Report

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A literature search on experiments with farm animals on food preference and performance with regards to GMO's and non-GMO's.

Irene Janssen

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The Animal Nutrition Group focuses its research programme on a few selected issues studies within WIAS, Wageningen Institute of Animal Sciences. Animal nutrition is considered as the transfer of nutrients in feed into animal products (milk, meat, eggs) and waste products (faeces, urine, fermentation end products). Heat production is also considered a waste product. In order to improve the sustainability in animal production systems, it tries to optimise the ratio between products and waste.

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The Science Shop of Wageningen UR acts as an intermediary on behalf of civil organisations with limited means for the realisation of applied scientific research. Science Shop projects fit into the central research themes of Wageningen UR: agriculture, environment, nature and food.

A summary with interviews and additional information on this research project has been published by Leonore Noorduyn under the title: **"Vreemde genen in het veevoer. Voorkeur van en effecten op het dier."** This summary is published in Dutch and available at the Science Shop of Wageningen UR.(brochure number 181)

Colophon

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Preface

This report is the result of a literature study on food preference of animals animal performance with regards to GMO's (genetically modified organisms).

The initial question, "Do animals have a different food preference for GMO's compared to non-GMO's?" came to us through the Dutch branch of the Natural Law Party. Farmers who observed a preference of animals for non GMO-feed alerted this political party. They wanted this observation to be verified and explained.

The Animal Nutrition Group and the Science Shop of Wageningen University and Research Centre were interested to study this question and a proposal for a four-month during literature study has been prepared.

At the start of the research project it became clear that only results of two small experiments on food choice with animals and GMO's were published. Therefore the projects Advisory Committee accepted to extend the initial research question. Factors that affect food preference indirectly, such as food intake and performance have been included as well.

Now four types of experiments with animals and GMO's could be identified:

?? Choice experiments

?? Comparison experiments with animals fed GMO and non-GMO with the same nutritional value

?? Comparison experiments with animals fed GMO and non-GMO with different nutritional values

?? Safety experiments

Referred literature, literature abstracts, grey literature have been studied and summarised. Additional personal information on farmer observations completed this study.

However, information related to our research questions seemed to be very limited. Essential methods and results of experiments were not always described. Crucial information was sometimes missing in the published abstracts. Not all farmer observations could be traced back to its sources. A complete interpretation of the collected information was therefore not always possible.

With the information actually available it is not possible to give an answer to the initial question on the effects of GMO's on food preference. Nor is possible to answer the question on the effects of GMO's on animal performance. This lack of information is remarkable, because of the rapid and important increase of the use of GMO's in animal feed worldwide. We consider this conclusion as the main merit of this study. This study emphasises clearly the initial call for more research on the effects of genetically modified feed on animals.

We would like to thank the Advisory Committee. Its support and valuable contributions are highly appreciated. Its diversity led to rich discussions, reflecting the on going public debate on genetic modification in the Netherlands. These discussions stimulated the Animal Nutrition Group to take into account different approaches and opinions on this sensitive issue.

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Summary

GMO's (Genetically Modified Organisms)

Genetic modification is a new technology applied to change specific properties of organisms. For example, colours of flowers and digestibility of feeding crops can be changed by this technology. More and more, genetically modified organisms are used in agriculture and in different parts of industry. This technology gives quicker results than traditional breeding, because some steps can be skipped.

However, the use of GMO's is criticised. The new technology is in full development, but its long-term effects on human health and on the environment are not yet fully known. Because of these concerns on the one side and the advantages of using GMO's on the other, a public and scientific debate is going on.

Insect protected (IP) and herbicide resistant (HR) GM crops are used on a large scale as animal feed. The nutritional value of these GMO's is not changed purposefully.

The research question

According to alarming farm observations from the US, animals would not eat GM crops as good as control food. If possible, they even try to avoid eating it.These observations combined with the public concerns on the use of GMO's in general, raised the main question for this study: "Do animals have a different food preference for GMO's or non-GMO's?"

We considered it wise not to limit ourselves to food preference only and to extend the main question by including other parameters in this study. Food preference is only one, and not always the best parameter to measure food quality. Food intake, animal performance and other parameters should be considered as well. Secondly, only two little articles on food preference were published. The extension of the question may contribute to a better understanding of the underlying mechanisms explaining a possible food preference for GMO or non-GMO.

This report is the result of a literature study including background literature on GMO's and on foodintake and reports describing experiments with animals and with GMO's. A serious food experiment on animals could not be realised within the budget and time limits of this study.

Food intake

Food intake and the control of the energy balance are complex systems. The factors that affect the intake are related to the animal itself, to the properties of the food and to the environment in which the animal lives.
The food intake is controlled by the central nervous system, (CNS). Neural, hormonal and metabolic signals inform the CNS about the body's internal environment.
Animals learn to associate the sensory properties of foods to the metabolic consequences. Taste, smell, vision, texture and palatability are the sensory properties of food, which are important for these associations. Food preference is species-specific as well dependent on

the individual animal. Within the limits of their evolutionary determined food preference, individual animals learn to prefer particular foods. Also signals from the environment where the animal lives in and the motivation of the animal can affect the food intake. An animal is able to choose a correctly balanced diet, adequate according to the animal in the given circumstances.

Experiments

Most experiments done with animals and GMO's are comparison experiments with animals fed GMO and non-GMO. Only two choice experiments as a small part of another experiment were done. In these experiments is concluded that animals do not avoid GMO's, and that no clear preference was shown.

Most experiments described in abstracts provide little information. The main conclusion of these experiments, based on the articles, is that the animals perform the same, whether fed GMO or non-GMO.

The chemical analyses are proximate (Weende analyses). In these limited analyses, no differences are found between GMO and non-GMO. The moisture and the fibre fraction seem somewhat higher in the GMO's, but this is not significant.
One article mentions slight differences between GMO and non-GMO corn with regards to chemical composition during different growth stages.

The experiments were compared per animal type. The experiments with dairy cows and beef cattle were carried out during a short period. Broilers were tested during most of the growing cycle. The duration of the experiments with pigs was very variable.

In one experiment increased fat depth and marbling has been found in pigs fed non-GMO. This is probably due to increased feed intake. Another experiment showed improved breast muscle yield in GMO fed broilers. Two experiments showed the opposite effect: a better feed efficiency with non-GMO fed pigs and cattle, compared to GMO-fed animals.

In descriptions of arm observations wild and farm animals refused to eat GM food or tried to avoid it, if possible. This might be a sign that something is wrong with GMO's.

The above mentioned experiments do not confirm the farm observations. It may be possible that wild animals are able to choose more adequately their food than farm animals. Wild animals are selected by nature on efficient foraging. Farm animals might have lost this capacity during their domestication.

Summarising it is not possible to draw firm conclusions on the data presented in this report. This study shows that an important lack of knowledge exists on food preference and performance regarding genetically engineered animal feed.

Recommendations for further research.

?? Different GM feed crops and non-GM feed crops, grown under similar conditions and comparable in nutritional value, should be studied.
?? Different species of animals (wild and farm animals, ruminants and monogastrics) should be included in the research, covering preferably more generations.
?? The history and the motivation of the animals should be taken into account.
?? Not only regular scientific methods but also biophysical methods should be applied.

1. Introduction

A new technique for changing the genetic properties of an organism is genetic modification. A piece of DNA, a specific gene, is added to the cell of an organism that is in the one-cell stage. The organism starts growing and the new gene provides the organism with a specific quality. Because of the novelty of this process, not everything is known yet, and the modification is still partly trial and error. The idea is that organisms are changed towards specific selected properties. Not only the qualities of microorganisms as bacteria and fungus but also qualities of plants, animals and even humans can be changed. Many steps of the traditional breeding are not necessary anymore. Even parts of genetics of one species are available for other species (Kleter 2000).

1.1. Reasons for using GMO's (Genetic modified organisms)

GMO's are used in many different parts of industry, like food industry, pharmaceutical industry and other industrial uses (for example in detergents and for purification of water). In agriculture, it can also be used in many ways, like for improving digestibility, changing the colours in flowers, or improved tolerance to drought.
Commonly used GMO's in agriculture are insect protected (IP) and herbicide resistant (HR) crops. These are the most important GMO's for this study, as also these crops are main GMO's used to feed animals. HR plants are made resistant to a specific herbicide by a new gene, so this herbicide can be sprayed after emergence of a crop, to control weeds. For IP, a new gene for producing toxins for certain insects is inserted in the plant. Therefore the insect damage is less and the farmers can use less insecticide.

1.2. Concerns about GMO's

Besides the benefits of the GMO's, there are also many concerns. Because the lack of knowledge about GMO's, and the concerns about long term effects on human health and the environment, a strong public and scientific debate on GMO's is going on.
One of the concerns from people about GMO's is the possibility that the toxins of IP crop may harm non-target organisms, such as the Monarch Butterfly (Calvin 2001, Powell 2000, Losey 1999). A second problem is the chance that the specific insect will develop resistance against this toxin. Besides that, the escape of the foreign genes through pollen dispersal to their weedy relatives creating super-weeds, or causing gene pollution to other species could become a problem (Meyer 1999a, Daniell 1999). In addition, there is a possibility that foreign DNA could be integrated with the cells of rodents (Einspanier *et al.,* 2001) and also possibly farm animals or humans. Another concern is the quality from GMO's as food and feed.

1.3. Introduction of a GMO on the market

Before a GMO is accepted and can be introduced on the market, the plant is tested for food safety and chemical composition to show that the composition of the plant has not changed. The rules for bringing GMO's on the market are strict.
The company, requesting permission for the new GMO must create a dossier about the new GMO according to the guideline 90/220/EEC for animal feed and regulation 258/97 for food. Guideline 90/220 will soon be replaced by guideline 2001/18. National and European commissions like the Scientific Committee on plants (SCP) evaluate these data. The data are mainly judged on:

- Nutritional value of the product
- Safety of the product
- Possibilities of detection the foreign DNA and protein in the final product
- Influence on the environment
- Safety of the new protein
- Known toxic connections in the original organism
- Possible allergenic reactions
- Origin of new genes
- Molecular characterisation

These safety reports mainly compare the GMO with parental crop and investigate the origin of the new protein. There is a lot of comment on these safety tests. The GMO's are not tested for long term effects on the environment. These tests neither deal with the long-term effect on animals and humans, nor with indirect production qualities.
This leaves space for more critical questions to be raised.

1.4. Facts and figures about the use of GMO's

The main GMO plants used all over the world, were HR soybean (54 % in 1999) and Bt corn (19%) (James, 1999). In the US, 27% of the corn, 54% of the soybean and 55% of cotton were biotech (GMO) crops (Faust 2000).
James (1999) showed that between 1996 and 1999, the area of GM crops grown globally increased from 1.7 to 39.9 million ha. While North America and Argentina were responsible for the vast majority of the area grown, (72 and 17 % respectively, in 1999) China, Australia, South Africa, Mexico, Spain, France, Portugal, Rumania and the Ukraine also grew transgenic crops. In the European Union, France and Spain grew 1,000 and 30,000 ha of Bt-maize, respectively, while Portugal grew 1,000 ha of Bt corn for the first time in 1999. The main IP crop, the Bt-crop, is protected against the European Corn Borer (ECB).
Infections with ECB result for farmers in the US and Canada in $1 billion yield loss per year loss (about 7% yield loss, (Bathia *et al.*, 1999)) and expenditures. The use of Bt corn is less in silage producing areas. Bt-hybrids are less adapted to these areas and there is less European corn borer (ECB) pressure in these states (Meyer 1999b).

1.5. The main question of this study

All kinds of alarming sounds came, and are still coming, from farmers, mainly in the USA. These farm observations indicate that farm animals won't eat the GMO food as well as control food, and try to do what they can to avoid eating it. Wild animals, such as deer, don't touch the GMO food, which results in less deer damage. Because animals are capable of choosing a balanced diet when they have a choice, they choose what is right for them (Forbes 1995, Emmans 1991). Therefore the choice of the animal might tell something about the quality of the food.
These observations combined with concerns about GMO's, raised the main topic for this study, "Do animals have food preference for GMO or non-GMO?".

The question is complicated and therefore not easy to answer. The preference of animals for a specific food is influenced by factors from the environment within the animal, the food and factors of the environment the animals lives in. it is also important to know what a preference means, when an animal does have a preference. Food preference can be a parameter for food quality.
Besides the complex part of the animal also one GMO is not the same as another GMO, and results for one GO cannot be extrapolated to GMO's in general. Before an experiment can be done to try to answer this question, all the available information about this subject should be collected. Therefore a literature study will be done to collect all necessary information as a base for experiments that may answer this question.

1.6. Methods of research

Background information about food intake and GMO's will be studied.
All the experiments done with animals fed GMO's will be studied, and compared, to investigate the knowledge about the performance and possible preference of animals for GMO's or non-GMO's.
The experiments will be sort by type of experiment, chemical analyses and by animal to compare the results and the design of the experiments.
Besides the published literature also farm observations will be collected and studied, for a possible indication of food preference for GMO's or non-GMO's.
By integrating the background information and the done experiments, recommendations for further research will be made.

2. Background information

2.1. GMO's, Genetic Modified Organisms

Genetic modification is changing the genetic structure of an organism by adding a specific gene. When the organism is in the one-cell stadium, a piece of DNA, (a specific gene) is added to the cell. The organism starts growing and the new gene provides the organism with a specific quality. Because of the novelty of this process, not everything is know yet, and since the modification is still partly trial and error, organisms can be changed with specific derived properties.
GMO's are used in very different production systems, both in industrial as well as in agricultural. In this study, the changes made in crops fed to animals are important.
Crops can be genetically modified for different reasons. Such as:

- Protecting crops from insects or natural stress such as drought, salt soil, or making them resistant to herbicides
- Decreasing substances that decrease the digestibility, like tannins, ANF's
- Changing substances in the crops, (amylase free potatoes, glucose instead of starch in maize), for more effective use in the industrial production-process

This study report experiments carried out with insect protected (IP) and herbicide resistant (HR) crops. Because these crops were changed genetically without the intention to change the nutritional value, and these are the most commonly used GMO's fed to farm animals. These changes, IP and HR, are encoded by a single gene, and called the first generation GMO's. In the last few years, more genes have been involved in the modification, the second generation.
In this report, the term GMO will mean the modified crops used in the experiments.

