of the seeds were defecated between 24 and 36 h after feeding. But 155 h after feeding small amounts of seeds were still defecated. Higher retention times were found for grass species (48 h, Barrow & Havstad 1992).

From the different retention times found it can be concluded that there is no unambiguous answer found for the retention time of grasses or Legumes. In generally we can say that most of the seeds will stay in the digestive tract for 2 till 4 days, except for the guanacast seed. It is not strange that there are differences in retention time because it is affected by different seed characteristics like size, gravity or seed coat.

In *in sacco* or *in vitro* digestibility tests the retention time of seeds in such tests could be manipulated (see chapter 3). In that way the effect of retention time on survival of seeds can be measured very well even though the conditions or retention times are not always the same between different experiments and not always comparable to real life conditions. Species respond differently when subjected to varying lengths in the digestive process, but most grass and weed species lost all their germination capacity during digestion. But for a few species the germination first rose at a maximum of 24 h or 51 h and then also decreased to almost zero (Gardener *et al.* 1993b). Blackshaw and Rode (1991) also concluded that the viability of all species tested declined with the increasing time in the rumen. Loss of seed viability did not appear to be a gradual process. Seeds appeared to tolerate a certain time in the rumen, but after 10 h there was a rapid decline in viability (Fig. 2). Gardener *et al.* (1993b) found something corresponding; the percentage of viable seeds of Legumes seem to reach a constant value during digestion. The lag period may have been caused by degradation of the seed coat or other protective layers before the start of embryo damage, although the periods found by these two authors were different. This difference can be caused by the difference species used. Simao Neto & Jones (1987) findings also show that viability of grass seeds was directly related to the time in the rumen. Ocumphaugh and Swakon (1993) even found a significant linear response when germination percentages were plotted against the total digestion time. It is hard to say something species specific about the percentage of decline because the different authors all use different methods and different species, Legumes as well as grass species. But in general it can be concluded that there is a negative relation between seed survival and exposure to digestive processes. And most of the authors conclude that there is also a relation with the thickness and hardness of the seed coat.

5.2 Seed characteristics

The effect of the seed characteristics size and composition of the seed coat on retention time will be evaluated in the next paragraph. Simao Neto *et al.* (1987) found that the seed size had no effect on recovery of seed by cattle, but the rate of passage of the large podded seed and longer grass seed was usually slower than for the other species. Other authors found different retention times for different seed sizes. Janzen (1982) also found for the relatively big seeds of *Enterolobium cyclocarpum* (25 x 11 mm) a long retention time (5 to 10 d) in cattle. The bulk of the small-seeded species was excreted sooner than bigger-seeded species (Gardener *et al.* 1993a). Concluding can be said that seed size had a small positive effect on the retention time, increasing seed size slows the rate of passage. This suggests that it is the position of particles in the reticulo-rumen, rather than their size that determines the passage rate (Gardener *et al.* 1993a). This corresponds with Dyce (1996), but the assumption that seed characteristics affect the retention time is not excluded because the position in the rumen is in fact determined by the specific gravity (see 5.3) of the seed which is subsequently dependent on the size, chemical composition and shape.

During passage through the digestive tract the permeable seeds imbibe large amounts of water, become swollen and the seed coat will be ruptured. Once the seed coat is broken the cotyledons and embryo will be rapidly affected by the digestive processes and the seed will die. Few hard seed will usually be softened towards the end of the digestive process but were not exposed to digestive processes long enough to be killed. Most of the seeds with a hard seed coat are valued as dormant seeds (see chapter 1).

Hard seed content of seeds can be measured by soaking the seeds first for 7 days. The seeds that imbibed within this period were said to be soft seed. Storing by high temperatures

(55-60^{0}C) can soften the remaining hard seed. After placing the seeds in Petri dishes at 28^{0}C for two weeks the numbers of germinated, hard, imbibed or rotten was recorded.

