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Memorandum on "The feeding of genetically modified glyphosate resistant soy products to livestock"

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Summary

Background

DCA has been commissioned to prepare a memorandum to enable the Danish Ministry of Food, Agriculture and Fisheries to assess the need for further tests or studies of the potential adverse effects of genetically modified (GM) crops, including crops treated with glyphosate. The request results from, among other things, the public debate on this issue and from a few observations in practice. The Danish Veterinary and Food Administration has specifically requested that the memorandum includes:

1. A summary of published studies on the growth and health of livestock fed GM vs. non-GM feed, including whether the studies include information about the spraying of crops or pesticide residues in the feed.

2. A summary of published studies on pesticide residues in GM vs. non-GM feed crops or feed.

3. An assessment of the need to carry out feeding experiments with GM. vs. non-GM feed, primarily soybean meal and, if so, how.

4. An assessment of the need and possibility for exploring whether GM feed, primarily soybean meal, differs from corresponding non-GM feed regarding the content of pesticide residues or other substances or quality parameters that might have an impact on animal health.

5. An analysis of Danish farmers' observations (Appendix 1).

Based on this request, DCA has reviewed the literature in these areas in order to identify potential risks to animal health.

Conclusion
On the basis of the material reviewed, DCA estimates that the triggering factor is glyphosate rather than the GM crop itself, and two hypotheses have been established that would need experimental evidence to be rejected or accepted:

A. Glyphosate can affect the microbial populations (microbiota) in the animal gut with secondary effects on animal production and health

B. Glyphosate can affect animal mineral status with secondary effects on animal production and health

These hypotheses are based on the known effects of glyphosate, i.e., on bacteria (that are very numerous in the gut of livestock) and the binding of minerals (including essential micro minerals in livestock). The hypotheses are supported by the most recent literature on farm animals, i.e., laboratory studies where pathogenic bacteria were less inhibited by glyphosate than non-pathogenic bacteria and a study where glyphosate was found in the urine of Danish cows concurrent with low levels of micro minerals in the blood.

Based on the increasing use of glyphosate at global level over a number of years, on the glyphosate contents in GM soy, on the discovery of glyphosate in the urine of Danish cows and on the disparate inhibition by glyphosate of pathogenic and non-pathogenic bacteria, it is estimated that there is a need to test the two stated hypotheses. Similarly, there is a need to verify how much glyphosate there is in GM soy products imported for animal feed.

The studies should include both *in vitro* laboratory experiments with relevant bacterial strains, and experiments with animals. An optimal and detailed protocol must be carefully designed, but could include soybean meal and cereals with known concentrations of glyphosate.

Background

The commissioning of the memorandum is underpinned by the following statement from, the Danish Veterinary and Food Administration: "A number of genetically modified (GM) plants have been approved in the EU for food and feed purposes following a thorough risk assessment and can be used in a similar way to the non-GM plant products. Products derived from GM plants, primarily in the form of protein sources such as soybean meal from herbicide-tolerant soy, are widely used for feeding farm animals such as pigs, cattle and poultry. Public debate in this area often turns to whether such products are less suitable as animal feed than similar products from traditional non-GM crops. Some Danish farmers have observed improvements in animal production after having switched from GM to non-GM soybean meal.
There have been speculations that there may be potential adverse effects on animals from residues of the active ingredient or carrier in Roundup, particularly with regard to Roundup Ready crops. A memorandum is required that appraises the existing knowledge in the area to provide a sounder basis for the Danish Ministry of Food, Agriculture and Fisheries to assess the need for further tests or studies in the area."

The Danish Veterinary and Food Administration requires inclusion of the following items in the memorandum:

1. A summary of published studies on the growth and health of livestock fed GM vs. non-GM feed, including whether the studies include information about the spraying of crops or on pesticide residues in the feed.

2. A summary of published studies on pesticide residues in GM vs. non-GM feed crops or feed.

3. An assessment of the need to carry out feeding experiments with GM. vs. non-GM feed, primarily soybean meal and, if so, how.

4. An assessment of the need and the possibility for exploring whether GM feed, primarily soybean meal, differs from the corresponding non-GM feed regarding the content of pesticide residues or other substances or quality parameters that could have an impact on animal health.

5. An analysis of Danish farmers' observations (Appendix 1).

Introductory remarks

The GM plants most relevant for use in livestock feed
Among GM plants the herbicide-tolerant GM plants are the most widespread, and among these glyphosate-tolerant GM plants are the most common. The herbicide glyphosate is the active ingredient in Roundup and similar products. Plants can also be genetically modified for insect resistance and for other traits or a combination of traits. The glyphosate-tolerant GM plants from Monsanto are called Roundup Ready. Glyphosate-tolerant soybean is the most widespread GM crop.