2.1.1. Herbicide resistant crops

Herbicides are used for weed control. Weeds can account for great yield losses. A variety of non-selective or selective herbicides are used. Some herbicides that could not be used after emergence of a crop because of toxicity to the plant, can be used if the crops are made resistant to these herbicides (Kleter *et al.,* 2000).
An explanation of the modifications used more frequently in herbicide resistant GMO's will be given below.

Glyphosate resistance (Roundup Ready)
Glyphosate acts by inhibition of a plant enzyme, 5-enolpyruvyl-3-phosphoshikimic acid synthetase (EPSPS). This enzyme is involved in the biosynthesis of aromatic amino acids, vitamins, and other secondary plant metabolites in plants and microorganisms (Kleter, 2000). EPSPS is present in plants, bacteria and fungi, but not in animals (Levin and Sprinson 1964, by Sihdu 2000). In plants, EPSPS is localised in the chloroplasts or plastids (Della-Cioppa *et al.,* 1986, by Sihdu 2000). The introduction of a glyphosate-tolerant EPSPS gene, derived from the common soil bacterium *Agrobacterium* sp. CP4, forms the basis of the Roundup? tolerance when expressed in GM plants.
Expression of mEPSPS from the corn, fused to an optimised transit peptide enables targeting of this protein to chloroplast, thereby conferring glyphosate tolerance to the

corn plant while meeting the plant's needs for the production of aromatic amino acids. (Sihdu *et al.,* 2000).
Glyphosate resistance is already tested in beets (fodder beets, sugar beets) canola, cotton, maize, oilseed rape and soybeans.

Glufosinate resistance (Basta, Liberty and Finale)
Glufosinate ammonium is an industrially produced herbicide, stereoisomeric mixture of ammonium salts of l- and d-phosphinothricin (PPT). Glufosinate ammonium inhibits the plant enzyme glutamine synthetase (GS) an enzyme essential for the assimilation of ammonia. Inhibition leads to the build-up of toxic levels of NH_4^+ in plant tissues.
In modified crops, so-called PAT or BAR genes, are inserted. These genes encode an enzyme called phosphinothricin acetyl transferase (PAT), which inactivates PPT by acetylation, and protects the plant against the toxic action of PPT.
The PAT gene is derived from the soil bacterium *Streptomyces viridochromogenes*, and the similar BAR gene is isolated from *Streptomyces hygroscopicus*.
Glufosinate resistance is already tested in green/red chicory, maize, oilseed rape, rice, sugar beets, sunflower, tomatoes and wheat.
(Kleter *et al.,* 2000, Wilmink and Dons, 1993, Flachowsky 2000)

2.1.2. Insect protected crops
Bacillus thuringiensis
Insects, bacteria and fungi are the greatest sources of predation in agriculture. To reduce losses, crops are regularly sprayed with insecticides. The ECB, *Ostrinia nubilalis*, is one of the most damaging insect of corn throughout the United States and Canada. The EBC pressure can be different every year. In 18 tests over the last six years, Iowa State University researchers saw losses of 4 bu/acre or more from 94 percent of the fields they examined due to the EBC (Dekalb, 1998, by Powel 2000). *Bacillus thuringiensis* (Bt) is a naturally occurring soil bacterium used as biological insect control already since the 50's. The bacterium produces crystalline inclusions, consisting of Cry or Bt toxins. Different Bt strains produce different toxins for the control of certain insect species among the orders Lepidoptera (for example the ECB), Diptera and Coleoptera.
After ingestion by the insect the crystals are cleaved by proteinases, resulting in products with toxic activity. The receptors in the mid-gut of the insect recognise these ?-endotoxins. These receptors are found in many species of animals. After binding, pore formation may occur, after which lysis starts. This pore formation, causing the death of the insect, only happens in insects, targets of these bacteria, and not in all the animals who have these receptors (Schnepf et al 1998; De Maagd et al 1999, 2001).
The plasmids exist of many different genes that code for different proteins. In almost all of these crops, either the Cry1Ab gene to control the European Corn Borer (ECB) or the Cry1Ac gene to control larvae of the tobacco bud worm and cotton ballroom are present. (Kleter *et al.,* 2000)

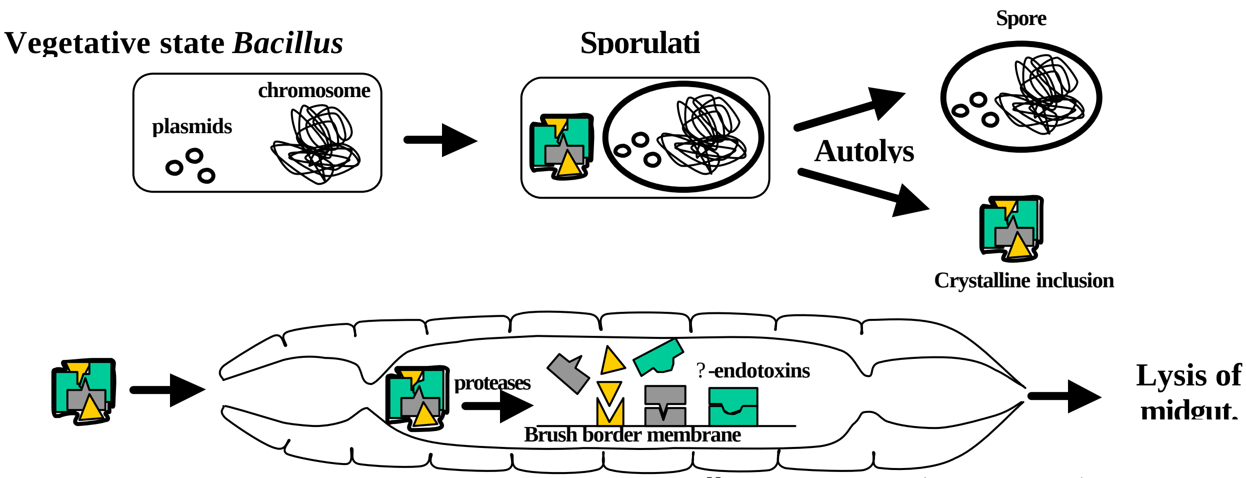


Figure 5: A schedule that explains the working of the *Bacillus turingiensis (Groot, 2001).*

Toxins for non-target insects

The toxin produced in Bt corn can also be toxic for non-target organisms.
Losey *et al.,* (1999) studied Monarch caterpillars fed milkweed leaves artificially coated with pollen from Bt corn or normal corn. The Monarch fed with Bt pollen coated leaves ate less, grew slower and suffered a higher death rate than larvae that consumed milkweed leaves free of corn pollen. Pollen from the Bt-corn could kill monarch caterpillars in laboratory tests. Also Powell (2000) found that the Bt-toxin from Bt-corn is active against the Lepidoptera family of moths and butterflies including the Monarch butterfly. There is some research going on about how the infestation of the milkweed is around Bt fields, and how it affects the Monarch caterpillars (Rice, 1999).
The volume of pollen falls sharply just a few feet away from the cornfields. Sears *et al.,* (1999) determined that 90 percent of pollen grains travelled less than 5 meters from the field edge. Also Rice (1999) found that the Bt pollen is relatively heavy, so doesn't drift too far from the Bt fields.

Bt pollen are toxic for Monarch caterpillars, (Powell 2000), but how far it is a risk in the environment is still investigated. Also other studies have been done on the effect of Bt toxin on other non- target organisms (Pilcher *et al.,* and Hilbeck *et al.,* both by Rice *et al.,* 1999) with positive and negative results.
However the use of Bt is replacing the use of insecticides for ECB control. A reduction in broad-spectrum insecticide use should be beneficial not only for the monarch but also for many other insect species (Rice 1999).

2.1.3. Events and expression

Table 1: The trademarks, events and expression in insect protected and herbicide resistant GMO corn (Mireles, 2000b combined with table Munkvold *et al.,* 2000)

Traits	Trademark	Events	Expressed protein	Promoter	Expression
Corn Borer Protection (IP)	Knockout	E176	Cry1A	PECP+Pollen	Green tissue+pollen
	NatureGard	E176	Cry1A	PECP+Pollen	Green tissue+pollen
	YieldGard	Bt11	Cry1A	CaMV	All Tissue
	YieldGard	MON810	Cry1A	CaMV	All Tissue
	BtXtra	DBT418	Cry1A	CaMV	All Tissue
	Attribute	Bt11	Cry1A	CaMV	All Tissue
	StarLink	CBH351	Cry9C	CaMV	All Tissue
Glufosinate tolerance (HR)	Liberty Link	T25	PAT Protein		
	GR	DLL25			
Glyphosate tolerance (HR)	Roundup Ready	GA21	MEPSPS protein		

In table 1 the different trademarks with the expressed new protein from GMO-corn are shown.
The events are the different genes brought into the plants. It seems that different genes encode for the same protein, but the protein name it is a generic term. There are more than 5 different Cry1A proteins and more than 20 Cry proteins (Schnepf *et al.,* 1998).
The promoter is a small piece of DNA, which regulates the expression of genes in the new plant.
YieldGard Bt11 and YieldGard MON810 use a cauliflower mosaic virus, CaMV, 35S gene promoter that results in a season-long expression of CryIA(b) in all plant tissues. CryIA(b) transformation 176 marketed as Knockout and NatureGard use a combination of two maize derived tissue specific promoters, PECP and pollen, that result in gene expression only in green plant tissues and pollen (Munkvold *et al.,* 2000).

Here is shown that even two hybrids of Bt-corn can be different in several factors.
In this chapter only a few herbicide resistance and insect protected GMO are discussed. There are many other GMO's also other GMO-crop. One GMO is not the same as an other GMO and results from experiments with one GMO cannot be extrapolated to other GMO's.

2.2. Food intake

2.2.1. Introduction

Feeding is a behavioural component of a physiological and physical process. Food selection and amounts eaten are responses to two types of metabolic demands. First supplies of carbohydrates, fats and proteins as interchangeable sources of metabolisable energy are required to cover expenditure. Essential amino acids, vitamins and minerals also must be provided by feeding in proportion to their rates of catabolism (Le Magnen 1992 by Forbes 1995).

Voluntary food intake is the amount eaten by an animal or group of animals during a given period of time during which they have free access to food (Forbes 1995).
Animals eat to optimise their comfort and to obtain a proper energy balance.
Food is a source of energy as well as material nutrients (Emmans in Kyriazakis 1999a).
Food intake and the regulation of energy balance are unlikely to be regulated by any single mechanism. Many factors influence food intake. Most factors act in a negative feedback manner. Factors like stomach distension, hypothalamic temperature, blood glucose concentration, body fat stores and plasma amino acids can all control intake to match requirements (Forbes 1995), so factors of the animal as well as factors of the food influence food intake.

2.2.2. General model

The effects of intake on stimulation of different groups of receptors are mostly additional and finally result in termination of the food intake. These receptors are metabolic or physiologic (Forbes 1995, Forbes 1996).
Several models have been developed to try to simplify the manner in which feed intake is regulated so as to try to understand it (Forbes *et al.,* 1977a, Forbes 1983, Vahl 1979, Steffens 1978, Morgan *et al.,* in Kyriazakis 1999).
Some models are shown here to explain the basics of food intake.
Forbes (1977a) (Figure 1) incorporated energostasis and physical limitation in a model of ruminant intake. It assumes that sheep (Forbes 1977a) and lactating cows (Forbes 1977b by Forbes 1995) will eat sufficient metabolisable energy to meet their requirements for maintenance, production and fattening, unless physical limitations interfere.

Figure 1 shows the flow diagram for the adult sheep model. The intake of food supplies energy to the body pool, which is utilised for maintenance, pregnancy, lactation and fattening. Food intake also leads to stomach distension and this is compared with the abdominal space available to determine a physical limit to feeding. Schettini *et al.,* (1999) found that mass and volume of ruminal contents have a significant effect on voluntary food intake of ruminants consuming low-quality forage diets. The limiting factor of the food intake is the metabolically controlled value, namely protein, fat and energy, or the stomach distension.

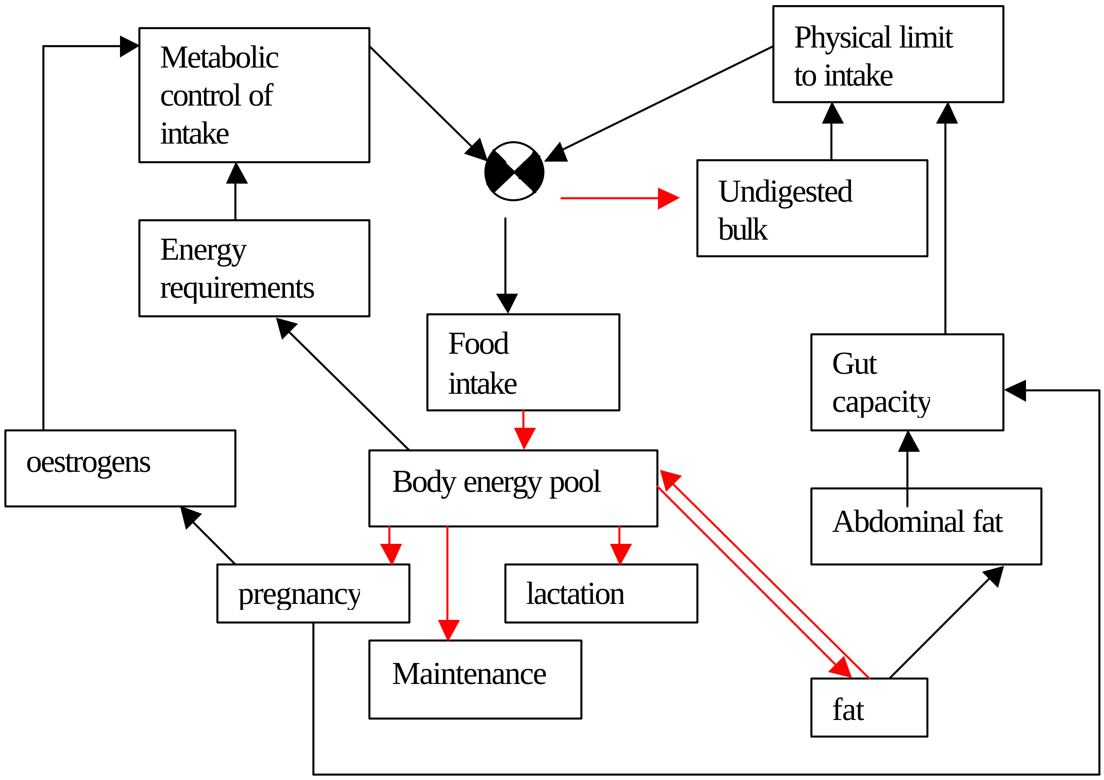
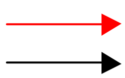


Figure 1: Systematic diagram of the model use to predict voluntary food intake of sheep (Forbes 1977a)
The sectored circle indicates a comparator, which uses whichever level of intake is the lower.