The extent of seed coat damage through digestive processes can be examined under a scanning electron microscope. The scanning revealed that a thin outer seed coat surface was partly or wholly removed during passage through the digestive tract. Changes in seed coat damage were more noticeable following digestion by sheep than by cattle (Simao Neto *et al.* 1987). Soft seed collected after short periods in the digestive tract tended to be dead but intact, while that retained for longer tended to disintegrate. Janzen (1982) found a high percentage of survival of seed for the hard-coated seed of the guanacaste tree. The amount of rotten seed recovered in the faeces depended on the amount of soft seed from the original seedlot.

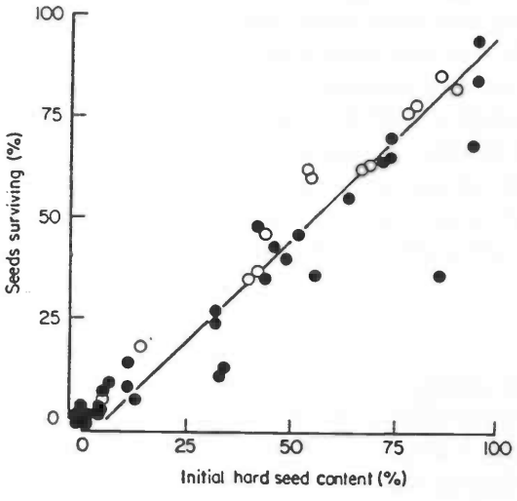

Figure 3. Relationship between the initial hard seed content and the fraction of legume seed surviving after 48 h in the rumen and 48 h in acid pepsin. Stylosanthes (o) and other legumes (o). r = 0.93 (From: Gardener *et al.* 1993b).

There was a highly significant correlation (r = 0.93) between the initial hard seed content and the survival of Legume seed (germinable + viable seed) (Fig. 3). The digestibility of the Legume seeds was significantly negatively correlated with the hard seed content (Gardener *et al.* 1993b). Hard seeds of Legumes seemed relatively resistant to breakdown in the rumen of cattle. This is emphasized by the finding that no seeds survived passage for the species with all soft seed (e.g. an imbibed seed coat) (Simao Neto & Jones 1987). Because soft seeds are broken faster than hard seeds it is not surprising that Janzen (1992) suggested that soft seeds past through faster than hard once (soft seeds have a lower specific gravity). Few grass and weed species germinated after digestion in contrast to Legumes this will suggest that there are specific differences between hard seed content between grasses and Legumes. This is in contradiction with what was found by Simoa Neto *et al.* (1987), they found that grass seed has a greater resistance compared with Legume seeds.

Whatever the ability of the seed coat is to withstand the degradation, the longer the seed is subjected to this process, the more likely it is to become permeable to water and initiate to germinate. Sometimes a higher germination percentage is found for species with a high hard seed content, it may be assumed this was due the breakdown of hardseededness during passage. (Simao Neto *et al.* 1987, Gardener *et al.* 1993b). So rumen digestion can break seed coat dormancy through scarification, partly mechanical during ingestion and rumination partly chemical during passage through the digestive tract. Some seed germinated already when placed in the rumen, despite that those seeds have no seed coat-imposed dormancy. Hardseededness of Legume seeds is known to vary with different environmental conditions during maturation, caution must be made in extrapolating results from one seed lot of each species, as usually used in experiments, to the species in general.

5.3 Specific gravity

The specific gravity of the seeds is important for the time spend in the reticulo-rumen, the longer the seed is present in this part of the digestion system the higher the change that the seed will lose its viability. The content of the reticulo-rumen exists of a mixture of feed, saliva, and micro-organisms with their fermentation products. The content can be divided in four macroscopic layers caused by the different specific gravity. Ventral in the rumen solid feed particles are present with a specific gravity higher than water. Then a thick layer of rumen fluid with fine suspended and soaked food particles with the same specific gravity as water. A fibrous mass of just ingested food, with a specific gravity lower than water is floating on the rumen fluid. The upper layer consists of rumen gasses (CO_2, methane, N_2, and H_2). The seeds with a higher specific gravity are ruminated till they have the right composition and can be transported to the next part of the digestion system. The seeds present ventral in the rumen can stack there and stay there for a long time. So depending on the specific gravity seeds stay for different periods in the reticulo-rumen (Syllabus). Particles with a specific gravity 1.17 and 1.42 have a higher passage rate than 0.90 and 0.96. Maximum rates were found at specific gravities of 1.20 and 1.34 (Des Bordes & Welch 1984).