Glyphosate-tolerant GM soybean plants require particular attention because of the large import to Denmark of animal feed based on such plants (http://www.foedevarestyrelsen.dk/Publikationer/Alle%20publikationer/2012106.pdf).

Glyphosate function – the EPSPS enzyme
Glyphosate works by binding to an enzyme (5-enolpyruvylshikimate-3-phosphate synthase, EPSPS) along the so-called shikimate pathway, which is involved in the formation of the aromatic amino acids (phenylalanine, tyrosine and tryptophan) in plants (Funke et al., 2006). The binding of glyphosate blocks the enzyme so that the formation of the aromatic amino acids is inhibited and the plant dies from lack of these amino acids.

Organisms affected by glyphosate
The shikimate pathway – and thus the EPSPS enzyme – is found not only in plants but also in a number of microorganisms (fungi and bacteria) and parasites. In 2003, Monsanto patented the use of glyphosate as an anti-parasitic remedy (against, for example, the malaria parasite). The patent specifically states that bacteria and other microorganisms are glyphosate-sensitive (https://www.google.com/patents/US7771736). Thus, it has been known for a long time that glyphosate can affect microorganisms, which means that the bacteria in the gut of animals and humans, for example, can be affected by glyphosate that enters the system via the diet. Despite this, the effect of glyphosate on microorganisms in the gut of farm animals and humans is not included in the risk assessment of glyphosate.

Exceptions in glyphosate sensitivity
There are exceptions in the sensitivity of the EPSPS enzyme to glyphosate. In a number of organisms there are variants of the enzyme that are not completely inhibited by glyphosate and the formation of the aromatic amino acids is therefore not completely blocked. Potentially pathogenic bacteria such as Salmonella and *E. coli* are some of the microorganisms that may harbour less sensitive EPSPS variants (see patent EP 2327785 A2, https://www.google.com/patents/EP2327785A2?cl=en).

Creation of glyphosate-tolerant GM plants
Such an exception to the functioning of glyphosate is precisely what is used in the production of glyphosate-tolerant GM plants. The bacterium *Agrobacterium* sp. strain CP4 contains a gene that codes for a variant of the enzyme wherein the binding of glyphosate does not block the functioning of the enzyme. The insertion of this gene into the plant results in a glyphosate-tolerant GM plant. These GM plants will, therefore, not die due to lack of these amino acids after being sprayed with glyphosate.

The EPSPS enzyme and glyphosate exposure to animals and humans
Animals and humans do not carry the EPSPS enzyme and therefore must get these essential amino acids from the diet. The absence of the EPSPS enzyme does not, however, mean that glyphosate cannot affect humans and animals via the EPSPS enzyme pathway, since animals and humans in a

multitude of ways are affected by the bacteria in the gut, which, in turn, may be affected by the glyphosate contained in the diet (Samsel & Seneff 2013).

Glyphosate's binding of metals
Glyphosate has chelating properties, which means that it binds to metals. Glyphosate binds not just to a single metal but to a wide range of metals. Several of these metals (manganese and zinc, for example) act as co-factors and are required for enzyme activity in livestock and other animals. Another example is the metal cobalt, which in animals is essential for the production of vitamin B12. Metals that are bound to glyphosate become inactive and can therefore not function and be used as building blocks in these biological systems.
Inactivation in farm animals and humans may take place both pre-absorption in the intestines, with the associated risk that the absorption of the minerals is reduced, and post-absorption where the absorbed glyphosate can bind minerals in the blood and possibly also in tissue. If some of the essential minerals are bound by glyphosate, there is a risk that livestock may develop deficiencies of, for instance, zinc. This can be a particular problem for animals in particularly sensitive phases (weaning, early pregnancy, birth, etc.) where the minerals are essential for several vital bodily functions.

There is no available data on whether glyphosate is present in farm animals in quantities that influence the availability of minerals. However, it is important to note that the concentration of glyphosate in blood and tissue is particularly crucial, since that is where minerals need to be available for the formation of enzymes, etc. Even a small reduction in the content of free metal ions in plasma and tissues can be critical. A small deficit in zinc, for example, can cause birth defects and death in animals and can also trigger diarrhoea. The potential effect on the mineral status of glyphosate absorbed via the residual content in livestock feed is not included in the risk assessment of glyphosate.