Energy flows
Passage of information

An important principle in this model is that fat deposition or fatness is seen to be driving food intake as it is assumed that a sheep tries to deposit 100 g of fat per day if the quality of the food is good enough to avoid physical limitation of intake. Also Scharrer (1991) reviewed that there is an important interaction between body fat and voluntary food intake. Changes in metabolic and physical factors are known to affect voluntary food intake in short-term experiments. This model was developed to see whether these factors effect the voluntary food intake in the long term (Forbes 1995).
The same kind of model for food intake for fish was shown in Vahl (1979).
Flachowsky (1989) found that roughage intake is primarily controlled by physical means, while the intake of more concentrated diets is controlled mainly by metabolic factors.
Feeding is essentially periodic in nature, but at cellular and whole-body levels the energy output is continuous. It requires an uninterrupted supply to the tissues of energy metabolites, and a constant level of one of the energy-yielding metabolites, namely glucose.
The discontinuous intake is made possible because the energy is drawn from three body energy stores, namely, hepatic and muscular glycogen store, a gastrointestinal store, loaded by each oral intake and the large capacity of body fats.
From experimental studies it has been concluded that the consumed food is not all used directly in the feeding supply or fuel to tissues, but it is involved in filling these stores periodically in response to their depletion (Le Magnen 1992 by Forbes 1995).

2.2.3. Feedback signals

Motivation is a reversible brain state induced by internal and external signals, which results in an increased tendency to perform a specific behaviour (Lawrence and Rushen, 1993 by Forbes 1995). An animal's drive to eat is primitive and powerful and this motivation increases as metabolic demands increase. Higher animals have satiety factors that can override this drive temporarily. Feedback signals and the central nervous system (CNS) regulate this energy balance.

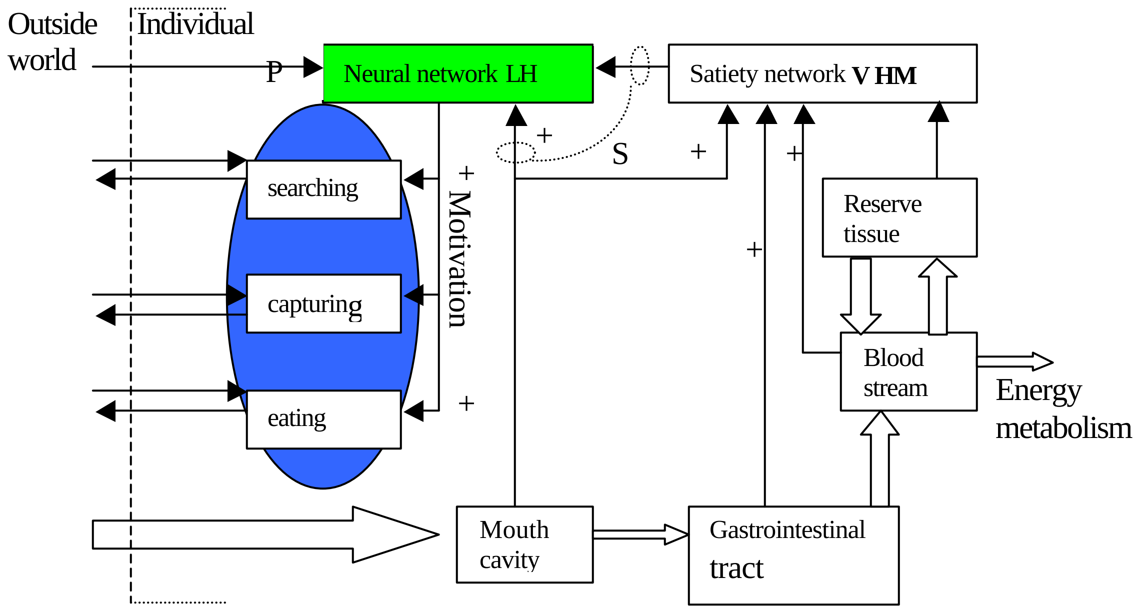
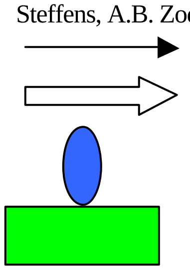


Figure 2: Feeding behaviour system sub-serving caloric regulation
Steffens, A.B. Zodiac Symposium, 1978 after L. de Ruiter, Progr. Brain Res. 41 (1971)



Messages (neural or hormonal)

Fuel transport in the body

Central nervous mechanisms

Neural network facilitating these elements in LH and elsewhere

P attractiveness of general external food situation
S net satiety, i.e. resultant of positive and negative feedback signals
Motivation = specific motivation for feeding behaviour
Performance of SCE (searching, capturing and eating), modifies sensory input from outside world, and on the other hand causes food enter to the mouth cavity. Interactions effects are disregarded in this model.

Neural, hormonal and metabolic signals inform the hypothalamus in the CNS of the body's internal enviroment and are integrated with information on conditions in the external environment. The resulting hormonal profiles provide a background on which behaviours and the relative activity of the sympathetic and parasympathetic nervous system contribute to the existing endocrine compliment (Savory 1982).

Figure 2 is a model showing the flow of information to the ventro-medial (VMH) and lateral hypothalamus (LH). The activity in the VMH, satiety area increases when insulin levels increase, provided normal amounts of glucose are present; whereas activity of the LH (lateral hypothalamus) neurones decrease.
During a meal a positive feedback mechanism is at work for the continuation of a meal, (Wiepkema 1971, by De Leeuw 2000).
Termination of a meal is probably due to an increase of insulin and glucose levels and these acts as negative feedback system. The duration of the meal depends on the balance between positive and negative feedback system at the hypothalamic level.

The stimulation of the oropharyngeal receptors during eating creates a positive feedback system. At the same time, stomach and gut wall receptors send information about distension to the hypothalamus. These are the same food intake limiting factors as seen in figure 1. The measuring of calories in the blood is also an important aspect that contribute to the nutritional homeostasis (Steffens, 1978).

One of the main hormones involved in the meal ending is the intestinal hormone CCK. Central endogenous CCK, produced in the duodenum, as a reaction on the presence of mainly fats and proteins, plays a role in the control of food intake.
Many functions of this hormone are already known, but the exact working of CCK for satisfaction is not clear yet (De Leeuw 2000).

2.2.4. A new theory of feed intake regulation: Oxygen Efficiency

The previous models are very common and generally accepted, but there is another approach. Oxygen and food consumption has a natural link in the release of energy for maintenance, growth and production. Both involve active processes to control the rate of uptake of oxygen and food. Feed consumption has positive and negative outcomes, both benefits and costs. Costs are represented by the total oxygen consumption of the animal. The ratio between benefits and costs is calculated as the oxygen efficiency of feeding behaviour. Voluntary energy intake corresponds to the feed consumption level at which oxygen efficiency is maximal (Ketelaars and Tolkamp 1996).

2.2.5. Metabolic illness

Animals learn to associate the sensory properties of foods with the metabolic consequences of eating those foods. When the animals do have the time and the choice between different sorts of food, they can choose a good diet by experience (Forbes 1995, Kyriazakis *et al.*, 1991, in Kyriazakis 1999a). Wild animals, and so the ancestors of our farm animals, can select food from a range of available foods and are able to select a mixture that allow them to grow and produce (Forbes 1995).

A common feature of all deficiencies is that they interfere with normal metabolism and lead to feelings of metabolic illness, and it seems likely that it is an innate response of animals to reduce their intake of a food which makes them feel unwell. In nature the animal could turn its attention to other sources of food but in the intensive husbandry no such opportunity is available. The only option for the animal to eat less in attempt to relieve the metabolic discomfort (Forbes 1995, Scharrer 1991).

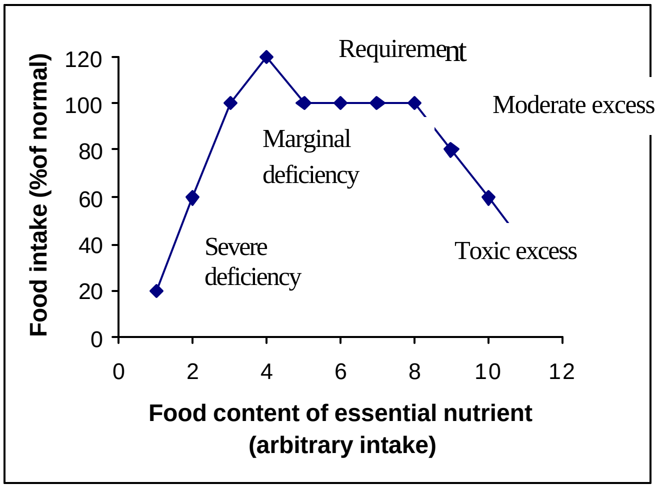


Figure 3: General diagram of the effects on voluntary intake of excesses and deficiencies of a nutrient in food (Forbes 1995)

Figure 3 is a general diagram of responses to changes in the content of an essential nutrient. For foods with a content of the nutrient just below the requirement there is sometimes a modest increase in intake as the animal tries to maintain its intake of the nutrient in question. Below this, metabolic illness occurs with consequent depression of intake. For foods with a content which is above the requirement there is little or no effect on intake until such a high content is reached that intake is depressed due to the toxic effects of the excess (Forbes 1995). Kyriazakis *et al.,* (1990) also showed that animals can select their diet. Young pigs restricted in energy or protein between 9 and 16 weeks had greater feed intake and selected higher levels of protein until body composition was similar to control animals.
Fairley *et al.,* (1993, by De Leeuw 2000) showed that pigs can select a diet based on amino acid concentration, but they were not capable of select a balanced diet for amino acid intake. They were capable of selecting a balanced protein diet (Kyriazakis *et al.,* 1990). The diet selected will be such that the animal meets its requirement change over time (Kyriazakis 1999a and Kyriazakis *et al.,* 1990).

If animals are satiated with i.v. glucose, they are not absolutely satiated. This is also called "sensory specific satiety". When a novel food is offered to rats, they immediately start to eat without the transient decline in blood glucose that precedes a spontaneous meal of familiar food (Campfield and Smith, 1986 by Forbes 1995). Animals choose to eat a variety of foods when none of them is aversive.

2.2.6. Learning

Food intake and feeding behaviour are affected by learning and the animal's internal state.

Since it is only the consumption of food that leads to a change in internal state, the animal is expected to learn quickly to differentiate between that which is good food or not.

It is generally accepted that animals have developed behavioural mechanisms to allow them to recognise foods on the basis of their nutritional, as well as other properties, and to include foods in their diet according to the post-ingestive consequences that they have. If the animal has a negative (or positive) experience around the time a novel food has been eaten, this food becomes aversive (or a preferred).

The rate at which animals learn about foods, and for how long this knowledge is retained, depends largely on the degree of the animals deficiency and on the extent of post-ingestive consequences by the foods. (Kyriazakis, 1999b). The learned associations are very important for the choice of food the animal makes.

Familiarity with a diet, it's accessibility, and choice among foodstuffs also influence intake of a given feed. Most animals exhibit neophobia, fear for new things, when confronted with a new diet, especially when in a new environment (Beverly 1997). Lambs fed an inadequate diet have stronger preference shifts than lambs fed a balanced diet (Early and Provenza 1998).

Internal State

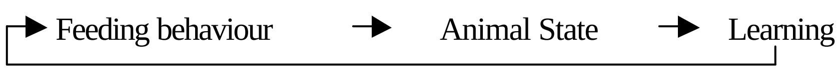


Figure 4: A framework considering the way which learning and animal state affect feeding behaviour (Kyriazakis *et al.,* 1999b)

Figure 4 shows a framework of feeding behaviour that shows both feed intake and diet selection as an outcome of the animals internal state and knowledge of feeding environment. There is little evidence that animals modify their diet selection in response to the very short-term fluctuations during the day. Long term changes in the internal environment, because of growing or reproduction cycles, lead to long term changes in their diet selection (Kyriazakis 1999b).

Learning from other animals

The food preferred by the newly weaned animals is affected by their mothers diet, possibly by transmission of flavours in milk. Young pigs accept food more readily when it has the flavour of their mothers milk.

When pigs, cows and chickens hear or/and see another animal eating (suckling) they will eat in synchrony, which is often called social facilitation of feeding.

Lambs retain dietary preferences learned through observation of their mothers or from other adults. By giving the ewes grain for 3 days before the lambs are weaned, the lambs will eat grain within 3 days when offered 18 months later, while inexperienced lambs needed a 3 week adaptation period before they ate the grain (Lynch and Bell 1987 by Forbes 1995), but it must be the same grain (Mottershead *et al.,* 1985, by Forbes1995).