The effective specific gravities can be obtained by determining if the seed floated or sank in a series of solutions of a known specific gravity. Gardener *et al.* (1993a) used the following solutions to measure the specific solutions of the seeds:
Water + sodium chloride
Water + ethyl chloride
Water + potassium chloride
They found a specific gravity from 18 species (grass and Legume species) varying between 0.88 and 1.36, and a significant ($P < 0.01$) negative correlation between mean retention time and true specific gravity ($r = -0.85$). For the Legumes specific gravity explained 49.3% of the variation in rate of passage. He also concluded that the less dense seed retained longer in the cattle. The specific gravity can be caused by other seed characteristics like fibrous pods surrounding the seed.

5.4 Damage during ingestion and rumination

Not only the different seed characteristics that might affect the retention time might effect the survival of seed, also the damage during ingestion and rumination were said to be effecting factors. During ingestion and rumination the seeds are exposed to mechanical and chemical damage. The mechanical damage is caused be the grinding action of teeth; the seed can be abraded or crushed. Chewing during rumination rather than during ingestion may cause most of the mechanical damage. There may be some differential species effects because large seeds would be more prone to chewing damage than small seeds. Chemical damage is caused by α-amylase; this enzyme is secreted by the salivary glands in the mouth of cattle. Starch, an important component of the seed coat will be broken down by α-amylase (Eckert *et al.* 1988). When a seed is chemical or mechanical damaged during ingestion or rumination the seed coat can not act as a barrier to acids, enzymes and microbes any more. The embryo will be exposed to these digestion processes and an acidified environment in the abomasum and will die. Some of the damaged seeds lost only their outer structures during passage and might still be viable. In real life most of the still viable seeds will die through activities of aerobic micro-organisms in the faeces.

6. Survival of seeds in cattle dung in "real life"

In permanent pastures or firmly established sods it is hard for seeds to establish. Most of the propagation will therefore be vegetatively, through stolones or rhizomes (Dore & Raymond 1942). But in bare soils propagation by means of seeds will also become a potential way for establishment. When bare soil is not present, it can be made up by large depositions of cattle that may kill vegetation and consequently providing a niche for opportunist species. Grazers also effect plant communities by trampling and defoliation (removal of biomass) (Bakker 1989). Dai (1998) supposed that the deposition of cattle dung should add to small-scale heterogeneity, which helps to maintain the high species richness of the grassland. It is known for a long time that herbivores transmit the seeds of many plant species in their dung, because these plant species produce seeds that withstand the digestion tract (Ridley 1930). Mainly seedlings of *Trifolium repens* were found in or on the dung of cattle. Dore and Raymond (1942) concluded from their experiment that cattle are the most important of the various dispersal agencies of seeds of pasture species.

6.1 Principle of the survival of seeds in "real life"

Until now we only looked at the artificial digestion or the forced feeding of seeds by cattle. Which seeds of plant species in "real life" will be eaten by cattle and will survive the digestive tract and are still able to germinate after defecation will be dealt with now. There are only few studies known about that examined the survival of seeds eaten by cattle in real life, most of these studies had the aim to study the spread of weeds by manuring. To know which species are present in the dung and have the potention to germinate in dung can be found out in two ways. The survival of seeds in real life can be monitored by counting the germination and colonization of seedlings in dung pats *in situ* (Welch 1985, Malo & Suárez 1995b) or the seedlings germinated in the dung brought into the greenhouse (Dore & Raymond 1942, Welch 1985, Malo & Suárez 1995b, Noot 1994, Dai 1998).