Conclusions on the general properties of glyphosate and secondary effects on animals
Higher animals do not have the EPSPS enzyme and therefore are not directly affected by the inhibition of the EPSPS enzyme. Higher animals can be affected by glyphosate through its effect on the gut microbiota, including the balance between different bacterial populations (also encompassing the pathogenic bacteria that to various degrees can utilise the glyphosate-insensitive variants of the EPSPS enzyme).

The ability of glyphosate to bind to metals can potentially lead to a mineral shortfall in important biological pathways.

Risk assessment of glyphosate
As previously mentioned, the risk assessment of glyphosate does not include the effect on microorganisms in the gut of farm animals or on their mineral status. It is our assessment that the general metabolic and feeding experiments included in the current risk assessment of glyphosate will not reveal the specific effects of glyphosate residues in feed, neither on the microorganisms in the gut of farm animals on the binding of circulating minerals in the animal, nor on any secondary effects on health. This opinion is based on the facts that specific parameters targeting the mineral status of farm animals and the microbial populations of the gut are not included in the assessment, and that the animals used are not sufficiently sensitive to the influence of these parameters. It is essential that the test animals used are in the relevant physiological and productive stages and sensitive to what is being tested – in this connection, sensitive to effects on mineral status and gut microbiota. A sensitive physiological phase with respect to gut microbiota would for pigs, for example, be just after weaning with the transition from milk to solid feed, and for dairy cows just after calving where the cow's milk production is so high that nutrients originate not only from the diet but also from her body reserves. A sensitive physiological phase with respect to mineral status could, for example, be early pregnancy with extensive cell division and cell differentiation in the

foetus, and after weaning. Such sensitive periods are experienced by farm animals but these conditions are not addressed in the risk assessment.

Hypotheses
The conditions described above lead to the following hypotheses:

• glyphosate can affect the gut microbiota of farm animals with secondary effects on animal production and health

• glyphosate can affect the mineral status of farm animals with secondary effects on animal production and health

Random testing of soy feed products
In the EU, the maximum residue level for glyphosate is 20 mg per kg soybeans/soybean meal; for comparison, the maximum residue level for barley and oats is also 20 mg per kg, but 10 mg per kg for wheat.

In 2009 the Danish Plant Directorate (now part of the Danish Veterinary and Food Administration) conducted a survey of soy-based feedstuffs in order "... to get an idea of the potential link between the genetically modified (GM) status and the level of pesticide residues in products" (http://www.foedevarestyrelsen.dk/Publikationer/Alle%20publikationer/2012106.pdf). Glyphosate residues were found above the permitted level of 20 mg per kg in three out of four lots of GM soy hulls from Brazil/Paraguay (all GM), while no glyphosate residues were found in 13 lots of soybean meal from Argentina and Brazil (9 GM, 3 non-GM, 1 not stated) above a value of 4 mg per kg, which in the investigation is called the limit of detection (i.e., the lowest concentration that can be reliably measured with the method used).

Consumption of glyphosate
Sales of glyphosate in Denmark were 1,314,958, 1,697,942, 812,661, 1,646,562, 1,941,310 and 1,402,520 kg in the years 2007 to 2012, respectively (Miljøstyrelsen (The Danish Environmental Protection Agency), 2013). Statistics on glyphosate consumption at the global level are not immediately available. In Argentina the consumption was 13.9 m litres in 1996 and in 2008 an estimated 200 m litres (Anonymous, 2008). In the USA, the consumption in the period 1996-2011 was estimated to have increased by 239 m kg, solely due to the spread of glyphosate-tolerant soybean, cotton and maize, of which soybean accounts for 70% (Benbrook, 2012). A significant part of the increase is attributed to the development and spread of glyphosate-resistant weeds (Benbrook 2012).

Item 1. Growth and health of livestock

A number of scientific reviews have summarised the existing knowledge on the growth and health of livestock fed non-GM vs. GM feed, the latest of which dates from 2012 (Flachowsky et al., 2012). This article summarises results from 47 experiments with ruminants, 21 experiments with pigs, 61 experiments with poultry and 8 experiments with other animals (including rabbits and fish). The authors conclude that there were no biologically relevant differences between animals fed GM or non-GM feed in any of the experiments. This conclusion is consistent with earlier reviews in this area (e.g., Aumaitre et al., 2002).