2.2.7. Motivation

Feeding motivation is a complex interaction of metabolic and environmental factors as shown above. The feeding motivation has been widely used in studies of feeding behaviour because it simplifies a complex interaction of both metabolic and environmental factors into one interfering variable (Day *et al.,* 1996).
The traditional method for quantifying an animal's preference for one food-item relative to another is the two-bowl test in which the animal chooses between eating from different bowls containing two foods. However this methodology depends on the animals consumatory behaviour very heavily (Rashotte and Smith, 1984).
The most widely used method is operant conditioning, where an association is formed between a behavioural response and a motivationally significant reinforcer.
It is possible to measure how hard an animal will work to obtain a food item using instrumental reward training. Skinner started this operant conditioning.

The intensity of the operant response is thought to closely reflect the motivation to obtain the reinforcer, mainly the level of operant responding that an animal is prepared to perform to gain access to food.

The animal's drive to eat, how hard an animal will work to obtain a specific food item, is a known parameter for the preference of the animal to that specific food. To measure this motivation different techniques were used
By using instrumental reward training, operant conditioning, like Skinner started, it is possible to measure this motivation (Rashotte and Smith 1984, Day *et al.,* 1996)

2.2.8. The sensory properties of food

When animals can chose between several foods, it is necessary that there are sensory differences, a clear clue, otherwise they cannot select their diet (Forbes1995). Natural stimuli are more effective than artificial ones.
The sensory properties of a food are as likely to encourage further feeding, work positively, as they are to discourage stop feeding, work negatively.

Olfaction

Odour of a single food does not affect the level of intake. Smell is used when selecting from range available foods. When given two bins of food, one tainted with odours of carnivore faeces, sheep took 95% of their intake from the uncontaminated pellets (Pfister *et al.,* 1990, by Forbes 1995). Bell and Sly (1983, by Forbes 1995) found that in cattle smell and taste are separate.

Texture

In conjunction with sight, smell and taste of a particular feed, texture is an additional clue in characterising food. Memories of chewing pressures and number of swallows help to recall how much food to eat for satiety (Miller and Teates, 1986, by Forbes 1995).
The form in which the food is presented also has an influence on the food intake.

Sheep selected more alfalfa pelleted than alfalfa long chop (Cooper *et al.,* 1996). Also Flachowsky (1989) found that the voluntary intake of ground and pelleted roughage increased with the decreasing particle size in growing bulls.
Pelleted forages are usually eaten in greater quantities than the same material in unpelleted form (Heaney *et al.,* 1963, by Forbes 1995). Chickens prefer food in pellet form (Lanson and Smyth, 1955, by Forbes 1995).
Although much of these increases are attributable to the reduction in particle size with pelleting, some improvement in palatability is also involved (Van Niekerk *et al.,* 1973, by Forbes 1995).

Studies of the chewing behaviour of pigs further confirm that an individual is able to respond to specific nutrient deficiencies, but also that a higher level of feeding motivation may increase the reinforcement value of a goal (Cabernac and Ferber 1987, by Day *et al.,* 1996a). This is strengthening the continuing argument that animals are able to closely assess the consequences of their feeding behaviour (Rushen and De Passile, 1995, by Day 1996a). The results from this study support the hypothesis that growing pigs can acquire nutritional information during exploratory chewing (Day *et al* 1996a).

Different species use different sensors to identify food.
Without visual or taste cues, animals cannot identify the appropriate diet. The same cues are important for learned preferences.
Rats use more taste than vision.
For *birds* vision and colour more important than taste and odour.
For *pigs*, taste as well as odour is an important cue, when more than one food is offered.
Sheep use smell, taste and tactile stimuli to discriminate between different plant species. When they discriminate on different hue, it is more to brightness rather than colour. Animals like sheep, goats, cattle and chickens can make quite complex discrimination between shapes. The cells that respond to the sight of food only respond to palatable food. These sites in the brain are probably associated with rewarding aspects of stimuli, but not their negative aspects.
Cattle use smell and taste as cues, but they only associate the feeling of illness with the new food as the consequences follow immediately after ingestion (Zahorik and Houpt 1981, by Forbes 1995)

2.2.9. Palatability
Gherardi *et al.,* (1991 by Forbes 1995) concluded that palatability effects are not important in determining the level at which a single forage is eaten, but can have marked effects on the relative intakes when two forages are offered. These effects are important as an indicator for the metabolic value of the food and will stimulate or stop the animal from eating the food.
Forbes (1995) presented ewes with three silages, A and C were of equal quality but B was of poor quality. B was eaten in the same quantities as A or C when the ewes had only access to one food. However the group that had access to all three ate equal amounts of silage A and C but very little of silage B (Forbes 1967, by Forbes 1995).

However it is quite possible that these sheep were eating according to learned rather than to innate preferences as they had experienced eating just hay or straw in some periods of the experiment.
Once an animal learns that a food item is unpalatable it avoids it. However, from time to time they sample food to which they averse, presumably so that they can be made aware of any changes in its properties (Forbes, 1995).

2.2.10. Taste

Taste is a powerful cue to associate the food with their nutritional properties, and contributes to the development of learned aversion to a diet (Houpt *et al* 1979, by Beverly 1997).
Taste receptors are mainly involved in food selection, and they do not seem to be important in the control of feed intake (Scharrer 1991).
Preference of a flavour can be very selective, but when the animal learns there is no nutritional implication of the different flavours, the influence on the intake by the flavour is rapidly gone (Forbes 1995).
Studies have indicated that animals have a strong preference for both nutritive and non-nutritive sweet solutions (Kennedy and Baldwin 1972, by Day *et al* 1996a) The effects of non-nutritive sweeteners such as saccharin are often relatively temporary (Aldinger *et al.,* 1959;Birritt and Provenza, 1992, both by Day 1996).
The preference for sweetness per se could reflect a strategy to obtain dietary energy. However, the absence of increasing reinforcement over time is consistent with theories of nutrition which suggest that animals are able to sense the efficiency of their foraging behaviour in correcting specific nutrient deficiencies present in their internal state (Kyriazakis 1994, by Day *et al.,* 1996a)

Rats

When a choice is given, the rats prefer fluid with oil. They also have a specific appetite for NaCl (Denton 1982, by Kyriazakis, 1999a).

Chickens

Chickens have only a few taste buds compared to other species, but they have a good sense of taste. Some flavour preferences are very strong; they will not drink saccharin solutions but take sucrose or glucose (Injidi 1981, by Forbes 1995)
Laying hens have specific appetites for Ca (Hughes 1979, by Kyriazakis 1999a).

Pigs

Sugar and other flavouring ingredients are widely used in weaning and creep foods for young pigs to attract their interest in solid food and encourage high levels of intake before and immediately after weaning (Forbes 1995).
Pigs are quite sensitive to taste and their preference to glucose or a sucrose solution increases up to 0,1 M (Kennedy and Baldwin, 1972 by Day *et al.,* 1996a).
Some flavours can be useful to overcome the stress of weaning (King 1979 by Forbes 1995).
When given a choice, pigs usually avoid, but not always, the foods that contain glucosinolates (Kyriazakis *et al.,* 1993 by Forbes 1995)

Cattle
Cattle are sensitive to bitter, salty, sour and sweet solutions (Goatcher and Church 1970 by Fores 1995), and they have greater sensitivity to taste than sheep.
Animal grazing preference is related to the concentrations of non-structural carbohydrates. Mayland *et al.,* (2000).
An increase of water soluble carbohydrate content of the grass leads to an increase of dry matter (DM) digestibility and DM forage intake (Miller *et al.,* 2000).
Sheep
Sheep and goats are sensitive to bitter, salty, sour and sweet solutions.
Goats are intermediate between sheep and cattle.
Following a change in flavour, even without changes in nutritive value of food, sheep sample the food cautiously (Provenza *et al.,* 1993a, by Forbes, 1995).
Cooper and Kyriazakis (1996) offered foods with different nutrient densities but similar metabolisable energy: crude protein (ME:CP) ratios to growing lambs: all choice fed lambs ate some of the poorer food and it was suggested that the better foods increased rumen osmolality or reduced pH to an uncomfortable extent.
There is ample evidence that ruminants do not eat for maximum efficiency, but strive to maintain sufficient intake of long fibre to ensure proper rumen function.
Sheep prefer feed supplemented with $NaHCO_3$, and it can stimulate the intake of high energy density feeds (Cooper and Kyriazakis, 1996). They also found that sheep prefer as dietary carbohydrate source barley above sugar beet/barley.

2.2.11. External signals that can affect the food preference

Environmental temperature
Every animal has a thermo-neutral zone. Below this zone the heat production must be increased to maintain body temperature and intake rises to provide substrates for increased heat production. Above this zone, the body temperature rises and food intake decreases in order to reduce the heat production associated with feeding, digestion, absorption and metabolism in order to prevent an excessive increase of body temperature (Flachowsky 1989).
The short-term results can be misleading because, as acclimatisation to hot weather usually gives the opportunity for compensatory intake in the cool of the night (Forbes 1995).

Photo periodicity
Poultry do not normally eat during darkness. However, photoperiodicity has a different influence on different species of animals.
Long daylight stimulates food intake (Forbes 1995).

Exercise
In the short term, exercise tends to reduce intake rather than increase it in conjunction with the increase in energy expenditure. The reduced time spent eating could be due to either fatigue or stress.
Long-term exercise must result in compensatory increase in intake. Otherwise hard-working animals would die of underfeeding (Forbes 1995).

Social structure

An animal's individual performance depends on its social rank and its access to critical sources (McBride 1968 by Curtis 1998). Because pigs want to have control over vital resources, a social order will establish (Curtis 1998). This social tension may reduces the voluntary food intake by pigs (Patterson 1985 and Gonyou *et al.,* 1992, both by Curtis 1998). Morgan *et al.,* (by Kyriazakis 1999) showed in a review food intake of individually penned animals being higher than those kept in a group. Also Forbes (1995) and Curtis *et al.,* (1998) mentioned that as group size increased, food intake declined in pigs.
Many different factors like physical activity, temporal environment, number of feeding spaces, different feeding behaviour, seeing other animals eating, are discussed. However they concluded that the considerable variation among group housed animals cannot explain the overall lower food intake. The effects probably a result of the many factors that are associated with group size (e.g. stocking density/space allowance) or are consequences of grouping *per se* (i.e. single pigs versus groups).
The diet selection from group housed animals is not as balanced as individually housed animals (Forbes, 1995,personal note Verstegen 2001).

2.2.12. Conclusion

In nature many animals are faced with a variety of foods, some of which they are able, and prepared to eat. Possibly, wild animals can select a diet more adequate than farm animals because they are selected already for a long time on efficient foraging.
As the foods may differ in their nutritional values the diet that the animal attains will vary with the selection made from the foods on offer. The relevant variables of diet selection are the animal, the characteristics of the feeds on offer, and the environment within which the animal is kept (Emmans 1991).
In this chapter all different factors of food intake and food preference are discussed, showing that the system is very complex.

Do eat animals what is good for them?

Above is described that animals can learn to associate the taste, smell or colour of a food with the feelings they experience when they have eaten that food. This shows the ability of animals to select from a range of foods to best meet their nutrient requirements.
The appetite is a first drive to eat a particular food, and the history of he animal, the learned associations, affect the food intake. The environment in which the animal lives also influences the food intake.
An animal can choose the right balanced diet, but many the circumstances affect the choice of the animals. So the animal will choose its own diet but this is not necessary the most balanced, but adequate according to the animal in the given circumstances. Even when an animal does not have a choice the animal will take the amount that is adequate, according the animal, in the given situation.

3. Experiments with animals and GMO's

Experiments to compare the performance of animals fed GMO or non-GMO, even as experiments to examine the performance and or safety of feeding GMO's to animals were studied. A summary where and when these experiments are done is given in table 2. Most of these experiments were done with insect protected or herbicide resistant plants. A few GMO's where the nutritional value of the corn was changed were tested.
The results from the experiments are sort by type of experiment, chemical analyses, and by animal, in different paragraphs in this chapter. This will help with the comparison of the results and designs of the experiments. Conclusions made in the articles, and conclusion made by comparing the articles within one table is shown in this chapter.

Table 2: Overview of experiments done with GMO's and animals

Species		Exp.	Research Institute	Place	Year
Cattle	Beef	9	Iowa State Univ., 2 Purdue Univ. 3 Univ. Nebraska, FAL, Iowa State Univ., Univ. Missouri,	8 US, Germany	1999, 5: 2000, 3: 2001
	Dairy	5	Monsanto, Univ. Nebraska, Purdue Univ. INRA, Iowa State Univ.	4 US, France	1997 2:1998, 2: 2000
Sheep		3	INRA, 2 FAL	France, 2 Germany	2:1999, 2001
Pigs		7	2: FAL, 4:Univ. Missouri, Purdue Univ.	2 Germany, 5 US	1999, 6:2000, 2001
Chickens	Layers	2	2:FAL	2 Germany	1998 1999
	Broilers	10	2 Monsanto, North Carolina State Univ., 2 Virginia Polytech. Inst. and Univ., 2 Foster Farm Feed Research, 2 FAL, ID-DLO	7:US, 2 Germany, Netherlands	1996, 3:1998, 1999, 4:2000, 2001
Rats/mice		5	2 Monsanto, Jiangsu Academy, Kyoto University, University of Aberdeen	China, 2 US, Japan, UK	2:1996, 1998, 1999, 2000
Rest,	Catfish, Quails	2	Monsanto, Jiangsu Academy	US, China	1995, 1996

Table 2 makes it clear that most of the experiments are done in the US. That is not surprising, because most of the GMO's are grown and used there.
Just a few of all the experiments done with rats and mice and GMO's are included here, to show that safety experiments are mainly done with laboratory animals. Studies to compare the performances of the animals, the most interesting for this study, are done with farm-animals.

Six studies have been published by Monsanto, and none by other GMO producing companies were found. There are more studies done by these companies, but not published. Also at universities or institutes, studies are done on behalf of these companies, but not always the name of the company is mentioned (shown in table 3 to 6).