6.2 Germination in greenhouse vs. *in situ*

Dung samples out of the field and taken into the greenhouse can be treated in different ways. Fresh intact dung pats or divided in small portions can be placed on sterilized soil. When placed in small portions sometimes the dung is stratified before drying and/or sieving the material. In all studies known about the seedlings germinated in cattle dung, many species were found to be present. The sample sizes varied much between the different studies from whole dung pats of about 3 l. (Welch 1985) to samples of 78,5 ml. (Dai 1998). Welch (1985) found on one intact cow pat 662 seedlings of 24 different species germinating in the greenhouse. Dai found a maximum of 18.5 seedlings per sample of 11 species, this difference is mainly caused by the difference in sample size. Because when we convert these two samples to the same size (11 * 38 = 684 seedlings), we find in the samples of Dai about the same number of seedlings as found by Welch. The other sample sizes used are represented in Table 3. From these seedlings more Graminoid than Dicotyledons species appeared. The Poaceae were the most abundant Monocotyledons but also many species and seedlings were found from the Juncaceae. Of the Dicots *Sagina procumbens* had the most seedlings occurring on the dung pats. Other authors (Dore & Raymond 1942, Noot 1994, Dai 1998) also found that most of the seedlings (37,2 to 63.7%) germinated in the dung samples placed

in the greenhouse were originating from the Poaceae (Table 3). Noot (1994) found also a high percentage of the Juncaceae (39,2%), but he examined a small number of dung pats. Families of the Dicotyledons found in considerable percentages were Leguminosae, Caryophyllaceae, Compositae, and Plantaginaceae.

In only one article the germination of seeds was monitored as well in the greenhouse as *in situ* (Welch 1985). In this germination site the Poaceae remained important, and also the seedlings of the Juncaceae had a percentage worth mentioning. There were many different kind of families found of the Dicotyledons with in total a higher percentage seedlings than the Monocotyledons. From these results it is concluded that the Dicots were exceeding the Monocots in *in situ* dung (Table 3). From these experiments it was indeed shown that the number of seedlings counted on the dung pats *in situ* were far less than in the greenhouse.

6.3 Establishment in dung compared with vegetation

Before conclusions can be drawn about which family or families have high percentages of seedlings present in the dung e.g. many seeds can survive the digestive tract of the cattle, it is important to relate these percentages to the percentage of the families in the species composition. Because one can imagine that when a species is abundant in the vegetation it will normally also be found in high numbers in the cattle dung. When a species is not abundant but present in the vegetation and relatively high numbers of seedlings in the cattle dung are found one may conclude that this species has a good survival of the digestive tract or is especially preferred by cattle.

The cover of plants spreading vegetatively into cattle dung or growing through it from below was much more than the seedlings that germinated in or on the dung. Welch (1985) found that the species dominant in the vegetation also was the most frequent colonizer. Most of the cover that arises from germination originates from Graminoids (*Agrostis tenuis*, *Poa pratensis* and *P. annua*, and *Festuca ovina*) from which the first and the latter were also the most dominant Graminoids in the vegetation. From the Dicotyledons *Calluna vulgaris* was dominant in the vegetation and had also the highest percentage covering the dung pats. This is in contrast with the conclusion that cattle dung pats are mainly colonized from seeds transported within the manure (Malo & Suárez 1995b). Desirable forage species, like *Trifolium repens*, *Poa pratensis*, *Agrostis stolonifera*, *Phleum pratense* and *Poa compressa*, are all well represented in the dung. Dore and Raymond (1942) found that seeds of these species represented more than 70% of the numbers of seeds in manure. The fruiting parts of these species are upright and easily accessible. The seeds produced are evidently quite resistant to digestion. *Festuca rubra* although highly abundant in the vegetation and palatable was not found in the dung. It is known that *Trifolium repens* produces seed with a hard seed coat and because of this it is able to withstand the damage caused by digestion throughout passing the alimentary tract. See also paragraph 3.4 although here it seemed that Leguminosae did could not withstand the passage through the digestive tact very well.

6.4 Seasonal effects on dung contents

Dung deposited in autumn gave rise (for most species) to significant more seedlings than in spring cattle dung (Welch 1985). Others (Dore & Raymond 1942) also found peak transmission in autumn. Malo & Suárez (1995b) found that the seed content of spring manure differed much from winter dung: 1373 seedlings from 46 species against only 17 seedlings

Table 3. The percentage seedlings per family found by different authors on dung pats in the greenhouse.