In the former Danish Institute of Agricultural Sciences (now part of Aarhus University) an experiment was carried out that compared GM fodder beet with non-GM fodder beet fed to dairy cows (Weisbjerg et al., 2001). The study concluded that there were no differences between GM and non-GM fodder beets in terms of milk production and milk quality; the same applied to a number of hormones and growth factors in blood and milk.

The review articles carry no information on whether the animal feed was based on crops that had been sprayed with pesticide or not. All the primary sources that have been examined in this context likewise carry no information on crop spraying or pesticide residues. Neither is there any information in the Danish study with fodder beets on whether the GM beet, which was Roundup-resistant, had been sprayed, and there is no information on pesticide residues. Due to the lack of information, no conclusion can therefore be drawn from these sources regarding the effect of pesticide residues in animal feed.

The vast majority of studies were of short duration where chronic conditions usually do not have sufficient time to become established. However, a few long-term studies do exist. These have been reviewed by Snell et al. (2012). Most of the experiments used rats and only a couple of them used farm animals, but the conclusion was that there generally are no differences in the health of animals fed GM vs. non-GM feed. A recently published experiment with fattening pigs found, however, that there was a higher incidence of inflammation in the stomach and heavier uterus in pigs fed a GM diet (including glyphosate-tolerant soybean meal and insect-resistant maize) than with a non-GM diet, whereas there was no difference in the incidence of gastric ulcers (Carman et al., 2013). In January 2013, VSP (Danish Pig Research Centre) concluded on its website that "GM soy does not affect gastrointestinal health", and further wrote that "the final results will be published on VSP's website at the turn of the year" (http://vsp.lf.dk/~/media/Files/PDF%20-%20Viden/Viden%20-%20Artikler%20fra%20SVIN/2013/SVIN%20nr%201/Nr1_LJ-NJK-GMO-soja_p%C3%A5virker_ikke_mavesundheden.ashx). VSP has indicated that a major study is under way; so far, no results have been published on their website. There was no information on glyphosate content in any of these studies on pigs.

Conclusion, Item 1
Almost all studies focus on performance, growth and feed conversion. It is to be expected that there will be no differences in these traits, since no genetic modifications have taken place that alter the composition of the feed based on the most commonly used GM crops, i.e., the glyphosate-tolerant crops.

Aspects relating to animal health are only included in a couple of experiments and there are no recordings tracking the most obvious potential effects of glyphosate – diseases that are related to the gut microbiota or micro mineral deficiencies.

There is generally no information on crop spraying or pesticide residues in the literature covering experiments with animals fed GM vs. non-GM feed.

Item 2. Pesticide residues in GM vs. non-GM feed

It has only been possible to find one article that directly compares glyphosate contents in soybean (Bøhn et al., 2014). Here the contents of glyphosate plus its breakdown product AMPA (aminomethylphosphonic acid) were compared for 10 lots of Roundup Ready GM soybeans, 10 lots of conventional soybeans and 11 lots organic soybeans from the United States. GM soybeans contained an average of 9 mg glyphosate incl. AMPA per kg while conventional and organic soybeans contained 0.0 mg per kg. All GM soybean lots contained measurable levels of glyphosate and AMPA. The method of analysis in this study was more sensitive than that used by the Danish Plant Directorate in the previously described study from 2009. Had the study by Bøhn et al. (2014) used the Danish Plant Directorate's maximum residue level of 4 mg glyphosate per kg, only one of the 10 GM lots would have been identified as having a glyphosate residue (not including AMPA), indicating that the choice of analysis method is essential for the study findings and conclusions.

Other studies show that pesticide residues are commonly found in sprayed crops (see for example http://www.fao.org/docrep/009/a0209e/a0209e0d.htm). Only the study by FAO and the above-mentioned study by Bøhn et al. (2014) included results for residual AMPA.

No studies have been found on the effect of AMPA on the EPSPS enzyme in bacteria or on binding to metals. In all likelihood, AMPA does not affect the EPSPS enzyme in either plants or bacteria, since FAO wrote in a statement that a gene which causes glyphosate to be degraded to AMPA is inserted into some GM plants (http://www.fao.org/docrep/w8141e/w8141e0u.htm). This genetic manipulation only makes sense if the GM plants' own EPSPS enzymes are not inhibited by AMPA. With regard to the binding of metals, AMPA presumably still possesses this property, since the section of the glyphosate molecule that binds to metals is also part of AMPA. It is therefore presumed that AMPA is not important for the assessment of the effect of glyphosate residues on bacteria, but that it may be important for the availability of metals.