A couple of studies are done at this moment. No information about these experiments was available for this study.

3.1. Types of experiment

The tables 3 to 6 give an overview of the experiments with animals and GMO's studied. The tables show that most articles found about this subject are abstracts. This means that other scientists did not review them, and that little information about the experiment is given. Some additional information about the abstract from Mireles *et al.,* 2001 came from a personal note, and for the abstracts of Bohme *et al.,* (1999) Aulrich *et al.,* (1998) and Aulrich *et al.,* (1999) came extra information from a conference proceeding (Flachowsky *et al.,* 2000). The abstracts of Hendrix *et al.,* (2000) and Petty *et al.,* (2001) are partly about the same experiment, but because they describe different parts of the experiment they are both included.

Choice experiments with animals and GMO's

The choice experiments with GMO's and non-GMO's, important for this study, are rarely done. In two abstracts (table 3) choice experiments were mentioned. Both were a small part of another experiment. The information given in the abstracts was not enough for any conclusions. Hendrix *et al.,* (2000) found that the animals were more in the normal field (46 % versus 56% P<0,01) but that the animals tended to graze as a group and the grazing pattern varied widely. Folmer *et al.,* (2000b) did not find any grazing preference.

Table 3: Overview of choice experiments with animals and GMO

Reference	Crop	IP/HR	Name	Animal	Silage/seed	Raw/ proc.	Place
Folmer *et al.,* (2000b) Abstract	Corn	IP (Bt)	N7333	Steers	Crop residue	Raw	University of Nebraska and Novartis Seeds
Hendrix *et al.,* (2000) Abstract	Corn	IP (Bt)	Pioneer 3489 or 34E79	Beef cows	Crop residue	Raw	Purdue University

When just one name is given the GMO is compared with the parental
Proc.= processed the used GMO is processed before fed to the animal

Comparison experiments with animals fed GMO and non GMO's

In table 4, the performances of animals fed on GMO or non-GMO, where the nutritional value of the plant was not changed is the largest group of experiments done with animals and GMO's. Because of the lack choice experiments and of information about the choice experiments in table 3, the experiments in table 4 are the most important experiments for this study.
Most of the experiments were done with Bt-corn. Many different varieties of Bt were used, only experiments done at one institute of University might have used the same GMO. The experiments were mainly done with farm animals.
In some the articles is mentioned that the enviroment where the crop grew, was the same (table 4 and 5). That means the crop is grown on the same or similar fields and also the used of herbicides and insecticides were the same.

Table 4: Survey of comparison experiments with animals fed GMO and Non-GMO food with the same nutritional value

Reference	Crop	IP/HR	Name	Animal	Silage/seed	Raw/proc.	Place
Aulrich *et al.*, (1998) Abstract	Corn	IP (Bt)	Cesar	Laying hens	Seeds	Processed	FAL Federal Agricultural Research Centre, Germany
Aulrich *et al.*, (1999) Abstract	Corn	IP (Bt) + HR (glufosi nate)	CG0025 6-176 , changed for Bt and Basta	Laying hens	Seeds (same envir.)	Processed	FAL
Barriere *et al.*, (2001)	Corn	IP(Bt)	Rh208 (Bt)	Sheep Dairy cows	Silage (same envir.)	Raw	INRA, Unite de Genetique et d Ámelioration des plantes Fourrages, France
Bohme and Aulrich (1999) Abstract	Sugar-beets, Corn	HR (Glufosi nate)	-	Pigs	- (same envir.)	-	FAL
Brake and Vlachos (1998)	Corn	IP (Bt)	5506BT X and G4665	Broilers	Seeds (same envir.)	Processed	North Carolina State University
Donkin *et al.*, (2000) Abstract	Corn	HR (glypho sate)	DK626 (RR)	Dairy cows	Silage + seeds	Raw	Purdue University and Monsanto
Faust (1997)	Corn	IP(Bt)	-	Dairy Cows	-	Raw	Iowa State University
Folmer *et al.*, (2000a) Abstract	Corn	IP (Bt)	N4242 Bt and N7333	Dairy Cows	Silage	Raw	University of Nebraska Lincoln and Novartis
Folmer *et al.*, (2000b)	Corn	IP (Bt)	N7333	Steers	Crop residue Silage	Raw	Univ. of Nebraska, and Novartis Seeds
Halle *et al.*, (1998) by Clark *et al.*, (2000)	Corn	IP (Bt)	-	Broilers	Seeds	Processed	FAL
Hammond *et al.*, (1996)	Soy-bean	HR (glypho sate)	40-3-2, 61-67-1, A5403	Rats, Broilers Catfish Dairy Cattle	Seeds Seeds Seeds Silage (same envi)	Both Processed Processed Raw	Monsanto, St. Louis and Mississippi State University
Hendrix *et al.*, (2000) Abstract	Corn	IP (Bt)	Pioneer 3489 or 34E79	Beef cows	Crop residue	Raw	Purdue University, US
Kan *et al.*, (2000) Abstract	Soy-bean	IP (Bt)	726 and 781	Broilers	Seeds	Processed	ID-DLO Netherlands
Kerley *et al.*, (2001) Abstract	Corn	IP (Bt)	CHB351	Beef cattle	Silage	Raw	University of Missouri

Continuation table 4

Reference	Crop	IP/HR	Name	Animal	Silage/seed	Raw/proc.	Place
Petty *et al.*, (2001) Abstract	Corn	IP (Bt)	3489 or 34E79	Beef cattle	Silage	Raw	Purdue University and Monsanto
Mireles *et al.*, (2000) Abstract	Corn	IP (Bt)	E176	Broilers	Seed (same envir.)	Processed	Foster Farms Feed Research, US
Reuter *et al.*, (2001) Abstract	Corn	IP (Bt) and HR (glufosi nate)	Zea Mays L Line CG 00256-176	Pigs	Corn	Processed	FAL
Russel *et al.*, (2001) Report	Corn	IP (Bt)	34R07, NX6236 and N64Z4	Beef cows	Crop residue	Raw	Iowa State University
Sidhu *et al.*, (2000)	Corn	HR (glypho sate)	GA21/D K580	Broilers	Seeds	Processed	Monsanto, US
Weber *et al.*, (2000)	Corn	IP (Bt)	Yield-gard?	Pigs	Seeds	Processed	Purdue University
Zhang *et al.*, (2000)	-	-	Phytase	Broilers	Seeds	Processed	Virginia Polytechnic Institute and State University

H.R. Herbicide resistant, I.P. Insect Protected
When just one name is given the GMO is compared with the parental
Proc.= processed the used GMO is processed before fed to the animal
(same envir.) Means the crop was grown in similar circumstances, the field and the spraying of herbicides

Comparison experiments with GMO's with a changed nutritional value fed to animals

Table 5: Comparison experiments with animals, GMO and Non-GMO food, whereby the nutritional value of the plant was changed

Reference	Crop	modification	Name	Animal	Silage/seed	Raw/proc.	Place
Denbow *et al.*, (1998)	Soybean	Improve phos-Phorus availability	M94550	Broilers	Seeds	Processed	Virginia Polytech Institute and State University
Hasimoto *et al.*, (1999)	Potatoes	Soybean glycinin	Ag877 Ag921	Laying hens	Seeds	Processed	Kyoto University
Momma *et al.*, (2000)	Rice	-	Soybean Glycinin	Rats	Seeds (same envi)	-	Kyoto University
Spencer *et al.*, (2000a)	Corn	-	Low phytase corn	Pigs	Seeds (same envir.)	Processed	University of Missouri
Spencer *et al.*, (2000b)	Corn	Low phytate	Zea mays L.	pigs	-(same envir.)	Processed	University of Missouri

When just one name is given the GMO is compared with the parental
Proc.= processed the used GMO is processed before fed to the animal
(same envir.) Means the crop was grown in similar circumstances, the field and the spraying of herbicides

In table 5 the nutritional value of the GMO's is changed, so the food intake will probably change as well. These experiments show that the animals don't refuse the GMO food. When the change in the plant is an improvement in a nutritional way, for the animal, they can even perform better by eating the plants made by genetic engineering.
These experiments will be involved in the rest of the study to compare the experimental design with the other comparison experiments from table 4.
The experiments in table 5 are done with processed seeds.

Safety experiments with animals and GMO's
Some experiments for safety tests are shown in table 6. These experiments are done with lab laboratory animals, and the protein produced by the new gene, not with the plant are a part of the plant. These experiments show, like the experiments in table 5, that the animals don't refuse the GMO food. Only a few safety tests are shown here. The other experiments, mainly done by or on behalf of GMO producing companies, are not published. These safety experiments cannot be used to compare the performances of the animals, so they will not be included in the rest of the study.

Table 6: Safety experiments with new protein from GMO plants in animals

Reference	Crop	Modification	Name	Animal	Silage	Raw/proc	Place
Ewen *et al.,* (1999)	Potatoes	Insect and nematode resistance	Snowdrop lectin, GNA	Rats	-	Both	University of Aberdeen, UK
Reed *et al.,* (1996)	Tomatoes	Fruit ripening	ACCd-protein	Mice	Seeds	-	Monsanto, Missouri, US
Song *et al.,* (1996) Abstract	Cotton	IP (Bt)	-	Rats, Quails	Seeds	-	Institute of Industrial Crops, Jiangsu Academy of Agricultural Science, China

H.R. Herbicide resistant, I.P. Insect Protected
Proc.= processed the used GMO is processed before fed to the animal

Ewen *et al.,* (1999) (Pusztai) carried out a GMO-non-GMO comparing experiment with rats and potatoes expressing a snowdrop lecithin, that increases the insect and nematode resistance. Because of comments on the experimental design, it took a long time before a Journal wanted to publish this article. It was published with a few review articles in the same journal. The same experiment with significant changes to the experimental design is now being repeated at the RIKILT, in The Netherlands (Ref.no.: SAFOTEST (QLK1-1999-00651)).

3.2. Plant growth

Only in a few articles, the way the crop grew was mentioned. Because the modification is the only difference between GMO and non-GMO, the growing conditions and the spraying should be the same, to be able find a difference between the GMO and the

non- GMO. Bohme and Aulrich (1999) and Sidhu *et al.,* (2000) compared non-GMO, GMO sprayed as conventional corn and GMO sprayed as GMO. But did not found any differences.
Only in Russel *et al.,* (2001) the growth and insect pressure was measured. This experiment was done over two years. The differences between the first and second year, what was a very dry year, were so large, no conclusions could be made. Besides Russel *et al.,*(2001) also Petty *et al.,* (2001) Hendrix *et al.,* (2000) (same experiment), did an experiment with crop from two different years. No differences between the crop were mentioned in these last two experiments.

3.3. Chemical analyses

In about half of the experiments done with GMO's and animals, the chemical composition of the crop was analysed. These results are shown in table 7 and 8. The analyses are proximate with sometimes extra information about essential amino acids or fatty acids and a few well-known anti-nutritional factors. These analyses are limited. Bohme and Aulrich (1999) and Sidhu *et al.,* (2000) compared non-GMO, GMO sprayed as conventional corn and GMO sprayed as GMO. In these experiments it can be shown if the modification or the way of spraying has influence on the crop or the animal.
The presence of the protein produced by the new gene was only analysed by Brake and Vlachos, (1998), <5 ppb Cry1A(b), and Spencer *et al.,* (2000) 0,10 versus 0,20 % for GMO versus non-GMO.
Sidhu *et al.,* (2000) treated the GMO with Roundup in one year and not in the other year, they did not found a difference in the chemical composition.
The insect damage, also a possible difference between GMO and non-GMO was only mentioned in Russel *et al.,* (2001). They found more insect damage on the non-GMO in both years. Further research was requested before definite inferences can be made.

The tables show that more information is available. Brake and Vlachos (1998), did the complete analysis of all the amino acids, and also the presence of mycotoxins. Padgette *et al.,* (1996) wrote a whole article about the composition of the GMO's used in the experiments of Hammond *et al.,* (1996), the amino acids as well as fatty acids, isoflavone, lectin, tripsin inhibitor and urease were analysed. Mireles *et al.,* (2000) gave the information about some more amino acids were analysed in his personal note. Flachowsky *et al.,* (2000) gave the analyses of the experiments done by Aulrich *et al.,* (1998) and Bohme *et al.,* (1999). The additional information is about the main fatty acids and minerals. Spencer *et al.,* (2000) gave some more data on the amino acids. Sidhu *et al.,* (2000) analysed all the amino acids and the fatty acids.
The data from the amino and fatty acids are shown in annex 1.

From the experiments of Russel *et al.,* two abstracts were written, Russel *et al.,* (2000a and 2000b), but the additional information is a leaflet, a research report (Russel *et al.,* 2001). The crop in Russel *et al.,*(2001) did not grow as well in the second year because of the dryness. The differences between non-Bt and Bt were significant in the first year but not in the second year. It seemed that the GMO plants were greener, grew faster and had a lower digestibility because of the higher amounts of fibre. The parental plant had

significant higher crude protein percentage. Because of the large amount of variation between the first and the second year, it is hard to draw a conclusion. The parameters used in the article are different from the ones used in table 7, and are therefore shown in annex 2.
In Hammond *et al.,* (1996) the crude ash percentage is significantly higher and the carbohydrates are significant lower for the GMO's (table 8). In the extended analysis (Padgette *et al.,* 1996) (annex 1), no more differences were found.

Conclusions in the articles
Most articles concluded that there were no significant differences in the composition between the genetically modified crop and the parental crop. When the analyses were extended probably more could be said about the composition as a result of the modification.

In the articles where the moisture is qualified, the moisture is higher in the GM plant than in the parental plant. In most articles the crude fibre fraction seems to be a bit higher in the GM plant, and the crude protein content seems a bit less.