Article *	Germination site	Family	Number of species **	% Seedlings
Dore & Raymond 1942 [a]	In Greenhouse	Poaceae	13	63.7
		Leguminosae	5	10.7
		Rosaceae	3	0.6
		Plantaginaceae	2	3.9
		Caryophyllaceae	2	3.2
		Scrophulariaceae	2	2.8
		Polygonaceae	2	0.9
		Other Dicots [1]	7	14.2
		Total	36	100.0
Noot 1994 [b]	In Greenhouse	Poaceae	5	41.7
		Juncaceae	2	39.2
		Compositae	2	4.6
		Onagraceae	2	1.4
		Polygonaceae	2	2.1
		Other Dicots [2]	3	11.1
		Total	16	100.0
Dai 1998 [c]	In Greenhouse	Poaceae	5	38.1
		Caryophyllaceae	5	27.4
		Compositae	3	2.1
		Juncaceae	2	4.7
		Lamiaceae	2	0.8
		Scrophulariaceae	2	1.3
		Other Dicots [3]	7	25.6
		Total	26	100.0
Welch 1985 [d]	In Greenhouse	Poaceae	8	37.2
		Juncaceae	5	1.7
		Cyperaceae	3	32.0
		Other Dicots [4]	16	29.1
		Total	32	100.0
	In Situ	Poaceae	9	30.8
		Juncaceae	2	7.7
		Other Dicots [4]	19	61.5
		Total	30	100.0

* Different sample sizes were used by different authors

[a] number of seedlings in 10 ounce (300gr) of dried cattle manure

[b] Mean number of seedlings found in 4 samples of 500 ml of dung pats

[c] Mean number of seedlings found in 30 samples 78,5 ml dung pats

[d] Mean number of seedlings found in 50 samples of 2600 ml germinated in the greenhouse and 75 samples of 3880 ml germinated *in situ*

** Species of which only one seedling was found in all kind of dung pat samples are not recorded in this table

Other Dicots: Only one species was found per family

[1] Junceae, Cyperaceae, Chenopodiaceae

[2] Caryophyllaceae, Plantaginaceae

[3] Cyperaceae, Leguminosae, Rosaceae, Plantaginaceae

[4] Leguminosae, Caryophyllaceae, Scrophulariaceae

from 5 species. Also the number of germinations and species per sample differed between spring and winter dung. Looking at species-specific numbers of seeds it was concluded that a maximum of seeds appears at the start of the summer (Malo & Suárez 1995a). Plant cover on dung increased most rapidly in summer, and spring dung was colonized sooner than autumn dung (Welch 1985).

6.5 Discussion survival of seeds in cattle dung in "real life"

Through different abiotic (temperature, moisture and nutrients) and biotic (competition with surrounding plants or disturbance from small animals e.g. dung beetle) conditions it is not surprising that a difference will be found for the number of seedlings found in the greenhouse and *in situ*. In greenhouses the conditions are kept most of the time optimal for seed germination and seedling development. It is subsequently not surprising that much fewer seedlings were found on the dung left *in situ* than in the greenhouse (Welch 1985). He found eight times more seedlings in the greenhouse than *in situ*. Another possible reason for this difference is that the monitoring of the germinated seedlings is more accurate and controlled in the greenhouse than in the field. Because the germination in the field takes more time than in the greenhouse, more death due to unfavourable conditions would be more likely because the seed were present in the dung longer.

Not only the germination site (*in situ* or in the greenhouse) but also the season when the dung is deposited will influence the number of seedlings found. Several authors found more seedlings in autumn dung than in spring dung (Welch 1985, Dore & Raymond 1942). This is in contrast with what was found by Malo & Suárez (1995b) in a Mediterranean pasture. The seasonal difference can be caused by the variance in seed supply between the seasons. Most of the species have not produced many seeds in spring already, but in late summer and spring most of the species have mature seeds. Another explanation can be the seasonal variation in feeding preference.

The species found in or on cattle dung often highly correlated with the species composition of the grazing area of the cattle. The Poaceae were the most abundant in the vegetation as well as in the dung. It is presumable that the species present in the dung are representing the supply of seeds in the vegetation but it is not impossible that the Graminoids indeed withstand the passage through the digestive tract better than other species.