No records have been found on Roundup quantities used in glyphosate-tolerant GM crops, but an estimated 8 litres per hectare is used in the soy production in Argentina (DanWatch, 2011).

Pesticide residues in non-GM experiments at Foulum
The former National Institute of Animal Science (now part of Aarhus University) sprayed barley fields with Roundup in 1985-87 and found glyphosate residues in barley grain of between 0.8 and 4.2 mg per kg at a Roundup dose of 3 litres per hectare 9-28 days before harvest, and between 2.7 and 16.0 mg glyphosate per kg at a dose of 6 litres Roundup per hectare 2-8 days prior to harvesting. Residues in straw, sprayed with 3 litres of Roundup per hectare 9-13 days before harvest, ranged from 10.0 to 22.0 mg glyphosate per kg. Straw sprayed with 6 litres of Roundup per hectare 3-8 days before harvest contained glyphosate residues of between 42.0 and 51.7 mg per kg (Andersen et al., 1990; Danielsen and Larsen, 1990). It is still common practice to spray crops with Roundup before harvest. It should be emphasised that the high dose of Roundup described above was used in an experimental context. For normal agricultural practice, a dose of around 1000 g of active substance is recommended, corresponding to approx. 3 litres of Roundup per hectare (https://www.landbrugsinfo.dk/Planteavl/Plantevaern/Ukrudt/Kemisk-bekaempelse/Sider/pl_pn_11_408.aspx). It is estimated that in Denmark approx. 10 % of the area with cereal and up to 25 % of the area with rapeseed is sprayed (http://ing.dk/artikel/landmaend-sproejter-med-roundup-kort-foer-hoest-modne-korn-161055). For crops close to maturity and where the chemical is therefore not actively transported into the plant, glyphosate residues will be found on plant surfaces. Cereal grain will therefore likely contain more pesticide residue than the seeds of legumes such as rape.

Conclusion, Item 2
Pesticide residues, including glyphosate, can be expected in animal feed from sprayed crops. Data on glyphosate residues in GM vs. non-GM feed crops and animal feed are scarce and are usually not included in the published results from experiments with GM feed to livestock.

Supplementary literature

It is only in the last few years, that studies have been carried out on the effect of glyphosate on farm animals following the two most obvious pathways: the effect on livestock gut microbiota and the impact on livestock micro mineral status.

Articles on gastrointestinal microorganisms from poultry and cattle
There are only two articles on the effect of glyphosate on gut microorganisms of farm animals: one covering poultry (Shehata et al., 2013) and one covering cattle (Krüger et al., 2013b). Both articles show that several of the pathogenic bacterial groups were less inhibited by glyphosate than the commensal (harmless, non-pathogenic) species. The less inhibited pathogenic bacteria included

Clostridium perfringens, *Clostridium botulinum* and Salmonella, all of which, besides being harmful to animals, include zoonotic strains (strains that can be transmitted from animals to humans by direct contact or through contaminated food). The results from these two articles thus indicate that glyphosate in the feed can result in adverse changes in the gut microbiota.

It should be noted that the two articles are based on *in vitro* studies, i.e., the bacteria are cultured in the laboratory where they were exposed to various concentrations of glyphosate. It is a well-known and widely used technique, but the results here are mainly theoretical and only an indication of what may be going on in the host animals (cattle, pigs and poultry) *in vivo*. It cannot be assumed that the concentration required to obtain an effect is the same for an *in vitro* technique in the laboratory technique and an *in vivo* experiment with the host animal.

Article on the mineral status of Danish cows
Blood and urine from 30 dairy cows from eight herds were tested (Krüger et al., 2013a). Glyphosate was found in the urine of all cows and the herd averages ranged from 10 to 100 ng per ml. Blood concentrations of the micro minerals cobalt and manganese were below the specified minimum reference levels. The low level of cobalt could indicate a deficiency in vitamin B12, of which cobalt is a central element. The study shows that there is widespread use of animal feed with glyphosate residues. Due to the concurrence of glyphosate in the urine with low levels of cobalt and manganese in the blood, a link between glyphosate and micro mineral status cannot be ruled out, but this relationship has not been directly documented.

Conclusions on supplementary literature
It has only been very recently that studies have been published on the effect of glyphosate on farm animals via the two most obvious pathways, i.e., the gastrointestinal pathway and the micro mineral pathway. These studies suggest that glyphosate can have an adverse effect via both of these pathways.

The above-mentioned studies on the effect of glyphosate on farm animals support the hypotheses formulated in the "Introductory remarks".