3.4. Diet composition

The test crop was used in the diets as the crops are normally used, so pigs and chickens got processed crops and beef and dairy cows mainly silage.
For the cattle the test crop was a large part of their diet, mainly 70 % for dairy cows and about 100% for beef cattle. Not all articles about pigs and chickens gave these numbers, mainly 33-60 % for chickens and about 70 % for pigs.
In Hammond *et al.,* (1996) where soybeans, an protein source, were used, the test crop-part of the diet was low compared to other experiments where corn, an energy source was used.
Reuter *et al.,* found a better feed/gain for animals fed non-GMO. The experiment of Bohme and Aulrich (1999) seemed very short but in this experiment the digestibility was the main aim and not the performance of the animals.

In most articles a comparison is made between the GMO and the parental non-GMO (table 13). In the article of Mireles *et al.,*(2000) the corn is compared, GMO and non-GMO, but in addition the soybean meal was probably partly or completely from GMO's as well. That means that they compared the two corns as different hybrids here but not a GMO and a GMO-free food.

Table 7 : The chemical analyses from comparison experiments done with animals fed GMO and non-GMO corn

Reference	GMO vs. non-GMO	Moisture %	Crude ash %	Crude protein %	Crude fibre %	Crude Fat %	Starch %	Lysine	Methionine	NSP	More information
Aulrich *et al*., 1998	Bt-maize	-	1,6	9,8	2,5	5,6	70,8	0,30	0,21	6,43	Yes
	Cesar	-	1,5	10,8	2,3	5,4	71,0	0,29	0,22	6,21	
Aulrich *et al*., (1999)	Bt	-	1,41	9,85	2,35	4,94	72,53	0,30	0,21	6,43	Yes
	Cesar		1,35	9,86	2,31	4,42	73,37	0,29	0,22	6,21	
Barriere *et al*., (2001)					(NDF)						No
	Rh208Bt (sheep)	-	3,7	5,4	20,0(44,5)	-	30,3	0,65	0,20	-	
	Rh208(sheep)+ 3 control	-	3,8	5,7	20,0(45,8)	-	26,8	0,63	0,19		
	Rh208Bt (cows)		4,3	6,7	19,5(36,9)		33,0	-	-	-	
	Rh208(cows)		4,1	6,0	20,2(38,5)		33,2	-	-		
Bohme and Aulrich (1999)	Basta	-	-	11,7	3,3	3,3	-	0,28	0,21	-	Yes
	Conventional[2]	-	-	11,9	3,0	3,5	-	0,27	0,26	-	
	Isogenic	-	-	12,0	3,4	3,1	-	0,28	0,22	-	
Brake *et al*., (1998)	5506BTX	12,13	1,02	8,43	2,20	3,19		0,26	0,21		Yes[3]
	G4665	11,62	0,93	8,87	2,10	3,00		0,25	0,21		
Daenicke et al., (1999)	Bt Maize (silage)	-		8,7	19,1	2,8	-	-	-	-	Yes
	Cesar (silage)	-		8,4	18,6	2,9	-	-	-	-	
Mireles et Al., (2000)	*Bt*	12,50	1,30	8,85		3,80	-	85,4[1]	91,5[1]	-	No
		14,45	1,20	8,00	-	3,70	-	86,2[1]	93,8[1]	-	
Russel *et al*., (2001)					NDF/ADF						Yes
	34R07	(crop residue)		7,2[y]	69,1/39,8						
	NX6236			6,8[y]	70,1/40,4						
	N64Z4			6,5[y]	69,5/40,2						
	3489 (parental)			8,5[x]	66,1/38,4						
Spencer et al., (2000)	Low phytate	12,93	-	8,5	1,5	3,4	-	0,23	0,15	-	Yes
	Zea mays L.	12,03	-	8,8	1,4	3,1	-	0,22	0,15	-	
Sidhu *et al*., (2000)	GA21	16,86	1,38	11,05		3,90		0,31	0,22		
	Parental	16,21	1,56	10,54		3,98		0,30	0,22		

% reported on dry weight basis, except for % moisture

X and Y means that there is a significant difference (P<0,05) in that Column

[1] amino acid digestibility coefficients in %

[2] conventional here means the way of spraying, the GMO is sprayed the same as the non-GMO crop

[3] The mycotoxins were analysed Bt Vs Non-Bt: Aflatoxins < 2 Vs 4 ppb, deoxynivalenol ND Vs 30 ppb, Fumonisin B_1 , both < 1 ppm.

Table 8: The chemical analyses from comparison experiments done with animals fed GMO and non-GMO soybeans and sugar beets

Reference	Crop	GMO vs. non-GMO	Moisture %	Crude ash %	Crude protein %	Crude fibre %	Crude Fat %	Carbo-hydrates %	Lysine	Methionine	More information
								(Sugar)			
Bohme and Aulrich (1999)	Sugar beets	Basta	-	-	63	47	4	738	-	-	No
		Conventional[1]	-	-	60	46	4	744	-	-	
		Isogenic	-	-	72	56	3	736	-	-	
Hammond *et al.*, (1996) / Padgette *et al.*, (1996)	Soy bean	GTS 40-3-2	8,12	5,24[x]	41,4	6,87	14,04	37,1[x]	2,56	0,55	Yes
		GTS 61-67-1	8,20	5,17[x]	41,3	7,08	16,09	37,5	2,58	0,54	
		A5403	8,12	5,04[y]	41,6	7,13	15,52	38,1[y]	2,61	0,55	

X and Y : a significant difference (P<0,05) in that Column

[1] conventional here means the way of spraying, the GMO-crop is sprayed the same as the non-GMO crop

Table 9: Survey of comparison experiments done with dairy cows fed GMO or non-GMO

Reference	Plant	Parental/ commercial	Number of animals	Raw/proc.	Part of diet	Exposure to food	Duration exp.	Results	Dry Matter intake	Performance	Notes
Barriere *et al.*, (2000)	Corn	Parental	24	Silage	73 %	-	13 w.	No diff.	21,8 Vs 20,6 kg/d (P<0.01) for GMO	Milkprod. 31,8 Vs 31,5 kg/d for GMO	No diff. in lactation performance
Donkin *et al.*, (2000)	Corn	Parental	16	Silage	62 % corn silage +17 % grain	Ad Lib.	3*28d	No diff	21,5 vs. 21,9 Kg/d for GMO vs. non-GMO	Same	All the information about lactation is given 29,4 vs. 29,5 GMO vs. isogen
Hammond *et al.*, (1996)	Soy-bean	Parental	36	Whole raw soybeans	17,5 %	Ad lib.	29 d.	No diff	23,8 – 25,7 Kg/d for non-GMO	3,5% fat-corr. milk 36,7 vs. 34,1 Kg/d (P<0.05) for GMO	Avg. 2,4 Kg/d soybeans
Faust and Miller (1997)	Corn	Parental	-	Green chopped corn plants	-	-	14 d.	No diff.	-	Same	Feed intake 43,4 vs. 44,8 – 47,0 non-GMO vs. Bt 176 – Bt 11
Folmer *et al.*, (2000)	Corn	Parental	16	Silage	40 % corn silage +28 % corn	2 daily	21 d.	No diff	22,4 vs. 22,8 Kg/d non-GMO vs. GMO	Same	Milk production 28,6 vs. 29,2 Kg/d non-GMO vs. GMO

No diff. = no differences were found in the results
Parental means the GMO was compared with the parental crop
Proc.= processed the used GMO is processed before fed to the animal

3.5. The experiments reviewed by animal

In tables 9 to 13, the experiments are reviewed by animal species to compare the experiments and the performance of the animals.

Experiments done with dairy cows
Table 9 shows the experiments with dairy cows, and the experiments are done over a short time-period compared with the lifetime of the animals. All the GMO's are compared with their parental non-GMO, so the only difference between the crops should be the modification. The test crop is in three articles about 70% of the diet, very high compared to the 17,5 % of Hammond *et al.,* (1996).
The dry matter intake seemed higher for the GMO, except in Donkin *et al.,* (2000). The milk production is about the same. The 3,5 % fat-corrected milk was significantly higher for GMO vs. non-GMO in Hammond *et al.,* (1996).

Experiments done with beef cattle
The experiments with beef cattle (table 10) are done over a longer term than the experiments with dairy cows. The animals are mainly fed with 100% test crop. In Kerley *et al.,*(2001) the GMO was compared to a conventional crop instead of a parental crop as in the other experiments.
The feed intake seems higher for animals fed GMO, but it is more due to the hybrid effect than the GMO, according to Aulrich *et al.,* (1999) and Folmer *et al.,* (2000).
The feed efficiency is better for non-GMO in Folmer *et al.,* (2000) and Hendrix *et al.,* (2000). In Russel *et al.,*(2001) a greater intake of crude protein was found, ($P<0,05$) for cattle grazing corn crop residues from the non-Bt hybrid than those grazing the Bt-residues. In the first year there was a difference for feed/gain, (7,08 versus 6,40) for GMO versus control ($P<0,05$) respectively. The parameters used for study were to rough to show the performance of the animals.

Experiments done with sheep
The experiments with sheep (table 11) did not show any differences between GMO and non-GMO. The experiment from Barriere *et al.,* (2001) was over a short-time period. No information about the chemical analysis done was given, no differences were found. The other experiment was mentioned in an abstract with a few other experiments, and very little information was given.

Experiments done with pigs
In three articles nothing is said about the amount of test crop in the diet. The experiment of Bohme and Aulrich seemed very short but in this experiment the digestibility was the main aim and not the performance of the animals.
Weber *et al.,* (2000) suggested (table 12) that the increased fat depth and marbling of the pigs fed with the isogenic control corn could partially be explained by the increased the feed intake (0,10 lb./day). He used a large group of animals and the main part of the diet was test crop. Reuter *et al.,* found a better feed/gain for animals fed non-GMO.

Experiments done with chickens

In most articles a comparison is made between the GMO and the parental non-GMO (table 13). Chickens perform well fed GMO and non-GMO.
In the articles the test-crop was processed like conventional feed. The duration of the experiments was the main growing period for broilers and the main production time of the laying hens.
Because of their fast growth, 5000%,broilers will be sensitive to changes in the nutrient value of diets (Hammond *et al.,* 1996, Sidhu *et al.,* 2000). Deficiencies or reduced bio-availability of key nutrients in the diet are readily manifested by broilers.
It seems that the feed efficiency is higher for animals fed non-GMO's.
In the article of Mireles *et al.,* (2000) corn is compared, GMO and non-GMO, but in addition the soybean meal was probably partly or completely from GMO's. That means that they compared the two corns as different hybrids here but not a GMO and a GMO-free food.
Brake and Vlachos (1998) found a better feed-conversion for animals fed GMO, the feed intake was a bit higher for the animals fed non-GMO with the same gain. Probably the reason for the improved muscle yield from animals fed Bt.

Table 10: Comparison experiments with Beef Cattle fed GMO or non-GMO

Reference	Plant	Compared with	Number of animals	Raw/ proc.	Part of diet	Exposure to food	Duration experiment	Results	Dry Matter intake	Performance	Notes
Aulrich *et al.*, (1999)	Corn	Parental	40	Raw, corn silage	100 %	Ad Lib.	246 d	No diff.	-	-	Also done with sheep
Folmer *et al.*, (2000)	Corn	Parental	83 and 128	Raw Corn residue corn silage	100%, 90 %	Ad Lib.	70 d. 101 d.	No diff.	8,61 vs. 8,32 kg/d, for GMO vs. non-GMO P<0.05	Feed efficiency, 6.33 vs. 6.81 P<0.05 N7333 non-Bt	More a hybrid effect, not clear whether these results are from 1st or 2nd experiment, or both. ADG was greater for steers fed the GMO early maturing corn silage.
Hendrix *et al.*, (2000)	Corn	Isogenic	56* 38** 20***	(WPS)Whole plant silage, corn residue, Free choice	100 %	Ad Lib.	89/ 85 d. -/- 1st /2nd year -	No Diff.	**88,8 vs. 8,67 Kg/d for GMO silage	*Feed/gain 6,86 vs. 6,48 (P< 0.05) Bt vs. Non-GMO Fed WPS	***Cows tend to graze like a group, great variety of grazing pattern.
Kerley *et al.*, (2001)	Corn	Conventional	36	Raw	75 %	-	49 d	No diff.	-	Same	
Petty *et al.*, (2001)	Corn	Parental	56	Raw, whole plant silage	100 % in the end 90 %	Ad Lib.	101 d (84 d 2nd year)	No diff.	Feed/gain 1st year: 7,08 vs. 6,40 P<0,05 GMO	Same	Over 2 years no sign. Differences
Russel *et al.*, 2000	Corn	Parental	30	Raw crop residue	100%	Ad Lib.	126d	No diff.	31,0 vs. 35,4 [27,7*] lb./d Non-Bt-yieldgard, knockout*	The variation in animal performance is hard to explain	Big differences over the two years.