Malo & Suárez 1995 concluded that the frequency of the species in pasture increases due to its recruitment on the cattle dung in which the seeds are dispersed. This was according to them the first evidence that the dispersal by herbivore in dung of seeds of a herbaceous species leads to an important increase in its frequency in vegetation, as postulated by the Foliage-as-Fruit hypothesis of Janzen (1984). But Dai (1998) concluded that dung deposition not influences the small-scale pattern of plant species except for the fertilizing effect of cattle dung.

Former we concluded that most of the seeds are retained for 2 or 3 days in cattle, since cattle can walk up to 14 km/day (Gardener *et al.* 1993a) (when they have the possibility to walk these distances). Seeds, which are able to withstand cattle digestion, can be disseminated over large areas.

7. Final discussion and conclusion

From the different studies reviewed in this essay we can conclude that some species have the potention to survive the passage through the digestive tract. Laboratory tests (*in sacco* and *in vivo* tests) can simulate the passage through the digestive tract of cattle in a certain way. This simulation will never be fully satisfactory due to different reasons. First the seeds are not exposed to mechanical and chemical processes during ingestion or rumination, which are often the most damaging processes. Because of this, probably an overestimation in survival of seeds will be made, which will even be higher in large seeds; for these seeds it is harder to escape from the chewing action of the teeth. A second reason is that in laboratory tests the time that seeds are subjected to digestive processes is manipulated and is never the same as *in vivo* retention times. Thirdly, *in vivo*, all species have different retention times depending on seed characteristics, but in laboratory tests equal retention times set up by men were used for all species tested. This manipulation can, however, be used to measure the effect of different retention times and to compare the survival of species as a relative measure to say something about the survival of seed.

Results found in the feeding tests will compare more to the real survival of seed after being eaten by cattle than laboratory tests. But when large numbers of species have to be screened for their survival after being eaten by cattle, e.g. to know which species have the potention to be dispersed by cattle, laboratory tests are preferred above feeding tests. For three main reasons, namely, in feeding tests large numbers of seeds are needed and laboratory tests are less time and space consuming than feeding tests.

In screening seeds it is not taken into account which species are present in the forage (area) or if cattle will eat them.

The different species tested in laboratory tests and in feeding tests showed different degrees of survival. Most of the seeds did not germinate after digestion and were rotten or totally digested and not found again. These seeds were mostly originating from species with a soft seed coat, which imbibed in the digestive tract. But also species with a hard seed coat died because of abrading or damaging of the seed coat. As soon as the seed is imbibed the embryo will die due to the damaging actions or conditions in the digestive tract. Sometimes the seed even germinated in the digestive tract but died after excretion. Other seeds were found to be still viable but did not germinate. Most of the time these seeds had a hard seed coat, which had not been imbibed yet. The lack of germination after digestion can be caused by seed coat-imposed dormancy (Baskin & Baskin 1998). There were also species found that have a higher germination percentage after digestion than before digestion. A logical explanation for this result is that the seeds were probably dormant seeds, where dormancy was broken during the passage through the digestive tract due to scarification by chemical or mechanical processes. Most of the seeds that germinated before digestion, and were easy to imbibe, were dead after digestion. This was especially found for the Poaceae in the *in sacco* and *in vitro* tests. Most of the seeds of the Dicots that were viable did not germinate but were still viable after digestion. Many species of the Dicots have hard seed coats and most species of the Poaceae have soft ones.

The composition of the seed coat is an important characteristic related to the survival of seed but not the only one. Also specific gravity, size and the presence of appendages are characteristics that are more or less related with the survival of seeds after consumption. Specific gravity of seeds seems to correlate highly with seed survival (Gardener *et al.* 1993a). These seed characteristics are related with seed survival because they determine the retention time and the longer the retention time the lower the seed survival. Seeds with the same specific gravity as water have the shortest retention time. It takes more time to digest larger

seeds so they stay longer in the reticulo-rumen. Seeds with a hard seed coat are difficult to digest and consequently stay longer in the digestive tract but are also more resistent to digestive processes than soft seeds, which have a shorter retention time. Also the forage in which the seed is ingested influences the digestion of seeds.