Item 3. The need for new feeding experiments

Against the background of (i) the known effects of glyphosate, i.e., the impact on microorganisms and binding of minerals, (ii) the growing consumption and documented content of glyphosate in GM soybeans and in the urine of Danish farm animals and (iii) the results from new *in vitro* laboratory studies, we deem that there is a need for experiments to test the two hypotheses. The experiments should include both *in vitro* laboratory experiments with bacterial strains and experiments with animals. An optimal and detailed experimental protocol must be carefully designed, and should include GM soybean meal with known concentrations of glyphosate and cereal sprayed before harvest with known concentrations of glyphosate and unsprayed cereals.

It is essential that experiments with farm animals include measurements of glyphosate in feed, blood, gastrointestinal contents and urine. Likewise, it is essential that potential disruptions to the microbiota of the gut are measured and that the micro mineral status (content and enzyme activity) of the animals is measured, especially manganese and cobalt, but also others such as zinc.

Item 4. The need for studies on pesticide residues and quality parameters in GM vs. non-GM feed

There is very little available information on pesticide residues in feed based on herbicide-resistant GM plants. There is a considerable need to provide this information, particularly information on glyphosate in feed from the very widespread use of glyphosate-tolerant GM soy, and there is a need for more information on glyphosate residues in cereals sprayed before harvest.

It is uncertain to what extent glyphosate-treated crops have a lower mineral content and thus contribute to a declining mineral supply for livestock. In a recent review, Duke et al. (2012) found that 11 out of 21 studies reported no effect of glyphosate on the mineral status of the plant, while the remaining 10 studies reported the opposite. A potential effect of glyphosate on the mineral content of forage plants is not included in the risk assessment of glyphosate. On this basis, and bearing in mind the importance of minerals for vital functions in livestock, there is a need to generate knowledge about the contents of micro minerals such as manganese, cobalt and zinc in crops sprayed with glyphosate.

Literature

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Appendix 1

Item 5. Observations of Danish farmers

As requested by the Danish Ministry of Food, Agriculture and Fisheries, observations from two farmers and one veterinarian have been included and commented below:

Farmer 1: Egg producer

The observations of the egg producer were recorded during a farm visit. This farm has produced consumer eggs since 2004, and before then brood eggs. The production is conventional, and there are no plans to change to organic production.
The egg producer had problems with dirty eggs, maybe because of diarrhoea. He mixes his own feed in a home mixer. The main ingredients in the feed are besides soy meal approx. 30 % maize, approx. 40 % wheat, soy oil, roach and vitamin/mineral mixture. Soy oil, maize and wheat have always been non-GMO. The egg producer changed from GMO soy meal to non-GMO soy meal as protein source in the feed mixtures in February 2012 and has since then only used non-GMO soy meal. The change in soy source was the only change in feed source, but the percentage of soy meal in the feed was simultaneously reduced from 20.0 to 18.5. Since then the egg producer has gradually reduced the content of non-GMO soy meal to 14 % of the feed without any effect on the egg production (average number of eggs per laying hen). There are no data on the residual content of glyphosate in the used lots of soy meal, and no feed samples have been saved. Therefore, analyses for glyphosate cannot be made. The egg producer explained that management, housing etc. had not been changed when switching from GMO to non-GMO soy meal.

Observations in connection with change from GMO to non-GMO soy meal

- The change was made on a Thursday and a change in the eggs was observed – cleaner – already on the following Sunday
- Subsequently it was observed that the bedding became drier/less spongy over a 1 to 2-month period
- A lower water consumption after the change was observed, from approx. 1.7-1.9 litres of water per kg feed to approx. 1.5-1.6 litres per kg feed
- The chickens were less stressed after the change – were e.g. not disturbed by noise and shouting
- Less feather pecking after the change. An employee at the Knowledge Centre for Agriculture (section for poultry) has evaluated the plumage of 72-week-old chickens and given the mark 20 on a scale from 0 to 20, where a mark of approx.12 was expected
- It was observed that the average number of eggs per laying hen increased after the change
- The chickens are culled at 95 weeks after the change against 72 weeks before the change. The culling happens later because the egg production per laying hen can now be maintained at a sufficiently high level until the age of 95 weeks

Comments concerning the egg producer
The egg producer has really made two changes, namely first the change from GMO to non-GMO soy meal and subsequently a reduction in the protein content of the feed. The reduction in the percentage of soy meal in the feed from 20.0 to 18.5 simultaneously with the change from GMO to non-GMO soy meal is based on the fact that non-GMO soy meal contains more protein than GMO soy meal (according to information from the feedstuff industry typically approx. 3 per cent more). The extra reduction to 14 % without a decrease in the egg production cannot be directly explained from feed analyses. Feeding plans both before and after the change to non-GMO soy meal do not give rise to believe that feeding in itself could have caused the problems observed.