*** reference to the different experiments and results in the same abstract

* differences are significant P<0.1
No diff. = no differences were found in the results
Parental means the GMO was compared with the parental crop
Proc.= processed the used GMO is processed before fed to the animal

Table 11: Comparison experiments done with sheep fed GMO and non-GMO

Reference	Plant	Compared with	Number of animals	Raw/ proc.	Part of diet	Exposure to food	Duration experiment	Results	Dry Matter intake	Performance	Notes
Barriere *et al.*, (2001)	Corn	Parental + 3 control hybrids	30	Silage	100%	Ad lib.	1 w.	No diff.	40,2 vs. 40,3 for non-GMO	Same	No diff. chemical analyses
Daenicke *et al.*, (1999)	Corn	Parental	4	Raw	-	-	-	-	-	Same	-

No diff. = no differences were found in the results
Parental means the GMO was compared with the parental crop
Proc.= processed the used GMO is processed before fed to the animal

Table 12: Comparison experiments done with pigs fed GMO and non-GMO

Reference	Plant	Compared with	Number of animals	Raw/ proc.	Part of diet	Exposure to food	Duration exp.	Results	Performance	Notes
Bohme and Aulrich (1999)	Sugar beets, corn	Parental	-	Proc.	-	Ad Lib.	8 d.	No diff.	-	-
Reuter *et al.*, (2001)	Corn	Parental	12	Proc.	70 %	-	91 d.	No diff	Feed:gain 2,59 vs. 2,55 For Bt	Daily gain 804 vs. 815 g/d for Bt
Spencer *et al.*, (2000a)	Corn	Low phytate	50	Proc.	-	Ad Lib.	35 d.	No diff	-	No extra phytase is necessary
Spencer *et al.*, (2000b)	Corn	Low phytate	20	Proc.	-	Ad Lib.	± 100 d.	No diff.	-	
Weber *et al.*, (2000)	Corn	Parental conventional	180	Proc.	70-80 %	Ad Lib.	± 100 d.	increased fat depth and marbling for isogenic control	0,10 lb./day more feed intake by fed isogenic,	ADG 1,92 –2,20 lb. in different stages of life, no diff between Bt and non- Bt

Dry Matter intake wasn't mentioned in these articles
No diff. = no differences were found in the results
Parental means the GMO was compared with the parental crop
Proc.= processed the used GMO is processed before fed to the animal

Table 13: Comparison experiments done with chickens fed GMO and non-GMO

Reference	Plant	Parental/ commerc	Number animals	Raw/ proc.	Part diet	Exposure to food	Duration exp.	Results	Performance	Feed conversion	Notes
Broilers											
Aulrich *et al.*, (1999)	Corn	Parental	18	Proc.	50 %	Ad Lib.	35 d.	No diff.	-	-	
Brake and Vlachos, (1998)	Corn	Parental	1280	Proc.	60 %	Ad lib.	38 d.	No diff.	Females fed GMO had a higher % of fat pad, breast skin and P.*minor*	1.50 vs. 1.54 (28 d) and 1.72 vs. 1.75 (38 d) for Bt P< 0,05	GMO, seems to better than normal
Denbow *et al.*, (1998)	Soy bean	Parental	416	Both	-	Ad Lib.	21 d	No diff.	-	Improves with the amount of phytase	Basal feed intake 618 g
Hammond *et al.*,(1996)	Corn	Parental	360	Proc	33 %	Ad Lid.	42 d	No diff.	Daily gain 51 vs. 51 and 50 g/d, for non-GMO vs. GMO and GMO	Feed:gain 0,551 vs. 0,548and 0,546 for non-GMO	-
Halle *et al.*, (1998) by Clark *et al.*, (2000)	Corn	Parental	12 per treatment	Proc.	50 %	-	35 d.	No diff.	Feed intake 2522 vs. 2627g for GMO	Feed:gain g/g 1,63 vs. 1,61 for Bt	-
Kan *et al.*, 2000	Soy bean	Parental and comm.	508	Proc.	-	Ad Lib.	41 d	No diff.	Avg. bodyweight above 2400 gr. at day 41.	Below 1,60	
Mireles *et al.*, (2000)	Corn	Iso-caloric and nitro genous	28	Proc	-	Ad Lib.	21 d	No diff.	Feed per gain 1,62 vs. 1,63 for GMO	Weight gain 1,123 vs. 1,056 g/chick for GMO	Not a real GMO-Non-GMO experiment

Sidhu *et al.*, (2000)	Corn	Parental	560	Proc.	-	Ad Lib.	38 d	No diff.	Terminal weight at day 38: 2 kg (male) 1,9 kg (female)	-	Feed efficiency 1,75 vs. 1,66 GMO vs. non-GMO for males

Continuation table 13

Reference	Plant	Parental/ commerc	Number of animals	Raw/ proc.	Part of diet	Exposure to food	Duration exp.	Results	Performance	Feed conversion	Notes
Laying hens									-		
Aulrich *et al.*, 1998	Corn	Parental	18	Proc.	50 %	Ad Lib	30 w	No diff.	-	-	Food intake 115 gr./d
Aulrich *et al.*, (1999)	Corn	Parental	12	Proc.	-	Ad Lib.	30 w.	No diff.	-	-	

No diff. = no differences were found in the results
Parental means the GMO was compared with the parental crop
Proc.= processed the used GMO is processed before fed to the animal

3.6. Farm Observations

Table 17: Farm observations, wild and farm-animals refuse to eat GMO's

Source	Animals	Plant	Land	Observation
	Mice, wildlife	Roundup ready soybeans	Missouri US	By switch to RR soybeans the mice and the wild animals did not eat the soybeans as they used to do.
T.S. Hoekstra 2001	Cows	GMO concentrates of compound feed	Drogeham, Netherlands	Before changing the feed from GMO to GMO-free there were many claw problems, after, these were disappearing.
M. Newhall 2000	Geese	Roundup ready Beans	Illinois, US	The geese ate only the conventional beans when in two the RR-beans in the adjoining field weren't touched
S. Sprinkel 1999	Cows	Roundup Ready corn	Nebraska US	Livestock was not grazing as in the past
S. Sprinkel 1999	Deer	Bt corn	North Dakota	Less deer damage, they don't go into the Bt field
S. Sprinkel 1999	Cattle	Pioneer 3477		Cattle broke through the fence walked through the GMO to get to the Pioneer 3477
S. Sprinkel 1999	Cattle			Cattle will go of their feed when they are switched to a GMO silage
S. Sprinkel 1999	Racoon	Bt corn		By dozens they playing in and eating from the normal corn, but down the road the Bt field is untouched

Farm observations are a sign that something can be wrong. This monitoring in the field is an important source of questions for scientific research.
The observations are done on farms, not under certain conditions, so there is also a possibility that other factors not related to the feeding of GMO's caused these changes.
The farm observations from Sprinkel (1999) and Newhall (2000) are stories from farmers in the US. These observations are hard to track down, the sources of the observations were not found, but it seems that mainly wild animals avoid the GMO fields, and the livestock eats less from the GMO than the non-GMO food.

T.S. Hoekstra thinks about the things that happen on the farm already for a long time and writes it down. The many claw problems of his cows he partly blamed on the GMO's in the concentrates of compound feed. After changing to GMO free feed, and changing other things the claw problems decreased.

4. Conclusion

4.1. The extension of the question

The research question "Do animals have a different food preference for GMO or non-GMO's" was extended. Not enough choice experiments were done, and the results of a food preference experiment won't give an unequivocal answer to the question when only the behaviour of the animal is studied. Food preference is a parameter of food quality, so are foodintake, like speed and amount, and performance, like growth and production.
By also studying these parameters conclusions can be made about the meaning of a possible food preference. By extending the question a more complete answer to the question can be given.
By studying experiments done with animals fed GMO's or non-GMO's, a summary of knowledge about this topic is made, and recommendations for further research could be formed.

4.2. Existing literature

Most articles found are abstracts. These are not reviewed and a very limited information is given. Even when some additional information was given by a personal note, or congress proceedings, not much can be said about the way these experiments are done. Some experiments, not only the ones where mentioned, were done in behalf of a GMO producing company. Because of the public debate and political sensitivity of the subject, the information from the articles was interpreted with caution by the authors. So the articles were for this report interpreted from the limited data and with caution.

4.3. Conclusions from the articles

The main conclusion in the articles is that no differences have been found between the chemical composition of GMO's and non-GMO's and the foodintake and performance of the animals fed GMO's and non-GMO's, as far as the research is done.
There were no reports on important factors about foodintake and the growth of the plants.
The chemical analyses were limited. The standard analyses were done, in a few articles extended with information about the (main) amino and fatty acids.
Expected differences, like the presence of the protein produced by the new gene, were not analysed.
The performance of the animals was mainly the same, no major significant differences were found.
When differences were found, only small differences, these were in the natural range of the hybrids of the plants according to the author, or further research was requested. The more specific comments and found differences are given below.

Comments on the experiments

In Kerly et al., (2001) the GMO was compared with conventional crop, instead of parental crop like in other experiments. Because hybrid effects are larger than effects between GMO and parental crop, no differences caused by the modification could be found.

The experiments are mainly done over a short time period compared to the life span of the animals. Only broilers were tested during their main growing period.
Animals can respond differently on food in the short term than in the long term.
No experiments were done with more than one generation of animals.

The numbers of animals in the experiments were very different. Reuter *et al.,* (2001) used 12 pigs and Weber *et al.,* (2000) used 180 pigs. In experiments with dairy cattle experiments are done with 20 to 128 animals, and for chickens 18 to 1280. Of course there are many different reason for the number of animals, like the number of GMO's tested and the main question of the experiment, but these differences are very large

The history of the animals, what they ate before the experiment, GMO or non-GMO was not mentioned in the articles. In a few articles something was said about an adaptation period.

Poor performance or a reduced food intake of the GMO was not seen in these experiments. This means that when the animals don't have a choice they will eat the GMO in the same way as the conventional food.

The difference Weber *et al.,* (2000) found, increased fat depth and marbling for pigs fed non-GMO, probably due to the increased feed intake, was also found by Brake and Vlachos (1998). They found that broilers fed Bt had improved breast muscle yield.

Differences found:

?? Russel *et al.,* (2001) found that the GMO plants were greener grew faster and had a lower digestibility because of the higher amounts of fibre.

?? The moisture and the fibre fraction seem to be higher in the GMO than in the non-GMO (table 7, annex 2) and crude protein seems less.

?? Hammond et al., (1996) found that the crude ash percentage is significantly higher and the carbohydrates are significant lower for the GMO's

?? Only Blake and Vlachos (1998) and Weber *et al.,* (2000) found similar a difference. The pigs fed non-GMO increased carcass fat depth and marbling (Weber *et al.,* 2000). Broilers fed Bt-corn improved adjusted feed conversion ratios and breast muscle yields over birds fed parental corn (Brake and Vlachos 1998).

?? The opposite effect, better feed efficiency for animals fed non-GMO is shown in Folmer *et al.,* (2000) and Hendrix *et al.,* (2000) (beef cattle), as well in Reuter *et al.,* (2001) (pigs). This last effect would be predictable because the GMO's seem to have higher levels of fibre. These experiments were done with large groups of animals, only Reuter *et al*, (2001) had only 12 pigs.

?? In dairy cows, the dry matter intake seems higher for the GMO, except in Donkin *et al.,* (2000). Also same for beef cattle, where the feed intake seems higher for animals fed GMO, but it is more due to the hybrid effect than the GMO, according to Aulrich *et al.,* (1999) and Folmer *et al.,* (2000).

4.4. Farm observations

The farm observations are a sign that something can be wrong, and therefore an important source of questions for research.
The observations about animals that refused GMO's were told often, but hard to track down. The sources of the observations were not found.
The scientific experiments and the farm observations seem to be opposite. The experiments done with the animals who had a choice, (Hendrix *et al.,* 2000 and Folmer *et al.,* 2000) should have shown a clear preference. In Hendrix *et al.,* 2000 the animals were in the Bt-field 46% versus 56 % in the normal field, of the entire observation period (P<0.01). The animals tended to graze as a group and the grazing pattern varied widely. Folmer *et al.,* (2000) did not found grazing preference.
In both abstracts little information was given about these preference experiments. Both experiments were done with domesticated animals in a group.
According to Dawkins (by Rose and Kyriazakis,1991) natural selection has probably favoured the individuals within species who forage more efficiently and so their genes have been dominated the population. Recent advances in the selection of domestic animals have profound changed their productive performance, but it is likely that their ability to select an adequate diet has been significant altered in relation to the rigorous selection which had operated on the wild ancestor for thousands of years. (Rose and Kyriazakis 1991). Maybe wild animals can choose better diet than farm animals. Further research is required before drawing conclusions.

4.5. Do animals eat what is good for them?

In paragraph 2.2. it is shown how the food intake is regulated in animals, and the factors that affect the food intake are discussed. The conclusion in the end of that paragraph is that an animal will choose his own diet but this is not necessary the most balanced, but adequate according to the animal in the given circumstances. In experiments where the animals did not have a choice in foods, they ate the same amount GMO as non-GMO food, and performed the same. The animals performed well in the given situation.

4.6. Overall conclusion

This report is a summary of the available literature on experiments done with animals fed GMO's. The amount of literature was limited, and the found literature were mainly abstracts, a limitation on information about the experiments. No conclusions could be made from this information. By combining the information with the background information some recommendations for further research could be made.
The need for more public knowledge on this subject became clear, the limited amount that was found and the many questions there are on this topic, also due to the political side of the use of GMO's.

Whether animals have a different preference between GMO and non-GMO feed, extended to what does this possible preference mean, should be a question for further research. The answer could not be given by the studies done already with GMO's and non-GMO's.
The experiment that should be done to answer this question is not very simple. The subjects discussed in this report should be included. In chapter 5 recommendations for further research based on the information collected for this study are given.

5. Recommendations for further research

Based on the experiments done with animals fed GMO's (chapter 3) and the background information about GMO's and about foodintake (chapter 2) some recommendations for further research on the food preference of animals for GMO's or non-GMO's can be done.

5.1. Recommendations for a regular experiment

The research question

In paragraph 1.5. the question, do animals have a different food preference for GMO's or non-GMO's, and the extension of the question, from only preference to food intake and performance was explained.
It is important to ask the question to the animals to get an answer without a political view. That animals can choose a balanced diet they consider being adequate, in certain circumstances, is also important to know. When the animals have a preference one can expect a difference between the GMO and non-GMO.
The results of a food preference experiment won't give an unequivocal answer to the question when only the behaviour of the animal is studied. Besides the behaviour the food intake and the performance is important. These parameters will tell something about the food quality and so indirect about the food preference. The history of the animal, the associations the animal has made between cues from the food and the feeling after eaten it, can affect the preference as well.
Another problem is that one GMO is completely different from the other GMO. So food preference for or against one GMO cannot be extrapolated to other GMO's. Because the question is for GMO's in general, some compromise should be found.
It is very difficult to achieve a correct set-up, taking all variables into account.

The experiment

A choice experiment in which the animal can choose between GMO and non-GMO in different circumstances should be done. Also some comparison experiments with animals fed GMO and non-GMO over more generations should be done, to compare the performance of the animals and to detect possible problems from feeding GMO's.