The kind of species and the age of the animal that ingests the seed also influence the survival of seeds. A comparison between seeds after being fed to cattle, sheep and goat of the survival of was made by Simao Neto *et al.* (1987). It was found that cattle digested less seed than sheep and coat. Also horses were found to digest a higher percentage of seed than cattle (Janzen 1982). There is much known about the survival of seeds in birds (Traveset 1998). It was concluded that an enhancement of the germination of seed, after passage through the digestive tract, occurred as twice as often as inhibition. This was found when the results of all species tested were combined.

The survival of seeds is different between species but also within species. This variation is related to the quality of the seeds. Factors such as the environmental conditions during development or the age of a seed can determine the quality or viability of the seeds that are used in the experiments.

The capacity of seeds to germinate after digestion is important for the population-dynamics of some plant species. For successful dispersal of seeds by cattle, the seeds must have the possibility to withstand the damaging processes during ingestion and digestion, which is the case in seeds with durable seed coats and some form of dormancy. But when the seeds are defecated they must again withstand a range of biotic and abiotic factors that can affect their viability. The biotic factors that can be thought of are aerobe micro-organisms that are active in the faeces or vertebrates like dung beetles and birds that can eat or take away the seeds. The drying out of seeds is an abiotic factor that can cause the dead of seeds.

At last some recommendations for screening plant species will be given. The best way to test the survival of seeds after digestion by cattle is the subjection of seeds in rumen fluid (because most of the damage takes place in the reticulo- rumen); an easy way to do this is with tapped rumen fluid from adult cattle. It is important to keep the conditions comparable to the *in vivo* conditions. Incubation in only a solution of cellulase or IADF gives a restricted simulation of the digestion processes. To simulate the little digestion that takes place in the rest of the digestion tract, incubation in an acidified pepsin solution can be added after subjection to rumen fluid. The simulation of the retention time in the last part of the digestive tract, that occurs *in vivo*, seems to have small influence on the survival of seeds.

It is still not obvious if there is one seed characteristic that determines the survival retention time in the digestive tract and so the survival of seed. Although the specific grafity of the seed seems to be highly correlated with the survival of seed after digestion. This relation was tested and found in only one experiment. Also size of the seed and thickness and hardness of the seed coat are still important factors for the degree of digestion, because these factors have effect on the retention time. In future it is important to measure all these characteristics before doing digestion tests to know more about this relation with survival.

A lot of species have not been tested yet in either *in sacco*, *in vitro* or *in vivo* tests, so for a lot of species it remains unclear if they have the potention to survive the digestive tract and to what extend. Only small numbers of species and most of the time tropical grasses or Legumes were tested. There is not much known about the survival of seeds after digestion of West-European plant species. In an overview given by Poschlod & Bonn (1998) the survival of serveral grassland species after digestion (*in vitro* and *in situ*) was given. They only used data of unpublished intern rapports. Most of the seeds that survived belonged to the Poaceae, this agrees with the results found in this assay. From the results of the West-European species

found in the dung pats (Table 3) it can be concluded that the Poaceae are more abundant in the dung than the Juncaceae. Comparing these results with the *in vitro* test done by Knevel (1997), contrary results were found; a higher percentage of germinable seeds from the Juncaceae than from the Poaceae. A possible explanation for this difference is that cattle have been eaten the Juncaceae much more than the Poaceae, so the seedlings of the Poaceae were more representative in the dung, but the Juncaceae have a higher change to survive. It is useful to test large numbers of species for their potention to survive the digestive tract, especially those species that have been found in dung pats. And after testing all present species in one vegetation type or species community it might be possible that something can be said about why a species is abundant in the dung pats (either the seed has been eaten much by the cattle or is just a good survivor). Nevertheless the possibility for seed dispersion by cattle seems to be very important in restoration ecology, because grazing cattle is regular used in restoration projects. So it is important to know which species potentially can be reintroduced by cattle, and this can be determined with *in vitro* digestion tests.

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