Diarrhoea

The quick change in the cleanness of the eggs could be related to less diarrhoea immediately after the first of the two changes, i.e. the change from GMO to non-GMO soy meal. The subsequent development towards drier bedding could be related to the lower water consumption. As far as the lower water consumption is concerned this could be related to the further gradual reduction in the allocation of soy meal since a decrease in the feed content of raw protein in itself reduces the water absorption.

The observation of more diarrhoea before the change to non-GMO soy meal could be a result of unfavourable bacteria with non-glyphosate sensitive variants of the EPSPS-enzyme being enhanced in the gastrointestinal system of the chickens, thus affecting the balance between the different bacterial types favourably. This interpretation of the observations in connection with diarrhoea is supported by the previously mentioned laboratory experiment where unfavourable (pathogen) bacteria were less hampered by glyphosate than favourable bacteria (Shehata et al., 2013). However, the interpretation depends on there having actually been a residual content of glyphosate in the GMO soy meal used.

Stress
Observations concerning stress and feather pecking could be related to the fact that less stress results in less feather pecking. An improved gastrointestinal health together with the lower amount of stress after the change can explain the higher egg yield and increased longevity. Foster and Neufeld (2013) state that microorganisms in the gastrointestinal system can affect the nervous system and behaviour via the gastrointestinal/brain axis.

Conclusion
It is always difficult to be definite about actual effects when observations in practice are concerned, where seasonal effects, for instance, cannot be taken into account. Thus, one cannot preclude the possibility that conditions observed by the egg producer can change over time from unknown and undescribed reasons. It cannot be immediately rejected, however, that there is a correlation between some of the observations made by some of the egg producers and the change from GMO to non-GMO soy meal.

Farmer 2: Pig producer

Observations, registrations and analyses from the pig producer come from material received from a conventional sow herd with approx. 450 animals that changed to GMO-free soy in April 2011 – later, however, for the weaned pigs in order to use up stock. The farmer read specialist literature on the subject, and the article on damage to frog and chicken foetuses (reference below) convinced him that glyphosate caused the deformities he observed in his new-born pigs. To keep an eye on potential glyphosate in the feed, the farmer subsequently had feed samples analysed, but only in the period after having changed to non-GMO feed. The measurements were extended to also include tissue samples from deformed foetuses and manure and urine samples from sows. The analyses were made in a German university laboratory. The farmer has taken photos of the deformed pigs at birth and also has the usual registrations of litter size and other reproduction measurements.

The pig producer has had a Norwegian scientist (Thomas Bøhn from the University of Tromsø) organise and carry out the statistical analyses of the registrations concerning glyphosate content in the feed, litter size and deformities in new-born pigs.

Observations and registrations/measurements in connection with the change from GMO to non-GMO soy meal
- A year after the change to non-GMO feed the use of medication has decreased to a third compared to before the change

- Reduction in diarrhoeal problems in piglets and weaned pigs
- No gastric problems (swollenness) in sows
- Increased longevity, i.e. the sows can maintain a herd average of live born pigs in 8 litters against previously 5 litters
- Weans 1.8 pigs more per litter after change to non-GMO feed

Observations and registrations in relation to glyphosate content in the feed
- Has received a lot of barley with a glyphosate content of 2.8 mg per kg and lots of non-GMO soy meal with up to 1.2 mg glyphosate per kg after the change to non-GMO feed
- Is now very careful not to get grain from fields sprayed with Roundup before harvest
- More cases of abortion in sows at a feed content of glyphosate above 1 mg/kg compared to a content of 0.2 mg/kg
- More pigs born per litter
    - 0.8 pigs more per litter when glyphosate in the feed was below 0.6 mg/kg compared to a content of above 0.6 mg/kg (statistical analyses carried out by Thomas Bøhn)
- A number of deformities registered in new-born pigs
    - The frequency of deformities was approx. twice as high at a feed content of glyphosate above 1 mg/kg compared to a content of 0.12 mg/kg (statistical analyses carried out by Thomas Bøhn)
- Glyphosate (ng/g) was measured in a number of tissues from deformed pigs (among others lungs, liver, kidney, heart)
- Glyphosate concentrations in the urine from 2.8 to 44.8 ng/kg were measured in sows fed glyphosate concentrations from 0.21 to 1.13 mg/kg
- Glyphosate concentrations in the manure of 0.25 mg/kg were measured from a sow receiving feed with a glyphosate concentration of 1.13 mg/kg

Comments concerning the pig producer
There are two sets of observations, namely (1) those concerning before versus after the change to non-GMO feed and (2) those concerning glyphosate in non-GMO feed. It is likely that a potential effect of glyphosate consumed with the feed is the same, whether it is consumed with GMO or non-GMO feed. Possible correlations to glyphosate in the feed can thus be considered generally valid, whether the feed is GMO or non-GM.