The crop, GMO's and non-GMO's

The GMO should be compared to the parental crop. Hybrid effects are probably larger than the differences between parental and GMO-hybrid.
The nutritional value of the crop should not be changed, so IP and HR crop are both possible. Also because these are the main GMO's fed to animals. There are two main types HR GMO's, glyphosphate and glufosinate resistant, both can/should both be used. By using different GMO's maybe more can be said about changes due to the modification than the function of the new gene.
Not only one crop should be taken. A protein source, like soybeans, as well as an energy source, like corn, should be used. Also grass as complete diet should be involved.

The growth

The crop should be grown under the same conditions. The same field will not be possible because of cross contamination, but the differences should be minimal. The spraying of herbicides and insecticides should be tested as well. One part of the GMO the sprayed the same as the non-GMO and the other part sprayed as done in practice. The effects of the environment can be larger than the differences between GMO and non-GMO. With IP different treatments by changing the pressure of the specific insect would be interesting.
The growth of the GMO may be different from the non-GMO. More literature should be searched about this subject to be able to give more recommendations about what to measure during the growth of the plants.

The analysis of the crop

The proximate analysis should be done, but is limited so should be extended.
The expected differences, like the protein of the new gene, presence of mycotoxins and the fibre fraction should be analysed.
The bio-availability of nutrients can influence the food intake of the animals, and therefore analysed.
Further literature study is necessary to know how the Weende analysis should be extended.

The species of animals

Ruminants may react different than monogastrics, so both should be tested.
To make the experiment as sensitive as possible wild animals or not selected animals should be compared to farm animals, because wild animals are possibly better in diet-selection.
By choosing animals with a long life span the possible problem from eating GMO's can be expressed, but the experiment can be done over more generations of animals with animals with a short life span.

The conditions of the animal

Young growing animals, as well as producing animals should be used.
Also animals with different motivations, satisfied and hungry.
And the environment of the animals rich or poor can also be tested.
All the animals should be housed individual.

The history

The food that animals have been eating or have seen eaten by other animals before the experiment may influence the experiment.
So it is important to know the history of the animals.

The diet composition

The animals should be fed the same kind of food they normally get.
For the differences between GMO and non-GMO it would be interesting to feed fresh crop, because Faust *et al.,* (2000) found differences in moisture contents and levels of

ammonia bound nitrogen in fresh material, but there were no differences between the silage from GMO or non-GMO as far as analysed.
The test crop can be the main part of the diet, depend on the plant and animal species used. Corn can be a larger part of the diet for cows, than for pigs and that soybean can be.
Also it is important is to know whether the other part of the diet is GMO or non-GMO. The rest of the diet should be non-GMO. Only the test crop should be different in the diets. When the nutritional value is different between the GMO and the non-GMO, it may be interesting besides feeding the same diet with only the test-crop as a difference, also to test an iso-caloric diet.

The motivation of the animals
The motivation for a preference can change when the animal has to work more for the preferred food. By changing the accessibility of the food something more can be said about a possible preference.

5.2. Possible differences between GMO's and non-GMO's that need to be studied

In the experiments mainly no differences in chemical composition and in animal performance were found between GMO's and non-GMO's. But farm observations tell about animals that refuse to eat the GMO food. The possible between GMO's and non-GMO's before fed to the animals, not a part of this study, should be studied for further research. Some possible differences are mentioned below.

?? Dove *et al.,* (1999) found that the nutritive value of the sprayed herbage was improved from annual pastures that were sprayed at seed head emergence with low rates of the herbicide glyphosate. The difference in spraying between GMO and non-GMO can affect possible preference.

?? Faust and Sprangler (2000) found that fresh whole plant material, the GMO hybrids maintained highest levels of moisture ($P<0.05$), but for silage there was no differences in the studied parameters between GMO and non-GMO hybrids. Possible differences between GMO's and non-GMO's will be seen better by feeding fresh plant material.

?? With the early harvest, whole plant moistures were high in both Bt and non-Bt varieties: 65% to 66% moisture. At the early black layer stage the Bt corn held its moisture content at 64%; Non-Bt hybrids dropped to 59% (Faust *et al.,* 1998). The plant can grow different because of the modification, this can be seen better in different growing stages of the plant than in the final stage. Also a possible higher fibre fraction show in table 7 can be explained by differences in growing.

?? Purrington (1997) found by comparing two plants, different by modification, at two levels of resource availability, to reduce the fitness. The modified plant produced fewer seeds than the counterpart. The fitness costs were greater for the GMO in nutrient poor conditions. Environmental stress can be different for GMO's and non-GMO's.

?? Kleter (2000) mentioned EPSPS the enzyme produced in glyphosate tolerant (HR) crops is involved in the producing plant material used for lignin among other things. Because the activity of the new enzyme a higher fraction of lignin could be the result. The new protein can have other functions in the plant than the specific wanted quality.

?? Mycotoxins are produced by fungi. The most important way for these fungi to reach damaged kernels, is carried by insects like the ECB. When the amount of insects decreases because of the modification, also the amounts of mycotoxins may be decreased.

?? Plants infected by insects produce a defence substance, often aromatic substances (Dixon and Steele 1999). Insects, mostly the natural enemies of the damaging insects, are attracted to this aroma and come to these plants. It would be possible that livestock reacts to these aromas as well, positively or negatively. Probably some research on this subject is already done. When the insect damage is changed because of the modification, the reaction of the animals can change.

5.3. Methods other than the regular science

Regular science has been already practised for a long time and doesn't seem to have an answer to many questions about health and environment, so people have started searching for other ways to investigate these problems. There are several complementary measure methods.

In the regular science, exclusive science according to Röling (2000), the research subject is put under the microscope in little pieces, the combination of this with the organism as one or as a part of an ecosystem is usually not a part of the research. This is something that deserves more attention. The regular science, and this should, in the best situation be changed to including the ecosystems, seeing the whole environment as one, and including the software of all these systems. The interactions between parts of the system are left out in the regular science (Röling 2000).

When an extra gene is brought into a plant, this can disturb the natural balance of the plant. This results in a different vibration, which can be measured with very sensitive equipment as described in Matthaei (2000). In further research this measurements should be applied alongside the classical trial.

Another way is, biophysical methods. These methods approach the whole system first and than investigate one subject in the context of the system. For example the crystallising of water or to check the structure of the ground to see if it is polluted. The ground that is used to grow the plants is often very polluted, and the results between

GMO and non-GMO will probably be different when the plants are grown in a biologically balanced condition (Van Bruchem, 2001, personal note).

From this biophysical point of view, the animal performance is a very rough parameter to detect a difference in plants that is so small. The resilience of the animal will keep the animal in balance as long as not too many factors can disturb the balance. The behaviour is more precise already, but a more sensitive parameter should be used.

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Annex I : Additional information chemical analyses

Table 19: Chemical analyses amino acids

Amino acid	Hammondet al., (1995) /Padgette et al., (1995)			Sidhu et al., (2000)				Spencer et al., (2000)		Brake and Vlachos (1998)		Mireles et al., (2000)	
	GTS 61671 **Soy bean**	GTS 4032 **Soy bean**	A5403 contr **Soy bean**	GA21 (1996)	Contr	GA21 (1997)	Contr	Low-phytate	Normal	5506BTX %	G4665 %	Bt	Non Bt
Alanine	1.71	1.67	1.71	7.62	7.64	7.64	7.62	0.36 [a]	0.36 [a]	0.69	0.70		
Arginine	2.94	2.85	2.94	4.13	4.30	4.48	4.51			0.39	0.38	79.8 [b]	87.2 [b]
Aspartic acid	4.53	4.42	4.53	6.71	6.78	6.63	6.65			0.55	0.55		
Cystine	0.60	0.62	0.60	2.10	2.11	2.22	2.28	0.18 [a]	0.20 [a]	0.23	0.23	88.2 [b]	85.1 [b]
Glutamic acid	7.34	7.10	7.34	19.27	19.06	18.78	18.70			1.65	1.66		
Glycine	1.72	1.67	1.72	3.72	3.78	3.83	3.89			0.34	0.33		
Histidine	1.06	1.03	1.06	2.81	2.84	2.67	2.74			0.27	0.27		
Hydroxylysine										0.00	0.00		
Hydroxyproline										0.02	0.02		
Isoleucine	1.78	1.73	1.78	3.60	3.58	3.53	3.57	0.26 [a]	0.30 [a]	0.29	0.29	86.4 [b]	86.2 [b]
Lanthionine										0.00	0.00		
Leucine	3.05	2.97	3.05	13.11	12.90	12.98	12.87			1.14	1.15		
Lysine	2.61	2.56	2.61	3.02	3.09	3.11	3.02	0.23 [a]	0.22 [a]	0.26	0.25	85.4 [b]	86.2 [b]
Methionine	0.55	0.55	0.55	1.98	2.03	2.16	2.17	0.15 [a]	0.15 [a]	0.21	0.21	91.5 [b]	93.8 [b]
Ornithine										0.02	0.02		
Phenylalanine	1.97	1.90	1.97	5.15	5.17	5.31	5.33			0.45	0.45		
Proline	2.03	1.98	2.03	8.69	8.69	8.98	9.00			0.84	0.85		
Serine	2.10	2.04	2.10	5.33 [x]	5.27 [y]	5.17	5.03			0.40	0.40		
Taurine										0.12	0.12		
Threonine	1.60	1.56	1.60	3.77	3.73	3.59	3.54	0.27 [a]	0.29 [a]	0.31	0.31	82.3 [b]	82.7 [b]
Trytophan	0.59	0.59	0.59	0.62	0.57	0.61	0.61	0.06 [a]	0.06 [a]	0.05	0.06		
Tyrosine	1.45	1.40	1.45	3.81 [x]	3.95 [y]	3.73	3.77	0.24 [a]	0.27 [a]	0.29	0.27		
Valine	1.85	1.80	1.85	4.58	4.64	4.57	4.62	0.39 [a]	0.42 [a]	0.42	0.41		

Values expressed as percent of the total amino acids
[a] expressed as content of corn
[b] animo acid digestibility coefficients in %

Table 20: Chemical analyses of fatty acids

Fatty acid	Hammond et al., (1995)/Padgette et al., (1995)			Sidhu et al., (2000)					Aulrich et al., (1999)	
	GTS6 1671	GTS4 032	A5403 contr	GA21	Contr	GA21	Contr	Contr	Bt	Cesar (contr)
	Soy bean	**Soy bean**	**Soy bean**	(1996)		(1997)				
(6:0)	0.11	0.11	0.11							
Palmitic (16:0)	11.14	11.21	11.19	9.94	9.92	10.70	10.72	11.5	12.5	12.4
(17:0)	0.13	0.13	0.13							
Stearic (18:0)	4.05	4.14	4.09	1.87	1.86	1.68	1.62	1.6	4.0	4.0
Oleic (18:1)	19.81	19.74	19.72	27.50	27.4	24.2	24.1	27.7	28.6	31.1
Linoleic (18:2)	53.48	52.31	52.52	58.56	58.72	61.40	61.51	57.0	51.2	50.0
Linolenic (18:3)	8.12	8.23	8.02	1.10	1.08	1.14	1.14	1.2	1.0	0.9
Arachidic (20:0)	0.35	0.37	0.36	0.40	0.41	0.37	0.36			
Eicosenoic (20:1)	0.17	0.17	0.17	0.28	0.29	0.30	0.30			
Behenic (22:0)	0.49	0.53^{x}	0.50^{y}	0.16	0.17	0.16	0.15			
(24:0)	0.18	0.19	0.18							

Value of the fatty acids expressed as a percentage of the total fatty acid

^{x,y} Significant difference (P< 0.05) in that experiment

The values are the same for Daenicke et al., (1999), Aulrich et al., (1998) and Aulrich et al., (1999).

Annex 2: The chemical composition of the plants used in Russel et al., 2000

	Name hybrid							
	3489 contr.		34R07		NX6236		N64Z4	
	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2	Year 1	Year 2
Yield, lb/acre								
OM	6230 ^{x}	3825	6569 ^{y}	3969	6906 ^{y}	3101	5796 ^{y}	344
IVDOM	2745 ^{x}	1670	2836 ^{y}	1813	3537 ^{y}	1498	2937 ^{y}	1684
DM%	71.9 ^{x}	84.8	68.7 ^{x}	85.8	56.6 ^{y}	80.7	58.4	81.6
OM % of DM	88.5 ^{x}	93.3	84.8 ^{x}	93.2	90.8 ^{x}	93.4	84.0 ^{x}	92.9
% of OM								
IVOMD	44.6 ^{x}	42.7	43.20 ^{x}	45.6	51.3 ^{x}	48.3	51.2 ^{x}	48.9
NDF	77.5 ^{x}	79.3	78.1 ^{x}	82.2	74.2 ^{x}	80.0	73.2 ^{x}	80.2
ADF	46.9 ^{x}	48.3	49.5 ^{x}	50.0	45.6 ^{x}	48.5	45.6 ^{x}	46.9
ADL	6.6 ^{x}	5.5	7.4 ^{x}	5.4	5.4 ^{y}	4.9	5.7 ^{y}	4.4
CP	4.4 ^{x}	4.1	4.7 ^{x}	4.0	4.8 ^{y}	4.3	5.1 ^{x}	3.7
ADL%of NDF	8.5 ^{x}	6.9	9.4 ^{x}	6.6	7.3 ^{y}	6.1	7.8 ^{y}	5.2
ADIN % OF N	25.8 ^{x}	14.4	25.6 ^{x}	13.6	17.0 ^{y}	12.1	21.4 ^{y}	13.2

^{x} and ^{y} mean a significant (P<0.10) difference between the control group and the GMO in that year (not between GMO's)
OM, organic matter
IVDOM, digestible organic matter
ADIN, acid detergent insolublenitrogen

The first year the pressure of the ECB was higher, than the secon year. The second year was very dry. The GMO's are all Bt hybrids, the 34RO7 is the near isogenic Bt hybrid to Pioneer 3489.