The observations concerning reduction in diarrhoea are in line with the observations made at the egg producer, and the explanation can be, as with the egg producer, that glyphosate affects the balance between different bacterial types unfavourably. The increased longevity of the sows can be explained by a generally higher health status, which is also reflected in the lower use of medication.

The observations concerning reproduction conditions, i.e. abortions, deformities and number of born pigs per litter indicate a possible correlation with concentrations of glyphosate in the feed via the previously explained binding of minerals. It has long been known that lack of zinc during pregnancy results in deformities in new-born mammals (Hurley & Swenerton, 1966). The suggested correlation could possibly be due to a low content of zinc or other micro minerals. Thus, Zobiole et al. (2010) have found that the contents of zinc and other minerals are lower in glyphosate-sprayed GMO soy beans compared to beans from non-sprayed GMO soy. Further, the occurrence of glyphosate in the tissue of deformed, new-born pigs and in the urine of sows shows that glyphosate is absorbed, circulates in the body and is deposited. Therefore, it does not preclude the possibility that undersupply of micro minerals happens because glyphosate binds these minerals, and that the bound minerals are not available for important biological processes in the animals.

The possibility of a correlation between glyphosate and deformities of foetuses/new-borns has been investigated in a laboratory study where it was found that the effect of glyphosate lead to an abnormal development of frog and chicken foetuses (Paganelli et al., 2010). This study further showed a low activity of a zinc-containing protein (transscription factor), which is necessary to decode the gene DNA. Zinc-dependent enzymes with key functions in cell division and differentiation also lose their function in case of zinc deficiency (Hambidge et al., 1986).

Conclusion
As in the case of the egg producer, it does not preclude the possibility that the conditions observed by the pig producer could have changed over time from unknown reasons. A correlation between the observations of the pig producer and the change from GMO to non-GMO soy meal cannot be rejected, however. Likewise it cannot be rejected that there is a correlation between the observations of the pig producer and the residue of glyphosate found in the feed. It is thus most likely that an effect is primarily connected with a residual content of glyphosate in the feed, rather than the GMO crop being the provoking factor.

Veterinarian

A pig specialist veterinarian has been consulted by phone. The veterinarian reports that more cases of piglet diarrhoea began to appear 5 to 10 years ago, particularly within the first 1 to 3 days – and especially in piglets from first-parity sows. These cases cannot be cured by the conventional antibiotics. In the scientific literature the disease is described as New Neonatal Porcine Diarrhoea – NNPD, and in Denmark the disease is called "the yellow death". The disease has been recorded in many countries. The veterinarian states that there are differences in management, feeding, breeding material etc. in the different countries where the disease has been recorded. On the face of it, according to the veterinarian, the most essential common feature seems to be the use of soy meal as protein source. The veterinarian further states that the appearance of the new piglet diarrhoea coincided with the prevalence of GMO soy.

Comments concerning the veterinarian
Documentation is necessary to verify the suspicion of a correlation between GMO soy and the new type of piglet diarrhoea and the role of glyphosate in this connection. A report from the former Danish Institute of Animal Science, where sows were fed cereals sprayed with Roundup (Danielsen and Larsen, 1990), contains a description of a litter of piglets with symptoms like those of "the yellow death" described by the veterinarian.

Overall evaluation of the observations of two Danish farmers

The observations cannot be regarded as documentation of a correlation with the GMO status and glyphosate content of the feed. The observations are, however, supported by literature on the subject. Nevertheless, this literature is scarce and has only recently focused on the two most natural pathways of the effect of glyphosate, i.e. the effect on microorganisms in the gastrointestinal system and the micro mineral status of farm animals. It is remarkable that the observations of the farmers have primarily been made in farm animals that are in a sensitive phase (e.g. weaning diarrhoea, deformities and abortions). The observations of the Danish farmers may thus contribute to illustrating the need for the investigations mentioned under points 3 and 4.